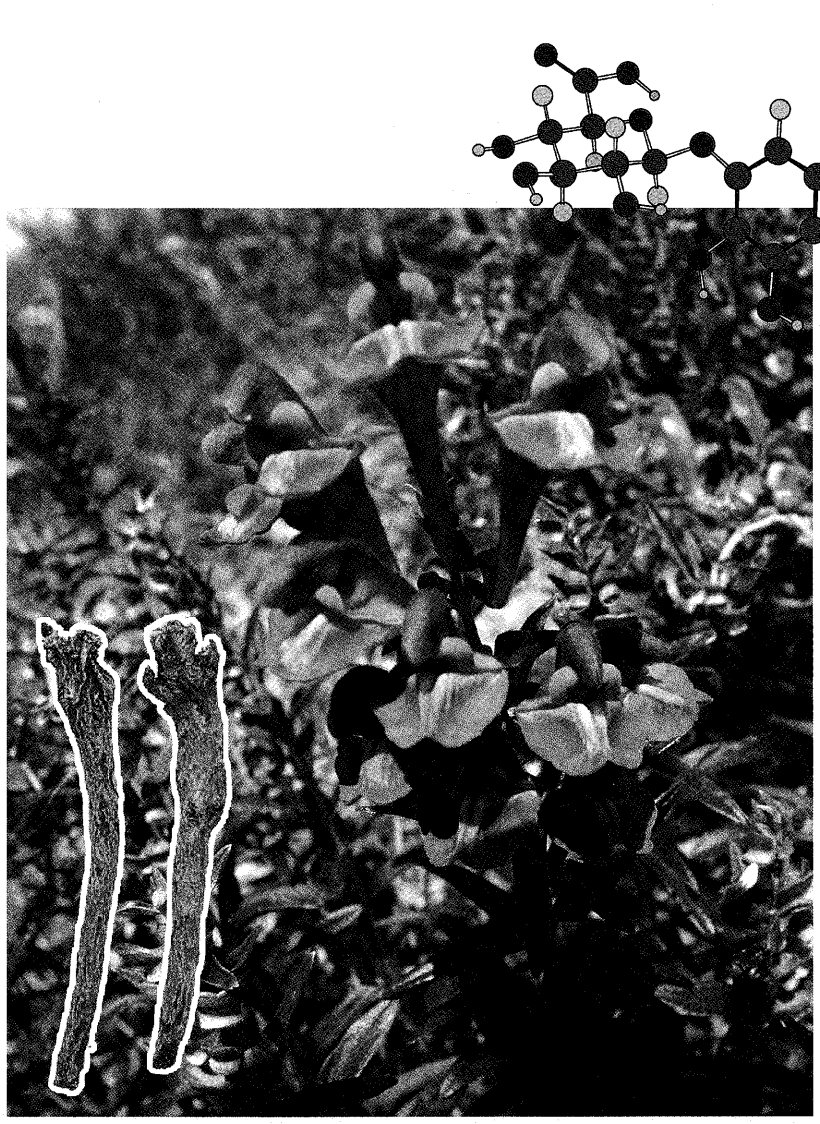
和漢薬研究所年報

富山医科薬科大学
第31巻 2004年



Annual Report
Institute of Natural Medicine
Toyama Medical and Pharmaceutical University

Vol. 31 2004

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富山医科薬科大学
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富山医科薬科大学
和漢薬研究所

富山医科薬科大学・和漢薬研究所年報　31巻 2004年

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表紙の写真

シソ科の *Scutellaria baicalensis* Georgi コガネバナ，黄芩，baicalin（モンゴル国ドルノド県にて，2004年7月15日，小松かつ子撮影）

黄芩は消炎，解熱薬などとして用いられる重要な漢薬で，柴胡と組み合わせて「柴胡剤」，黄連と組み合わせて「瀉心湯類」と総称される漢方方剤を形成します。小柴胡湯服用時の副作用が問題になった時に，黄芩の野生品と栽培品の品質の差異が議論されました。黄芩に多く含まれ，抗炎症・活性酸素捕捉作用のある baicalin は，腸内細菌により baicalein に代謝されますが，腸管で再度 glucuronide 抱合され，baicalin になります。この循環過程の生化学的検討が進んでいます。

研究所年報　巻頭の言葉

富山医科薬科大学が「国立大学法人富山医科薬科大学」に移行し，法人化にともなった変化と言えば，しきりと外部資金の獲得，特許に結びついた研究の奨励，企業人の受け入れや企業との共同研究の奨励など，一昔前ならば企業に癒着していると批判されたであろう事柄が常識化し，戸惑いの感じられる今日この頃である。和漢薬研究はどちらかと言えば地味な学問と考えられていたが，健康食品産業が和漢薬の素材に熱い関心を寄せており，韓国や欧米のベンチャー企業の研究所訪問も多くなってきている。また，ヒトの遺伝子発現やタンパク生成の網羅的解析が東洋医学の診断に応用できるか否かも，重大な関心事になりつつあり，先端科学との距離も徐々に縮まりつつある。

一方，欧米を中心にしたcomplimentary and alternative medicine（CAM）の動きはアジアの伝統医学にも刺激となり，澳門での国際中医学会会議，韓国大邱での中・韓・日の国際東方医学会議の開催と西欧型のCAMに対抗して，アジアでのイニシアティブ確立を目指した動きが目立つようになっている。和漢薬研究所はどちらからも勧誘を受けありがたい事とは思うが，クールな目で推移を見守っている状況である。

地域貢献の観点からは，今年度立ち上がった富山県の寄附講座「和漢薬製剤開発部門」（谿　忠人教授）はオリジナルブランド配置薬の蓼王（パナワン）の開発に成功し，富山県配置薬業再興の切り札と目されている。研究所40年の歴史のなかでも特記すべき事と思われる。

本年度は門脇　真（消化管生理学分野），松本欣三（複合薬物薬理学分野），小松かつ子（生薬資源科学分野）の新教授が誕生し和漢薬研究所の若返りが図られたが，今後の研究の発展を期待したい。また，平成17年10月に行われる富山県の3国立大学の合併を機に「和漢医薬学総合研究所」と研究所名を改める予定である。総合的観点から伝統医学の長所を解き明かし，現代医療に役立てることを理念とする研究所を目指すつもりである。

平成16年12月

和漢薬研究所　服部征雄

総　　説

コカインと創薬
—麻薬からイノヴェイティブな新薬へ—

門脇　真
和漢薬研究所　消化管生理学分野　教授

1．コカイン[1,2,3]

コカイン cocaine は，アンデス山脈原産のコカ Erythroxylon coca という喬木の葉に多量（0.6-1.8%）に含まれているアルカロイドである。古代ペルーのインディオ達にとって，「コカの木は飢えた人を充分満足させ，疲れた人に新たな力を与え，不幸な人々に悲しみを忘れさせる神からの授かり物である。」と考えられ，何世紀もの間用いられてきた。

コカインは無色の結晶又は白色の結晶性粉末で，無臭で苦みがあり，現在では「麻薬及び向精神薬取締法」で麻薬として規制されている。コカインには覚醒剤と同様の神経興奮作用があり，ヒトでは，まず気分が高揚し多幸感が現れ，眠気や疲労感がなくなり，体が軽く感じられ，腕力，知力がついたという錯覚が起こる。しかし，中枢興奮作用の後，まもなく抑制が認められ，乱用を続けると幻覚などの精神障害が現れたり，虫が皮膚内を動き回っているような不快な感覚に襲われて，実在しないその虫を殺そうと自らの皮膚を針で刺したりすることもある。また，コカインを大量に摂取すると，延髄中枢が抑制され呼吸困難により死亡することがある。

コカイン摂取の一番の問題は，中枢神経興奮作用によって幻覚や妄想などの精神症状（精神毒性）を呈し攻撃行動を起こすことから，凶悪犯罪の原因になることである。この意味で，コカインは社会問題惹起薬物のトップにランクされているのである。

2．コカインの局所麻酔薬としての臨床応用[1,2,3]

古代ペルーのインディオ達は，コカの葉に麻酔作用のあることを知っており，開頭術などの難しい外科治療の際に用いていた。コカの有効成分であるアルカロイドは，1855年にドイツの Gadicke よって分離された。さらに1860年ドイツの A. Niemann が純粋な分離に成功してコカイン cocaine と命名し，舌の表面を痺れさせ感覚を麻痺させる作用があることを報告したが，この重要な発見は当時，あまり注目されなかった。1880年になり，Von Anrep はコカインの希釈液を自身の腕の皮下に注射し，注射部位の皮膚が刺激に対して無感覚となることを観察し，局所麻酔薬として臨床応用を推奨した。

コカインを初めて臨床に応用したのはウィーンの外科医の Karl Koeller である。1884年に，彼は蛙とイヌの眼にコカインを一滴たらし，刺激を加えて局所麻酔作用を確認した。それからまもなく，1884年9月11日立会人なしで秘密裏にコカインを用いて，白内障の手術に行い，その成功の喜びに浸ったとされている。その後，眼の局所麻酔法としてコカイン麻酔は広く受け入れられた。

Koeller の局所麻酔法の発見の話はすぐに米国に伝えられ，Johns Hopkins 大の W. Halsted はコカインが神経幹での伝導を止めることを実証し，外科学における神経遮断麻酔の土台を作った。1885年の終わり頃，Halsted は，共同研究者が激しい歯痛に襲われたとき，コカインを彼の下歯槽神経に注射したところ，顎は局所的に約25分間無感覚になり，その間に歯を痛みもなく抜くことができた。

さらに，New York の L. Corning は，1885年，コカインをイヌの胸椎下部の棘突起間より注入して脊椎麻酔の実験を行い，後肢の麻痺を観察した。これらの発見の後，コカインに関する研究が広範囲に進められ，歯や下顎の手術，手足の手術，ヘルニアの手術などがコカイン麻酔のもとで行われた。しかし，同時にコカインの中毒症状から沢山の患者が死亡する結果を招いた。

3．合成局所麻酔薬プロカイン及び抗不整脈薬プロカインアミドの登場[1,2,3]

局所麻酔薬の作用機構である電位依存性 Na^+ チャネルに対する直接的阻害作用を持つコカインの合成代用品に関する研究は，分子修飾の典型的な例として，しばしば取り上げられる。ドイツの Einhorn らにより，コカイン分子のメトキシカルボニル基をはずし，七員環構造を開裂させるという段階的な操

作によって，中枢神経系にダメージを与えずに局所麻酔活性にかかわる分子の活性部位が見いだされ，エステルのアルコール部分の末端に第三級アミノ基をもつ数百の安息香酸エステルが合成され，1905年，プロカイン procaine が得られた。プロカインは，歴史上初めて使われた合成局所麻酔薬である。

1936年になり，Mautz はプロカインを塗布すると心室筋の電気刺激閾値が上昇することを示し，その後，プロカインの心臓作用は Na^+ と K^+ の膜透過性を直接変化させるクラス IA 抗不整脈薬であるキニジン quinidine の作用と類似していることが明らかとなった。そこで，プロカインの臨床上の問題点である，速やかに酵素的加水分解を受けること及び中枢神経系に強い作用があることを克服するため，プロカイン類似化合物の系統的研究が行われた。そして，1951年 Mark らにより，プロカインのエステル結合部分をアミド結合に置き換えた化合物であるプロカインアミド procainamide が見出された[4]。現在，プロカインアミドは抗不整脈薬として臨床で使用されている。

4．消化管運動亢進薬メトクロプロミドの発見[5,6,7]

抗不整脈薬の改良研究のため，フランス Delagrange 社の研究陣はプロカインアミドのベンゼン環に修飾を加え，2位にメトキシ基を5位にクロル基を入れることにより，1964年に偶然にも消化管運動亢進薬メトクロプロミド metoclopromide を開発した。このようにして生まれたメトクロプロミドからは局所麻酔作用や抗不整脈作用は殆んど失われ[8]，それまで全く予想もしなかったことに，ドパミン dopamine 受容体アゴニストであるアポモルフィン apomorphine を含む様々な催吐刺激による嘔吐を抑制することが明らかとなった[9]。

さらに，上部消化管の不定愁訴を改善することが明らかとなり[10]，様々な原因による嘔吐，逆流性食道炎，胃運動不全，Non-ulcer dyspepsia，術後胃運動不全，慢性胃炎などを改善する全く新しいタイプの消化管運動亢進薬として，広く臨床応用されるようになった[11]。しかも，その後メトクロプロミドに化学的修飾を加えることにより，数多くの新薬が誕生した[5,6,7]。メトクロプロミドは日本ではプリンペランの商品名で長く親しまれている。

5．新規消化管運動亢進薬を求めて[5]

広く臨床応用されるようになったメトクロプロミドであるが，その作用機序は不明な点が多かった。当時，ドパミンが消化管運動を抑制することは知られており，メトクロプロミドは胃及び上部小腸におけるドパミンの運動抑制作用に拮抗すること[12]，アポモルフィンを含む様々な催吐刺激による嘔吐を抑制することから，ドパミン受容体を阻害することにより消化管運動亢進作用が発揮されると考えられた。しかし，消化管におけるドパミン受容体及びドパミン神経の存在が現在でも良くわかっていないことなどより，さらに薬理学的な検討が行われた。そして，メトクロプロミドが直接的なアセチルコリン acetylcholine 様作用を示さないこと，運動亢進作用がアセチルコリンのムスカリン受容体アンタゴニストであるアトロピン atropine により抑制されること，運動亢進作用が迷走神経切除により影響されないこと[13]などにより，メトクロプロミドが腸管神経系に作用して内在性のアセチルコリンの放出を促進することが，作用機序の本体と考えられた[14]。作用機序の解明と平行して，消化管運動亢進作用を optimize する研究も精力的に行われた。その過程で合成されたのが celebopride であるが，celebopride はメトクロプロミドよりも強力なドパミン受容体拮抗作用を持つが，その消化管運動亢進作用はほぼ同等であった[5]。

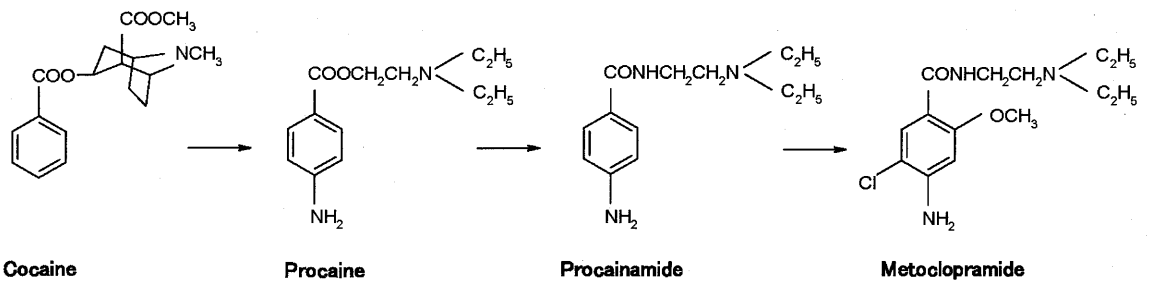

図1　コカインから誘導された医薬品

さらに研究が進められた結果，1985年，ベルギーのJanssen社で，強力な消化管運動亢進作用を持つシサプリドcisaprideが見出された[15]。シサプリドはreceptor binding assayで，ドパミン受容体と殆んど結合せず，しかもアポモルフィン誘発嘔吐に抑制作用を示さなかったことより，ドパミン受容体拮抗作用は殆んどないと考えられた[5]。さらに，シサプリドは直接的なアセチルコリン様作用を示さないにもかかわらず，消化管運動亢進作用はメトクロプロミドよりも20-200倍も強力であり，しかもメトクロプロミドはじめとする今までの消化管運動亢進薬が作用を示さなかった下部消化管にも運動亢進作用を示すことが明らかとなった[5]。しかしながら，その詳細な作用機序は不明のままであった。

その後，シサプリドは世界各国で臨床応用され（日本での商品名はアセナリン及びリサモール），消化管運動亢進薬のstanderd drugになり，日本でも発売以来11年間使用された。しかし，2000年に米国でQT延長を起こす可能性のある重篤な心血管系副作用が相次いで報告され，ついに発売中止となってしまった。結局，シサプリドは，その基本骨格とした抗不整脈薬プロカインアミドに稀に認められる副作用を克服できなかったのである。しかしながら，日本で開発されたシサプリドを修飾したモサプリドmosapride（商品名ガスモチン），メトクロプロミドを修飾したイトプリドitopride（商品名ガナトン，ドパミン受容体拮抗作用及び抗コリンエステラーゼ作用）は，消化管運動亢進薬として現在も臨床応用されている。

6．セロトニン（5-HT）受容体とメトクロプロミド

セロトニンserotonin（5-hydroxytryptamine, 5-HT）はバナナ，アボガド，ナスなどの植物や動物など，広く天然に存在するインドールアルキルアミンである。動物では，約90%が消化管，特に腸クロム親和性細胞（EC細胞）にあり，約1-2%が肥満細胞や神経組織に存在する。1965年，米国コロンビア大学のMichael D. Gershon教授により，セロトニンが腸管神経系の神経伝達物質であること[16]が証明されて以来，第三の自律神経系である腸管神経系の研究が急速に展開し，神経消化器病学というニューフロンティアが切り開かれた[17]。Gershonらの精力的な多くの研究[17]によって，腸管神経系での神経伝達におけるセロトニンの重要性が解明され，さらにEC細胞及び肥満細胞でのセロトニンの生理学的，病態生理学的役割が明らかにされると，セロトニンが腸管の恒常性維持において最も重要な役割を果たしているmolecularであることに異論を挟む研究者はいなくなった。

1970年代まで，セロトニン（5-HT）受容体はGaddumらにより，D受容体（後の5-HT_{2A}受容体）とM受容体（後の5-HT_3受容体）に分類されていた。D受容体は消化管や子宮の平滑筋に存在し，その反応はLSDおよびdibenzylineによって遮断された。また，M受容体は副交感神経（コリン作動性神経）に存在し，その作用は節後神経からのアセチルコリンの遊離を介して発揮されると考えられ，モルヒネmorphine及びコカインによって拮抗された。

その後，放射性リガンドを用いた結合実験により，5-HT_1及び5-HT_2の2種類の受容体サブタイプに分類された[18]が，M受容体はこれには分類されない第3の受容体として残った。

一方，メトクロプロミドの作用機序の研究は精力的に続けられ，ついにはセロトニン受容体との関連も検討され始めたが，明快な答えは得られなかった。1970年，Bianchiらはモルモット腸管を用いて，セロトニンのコリン作動性神経に対する作用がメトクロプロミドで遮断されることを見出した[19]が，この結果はしばらく注目されることはなかった。その後，Fozardらは，コカイン及びメトクロプロミドを含む関連物質が神経原性のセロトニン反応を抑制することを見出し[20,21]，メトクロプロミドがセロトニン

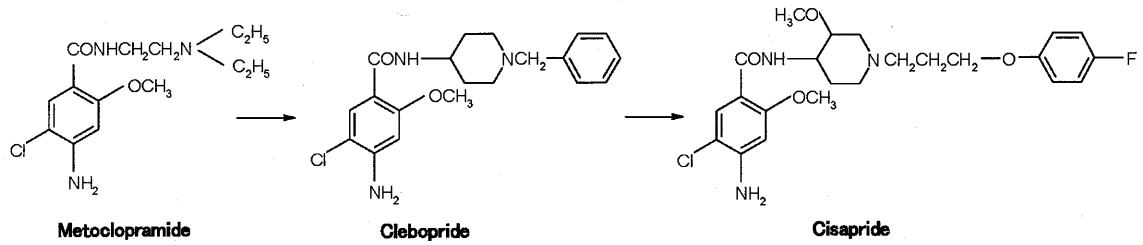

図2　メトクロプロミドから誘導された消化管運動亢進薬

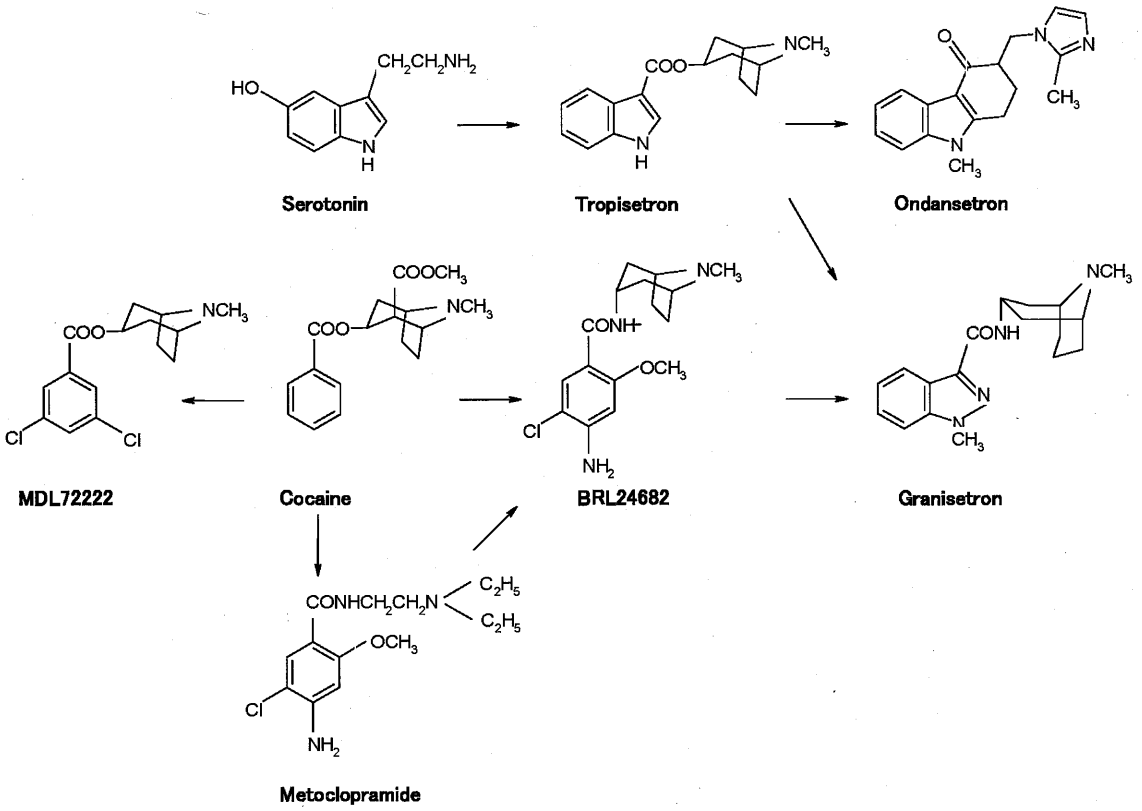

図3 コカイン，セロトニン，メトクロプロミドから誘導された5-HT$_3$受容体拮抗薬

受容体，特にM受容体を阻害することを示唆した。

これとは対照的に，Kilbingerらはモルモット腸管を使った実験で，メトクロプロミドがセロトニンと類似の運動亢進作用を有していること，及びメトクロプロミドの作用がセロトニン受容体の脱感作により消失することより，メトクロプロミドはセロトニン受容体のアゴニストであると主張した[22,23]。当時は，このようなメトクロプロミドの作用の矛盾を説明することはできなかった。

7．5-HT$_3$受容体と5-HT$_4$受容体

7.1. 5-HT$_3$受容体

M受容体に関する研究は，コカインもメトクロプロミドもその選択性に大きな問題があったため，選択的拮抗薬の研究が精力的に進められた。そして，ついにコカインを出発原料として米国のMerrell Dow社からMDL72222[24]が，スイスのSandoz社からICS205-930（トロピセトロンtropisetron）[25]が選択的M受容体アンタゴニストとして相次いで発表され，M受容体は5-HT$_3$受容体と呼ばれるようになった[5,6,7]。

この5-HT$_3$受容体がさらに脚光を浴びるのは，それから間もなくであった。「悪心・嘔吐」は，食中毒やアルコール類の飲過ぎ等による一過性の症状だが，各種薬剤による副作用としても生じる。臨床で大きな問題となっていたのは，「癌化学療法剤投与に伴う消化器症状（悪心，嘔吐）」であった。癌化学療法を受ける癌患者の約85%に悪心・嘔吐が発生し，患者にとって最も耐えがたい苦痛であったが，その抑制は極めて困難であり治療の大きな妨げとなっていた。すなわち，癌化学療法剤シスプラチンcisplatin誘発嘔吐は，制吐剤であるメトクロプロミドの通常用量投与，ドンペリドンdomperidoneなどのドパミン受容体拮抗性制吐剤の通常用量投与及び大量投与では全く抑制されなかったが，一筋の光明としてメトクロプロミドの大量投与で始めて不十分ながらコントロールされることが臨床的に見出された[26]。

そこで，イギリスのBeecham社の研究陣はメトクロプロミドが高用量でM受容体，すなわち5-HT$_3$受容体を弱いながらも阻害すること，及びドンペリドンには5-HT$_3$受容体阻害作用が全くないことより，5-HT$_3$受容体拮抗薬が癌化学療法剤誘発嘔吐に効果があるのではないかと考えた。そこで，フェッレット（いたちの仲間。マウス，ラット，モルモットは吐くことができない動物であるが，いたち，イヌ，ハトは吐くことができるので嘔吐の薬理学的実験に用いられる）を用いてシスプラチン誘発嘔吐に対する薬物の効果を検討したところ，

metoclopromide（大量投与）と5-HT_3受容体選択的拮抗薬MDL72222に，強力な制吐作用を見出した[27,28]。従って，この結果より癌化学療法剤誘発嘔吐は，癌化学療法剤の毒性により腸クロム親和性細胞から遊離したセロトニンが腸管粘膜にある迷走神経求心路神経終末上の5-HT_3受容体に結合し，その刺激が延髄にある嘔吐中枢に達し嘔吐が発現すると推定された[29]。

この劇的な効果が公表されると各社は直ちに臨床試験を開始し，翌年にはLancetにトロピセトロン[30]とオンダンセトロンondansetron[31]の臨床試験結果が発表された。5-HT_3受容体拮抗性制吐剤が登場して以来，癌化学療法剤誘発急性嘔吐症状は80%以上制御可能となり，副作用発現頻度も1～8%と劇的に少なくなった。悪心・嘔吐治療剤の市場規模（2003年度）は，世界全体で推定約2100億円で，オンダンセトロン（商品名ゾフラン，売上高1483億円）が約70%のシェアを占めている。

7.2. 5-HT_4受容体

コカイン及びメトクロプロミドのセロトニンM受容体拮抗作用（benzamide系の消化管運動亢進薬メトクロプロミド，シサプリド，BRL24924，モサプリドなどには弱いながらも5-HT_3受容体拮抗作用がある）から，5-HT_3受容体拮抗薬オンダンセトロンやグラニセトロンgranisetroneが誕生し，癌化学療法剤治療に画期的な変化をもたらした。しかしながら，メトクロプロミドはセロトニン受容体アゴニストとしても作用すると考えられていた[22,23]。

5-HT_3受容体拮抗性制吐剤の華々しい成果による騒々しい狂想曲がまだ鳴り止まない1988年，フランスのDumuisらにより，マウス初代神経培養細胞においてアデニル酸シクラーゼを活性化しcAMP産生を促進する新しいタイプのセロトニン受容体が発見され，5-HT_4受容体と命名された[32]。さらに，翌1989年，彼らは，驚くべきことに，benzamide系の消化管運動亢進薬メトクロプロミド，シサプリド，BRL24924が5-HT_4受容体のアゴニストであることを報告した[33]。これに対し，コカイン及び5-HT_3受容体拮抗薬グラニセトロン，MDL7222，オンダンセトロンには5-HT_4受容体に対する作用は認められなかった[33]。

ここに，メトクロプロミドのセロトニン・パラドックスは解け，メトクロプロミドは，5-HT_3受容体拮抗薬であるとともに5-HT_4受容体刺激薬であり，癌化学療法剤誘発嘔吐に対する抑制作用は5-HT_3受容体拮抗作用により，消化管運動亢進作用はドパミン受容体拮抗作用及び5-HT_4受容体刺激薬によることが明らかとなった。

7.3. 腸管にあるセロトニン受容体

現在，セロトニン受容体は，5-HT_1受容体から5-HT_7受容体までの7つに分類されているが，サブタイプは15種類以上あると考えられている。このうち，腸管には5-$HT_{1A,\ 1p,\ 2A,\ 2B,\ 3,\ 4,\ 7}$受容体の7つのサブタイプがあるとされており，それぞれの受容体に関する生理学的及び病態生理学的研究，さらには創薬科学研究は世界中で精力的に行われている。

なかでも5-HT_3及び5-HT_4受容体は，腸管機能において特に重要な役割を果たしていると考えられている。5-HT_3受容体アゴニスト及び5-HT_4受容体アゴニストは共に腸管運動を強力に亢進させ[34,35]，両受容体を同時に阻害することにより腸管運動は強く抑制される[35,36,37]。また，5-HT_3受容体はイオンチャネル内臓型受容体で，アゴニストは腸管神経に活動電位を発生させ[38,39]，5-HT_4受容体は腸管神経系で速い興奮シナプス後電位（fast EPSP）を増大させる[40]。

近年，消化管疾患で最も大きな問題となっている過敏性腸症候群に，セロトニン受容体関連物質が効果を示すのではないかと，1980年代末より世界中の製薬メーカが開発に乗り出した。米国では2000万人が罹患していると言われる慢性疾患である過敏性腸症候群は，米国において医師によって最も頻繁に診断されるトップ10の疾患の1つである。

熾烈な開発競争を勝ち抜いたGlaxo Smith Kline社が開発した選択性の高い5-HT_3受容体拮抗薬alosetronの過敏性腸症候群を適応症とする新薬承認申請は，1999年6月にFDAへ提出された。そして，既存の治療法に比べ治療効果の向上が見込まれる画期的薬剤として優先審査品目に指定され，2000年3月には発売されて現在米国で臨床応用されている[41,42]。

終わりに

古代ペルーのインディオ達のひとつの伝統医薬「コカの葉」が次々と画期的新薬を生み出し，病気に苦しむ患者たちを絶望の淵から救い出し，彼らに福音をもたらしたことは，私たち創薬研究者に勇気を与え，また創薬研究者として進むべき道を暗示し

ているように思える。すなわち，次々と生み出された画期的新薬は偶然の産物ではなく，それぞれの研究者が充分な準備を怠らなかったために，偶然から真実を見つけだす能力―「セレンディピティ」を発揮することができたのであると考えるからである。セレンディピティは，個人レベルでは各研究者の心構え次第であり，組織レベルでは偶然を必然に変えるシステムの構築次第であると思う。本研究所も広くアジア（モンゴル，中国，韓国，ミャンマー，タイ，ネパールなど）で調査研究活動を展開し，富山においては各研究室で日夜，真摯な研究活動が展開されている。私たちの日々の研究活動により，最後には「セレンディピティ」が発揮されて画期的新薬に結び付き，患者さんに笑顔をもたらすという「夢の途中」に今，私たちはいるということを信じてやまない。

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Ayurvedic Pharmacopoeia Databases in the Context of Revitalization of Traditional Medicine

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Traditional medicine in India includes 'codified' systems like Ayurveda, Siddha, Unani, and Tibetan medicine and the 'non-codified' oral traditions. Codified systems are grounded in a theory of physiological functioning, disease aetiology and clinical practice. They have formal traditions of training and possess a vast array of written documents recording the *materia medica,* specialized subjects related to medicine and surgery, clinical procedures and medical ethics. The non-codified or folk traditions, such as those represented by bonesetters, birth attendants, paediatric specialists, veterinary healers, poison healers, healers specialized in specific diseases like jaundice, eye diseases, gastro-intestinal diseases etc., have been transferred as oral traditions for generations through a person-to-person process. Another feature of folk traditions is that they are ethnic community and eco system specific, and thus embody tremendous geo-cultural diversity. Folk medicine also includes what is popularly known as grandmothers' remedies, the household knowledge about primary healthcare, different health food recipes, seasonal health regimens and health customs, rituals etc.

Though local health practices have evolved in parallel with the codified knowledge system they have closely interacted and have been influenced mutually. Ayurvedic texts have many examples stating this relationship. At times one can find detailed explanations for a local health practice in the classical texts. One could say that local health practices are living expressions of the theories and concepts mentioned in the texts. However since this relationship is a complex issue only a detailed study of this would give exact nature of it.

Historical Context

Folk and the classical systems of medicines have had two different ways of historical evolution in India. In the post independence period Ayurveda and other systems of medicines such as Siddha and Unani were professionalized with the establishment of university programs and medical and research councils. Where as the folk medicine did not find their mention in the policies till recently as the year 2002. Except for some stray, vain attempts to integrate the traditional birth attendants (TBAs) to the national health program, folk practitioners were being completely neglected in the past. Nationalistic and pluralistic ideology of the independence movement has contributed to assuring a respectable place for Ayurveda and other medical systems in India today. However even with more than fifty years of government support, though marginal, the situation of Ayurveda is still not very healthy at present (Shankar 2004). Let us look at some of the historical aspects that have led to this state of affairs.

During the independence movement there was an ideological debate regarding approach of revitalising Ayurveda, which had its roots in the "Swadeshi" movement. This led to two distinct ideological positions such as a classical or *suddh* approach as well as an integrated approach. Those who were overwhelmed by the advancement of modern science and had a pragmatic and liberal view, held the position that the tradition has to be enriched by the modern technology and methods where as the other group held a purist view. However no systematic study of the influence of modern concepts and methods on the epistemology of traditional medicine was carried out. This has led to changes in the nature of basic aspects such as

education, research and clinical practice (Shankar 2004). Even today this foundational ideological confusion exists in Ayurveda and other traditional medical systems.

Till late 19th century the education of Ayurveda was purely through guru-sishya parampara i.e. an experiential learning where the student stays with the teacher. But around the period of Indian independence Ayurvedic university programs were established. At present there are more than 300 Ayurveda colleges in the country for graduate and post-graduate courses with a syllabus, which is designed on similar lines to that of modern medical education. At present there are Indian medicine councils in every state that give registration to graduates and only those who are licensed by these councils are allowed to practice. Even though the institutionalization was thought to improve the quality of medical education, it has deteriorated after the introduction of formal courses (Shanker & Manohar 1995). For example *nadi pariksa* (pulse examination), a method of diagnosis acquired through an experiential learning process, is widely practiced even today by the traditional physicians, yet in the university system many such fascinating methods and practices are not being taught. Many Ayurvedic graduates consider Ayurvedic education as an entry to privately practise allopathy.

In the area of research, after the independence, Indian Council of Medical Research (ICMR) was responsible for the pharmacological and clinical evaluation of Indian Systems of Medicine (ISM) drugs. After the formation of Central Council for Research in Ayurveda and Siddha (CCRAS), this responsibility was handed over to them. Today most Ayurvedic research programs are based on modern medical methods and parameters. Even after 30 years of research in ISM, these bodies have not come out with any comprehensive publication that can advise Indian medical professionals on how and what aspects of ISM can be integrated with mainstream Indian medical practice (Shankar 2004). In the area of research for want of a proper intercultural research methodology Ayurveda is loosing itself in the struggle of proving itself to the modern science. Lack of peer reviewed publications on Ayurveda meeting international standards is yet another lacuna in the area of research.

With respect to economic resources ISM still suffers from government neglect. During the Seventh, Eighth and Ninth National Plan periods, ISM (including Homeopathy, Naturopathy and Yoga) received around 3 per cent of the national health budget. State allocations varied, with only the government of Kerala allocating 13 per cent of its medical budget for ISM and Bengal allocated less than 0.5 per cent. ISM health delivery services in rural and urban areas are not in any way linked to the national primary health care services. There are around 23,000 Ayurvedic, Unani, and Siddha dispensaries, fully funded by state governments operational in various parts of the country. However they are effective and popular in some states like Kerala and Tamil Nadu, in other states their impact is negligible. They function without any orientation to national health goals, and there has been no review in post-independent India of how to make the ISM health services sector more effective (Shankar 2004).

On the whole, as described above, development in the field of Ayurveda and other traditional medical systems is facing an epistemological crisis along with a social and political disregard. Poor quality of education, research and clinical practice, lack of appropriate political and social support, loss of self esteem among the practitioners, marginalization by mainstream knowledge systems, issues related to intellectual property rights, lack of serious efforts to the fundamental as well as collaborative research, large scale depletion of the natural resources are some of the major issues confronted by the traditional medicine in India today.

In this context, Foundation for Revitalisation of Local Health Traditions, a nongovernmental

organization was set up with two broad objectives such as revitalization of Indian medical heritage and conservation of natural resources used by traditional medical systems. Over the last ten years, FRLHT has established an effective medicinal plant conservation program through public-private partnership in many states in India. It has also developed a number of comprehensive multidisciplinary databases of medicinal plants of India and the related traditional knowledge. Partnering with the government organizations in each of the projects FRLHT has been able also play a key role in advocacy for traditional medicine. It has been involved in training, extension activities as well as development of products to serve various interests groups of traditional medical community (i.e. practitioners, professors and students) as well as scientists, public health workers and the common public. The following section illustrates one of the successful projects such as the Ayurvedic databases.

Ayurvedic Pharmacopoeia Databases

It is estimated that traditional medicine in India uses around 7,500 medicinal plants in various health practices (AICRPE report). Out of this, Ayurveda uses around 1,750 medicinal plants and around 880 are in trade. According to a study, nearly 300, plants used in traditional medicine have some threat status (rare, endangered, threatened etc.) due to over exploitation, unsustainable harvest etc.

A project for to in-situ and ex-situ conservation of medicinal plants was launched in three Southern states of India in 1993. As part of the conservation project, to prepare a comprehensive checklist of medicinal plants, and their related information, a database project was initiated. This database project included various topics like distribution mapping, trade, threat status, propagation, traditional medical information and so on. Under the traditional medicine databases Ayurvedic, Siddha, Unani databases were built. A number of challenges were faced while building these databases, some of which remain unsolved even today. One of the major challenges was the nomenclature correlation i.e. developing linkage between Sanskrit plant names and their botanical equivalents. Another major challenge was related to identity of certain percentage of plants used in Ayurveda. Following pages highlight some of the experiences and the insights gained during this process.

The objective of the traditional medicine database was to understand which are the medicinal plants according to the codified medical traditions such as Ayurveda, Siddha and Unani. In the Ayurvedic database, first effort was to build a database on a state of the art correlation of Sanskrit names with botanical names mentioned in the secondary literature (non-classical). The textual sources for this database included 21 books belonging to last 100 years of works by Ayurvedists, botanists and pharmacognosists that correlated Sanskrit names with botanical names. This work culminated in a correlation of more than 20,000 Sanskrit names to 1,750 Species belonging to 830 genera. Following were the Ayurvedic sources selected.

A number of nomenclature correlation related issues were understood during the building up of the Ayurvedic part of this database. Ayurveda follows polynomial system of nomenclature where a plant is described with multiple names. Each of these names pertains to a specific character or feature of the plant. By grouping the names together (as usually found in the *nighantu* Sanskrit verses), an indicative picture of the identity and uses of a plant can be arrived at. For example, *Guduci* which is correlated to *Tinospora cordifolia* has around 70 names described in the classical literature. *Amrta, somavalli, somalatika* (climber with nectar like properties), *cakralaksana* (having wheel like appearance in cross section), *mandali* (circular), *kundali* (with an entangled nature), *nagakumari* (young snake like appearance), *tantrika* (spreading nature), *cchinnaruha, cchinnodbhava, cchinnangi* (grows when cut and put in soil), *syama* (with a bluish black colour), *rasayani* (rejuvenative), *vayastha* (age regulating), *jivanti* (life promoting),

No	Name of the work	Author	Year
1	Pharmacognosy of Ayurvedic drugs vol 1,2, 3, 10	K.N.Iyer, A. N.Namboodiri and M.Kolammal	1951 1957 1979
2	La-Harita Samhita	Alix Raisom	1974
3	Astanga Hrdaya Kosha	Anonymous	1936
4	Ayurvedic Pharmacopoeia of India vol 1	Ministry of Health and Family Welfare	
5	Ayurvedic Formulary of India Part 1	Controller of publications	1978
6	A Dictionary of Economic Products of India	George Watt	1889
7	Indian Medicinal Plants 4 volumes	Kirtikar and Basu	1935
8	Handbook of Medicinal plants	P.N.V.Kurup	1968
9	A Catalogue of Indian Synonyms	Moodeen Sheriff	1988
10	Single Drug Remedies	N.S.Moos	1976
11	Ganas of Vahata	N.S.Moos	1980
12	Indian Pharmaceutical Codex vol 1	B.Mukherji	1953
13	Indian Materia Medica 2 vols	K.M.Nadkarni	1954
14	Indian Medicinal Plants 1-5 vols	S.Raghunath Iyer	1993-96
15	Dravyagunavijnana vol 2&5	P.V.Sharma	1994
16	Ayurvedic Drugs and their Plant Sources	Sivarajan and I.Balachandran	1994
17	Glossary of Vegetable Drugs in Brhattrayi	Thakur Balwant Singh & Chunekar	1972
18	Nighantu Adarsha vol 1 & 2	Vaidya Bapalal	1968
19	Some Controversial Drugs of India	Vaidya Bapalal	1982
20	Studies on Medicinal Plants in Dhanvantariya Nighantu vol 1	Vaidya D.K.Kamat	1972
21	Materia Medica	Whitelaw Ainslie	1984

jvarari (pacifying fever) etc. (Manohar 1994).

Another peculiarity of this system is that the same names are used for different plants as well. For example, krsna is a name for *pippali* (*Piper longum*) as well as *arjuna* (*Terminalia arjuna*). As there are multiple names and common synonyms there are certain advantages as well as problems. It is difficult to decide the basic name for a plant. The textual sources that we used did not address this issue. Thus, we found that many of the correlation between Sanskrit and botanical names were casual and non-critical without having sufficient substantiating references or voucher specimens. Confirming the identity through textual descriptions was also not done in majority of these works.

Plants that are in contemporary use in different regions are correlated to the Sanskrit names by these authors. For example for the plant Sankhapuspi, a Kerala author gives *Clitoria ternatea* as the candidate where as a North Indian author correlates to *Convolvulus microphyllus*. Thus, there is a problem of multiple botanical species correlated to a single plant. It is estimated that nearly 50 % of the plants used currently have multiple botanical sources. The following are two examples of multiple numbers of botanical species correlated to the same name. Various reasons for these multiple correlations were identified.

Another problem was relating to transliteration of Sanskrit names. Different authors spelled/transliterated the Sanskrit names differently. For example the entry for botanical name *Acalypha indica* L. reads like this:

> Aristamanjari (VB), Arittamanjarie (KM),
> Arittamunjariye (KB), Arittamunjari (IP),
> Arittamunjayrie (GW, WH),
> Haritamanjari (VA), Manshinka (WH),
> Muktavarcca (VB).

At times, slight variation caused a completely

✣ *Sankhapuspi*	✣ *Pasanabheda*
1. *Clitoria ternatea*	1. *Aerva persica*
2. *Evolvulus alsinoides*	2. *Aerva lanata*
3. *Convolvulus microphyllus*	3. *Ammania baccifera*
4. *Canscora decussata*	4. *Bergenia ciliata*
5. *Canscora diffusa*	5. *Bergenia stracheyi*
6. *Lavendula bipinnata*	6. *Bridelia montana*
7. *Cannabis sativa*	7. *Bridelia retusa*
8. *Xanthium stumarium*	8. *Didymocarpus pedicellata*
	9. *Homonoia riparia*
	10. *Kalanchoe pinnata*
	11. *Nothosaerva brachiata*
	12. *Ocimum basilicum*
	13. *Plectranthus amboinicus*
	14. *Rotula aquatica*

different entity. There needed some standardization in the transliteration. This was possible only by checking the classical literature, contextual variation in description, *nirukti* of each names etc.

In general, an in-depth understanding of Ayurvedic plant nomenclature was lacking among these authors. Thus, we understood that a detailed study of classical texts with their chronological linkages was necessary to address issues related to nomenclature correlation and multiple identity. This led us to building of a primary literature source based database.

Nomenclature Correlation Database Based on Primary Sources

The effort followed was to develop a nomenclature database based on primary sources i.e. from the classical texts of Ayurveda. For this purpose, 20 classical texts covering a chronological period of around 2,200 years were selected. The texts that are major milestones in the area of *dravya guna* (Ayurvedic pharmacology) and are in contemporary use, belonging to various geographical locations were choosen. This also covered various types of texts like *samhita* (treatises), *samgraha* (compendiums), *nighantu* (lexicons). The purpose of this selection was to get maximum variation in the usage of plants.

Structure of the Classical Nomenclature (*Namajnana*) Database

Though this was primarily a plant database, it covered animals, minerals and metals from select texts.

Following are the fields in the database.

- Sanskrit name
- Plant/animal/mineral/metal
- Gender
- Whether plant, plant part, product, group, relation with time, space
- Chapter
- *Sthana* (Section)
- Verse

The field "Sanskrit names" pertain to resource name, which is classified into plant, animal and minerals and metals. Gender of the Sanskrit name is important as some names in female gender pertain to tender climbers and the same name in male gender meant trees. For example, *amrta* (*Terminalia chebula*), *amrtaa* (*Tinospora cordifolia*). To differentiate this, "gender" is taken as a separate field. Plant part, products, groups are differentiated by tags. Similarly, time relation, for example *kadali phalam - ama* (unripe) *pakva* (ripe), or space relation for example *jalam - sahyaja* (water from Western ghats) etc. are classified.

At present, this consists of around 23,000 Sanskrit names mentioned in 122,000 references across 20 texts. Tentative botanical correlation with pictures based on the earlier database and interface facility of searching references from individual texts, across texts, synonym and basionym search

have been created.

This database now helps in analyzing and searching the data for various research purposes. For example, through an analysis of nighantus between 8th and 19th Century it was found that around 70% of the materials used in Ayurveda are plants 20 % animals and 10% minerals during this period (Unnikrishnan 1997).

One of the challenges for building this classical text based database was selection of classical texts. It was difficult to define a classical text. There are many regional language texts those are in high contemporary use. For example the Kerala tradition uses *Cikitsa manjari, Sahasrayogam, Yogamrtam, Vaidyamanorama, Arogyaraksakalpadrumam* etc., which are not used elsewhere in India. These are either in Sanskrit or in a mixture of Sanskrit and regional language. As a first phase, we selected only Sanskrit texts that have not incorporated modern views or botanical correlation. Another criterion was that the text has to be in mainstream and used or known in different parts of the country. This also tried to cover major chronological milestones (to incorporate various temporal ideas) and different geographical locations (to cover variations spatial ideas).

Ascertaining chronology of these classical texts was difficult, as there are differences of opinion. Another issue is the lack of critical editions. Different publications of these texts have variations in *sloka* numbers or there are even differences in the verses. For standardization purpose, *mula granthas* (text with only verses) or those with Sanskrit commentaries and widely accepted publications were selected.

There were grammatical issues that needed to be solved. As mentioned earlier, slight variations in the word, gender or a *pratyaya* (suffix or prefix) changed the identity of the plant name completely. For example *pippali, pippala, mrnala, mrnali, amrnala, venu, veni, madhuka, madhooka, patanga, pattanga, padma, padmaa, arishta, arishtaka, nygrodha, nygrodhee, parpata, parpatakee, palasa, palaasaa, sarala, saralaa, sala, Sali* are some of the typical examples.

Same plant names are used in different variant forms. For example *yasti, yastika, yasteeka, yastiahvika, yastimadhuka, yasteemadhuka, madhuka, madhuyastika, madhuyasti* etc. All these pertain to the same name *yastimadhu.* These variations had to be considered.

Differences of names across different time periods, was another problem. For example *kokilaksa* is the name used for *iksuraka* by Susruta, *bhumyamalaki* is the name for *tamalaki* and *caksusya* for *kulathika* by nighantu authors. Yet another issue was collecting commentators` views on a plant in case of doubt. In number of instances, there are differences of opinion among commentators and there are scant descriptions, which made the issue complex.While compiling the references contextual differences had to be considered. For example, in some context *kustha* means a skin disease where as elsewhere it means a plant. Similarly the name tikta means bitter as well as the plant *katuka* and *kiratatikta.* So these references had to be screened carefully.

There were a number of synonyms used in the same text in different contexts. Since the effort of this database was to prepare a unique list of plant names from each of these texts, it was necessary to group the variants and synonyms of each plant and link it with a basic name. This had to be done by grouping the number of references. This was found difficult without having complete descriptions and commentators' view on each plant.

In grouping, another difficulty was that of classification of the parts used. At times the part used has a different name altogether which is considered as a separate entity. For example - *mocarasa* is the exudate of *Salmali* (*Bombax* sp.) and it is mentioned as a different entity. *Kutaja* (

Holarrhena pubescens), *Indrayava* (Seed of the same plant) is another example. Similarly, there are differences of plant names in different stages of maturity. For example *ardraka* (fresh ginger) and *sunthi* (dry ginger). Such references had to be identified and grouped separately. As mentioned earlier, same names are used in the classical texts for denoting different plants. For example, *"usna"* is a name used for pippali and marica, *"krsna"* for *pippali* and *arjuna.* Similarly *"citra"* is used for three plants such as *urubuka, eranda* and *danti, "tikta"* for *katurohini* and *kakatikta*, *"amrta"* for *guduci* and *haritaki.* In this case each reference had to be searched for such common names and synonyms.

There are some broad general rules available in the texts for understanding the nomenclature. According to Dhanvantari nighantu, all these features are owing to the nomenclature system, which is designed based on *jatilinga* (reproductive characters), *akrti* (physicial characters), *varna* (colour), *virya* (potency), *rasa* (taste), *prabhava* (specific action) etc. It is mentioned that common synonyms have to be decided according to the meaning, context, tradition (*sampradaya. Parampara*) and reasoning (*tarka*). Thus each contextual reference became important.

The references selected for this work did not represent the regional literature in which a plethora of knowledge is available. Thus, this is not a comprehensive inventory yet. Thus some plants that are commonly used at present in Ayurveda did not appear in this database. For example - *saptacakra* (*Salacia oblonga*) is described in Kerala traditional literature but it did not appear in the database, as regional literatures were not included. Around 200 such materials that are unique in their usage in Kerala tradition is documented in Dhanvantari, a journal that was published from Arya Vaidya Sala, Kottakkal a few decades ago. But many of these materials may have not come in this database.

Lack of proper software in Sanskrit language also became a major hurdle. The GIST software for Sankrit language was not Windows compatible for database operations. Lack of good software for database operations in Sanskrit remains an unsolved issue even today.

Even after completion of this database, the major issue of critical nomenclature correlation remained unsolved. It was learned that the issue of nomenclature correlation could only be progressed by a combination of approaches such as in-depth studies of classical literature, documentation of the understanding of vaidyas/hakims (physicians) and pharmacognostic and pharmacological/clinical studies.

For such an in-depth study, a mere reference database was not sufficient. All the contextual details in each of the references were necessary. Thus detailed individual text databases were prepared. This included databases on brhatrayi - Caraka samhita, Susruta samhita, Astanga hrdaya, database of nighantus - Dhanvantari nighantu, Madanapala nighantu, Bhavaprakasa nighantu, Raja nighantu.

In this bilingual (Sanskrit and English) database, the references are grouped into *nama* (name), *rupa* (form/identity), *gunakarma* (quality and action), *varga, yoga, gana* (classification/formulation), *kalpana* (pharmacy preparation), *prayoga* (clinical application) groups. A section includes views of major commentators like Cakrapani, Dalhana. Nomenclature is classified into *svarupabodhaka* (revealing form), *gunabodhaka* (revealing quality), *karmabodhaka* (action) and so on. A glossary is prepared for the Sanskrit terms. As there are similar problems of nomenclature correlation in disease names, and as we have not attempted a detailed study in this area, secondary sources were used for preparation of this glossary. After building individual text databases, following methodology was used to group the synonyms and to find out the unique plants in each of these texts.

- Collection of plant references
- Collection of commentators' views on references
- Fix tentative basionym - based on commentary, frequently used names

- Mark grammatical variants linked to basionyms
- Mark synonyms based on suggestions of commentators
- Mark gender variations, if any
- Mark plant names which pertain to groups e.g. *triphala, dasamula*
- Mark plant names as basionyms, if the plant name correlated by the commentator is not found marked under synonym or variant name
- Mark tentative basionyms as basionyms if they are not linked to synonyms or variant names
- Compare botanical correlations done by subject experts
- Fix status of identification by giving flags like non-controversial, controversial or unidentified based on these studies

This analysis has now culminated in the following data in Caraka samhita. There are 12,870 references related to plants in Caraka samhita. After grouping the synonyms, there are 620 unique plant names. Out of this, 508 are identified and they are correlated to 630 botanical species. There were around 500 synonyms, 817 variants, and 56 group names in Caraka samhita (Venugopal 2001).

Out of this, 305 plant names are non-controversial, 203 are controversial and 112 are unidentified. There are around 1,630 formulations recorded in Caraka samhita. Now this has become a unique inventory of plants of Caraka samhita. Based on the conservation databases at the foundation, it is understood that 56 plants in Caraka samhita are having some threat status.

Now similar works are carried out in other texts also and it is expected that in a few years time we will be able to arrive at a better picture of plants of Ayurveda.

Apart from this, as part of this project, similar works are being done on Siddha and Unani systems of medicine. A number of other databases on traditional quality standards, malaria, home doctor,

Reference books:

S No.	Text name	Chronology	Author	Region	plant ref.No
1	Caraka Samhita	1500BC-400AD	Agnivesa Caraka Drdhabla	Himalaya Kashmir	12850
2	Susruta Samhita	1500BC-500AD	Susruta Nagarjuna	Kasi Sindhudesa	9650
3	Astanga Sangraha	500 AD	Vagbhata	Sindhudesa	20500
4	Astanga Hrdayam	600 AD	Vagbhata	Sindhudesa	9900
5	Astanga Nighantu	800 AD	Vagbhata		2100
6	Paryayaratnamala	900 AD	Madhava	Silahrda	1900
7	Dhanvantari Nighantu	200AD-1000AD	Unknown	Unknown	3250
8	Cakradatta	1075 AD	Cakrpanidatta	Vanga desa	12300
9	Dravyagunasangraha	1075 AD	Cakrapanidatta	Vangadesa	320
10	Madhavadravyaguna	1250 AD	Madhava	Unknown	750
11	Sarngadhara Samhita	1300 AD	Sarngadhara	Devagiri	4200
12	Nighantu Sesa	1200 AD	Hemachandra	unknown	2950
13	Siddhamantra	1210AD-1247AD	Kesava	Unknown	950
14	Hrdayadipaka Nighantu	1260AD-1271AD	Bopadeva	Unknown	820
15	Madanapala Nighantu	1374 AD	Madanapala	Kashthanagara	3000
16	Bhavaprakasa	1550 AD	Bhavamisra	Kasi Kanyakubja	11200
17	Bhavaprakasa Nighantu	1550 AD	Bhavamisra	Kasi Kanyakubja	2600
18	Raja Nighantu	1700 AD	Naraharipandita	Kasmira	7300
19	Saligrama Nighantu	1896 AD	Saligramvaisya	Muradabad	4200
20	Siddhabhesajamanimala	1896 AD	Krshnaramabhatta	Jayapura	620

clinically important plants of Ayurveda, rapid assessment of local health practices are also being developed. These are linked to the master nomenclature correlation database (secondary sources of Ayurveda, Siddha, Unani, Folk). Apart from supporting a detailed study on nomenclature correlation, these databases can serve purposes of conservation, education, research, clinical practice, pharmaceutical industry, intellectual property right aspects, and local health traditions assessment programs for primary health care.

Conclusion:

During the process of developing these databases it was understood that the nomenclature correlation of Sanskrit names of Ayurvedic classical literature to Botanical names is a complex task. Casual and non-critical approach in correlation has been one of the reasons for controversial and multiple botanical identities. Only a systematic approach taking into consideration the available classical textual literature, their critical commentaries, regional literature, experience of living traditions combined with pharmacognostic and pharmacological works can shed light on this and solve this issue to some extent.

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各部門・附属センターの活動と業績

和漢薬研究所の概要（研究部門と各分野の研究目的）

2004年12月現在

部門・附属センター・寄付部門	研究分野と研究目的
1．資源開発研究部門 **Department of Medicinal Resources** 教　授　小松かつ子 教　授　門田 重利 教　授　服部 征雄 助教授　手塚 康弘 助教授　横澤 隆子 助　手　山路 誠一 助　手　Suresh Awale 助　手　宮代 博継 助　手　中村 憲夫	**生薬資源科学分野：Pharmacognosy** 薬用生物および伝統薬物の調査とそれらの遺伝学的，生薬学的，成分化学的，薬理学的多様性の解析を行う。遺伝子多型に基づく和漢薬の同定法を開発する。 **化学応用分野：Natural Products Chemistry** 和漢薬及びそれに関連する動植物の生理活性成分の分離，構造解析を行うとともに，それらの有効成分の化学的合成法を開発研究し，さらに化学構造と生理活性との相関関係を究明する。 **薬物代謝工学分野：Metabolic Engineering** 和漢薬の薬効発現に関与する腸内細菌およびその遺伝子の解明。抗エイズ，抗C型肝炎ウイルス薬の開発研究。担子菌類の薬効評価。腎疾患における病態の解明と腎臓病治療薬の開発。
2．病態制御研究部門 **Department of Bioscience** 教　授　松本欣三 教　授　済木育夫 教　授　門脇　真 （客）教　授　奥山治美 助教授　櫻井宏明 助　手　東田道久 助　手　小泉桂一 助　手　村上孝寿 助　手　山本　武	**複合薬物薬理学分野：Medicinal Pharmacology** 和漢薬の薬効に関する計量薬理学的な評価およびその作用機序と作用本体の解明を行うとともに，和漢薬が薬効を発現する生体病態を解析する。 **病態生化学分野：Pathogenic Biochemistry** 和漢薬効果に対応する体質（遺伝的要因）ならびに病態に対する和漢薬の効果を遺伝学，生化学，分子生物学ならびに免疫学など多面的に解析する。 **消化管生理学分野：Gastrointestinal Pathophysiology** 消化管疾患，特に腸管免疫性疾患の病因および病態形成機序を解明し，それに基づき和漢薬等を含めた新規治療薬の創出を目指す。 **恒常性機能解析分野（客員）：Analysis of Homeostasis** 菜種油に含まれる脂溶性微量有害成分にはネズミの寿命を短縮する物質があるが，その成分は未だ同定されていない。そこでその成分を検索する。
3．臨床科学研究部門 **Department of Clinical Science** 教　授　浜崎智仁 助教授　渡辺志朗 助　手　長澤哲郎	**臨床利用分野：Clinical Application** 天然薬物（特に魚油中のEPA，DHA）の作用機序の解明とその臨床利用。
4．附属薬効解析センター ｾﾝﾀｰ長　小松かつ子(併任) 助　手　東田千尋 （客）教　授　Abdul Md. Gafur （客）助教授　Piyal Arunashantha Marasinghe	**Research Center for Ethnomedicines** 民族薬物資料館に保管される生薬についてデータベースを構築し，それらの薬物の品質並びに薬効に関する研究を通じて世界の民族薬の標準化を図る。
5．漢方診断学部門（寄） （寄）教　授　柴原直利 （寄）助教授　後藤博三 （寄）助教授　酒井伸也 （寄）助　手　中川 孝子	**Kampo Diagnostics** 経験が重視される漢方医学固有の診断体系を基礎的および臨床的研究により客観化するとともに普遍的な教育カリキュラムを確立する。
6．和漢薬製剤開発部門（寄） （寄）教　授　谿　忠人 （寄）助　手　何　菊秀	**Kampo-pharmaceutics** 和漢薬資源とその製剤を開発する基礎研究と漢方医療情報研究を通して地域連携研究と県民の健康福祉に貢献する。

（客）：客員；（寄）：寄付部門

漢方薬学分野（10月31日まで） Division of Pharmacognosy
生薬資源科学分野（11月１日から）

教 授	谿　忠人（６月30日まで）	Professor	Tadato Tani (Ph.D.)
教 授	小松かつ子（11月１日から）	Professor	Katsuko Komatsu (Ph.D.)
助 手	山路　誠一	Assistant Professor	Seiichi Yamaji (Ph.D.)

◇研究目的　Aims of the research projects

地球環境の変化により，薬用天然資源の減少が危惧される。そこで本分野では，生薬資源の現状の把握と代替生薬の開発，生薬の特徴を把握した効率的利用の促進並びに栽培薬用植物の選択と栽培拡充を目的にして，アジアにおける漢薬資源の調査と薬用生物の遺伝学的，生薬学的，成分化学的及び薬理学的多様性の解析を行う。また，生薬・漢方薬の品質管理と健康食品のレギュレーションを目的にして，遺伝子多型に基づく生薬同定法の開発並びに品質評価法の確立を行う。さらに，民族薬物データベースを拡充し，各国の生薬の標準化や適正使用に役立てる。

◇研究概要　Research projects

Ⅰ）薬用生物及び伝統薬物の調査研究

モンゴル国東部で *Ephedra* 属，*Glycyrrhiza* 属，*Astragalus* 属植物などの資源調査を行った。また，中国四川省及び甘粛省で野生及び栽培 *Rheum* 属植物の調査を行った。

Ⅱ）薬用植物・生薬の多様性の解析

モンゴル産 *Glycyrrhiza uralensis* の地下部の成分化学的多様性を薬理作用成分の含量から検討した。Glycyrrhizin含量は中国産甘草にほぼ匹敵したが，フラバノン類及びカルコン類が低含量であり，また産地間差が大きかった。

Curcuma 属７種に由来する鬱金類生薬の薬理学的多様性を，アジュバント関節炎に対する作用で検討した。莪朮の基源である３種の根茎に抗炎症作用が期待できた。とくに *C. phaeocaulis* メタノールエキスは，肢の腫脹と血清中の炎症マーカータンパク質の発現を有意に抑制し，また *in vitro* 実験においてCOX-2活性の抑制作用を有意に示した。

Ⅲ）生薬・健康食品の品質とレギュレーション

日本市場のガジュツについて，*trnK* 遺伝子の解析及び精油含量・エキス含量の定量を行った。四川省産ガジュツは *C. phaeocaulis* の根茎であったが，広西壮族自治区産は *C. kwangsiensis*（gl タイプ）が多かったものの，上記２種または *C. kwangsiensis*（pl タイプ）の１塩基または２塩基置換体が認められ，交雑が示唆された。

人参類及びウコン類健康食品の遺伝子解析を行い，同時に ginsenosides 6 成分または curcuminoids 3 成分を定量した。ウコン類には基源不明で curcuminoids 含量が4.5%の製品があり，成分組成も *C. longa* とは異なった。

Ⅳ）薬用植物の遺伝子多型に基づく生薬同定法の開発

Panax 属13分類群の 18S rRNA 遺伝子の塩基配列に基づいた合成オリゴを作成し，人参類同定用DNA マイクロアレイの試作品を開発した。

◇原著論文　Original papers

1）**Sasaki Y., Fushimi H., and Komatsu K.: Application of Single Nucleotide Polymorphisms Analysis of *trn*K Gene to the Identification of *Curcuma* Plants. *Biol. Pharm. Bull.*, 27: 144-146, 2004.**

Abstract: We previously found that *Curcuma* plants and drugs derived from *Curcuma longa, C. phaeocaulis, C. zedoaria,* and *C. aromatica* could be identified by the nucleotide differences at two sites and the existence of a 4-base indel on *trn*K gene. In this paper, based on species-specific nucleotide sequences, the application of a new method, single-nucleotide polymorphism (SNP) analysis was investigated to identify *Curcuma* plants more conveniently. First, three types of reverse primer were synthesized in different lengths, 34 mer, 26 mer, and 30 mer, to anneal the template DNAs from each species at sites immediately upstream from substitution positions 177 and 645, and at the site including the 4-base insertion from 728 to 731, respectively. After single-base extension reaction of these primers using fluorescent-labeled ddNTPs and PCR products of the *trn*K gene region as template, the resulting products were detected using an ABI PRISM 310 Genetic Analyzer. The electrophoretogram showed three or two peaks at different positions depending on the 27 mer, 31 mer, and 35 mer product lengths. Each peak was derived from the incorporated fluorescent-labeled ddNMPs complementary to template nucleotides at positions 645, 724, and 177, respectively. *C. phaeocaulis* showed three peaks of ddCMP, ddAMP, and ddAMP. The other three species showed two peaks derived from 27 mer and 35 mer products: peaks of ddCMP and ddAMP in *C. longa,* those of ddCMP and ddTMP in *C. zedoaria,* and those of ddTMP and ddAMP in *C. aromatica.* Thus SNP analysis to identify four *Curcuma* plants was newly developed.

2）**Zhao J., Nakamura N., Hattori M., Yang X. W., Komatsu K., and Qio M. H.: New Triterpenoid Saponins from the Roots of *Sinocrassula asclepiadea. Chem. Pharm. Bull.*, 52: 230-237, 2004.**

3）**Tohda C., Matsumoto N., Zou K., Meselhy M. R., and Komatsu K.: Aβ (25-35)-induced memory impairment, axonal atrophy and synaptic loss are ameliorated by M1, a metabolite of protopanaxadiol-type saponins. *Neuropsychopharmacology,* 29: 860-868, 2004.**

Abstract: We previously screened neurite outgrowth activities of several Ginseng drugs in human neuroblastoma, and demonstrated that protopanaxadiol (ppd)-type saponins were active constituents. Since ppd-type saponins are known to be completely metabolized to 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol (M1) by intestinal bacteria when taken orally, M1 and ginsenoside Rb1, as a representative of ppd-type saponins, were examined for cognitive disorder. In a mouse model of Alzheimer's disease (AD) by Aβ (25-35) i.c.v. injection, impaired spatial memory was recovered by p.o. administration of ginsenoside Rb_1 or M1. Although the expression levels of phosphorylated NF-H and synaptophysin were reduced in the cerebral cortex and the hippocampus of Aβ (25-35)-injected mice, their levels in ginsenoside Rb_1- and M1-treated mice were almost completely recovered up to control levels. Potencies of the effects were not different between ginsenoside Rb_1 and M1 when given orally, suggesting that most of the ginsenoside Rb_1 may be metabolized to M1, and M1 is an active principal of ppd-type saponins for the memory improvement. In cultured rat cortical neurons, M1 showed extension activity of axons, but not dendrites. The axon-specific outgrowth was seen even when neuritic atrophy had already progressed in response to administration of Aβ (25-35) as well as in the normal condition. These results suggest that M1 has axonal extension activity in degenerated neurons, and improve memory disorder and synaptic loss induced by Aβ (25-35). M1 was shown to be effective in vitro and in vivo, indicating that Ginseng drugs containing ppd-type saponins may reactivate neuronal function in AD by p.o. administration.

4） **Zhu S., Fushimi H., Cai S. Q., and Komatsu K.: Species Identification from Ginseng Drugs by Multiplex Amplification Refractory Mutation System (MARMS). *Planta Med.*, 70: 189-192, 2004.**

Abstract: The multiplex amplification refractory mutation system (MARMS) was applied to the identification of 5 *Panax* species (*P. ginseng, P. japonicus, P. quinquefolius, P. notoginseng* and *P. vietnamensis*). A set of specific primers, including 2-pair primers on chloroplast *trn*K gene and nuclear 18S rRNA gene regions, respectively, was designed and synthesized for each species on the basis of species-specific sequences of the 2 genes. By using 5 sets of specific primers, in turn, PCR amplifications were performed with total DNA extracted from 5 *Panax* species as template under appropriate condition, and each resulting product was detected by agarose gel electrophoresis. The results showed that two expected fragments, one from *trn*K gene and another from 18S rRNA gene regions, were observed simultaneously only when the set of species-specific primers encountered template DNA of the corresponding species. This assay could give more reliable results for identification of not only 5 *Panax* species but also corresponding Ginseng drugs by simultaneous detection of 4-site nucleotide differences on 2 completely different genes.

5） **Yang D. Y., Fushimi H., Cai S. Q., and Komatsu K.: Molecular Analysis of *Rheum* Species Used as Rhei Rhizoma Based on Chloroplast *mat*K Gene Sequence and Its Application for Identification. *Biol. Pharm. Bull.*, 27: 375-383, 2004.**

Abstract: Rhei Rhizoma (Dahuang in Chinese) is widely known as a purgative and antiinflammatory agent. In the Japanese Pharmacopoeia, Rhei Rhizoma is prescribed for four *Rheum* species, *Rheum palmatum, R. tanguticum, R. officinale,* and *R. coreanum,* while the first three species are prescribed for Dahuang in the Chinese Pharmacopoeia. Due to the morphologic similarity of the aerial parts and frequent occurrence of intermediate forms, the taxonomy of this genus and the correct identification of *Rheum* species and their derivative drugs are very difficult. To resolve taxonomic problems of the genus Rheum and develop an ultimate identification method for plants and drugs, molecular analysis of the chloroplast *mat*K gene and nuclear 18S ribosomal RNA gene were performed on nine species. The sequence comparison of the *mat*K gene revealed that most species had variable sequences not only inter- but also intraspecies. However, the specimens of the same species belonged to the same subclade in the phylogenetic tree constructed based on *mat*K gene sequences, except for *R. palmatum,* in which specimens belonged to three subclades related to their production areas. The nucleotide differences at positions 587, 707, and 838 distinguished official species from others, while specific nucleotides at positions 367 and 937 became identification markers for *R. palmatum, R. tanguticum,* and *R. officinale* (or *R. coreanum*). Moreover, three groups of *R. palmatum,* each belonging to three subclades, were characterized by the nucleotides at positions 619, 769, 883, and 1061. By detecting marker nucleotides, the botanical origins of Rhei Rhizoma were determined.

6） **Teerawatanasuk N., Nakamura E. S., Wangma-neerat A., Komatsu K., Saiki I: Anti-invasive and anti-angiogenic activities of *Curcuma* sp. extracts. *J. Trad. Med.*, 21: 27-33, 2004.**

7） **Yang D. Y., Fushimi H., Cai S. Q., and Komatsu K.: Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) and Amplification Refractory Mutation System (ARMS) Analyses of Medicinally Used *Rheum* Species and Their Application for Identification of Rhei Rhizoma. *Biol. Pharm. Bull.*, 27: 661-669, 2004.**

Abstract: Previously, we have determined marker nucleotides on the chloroplast *mat*K gene to identify *Rheum palmatum, R. tanguticum* and *R. officinale* used as Rhei Rhizoma officially. In the present study, we further developed a convenient and efficient identification method on the basis of marker nucleotides with Amplification Refractory Mutation System analysis. On the basis of the nucleotide substitutions at positions 367 and 937 among

the three species on the *mat*K gene, at each position two kinds of reverse primers with complementary 3'-terminal nucleotides were designed. Upon PCR amplification using three sets of primers and template DNA from each species, one or two fragments (202 bp or/and 770 bp) were detected. As the resultant three fragment profiles were species-specific, the procedure enabled us to classify the botanic origins of 22 drug samples of Rhei Rhizoma.

8) **Zhu S., Zou K., Fushimi H., Cai S. Q., and Komatsu K.: Comparative Study on Triterpene Saponins of Ginseng Drugs. *Planta Med.*, 70: 666-677, 2004.**

Abstract: A comparative study on the triterpene saponins of 47 samples of Ginseng drugs derived from 12 *Panax* taxa was conducted using a reverse-phase high-performance liquid chromatography (HPLC) method. Eleven ginsenosides, which represent 4 types of typical sapogenins, were chosen as standards for quantitative determination in order to characterize the chemical constituent pattern of each Ginseng drug and investigate the relationship between genetic varieties and chemical constituent pattern. The results showed that the ginsenoside compositions in Ginseng drugs of different origins were of considerable variability. Total saponin contents varied by 10-fold from the highest drug to the lowest one. Chikusetsu-ninjin derived from *P. japonicus* (Japan) was found to have the highest content (192.80 - 296.18 mg/g) and Ginseng from *P. ginseng* to be the lowest (5.78 - 15.63 mg/g).Two main groups (I and II) suggested by phytochemical data were clearly observed; group I mainly containing dammarane saponins consisted of *P. ginseng, P. quinquefolius, P. notoginseng, P. vietnamensis* and *P. vietnamensis* var. *fuscidiscus*; and group II containing a large amount of oleanolic acid saponins was composed of *P. japonicus* (Japan), *P. zingiberensis, P. japonicus* (China), *P. japonicus* var. *angustifolius, P. japonicus* var. *major, P. japonicus* var. *bipinnatifidus* and *P. stipuleanatus.* The ratios of the subtotal of dammarane saponins to that of oleanolic acid saponins (D/O) were found to be > 1.9 and < 0.25 for groups I and II, respectively. The drug samples derived from the same botanical origin revealed similar constituent patterns, in other words, each *Panax* taxon showed its own characteristic chromatographic profile,which appeared in the specific shape of an 11-direction radar graph constructed on the basis of the result of quantitative analysis. Similarities of chemical constitution were seen among the closely phylogenetically-related taxa, including *P. ginseng* and *P. quinquefolius, P. vietnamensis* and *P. vietnamensis* var. *fuscidiscus, P. japonicus* (China) and its varieties were demonstrated, except *P. japonicus* (Japan) and *P. zingiberensis.*

9) **Zhu S., Zou K., Cai S. Q., Meselhy M. R., and Komatsu K.: Simultaneous Determination of Triterpene Saponins in Ginseng Drugs by High Performance Liquid Chromatography. *Chem. Pharm. Bull.*, 52: 995-998, 2004.**

Abstract: A HPLC method for the simultaneous determination of 11 triterpene saponins with four-type aglycones (protopanaxadiol, protopanaxatriol, ocotillol and oleanolic acid types) in Ginseng drugs was developed and validated. Using a gradient of acetonitrile and 10 mM K-phosphate buffer (pH 5.80) as the mobile phase and UV detection at 196 nm, more than 18 ginsenosides with different aglycones were separated satisfactorily within 60 min. The detection limits (signal/noise> or =3) were 0.1 μg for ginsenosides Rb_1, Rc, Rd, Re and Rg_1, chikusetsusaponin III, and notoginsenoside R_2, 0.2 microg for gisenoside Ro and chikusetsusaponin IVa, 0.3 μg for chikusetsusaponin IV, and 3 μg for majonoside R_2. The calibration curve of each saponin had a correlation coefficient close to 1. Intra- and interday precisions were less than 2.1% (n = 5) and 3.3% (n = 15), respectively. The recovery rates of extraction were in the range of 96.4-102.7% for all ginsenosides. By adopting this method, the determinations of 11 ginsenosides in three Ginseng drugs derived from *Panax ginseng, Panax vietnamensis* var. *fuscidiscus* and *Panax japonicus* (Japan) were achieved.

10) **Ahn E. M., Akao T., Nakamura N., Komatsu K., Nishihara T., and Hattori M.: Screening of Medicinal Plant Extracts for Estrogenic Activity in Combination with a Glycosidase**

Treatment. *J. Trad. Med.*, 21: 81-86, 2004.

11) **Long C. F., Kakiuchi N., Takahashi A., Komatsu K., Cai S. Q., and Mikage M.: Phylogenetic Analysis of the DNA Sequence of the Non-Coding Region of Nuclear Ribosomal DNA and Chloroplast of *Ephedra* Plants in China. *Planta Med.*, 70: 1080-1084, 2004.**

Abstract: Twenty-four *Ephedra* plants belonging to 8 species grown in the northern and western parts of China were phylogenetically analyzed for their non-coding DNA sequences, internal transcribed spacers (ITSs) of nuclear ribosomal DNA as well as *trn*L intron and intergenic spacers between *trn*L and *trn*F (*trn*L/ *trn*F) of the chloroplast. Based on the ITS sequences, the 8 species could be divided into 3 groups: Group 1 (*Ephedra intermedia, E. sinica, E. przewalskii*), Group 2 (*E. equisetina, E. monosperma, E. gerardiana*), and Group 3 (*E. likiangensis, E. minuta*). The species classified into Group 1 grow mainly in the north, Group 3 in the south and Group 2 in the center, suggesting their genetic and geographic relationships. A specific primer set was designed to classify the 3 groups by routine PCR. Combined analysis of ITS and *trn*L/ *trn*F differentiated the 8 *Ephedra* species.

12) **Ahn E. M., Nakamura N., Fushimi H., Komatsu K., Batkhuu J., and Hattori M.: Constituents of the seeds of *Glycyrrhiza uralensis*. *Nat. Med.*, 58: 311, 2004.**

13) **Li J., Wang X., Ma F. Y., Jia X. H., Cai S. Q., Liang X. M., and Komatsu K.: Several Factors Affecting the HPLC-Fingerprinting of *Panax notoginseng*. *Chi. J. Nat. Med.*, 2 (1): 33-41, 2004.**

Abstract: **Aim**: To study how can the way and the degree of dryness and the period of storage of crude drug samples affect the HPLC-fingerprinting of *Panax notoginseng*. **Methods**: The HPLC-fingerprinting method: Luna C_{18} analytical column (250 x 4.6 mm, 5 μm); acetonitrile-water as gradient eluent with flow rate at 1.0 ml/min, detective wavelength at 200 nm. Notoginseng samples made in different ways of dryness and samples in different degrees of dryness and different moments of storage were analyzed to discover how they can affect the HPLC-fingerprinting of Notoginseng. Two shapes of Notoginseng, Notoginseng in integrity and pieces, were dried in shade and by baking (35℃) respectively. Notoginseng in integrity, in pieces and in powder which had been stored for 0 days, 10 days, 20 days, 30 days(1 month), 2 months, 4 months, and 6 months were analyzed individually. **Results**: ① Different drying ways work on the components of Notoginseng in different ways: some components were affected more by the temperature and period of dryness, which had higher area values of peaks under drying in heat (or in shade); some were affected more by the shape of drug, which had higher peak area value in the shape of pieces (or integrity); some were affected by the both factors mentioned above. ② In the experiment, peak No. 15, whose relative peak area is 12.7% - 28.1% in fresh and drying drugs, was discovered to be characteristic. Its relative peak area was keeping on declining during drying and dropped to 0.2% when the drug was dried completely. However the value rose to 0.7% - 1.4% when the dried drug was hydrated, so the reaction was concluded to be reversible. ③ The discipline of changes during storage was found: two peaks (No. 10 and No. 12), areas kept on going up during storage, while most others' went down; drug of different shapes changed differently, e. g. drug in powder changed more rapidly than that in pieces and integrity. **Conclusion**: The way and the degree of dryness and the period of storage all can affect the HPLC-FPS of Notoginseng in a certain extent.

14) **Liu J. H., Wang X., Cai S. Q., Komatsu K., and Namba T.: Analysis of the Constituents in the Chinese Drug Notoginseng by Liquid Chromatography-Electrospray Mass Spectrometry. *J. Chin. Pharm. Sci.*, 13 (4): 225-237, 2004.**

Abstract: **Aim**: To develop a HPLC-UV-MS method for identifying the constituents in the Chinese drug Notoginseng (the root of *Panax notoginseng*). **Methods**: A Phenomenex Luna C_{18} column (250 mm x 4.6 mm ID,

5 μm) was utilized. Water containing 0.005% formic acid (A) and acetonitrile containing 0.005% formic acid (B) were used as gradient eluents. UV spectra were recorded in range 195 - 400 nm. Both positive and negative ion ESI modes were used. **Results**: The constituents in Notoginseng were well separated and detected. Fourteen compounds were identified by comparing their retention time and ESI-MS data with those obtained from the reference compounds. Forty-one compounds were deduced by data analysis of MS and literature; among them, yesanchinosides-H and -E, chikusetsusaponin-L_5, malonyl-ginsenoside-Rg_1, the isomers of notoginsenosides-J, -A, -R_1, -G, -R_2, and ginsenoside-Rh_3 were discovered in Notoginseng for the first time. **Conclusion**: This method gives high sensitivity and good separation, and is suitable for identifying the constituents in Notoginseng. This result is helpful for further phytochemical research on Notoginseng. Based on this result, further quality control can be studied.

15) 牧野利明，山路誠一：薬用植物・生薬に関する副作用と薬害．薬用植物研究，26 (1) : 30-37，2004.

◇総説 Review papers

1) Komatsu K., Zhu S., and Sasaki Y.: Systematic Pharmacognostical Study on *Panax* Drugs and *Curcuma* Drugs - Phylogenetic Analysis, Molecular Authentication and Quality Evaluation -. *J. Trad. Med.*, 21: 251-270, 2004.

◇学会報告 Scientific presentation (*: 招待講演)

1) 田村隆幸，東田千尋，鄒 坤，小松かつ子：黄耆によるAβ25-35誘発性の神経突起萎縮に対する抑制作用—基源植物の差異および修治が及ぼす影響—．日本薬学会第124年会，2004, 3.29-31, 大阪.
2) 小松かつ子：フィールドワークの2つの視点—比較民族薬物学と生薬資源学，ミニシンポジウム「天然薬物のフィールドワークを考える」．日本薬学会第124年会，2004, 3.29-31, 大阪.
3) Rauchensteiner F., Matsumura Y., Yamamoto Y., Yamaji S., and Tani T.: Development of environmental friendly analysis of *Glycyrrhiza* species from Europe and China by capillary zone electrophoresis (CZE). The 124th Annual Meeting of Pharmaceutical Society of Japan. 2004, 3.29-31, Osaka.
4) Zhu S., Fushimi H., Cai S. Q., and Komatsu K.: Phylogenetic Relationship in the Genus *Panax*: inferred from Chloroplast *trn*K Gene and Nuclear 18S rRNA Gene Sequences. International Symposium on Asian Plant Diversity and Systematics, The Japanese Society for Plant Systematics, International Association of Plant Taxonomists, 2004, 7.29-8.1, Chiba, Japan.
5) Cai S. Q., Wang X., Ma F. Y., Li J., and Komatsu K.: Studies on HPLC-Fingerprinting of Notoginseng. JSP-KSP-CCTNM Joint Seminar 2004 -International Symposium on Natural Medicines-, The Japanese Society of Pharmacognosy, 2004, 8.9-11, Kaga, Japan.
6) 橋本斎，東田千尋，小松かつ子：Aβ25-35誘発性の神経突起萎縮に対するprotopanaxadiol系サポニンの腸内細菌代謝物M1による軸索伸展作用とそのメカニズム．第21回和漢医薬学会大会，2004，8.21-22，富山.
7) 東田千尋，畠中史幸，中山なつき，小松かつ子：NO産生系を指標とした鬱金類生薬の駆瘀血作用．第21回和漢医薬学会大会，2004，8.21-22，富山.
8) 高橋京子，松田秀康，松永和憲，隅田昭彦，木下香葉子，小松かつ子，服部征雄，高橋幸一，東純一：動物性生薬由来成分の肝薬物代謝酵素に及ぼす影響．第21回和漢医薬学会大会，2004，8.21-22，富山.
9) 西田裕子，高橋京子，上島悦子，小松かつ子，佐々木陽平，畠中史幸，高橋幸一，荒川行生，黒川信夫，東純一：ウコン属生薬の基源と品質：ヒト肝CYP代謝活性への影響．第21回和漢医薬学会大会，2004，8.21-22，富山.
10) 久保山友晴，東田千尋，小松かつ子：Withanolide A, withanoside IV, withanoside VIによる神

経突起再伸展とシナプス再形成作用．日本生薬学会第51年会，2004，9.9-10，神戸．

11）Zhu S., Zou K., Fushimi H., Cai S. Q., and Komatsu K.: Comparative study on triterpene saponins of Ginseng drugs. 日本生薬学会第51年会，2004，9.9-10，神戸．

12）佐々木聡子，佐々木陽平，伏見裕利，南雲清二，合田幸広，小松かつ子：日本市場に流通するガジュツの基原—*trn*K 遺伝子の塩基配列—．日本生薬学会第51年会，2004，9.9-10，神戸．

13）佐々木陽平，佐々木聡子，伏見裕利，南雲清二，合田幸広，小松かつ子：ガジュツ及びウコンの試験法に関する研究．日本生薬学会第51年会，2004，9.9-10，神戸．

14）久保山友晴，東田千尋，小松かつ子：神経突起伸展及びシナプス形成を機序とする withanolide 類の空間記憶障害改善作用．第27回日本神経科学大会・第47回日本神経化学大会合同大会 Neuro 2004，2004，9.21-23，大阪．

＊15）Komatsu K.: Recent Research on Genus *Curcuma*: Molecular Analysis, Identification and Quality Evaluation on Vasomotion Effect. The First International Conference presented by Western Pacific Regional Forum for the Harmoni- zation of Herbal Medicines, WHO/WPRO, 2004, 9.21-22, Shanghai, China.

◇その他　Others

資料等

1）小松かつ子，佐々木陽平，佐々木聡子，伏見裕利，南雲清二：平成15年度「日本薬局方の試験法に関する研究」研究報告—ガジュツ及びウコンの試験法に関する研究（第1報）—．医薬品研究，35：416-424，2004.

2）小松かつ子：富山県で栽培可能な生薬に関する総合的研究—優良種選抜を志向した和漢薬の品質の多様性に関する研究－人参類生薬の品質評価－．平成15年度受託研究「和漢薬・バイオテクノロジー研究」研究成果報告書，pp.37-44，2004.

3）小松かつ子：和漢薬研究所・民族薬物資料館②．（財）ヒューマンサイエンス振興財団，ヒューマンサイエンス，15(1)：38-39，2004.

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5）小松かつ子：生薬探訪シリーズ 大黄をめぐる．（株）ウチダ和漢薬，和漢薬，611：13-17，2004.

6）小松かつ子：「くすりの富山」の目指すもの（取材記事）．みゃあらく，創刊号，富山県広報課，2004，pp.10-11.

7）山路誠一：葉序の調節：明らかになったオーキシン極性輸送の役割．ファルマシア，40：1051-1052，2004.

講演等

8）小松かつ子：パネルディスカッション「慧海の旅とチベット」．東北大学総合学術博物館平成15年度公開講座【チベット探訪】第4回，東北大学，2004，2.7，仙台．

9）小松かつ子：モンゴルの伝統医薬と有用生物資源について．JBA・モンゴルワークショップ，（財）バイオインダストリー協会，2004，3.3，東京．

10）Komatsu K.: Molecular Analysis, Identification and Quality Evaluation of *Curcuma* Drugs from China and Japan. International Symposium on Oriental Medicine, Research Institute of Oriental Medicine, Dongguk University, 2004, 4.29, Kyongju, Korea.

11）小松かつ子：加賀・能登の『薬草シンポジウム』（第5回）薬草観察会．2004，5.22-23，輪島．

12）小松かつ子：野外で薬草を勉強する会．富山県薬事研究所，2004，7.4，富山．

13）小松かつ子：体験実習2．生薬方剤の鑑定．第9回和漢薬研究所夏期セミナー，2004，8.9-11，大山町．

14）小松かつ子：ウコン類生薬の遺伝子解析による基源解明と駆瘀血作用に基づく品質比較．第1回統合医療国際シンポジウム，富山県国際伝統医学センター，2004，10.24，富山．

15) 服部征雄，東田千尋，小松かつ子，土屋真澄，中村憲夫：コーヒー豆のトリゴネリンと脳神経細胞. 第7回「くすりと食物」シンポジウム－シーズ と ニーズ－，2004，11.19，東京.

16) 小松かつ子：遺伝子解析を応用した生薬および健康食品の評価. 21世紀COEプログラム・国際シンポジウム「薬用資源の保全とその有効利用」，21世紀COEプログラム（富山医科薬科大学），2004，12.4，富山.

17) Komatsu K. and Zhu S.: Workshop on Identification of Herbal Drugs by Molecular Methods. JSPS-NRCT Program, Faculty of Pharmaceutical Sciences, Chulalongkorn University, 2004, 12.7-9, Bangkok, Thailand.

18) Komatsu K.: Identification of Herbal Drugs by Molecular Technology: Background, Rationale, Significance, Impact. Workshop on Identification of Herbal Drugs by Molecular Methods, JSPS-NRCT Program, 2004, 12.7, Bangkok, Thailand.

19) Zhu S.: General Principle of DNA Technology for Authentication of Herbal Drugs. Workshop on Identification of Herbal Drugs by Molecular Methods, JSPS-NRCT Program, 2004, 12.7, Bangkok, Thailand.

20) Komatsu K.: Recent Research on Genus *Curcuma*: Molecular Analysis, Identification and Quality Evaluation on Vasomotion Effect. Workshop on Identification of Herbal Drugs by Molecular Methods, JSPS-NRCT Program, 2004, 12.8, Bangkok, Thailand.

21) Zhu S.: Pharmacognostical Study on Ginseng Drugs - Phylogenetic Analysis, Molecular Identification and Quality Evaluation. Workshop on Identification of Herbal Drugs by Molecular Methods, JSPS-NRCT Program, 2004, 12.9, Bangkok, Thailand.

22) 小松かつ子：民族薬物の謎を探る―くすりはいかに伝播したか. 九州厚生年金病院，（財）厚生年金事業振興団，2004，12.22，福岡.

◇海外調査　Oversea researches

1）小松かつ子：漢薬の資源をアジアに探る：モンゴル及びタイ産薬用植物の調査研究，科学研究費基盤研究(B)(2)，2004，7.5-7.24，モンゴル国.

2）小松かつ子：薬用資源（大黄）の調査研究，21世紀COEプログラム，2004，7.27-8.6，中華人民共和国.

◇共同研究　Co-operative researches

学内

1）柴原直利：富山医科薬科大学和漢薬研究所，「富山県で栽培可能な生薬に関する総合的研究」，2002～2004

国内

1）合田幸広：国立医薬品食品衛生研究所，「生薬及び漢方処方の科学的品質保証に関する研究」，2004～

2）高橋京子：大阪大学大学院薬学研究科臨床薬効解析学分野，「ヒト由来培養細胞を用いた和漢薬の吸収・代謝機構の解明」，2004～

海外

1）Javzan Batkhuu：国立モンゴル大学生物学部，蔡　少青：北京大学薬学院，Sitthithaworn Worapan : Srinakarinwirot 大学薬学部，服部征雄：富山医科薬科大学和漢薬研究所，東田千尋：富山医科薬科大学和漢薬研究所，「漢薬の資源をアジアに探る：モンゴル及びタイ産薬用植物の調査研究」，2002～2004

2）Vajrgupta Opa : Mahidol University,「DNA fingerprint of Thai Medicinal Plants」, 2004

◇非常勤講師　Part-time lecturer

1）小松かつ子：九州大学大学院薬学府・大学院担当科目「薬用植物育種学特論」，2004, 6.7，福岡.

2）小松かつ子：金沢大学教養的科目・総合科目「ヒマラヤ風土記」第11回「中国ヒマラヤの自然と文化」，2004, 12.16，第12回「チベット医学と仏教」，2005, 1.13，金沢.

◇研究費取得状況　Acquisition of research funds

1）21世紀 COE プログラム「東洋の知に立脚した個の医療の創生」（事業推進担当者：小松かつ子）「漢方薬資源の開発と基原や規格に関する基盤研究」

2）文部省科学研究費，基盤研究(B)(2)（第３年度）（代表：小松かつ子）「漢薬の資源をアジアに探る：モンゴル及びタイ産薬用植物の調査研究」，220万

3）（財）ヒューマンサイエンス振興財団（分担：小松かつ子）「生薬及び漢方処方の科学的品質保証に関する研究」：「生薬の科学的品質保証に関する研究」，100万

4）（財）田村科学技術振興財団（代表：小松かつ子）「各種ウコン属生薬の生活習慣病予防・治療薬としての有効性評価」，30万

5）富山医科薬科大学特別経費「戦略的経費」（分担：小松かつ子）「東洋の知に立脚した個の医療の創生」，70万

6）富山県受託研究「和漢薬・バイオテクノロジー研究」（分担：小松かつ子）「富山県で栽培可能な生薬に関する総合的研究：優良種選抜を志向した和漢薬の品質の多様性に関する研究」，50万

7）富山県受託研究（代表：小松かつ子）「富山産ヤマブシタケの抗痴呆作用の検討」，40万

◇研究室在籍者　Research members（11月から）

学部４年生：中山なつき，松山修二

大学院前期１年：市村真帆子，表　貴之，橋本　斎，劉　洪宇

大学院前期２年：佐々木聡子，杉山玲子

大学院後期３年：久保山友晴

研究機関研究員：朱　姝

技術補佐員（研究支援推進員）：幸　雅子，出口鳴美

技術補佐員：林　和子

◇学位（修士，博士）取得者　Academic degrees and theses

卒業論文：

中山なつき：病態モデルマウスを用いた鬱金類生薬の薬理作用の比較

松山修二：痴呆を治療する新しい漢方処方の開発およびその作用機序の解析

修士論文：

佐々木聡子：アジア産ウコン類を原料とする生薬及び健康食品の基源と品質に関する研究－遺伝子解析と Curcuminoids 含量－

杉山玲子：モンゴル産野生甘草の成分的多様性の解析と中国産甘草との比較

博士論文：

久保山友晴：インド生薬 Ashwagandha（*Withania somnifera* Dunal）成分による神経回路網再構築を機序とした抗痴呆作用の研究

化学応用分野 Division of Natural Products Chemistry

教 授	門田 重利	Professor	Shigetoshi Kadota (Ph.D.)
助教授	手塚 康弘	Associate Professor	Yasuhiro Tezuka (Ph.D.)
助 手	Suresh Awale	Assistant Professor	Suresh Awale (Ph.D.)

◇研究目的及び概要 Research projects

Ⅰ）伝統薬物から栄養飢餓状態で殺細胞作用を有する物質の探索

国立がんセンターとの共同研究で，膵臓癌 PANC-1 細胞株を用い，低栄養状態（IMEM）で PANC-1 に対する殺細胞活性を示し，通常培地（DMEM）では細胞の成育に活性を示さないような薬物を天然資源より探索している。これまでに，伝統薬物600種のエキス中，36エキスに PANC-1 細胞株に対する選択的な細胞毒性があることを見いだした。さらに36の伝統薬物のうち，特に強い活性を示した伝統薬物エキス 6 種を選び，これらについて現在研究を進めている。

Ⅱ）天然薬物から酵母 Ca^{2+} シグナル伝達阻害物質の探索と医薬への応用

広島大学で開発したポジティブスクリーニング法を用いて，新規医薬シーズ開発を目的に研究を行っている。中国及び東南アジア産生薬によるスクリーニングを行い，これまで約1000サンプルの試験を終え，25検体に阻害活性を見い出している。

Ⅲ）インドネシア産薬用植物から抗マラリア活性物質の探索

現代のマラリア流行地は，ほぼ熱帯・亜熱帯に限定され，それら地域では多剤耐性マラリアに有効な新しい抗マラリア薬が必要とされている。我々は東南アジア等で抗マラリア薬として用いられている薬用植物エキスについて多剤耐性マラリアに対する活性をスクリーニングを行い，活性を示した薬用植物中の活性物質を単離・構造解析を行っている。現在，インドネシア産薬用植物 *Caesalpinia crista* について抗マラリア作用を検討し，その活性物質の構造を解析した。

Ⅳ）天然薬物の薬物代謝酵素阻害に関する研究

和漢薬を始めとする天然薬物は，通常，合成医薬品と併用されている現状では，天然薬物が“薬物代謝酵素（シトクロームP450，CYP）”に及ぼす影響（薬物間相互作用）を系統的に検証しておく事が，天然薬物の有効利用の上で必要とされている。我々は，漢方生薬78種及びインドネシアジャムウ生薬30種について CYP3A4 及び CYP2D6 阻害活性を検索し，漢方生薬“五味子”およびジャムウ生薬“*Zingiber aromaticum*”の活性成分を明らかにした。

Ⅴ）骨粗鬆症に有効な天然薬物成分の開発研究

中医学において骨粗鬆症に類似の疾患（骨痿や骨痺）の治療に補腎剤や強筋骨剤が使用されている事に注目し，使用されている漢薬30種について抗骨粗鬆症活性をスクリーニングした。その結果，強い活性を示した漢薬“メンヒセン（*Dioscorea spongiosa* の根茎）”の成分の解明を行い，得られた成分の pQCT 装置による抗骨粗鬆症活性と合わせて，活性本体がステロイド配糖体である事を明らかにした。

また，紅豆杉の活性成分のリグナンが，抗骨粗鬆症活性がある事も判明した。

Ⅵ）ベトナム産生薬の Xanthine Oxidase 阻害活性物質の研究

痛風治療薬の開発を目的に，ベトナム産生薬（98種）について Xanthine Oxidase 阻害作用を指標にスクリーニングした。阻害作用の強かった *Caesalpinia sappan* 中の活性物質の構造を決定した。

Ⅶ）NO 産生阻害活性成分の検索

東南アジア各地で採集した *Orthosiphon stamineus* について，地理的な成分比較ならびに NO 阻害活性物質の構造を解析した。

上記の研究課題によって得られた本年度の成果（原著及び学会報告）は下記の通りである。

◇原著論文 Original papers

1）**Awale S., Tezuka Y., Kobayashi M., Ueda J., and Kadota S.: Neoorthosiphonone A; A Nitric Oxide (NO) Inhibitory Diterpene with New Carbon Skeleton From *Orthosiphon stamineus*. *Tetrahedron Lett.*, 45, 1359-1362 (2004).**

Abstract: From the aerial part of *Orthosiphon stamineus* from Hainan island of China, a diterpene named neoorthosiphonone A (**1**), having a novel carbon framework, has been isolated. Neoorthosiphonone A (**1**) possessed a unique unprecedented structural feature of eight membered ring C in its structure, which may be biogenetically derived from its isopimarane precursor, orthosiphonone A, through the insertion of vinyl group into the C_{13}-C_{14} bond. Neoorthosiphonone A (**1**) displayed potent inhibitory activity on the nitric oxide production in LPS-activated macrophage-like J774.1 cells with an IC_{50} value of 7.08 μM, more potent than the positive control L-NMMA.

2）**Nguyen N. T., Banskota A. H., Tezuka Y., Tran Q. L., Nobukawa T., Kurashige Y., Sasahara M., and Kadota S.: Hepatoprotective Effect of Taxiresinol and (7'*R*)-7'-Hydroxylariciresinol on D-Galactosamine and Lipopolysaccharide-Induced Liver Injury in Mice. *Planta Med.*, 70, 29-33 (2004).**

Abstract: The hepatoprotective effect of taxiresinol (**1**) and (7'*R*)-7'-hydroxylariciresinol (**2**), two tetrahydrofuran-type lignans isolated from the wood of *Taxus yunnanensis,* were investigated on D-galactosamine (D-GalN)/lipopolisaccharide (LPS)-induced hepatic liver injury in mice. Pre-administration of **1** or **2** at doses of 50 and 10 mg/kg (*i.p.*) at 12 and 1 h before D-GalN/LPS injection significantly inhibited hepatocyte DNA fragmentation and apoptotic body formation. Pre-treatment of these two lignans further suppressed hepatic necrosis which occur at later stage of D-GalN/LPS intoxication as demonstrated by the significant and dose-dependent reduction in serum glutamic pyruvic transaminase (sGPT) and serum glutamic oxaloacetic transaminase (sGOT) at 8 h after intoxication. The elevation of serum tumor necrosis factor-alpha (TNF-α) level by D-GalN/LPS toxication was significantly inhibited by **1** or **2** at doses of 50 and 10 mg/kg. Moreover, both of these lignans significantly protected hepatocytes from D-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes. These results suggested that **1** and **2** had protected the hepatocytes from apoptosis via an inhibition of TNF-α production by activated macrophages and a direct inhibition of apoptosis induced by TNF-α in D-GalN/LPS-treated mice.

3）**Iwata H., Usia T., Tezuka Y., Kadota S., Hiratsuka A., and Watabe T.: Inhibition of Human Liver Microsomal CYP3A4 and CYP2D6 with Extract from 78 Herbal Medicines. *J. Trad. Med.*, 21, 42-50 (2004).**

Abstract: The inhibitory effects of 78 herbal extracts on cytochrome P450 3A4 (CYP3A4) and P450 2D6 (CYP2D6) activity were investigated using human liver microsomes. The incubation mixture contained a methanol soluble fraction prepared from the powder of each herbal water extract (equivalent to 1.65 mg of extract powder per mL). Thirty-one herbal extracts inhibited over 50% of human liver microsomal erythromycin N-demethylation, a marker reaction of CYP3A4 activity. Among the 31 herbal extracts, 8 of them (Angelica Dahurica Root, Cassia Bark, Clove, Incised Notopterygium Rhizome, Moutan Bark, Rhubarb, Sappan Wood, Schisandra Fruit) inhibited N-demethylation by over 90%. Among the herbal extracts examined, the strongest inhibition of CYP3A4 was noted with Sappan Wood, which had an IC_{50} value of 43 μg/mL. Rhubarb, Schisandra Fruit, Incised Notopterygium Rhizome, and Angelica Dahurica Root had IC_{50} values of 77, 127, 144, and 185 μg/mL, respectively. Further, 28 of the herbal extracts inhibited over 50% of human liver microsomal dextromethorphan *O*-demethylation, which is a marker of CYP2D6 activity. Among the 28 herbal extracts, 13 (Cassia Bark, Clove, Coptis Rhizome, Ephedra Herb, Gambir Plant, Incised Notopterygium Rhizome, Magnolia Bark, Moutan Bark, Phellodendron Bark, Rhubarb, Sappan Wood, Sinomenium Stem, Zanthoxylum Fruit) inhibited *O*-demethylation by over 90%. The strongest inhibition of CYP2D6 was noted with Phellodendron Bark, which had an IC_{50} value of 4 μg/mL. Coptis Rhizome,

Sinomenium Stem, Sappan Wood, and Rhubarb showed IC_{50} values of 14, 40, 52, and 64 μg/mL, respectively. These results indicate that many herbal extracts have an inhibitory effect on CYP3A4 and CYP2D6.

4) **Yin J., Tezuka Y., Kouda K., Tran Q. L., Miyahara T., Chen Y., and Kadota S.: Antiosteoporotic Activity of the Water Extract of *Dioscorea spongiosa*. *Biol. Pharm. Bull.*, 27, 583-586 (2004).**

Abstract: After 60 MeOH and water extracts of natural crude drugs were screened for their ability to stimulate osteoblast proliferation, four MeOH extracts (*Cynomorium songaricum, Drynaria fortunei, Licium chinense, Rehmannia glutinosa*) and seven water extracts (*Cornus officinalis, Dendrobium nobile, Dioscorea spongiosa, Drynaria fortunei, Eucommia ulmoides, Lycium chinensis, Viscum coloratum*) showed that potent activities were evaluated for inhibition of osteoclast formation. The results indicated that the water extract of *D. spongiosa* not only showed the strongist stimulation of osteoblast proliferation but also possesed potent inhibitory activity against osteoclast formation, whereas it showed lower cytotoxicity in osteoblast and bone marrow cells. A further *in vivo* experiment determined the antiosteoporotic activity of this extract, in which it inhibited the decrease in cancellous bone mineral content, cancellous bone mineral density, and cortical bone mineral content of the proximal tibia in ovariectomized rats.

5) **Yin J., Kouda K., Tezuka Y., Tran Q. L., Miyahara T., Chen Y., and Kadota S.: New Diarylheptanoids from the Rhizomes of *Dioscorea spongiosa* and Their Antiosteoporotic Activity. *Planta Med.*, 70, 54-58 (2004).**

Abstract: Bioassay-guided fractionation of the water extract of the rhizomes of *Dioscorea spongiosa* led to the isolation and identification of new diarylheptanoids, diospongins A-C, together with three known lignans. Their structures, including absolute stereochemistry, were determined by analyses of NMR data, chemical conversions and CD spectrum. The isolated compounds, except for diospongin A, exerted potent inhibitory activities on bone resorption induced by parathyroid hormone in a bone organ culture system.

6) **Nakano H., Ogura K., Takahashi E., Harada T., Nishiyama T., Muro K., Hiratsuka A., Kadota S., and Watabe T.: Regioselective Monosulfation and Disulfation of the Phytoestrogens Daidzein and Genistein by Human Liver Sulfotransferases. *Drug Metab. Pharmacokinet.*, 19, 216-226 (2004).**

Abstract: Regioselective sulfation of the phytoestrogens daidzein (DZ, 7,4'-dihydroxyisoflavone) and genistein (GS, 5,7,4'-trihydroxyisoflavone) was investigated using human liver cytosol and purified recombinant human sulfotransferase (SULT) isoforms, SULT1A1, SULT1A3, SULT2A1, and SULT1E1. 7-Position-preferential sulfation of DZ and GS was observed in human hepatic cytosols from 3 male and 3 female subjects. Average ratios for 7- to 4'-sulfate formation were 4.5:1 from DZ and 8.4:1 from GS in these human liver cytosols. Apparent K(m) values for the 7- and 4'-sulfation of DZ and GS by these cytosols were similar and in a range from 0.46 to 0.66 microM. All recombinant human SULTs had activity for 7- and 4'-sulfation of these phytoestrogens except for 7-sulfating activity of SULT1A3. SULT1A1 and SULT1E1 exhibited much higher catalytic efficiency, k(cat)/K(m), for 7- and 4'-sulfation of these substrates than did the other two, SULT1A3 and SULT2A1. SULT1A1 showed K(m) values of 0.47 and 0.52 microM for the mono-sulfation of DZ and GS, respectively, which were very similar to those of human cytosol. The observed k(cat)/K(m) indicated that SULT1A1 catalyzed 7-sulfation of DZ and GS at rates 4.4- and 8.8-fold higher, respectively, than such 4'-sulfation. However, with SULT1E1, catalytic efficiency was very similar for the sulfation of both positions. These data strongly suggest that SULT1A1 plays a major role in monosulfation of the phytoestrogens and determines the regioselectivity of sulfation in human hepatic cytosol. A kinetic study for 7,4'-disulfate formation of DZ and GS from their 7- and 4'-monosulfates indicated that SULT1E1

most efficiently catalyzed both reactions among human SULTs.

7) **Nguyen M. T. T., Awale S., Tezuka Y., Chang C.-H., and Kadota S.: Staminane- and Isopimarane-type Diterpenes from *Orthosiphon stamineus* of Taiwan and Their Nitric Oxide Inhibitory Activity. *J. Nat. Prod.*, 67, 654-658 (2004).**

Abstract: From the MeOH extract of Taiwanese *Orthosiphon stamineus,* two new staminane-type diterpenes, staminols C (**1**) and D (**2**), and three new isopimarane-type diterpenes, orthosiphonones C (**3**) and D (**4**) and 14-deoxo-14-*O*-acetylorthosiphol Y (**5**), have been isolated together with 16 known diterpenes, orthosiphols A, B, D, K, M, N, O, X, and Y, norortho siphonolide A, neoorthosiphol B, orthosiphonone A, secoorthosiphols B and C, 3-*O*-deacetylorthosiphol I, and 2-*O*-deacetylorthosiphol J. Their structures were determined based on the spectroscopic data. All the newly isolated diterpenes exhibited dose-dependent inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated macrophage-like J774.1 cells, and 2-*O*-deacetylorthosiphonone A showed the most potent activity with an IC_{50} value of 35.0 μM, comparable to that of positive control N^G-monomethyl-L-arginine (L-NMMA; IC_{50}, 35.7 μM).

8) **Yin J., Tezuka Y., Kouda K., Tran Q. L., Miyahara T., Chen Y., and Kadota S.: *In vivo* Antiosteoporotic Activity of a Fraction of *Dioscorea spongiosa* and Its Constituent, Methyl Protodioscin. *Planta Med.*, 70, 220-226 (2004).**

Abstract: The antiosteoporotic activity of the 90% EtOH fraction of the water extract of rhizomes of *Dioscorea spongiosa* and methylprotodioscin, its major constituent, were examined in the model of postmenopausal bone loss using ovariectomized (OVX) rats or mice. After 6 weeks treatment, the proximal tibia of rats or mice and the distal femora of mice were scanned by peripheral quantitative computed tomography (pQCT). Both the 90% EtOH fraction (100 mg/kg/d) and methylprotodioscin (50 mg/kg/d) significantly inhibited bone loss in bone mineral content (BMC) and bone mineral density (BMD) in total, cancellous and cortical bones, and the decrease in bone strength indexes induced by OVX, without side effect on the uterus.

9) **Banskota A. H., Nguyen N. T., Tezuka Y., Tran Q. L., Nobukawa T., Kurashige Y., Sasahara M., and Kadota S.: Secoisolariciresinol and isotaxiresinol inhibit tumor necrosis factor-α-dependent hepatic apoptosis in mice. *Life Sci.*, 74, 2781-2792 (2004).**

Abstract: The effect of secoisolariciresinol (**1**) and isotaxiresinol (**2**), two major lignans isolated from the wood of *Taxus yunnanensis,* were investigated on tumor necrosis factor-α (TNF-α)-dependent hepatic apoptosis induced by D-galactosamine (D-GalN)/lipopolysaccharide (LPS) in mice. Co-administration of D-GalN (700 mg/kg) and LPS (10 μg/kg) resulted typical hepatic apoptosis characterized by DNA fragmentation and apoptotic body formation in mice. The serum glutamic pyruvic transaminase (sGPT) and glutamic oxaloacetic transaminase (sGOT) were also raised at 8 h after D-GalN/LPS intoxication due to severe necrosis of the hepatocytes. Pre-administration of **1** or **2** (50, 10 mg/kg, *i.p.*) at 12 and 1 h before D-GalN/LPS intoxication significantly reduced DNA fragmentation and prevented the emergence of chromatin condensation, apoptotic body formation and hepatitis of the mice. TNF-α secreted from LPS-activated macrophages is an important mediator for hepatocyte apoptosis in this model. Pretreatment of **1** or **2** significantly inhibited the elevation of serum TNF-α level. In a separate experiment, both lignans showed significant and dose-dependent hepatocyte protective effect towards D-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes. These results indicated that **1** and **2** protect D-GalN/LPS-induced hepatic injury by inhibiting hepatocyte apoptosis through blocking TNF-α production from activated macrophages and a direct inhibition of apoptosis induced by TNF-α.

10) **Usia T., Iwata H., Hiratsuka A., Watabe T., Kadota S., and Tezuka Y.: Sesquiterpenes and Flavonol Glycosides From *Zingiber aromaticum* and Their CYP3A4 and CYP2D6 Inhibitory Activities. *J. Nat. Prod.*, 67, 1079-1083 (2004).**

Abstract: Three new sesquiterpenes, (2*R*,3*S*,5*R*)-2,3-epoxy-6,9-humuladien-5-ol-8-one (**1**), (2*R*,3*S*,5*R*)-2,3-epoxy-6,9-humuladien-5-ol-8-one (**2**), and (5*R*)-2,6,9-humulatrien-5-ol-8-one (**3**), and two new flavonol glycosides, kaempferol-3-*O*-(2,3-di-*O*-acetyl-α-L-rhamnopyranoside) (**4**) and kaempferol-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside) (**5**), were isolated from the EtOAc-soluble fraction of the water extract of *Zingiber aromaticum,* along with 13 known compounds (**6-18**). The structures of the isolated compounds were elucidated on the basis of spectroscopic and chemical analyses. The isolated compounds were tested for their inhibitory activity on the metabolism mediated by CYP3A4 or CYP2D6 using [*N*-methyl-^{14}C]erythromycin or [*O*-methyl-^{14}C]dextromethorphan as a substrate, respectively. Kaempferol-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside) (**5**) showed the most potent inhibitory activity (IC_{50}, 14.4 μM) on the metabolism mediated by CYP3A4 and kaempferol-3-*O*-methylether (**14**) inhibited CYP2D6 most potently (IC_{50}, 4.63 μM).

11) **Nguyen M. T. T., Awale S., Tezuka Y., Tran Q. L., Watanabe H., and Kadota S.: Xanthine Oxidase Inhibitory Activity of Vietnamese Medicinal Plants. *Biol. Pharm. Bill.*, 27, 1414-1421 (2004).**

Abstract: Among 288 extracts, prepared from 96 medicinal plants used in Vietnamese traditional medicine to treat gout and related symptoms, 188 demonstrated xanthine oxidase (XO) inhibitory activity at 100 μg/ml, with 46 having greater than 50% inhibition. At 50 μg/ml, 168 of the extracts were active, with 21 possessing more than 50% inhibition. At 25 μg/ml, 146 extracts exhibited inhibitory activity, with 8 showing over 50 % inhibition, while 126 extracts presented activity at 10 μg/ml, with 2 having greater than 50% inhibition. The MeOH extracts of *Artemisia vulgaris, Caesalpinia sappan* (collected at the Seven-Mountain area), *Blumea balsamifera* (collected in Lam Dong province), *Chrysanthemum sinense* and MeOH-H_2O extract of *Tetracera scandens* (Khanh Hoa province) exhibited strong XO inhibitory activity with IC_{50} values less than 20 μg/ml. The most active extract was the MeOH extract of the flower of C. sinense with an IC_{50} value of 5.1 μg/ml. Activity-guided fractionation of the MeOH extract led to the isolation of caffeic acid (**1**), luteolin (**2**), eriodictyol (**3**), and 1,5-di-*O*-caffeoylquinic acid (**4**). All these compounds showed significant XO inhibitory activity in a concentration-dependent manner, and the activity of **2** was more potent (IC_{50} 1.3 μM) than the clinically used drug, allopurinol (IC_{50} 2.5 μM).

12) **Nguyen M. T. T., Awale S., Tezuka Y., Tran Q. L., and Kadota S.: Neosappanone A, a Xanthine Oxidase (XO) Inhibitory Dimeric Methanodibenzoxocinone with New Carbon Skeleton from *Caesalpinia sappan. Tetrahedron Lett.*, 45, 8519-8522 (2004).**

Abstract: A novel dimeric methanodibenzoxocinone, named neosappanone A (**1**), possessing a unique unprecedented novel carbon framework, has been isolated from the heartwood of *Caesalpinia sappan* L. of Vietnam, and its structure was elucidated on the basis of spectroscopic analysis. Neosappanone A (**1**) competitively inhibited xanthine oxidase in a concentration-dependent manner (IC_{50}, 29.7 μM; *Ki*, 16.3 μM).

13) **Iwata H., Tezuka Y., Kadota S., Hiratsuka A., and Watabe T.: Identification of Potent CYP3A4 Inhibitors in Schizandra Fruit Extract. *Drug Metab. Disp.*, 32, 1351-1358 (2004).**

Abstract: Schisandra fruit, a Schisandraceae family herb, is used as a component in Kampo medicines (developed from Chinese medicines, but established in Japan). It can act as a sedative and antitussive, improve hepatic function, and give a general tonic effect. An extract of Schisandra fruit has been shown with a potent inhibitory effect on human liver microsomal erythromycin *N*-demethylation activity mediated by cytochrome P450 3A4 (CYP3A4). The present study was conducted to identify Schisandra fruit components having inhibitory effects on CYP3A4 by

surveying the effect on human liver microsomal erythromycin *N*-demethylation activity. Known components of Schisandra fruit, gomisins B, C, G, and N and γ-shizandrin, showed inhibitory effects on *N*-demethylation activity. Among these components, gomisin C displayed the most potent and competitive inhibitory effect with a *K*i value of 0.049 μM. Furthermore, the inhibitory effect of gomisin C was stronger than that of ketoconazole (*K*i = 0.070 μM), a known potent CYP3A4 inhibitor. Gomisin C, however, inhibited CYP1A2-, CYP2C9-, CYP2C19-, and CYP2D6-dependent activities only to a limited extent (IC_{50} values > 10 μM). Moreover, gomisin C inactivated human liver microsomal erythromycin N-demethylation activity in a time- and concentration-dependent manner. The inactivation kinetic parameters k_{inact} and K_I were 0.092 min^{-1} and 0.399 μM, respectively. The human liver microsomal erythromycin *N*-demethylation activity inactivated by gomisin C did not recover on dialysis of the microsomes. Spectral scanning of CYP3A4 with gomisin C yielded an absorbance at 455 nm suggesting gomisin C inactivated the CYP via the formation of a metabolite intermediate complex. This pattern is consistent with the metabolism of the methylenedioxy substituent in gomisin C. These results indicate that gomisin C is a mechanism-based inhibitor that not only competitively inhibits but irreversibly inactivates CYP3A4.

14) **Iwata H., Tezuka Y., Kadota S., Hiratsuka A., and Watabe T.: Metabolism-Dependent Inhibition of CYP3A4 and CYP2D6 by Extracts from 26 Herbal Medicines. *J. Trad. Med.*, 21, 281-286 (2004).**

Abstract: A total of 26 herbal medicines were examined for their inhibitory effects on cytochrome P450 3A4 (CYP3A4) and 2D6 (CYP2D6). A methanol extract of each herbal medicine was prepared and then preincubated with human liver microsomes in the presence of an NADPH-generating system. Residual microsomal CYP3A4 and CYP2D6 activity was then determined by measuring the *N*-demethylation of erythromycin and the *O*-demethylation of dextromethorphan, respectively. Of the 26 herbal medicines tested, 16 were found to decrease the residual CYP3A4 activity in a preincubation time-dependent manner. The extract of Evodia Fruit caused the most dramatic decrease in residual CYP3A4 activity (i.e. 22.3% residual activity after 30 min preincubation). A substantial decrease in residual CYP3A4 activity was also observed from extracts of Sappan Wood, Incised Notopterygium Rhizome, Schisandra Fruit, Great Burdock Achene, Angelica Dahurica Root and Rhubarb (residual activity of 40.6, 41.2, 53.4, 47.1, 53.4 and 59.2% after 30 min preincubation, respectively). These results are comparable to those using troleandmycin, a known irreversible inhibitor of CYP3A4, which gave a residual activity of 49.4% under identical conditions. We found 5 herbal medicines that showed a preincubation time-dependent inhibition of CYP2D6. The extract of Incised Notopterygium Rhizome caused the most dramatic decrease in residual CYP2D6 activity (i.e. 61.9% residual activity after 30 min preincubation). These results suggest that extracts of herbal medicines contain metabolism-dependent inhibitors of CYP, especially CYP3A4.

15) **Kalauni S. K., Awale S., Tezuka Y., Banskota A. H., Linn T. Z., and Kadota S.: Cassane- and Norcassane-type Diterpenes of *Caesalpinia crista* from Myanmar. *J. Nat. Prod.*, *67*, 1859-1863 (2004).**

Abstract: From the CH_2Cl_2 extract of seed kernels of *Caesalpinia crista* from Myanmar, five new cassane-type diterpenes, caesalpinins MA-ME(**1-5**), and three new norcassane-type diterpenes, norcaesalpinins MA-MC (**6-8**), have been isolated, together with 12 known cassane-type diterpenes, 14(17)-dehydrocaesalmin F, caesaldekarin e, caesalmin B, caesalmin C, caesalmin E, 2-acetoxy-3-deacetoxycaesaldekarin e, 2-acetoxycaesaldekarin e, caesalpinin C, 7-acetoxybonducellpin C, caesalpinin E, norcaesalpinin B, and 6-acetoxy-3-deacetoxycaesaldekarin e. The structures of the isolated compounds were elucidated by analysis of their spectroscopic data.

◇総説　Review papers

1）門田重利，手塚康弘：プロポリス成分 CAPE およびその類縁体の癌転移抑制活性に関する研究.

ミツバチ科学 (*Honeybee Science*), 25, 107-112 (2004).

◇学会報告　Scientific presentation (*: 招待講演)

＊1) Shigetoshi Kadota, Arjun H. Banskota, Yasuhiro Tezuka, Nhan Trung Nguyen and Takahiro Nobukawa: Chemical Constituents and Biological Activities of the Wood of *Taxus yunnanensis*. IUPAC International Conference on Biodiversity and Natural Products: Chemistry and Medical Application, 2004, 1, New Delhi, India.

2) Suresh Awale, 手塚康弘, 上田純也, 門田重利：Study on the bioactive constituents from Brazilian medicinal plant *Tabebuia avellanedae* "Taheebo". 日本薬学会第124年会, 2004, 3, 大阪.

3) Surya K. Kalauni, Arjun H. Banskota, 手塚康弘, 門田重利：New Cassane- and Norcassane-type Diterpenes of *Caesalpinia crista* from Myanmar. 日本薬学会第124年会, 2004, 3, 大阪.

4) Thein Zaw Linn, 手塚康弘, Suresh Awale, Arjun H. Banskota, Faisal Attamimi, 門田重利：New Cassane- and 17-norcassane-type diterpenes of *Caesalpinia crista* from Indonesia. 日本薬学会第124年会, 2004, 3, 大阪.

5) 岩田　宏, 大沼友和, 金子哲也, Tepy Usia, 手塚康弘, 門田重利, 平塚　明, 渡部　烈：呉茱萸 (Evodiae fructus) に含まれる不可逆的CYP3A4阻害成分の検索. 日本薬学会第124年会, 2004, 3, 大阪.

6) Mai Thanh Thi Nguyen, Suresh Awale, 手塚康弘, 渡邉裕司, 門田重利：Xanthine Oxidase Inhibitory Activity of Vietnamese Medicinal Plants. 日本薬学会北陸支部第110回例会, 2004, 7, 金沢.

7) Suresh Awale, 手塚康弘, 小林光夫, 上田純也, 門田重利：Neoorthosiphonone A; A Nitric Oxide (NO) Inhibitory Diterpene with New Carbon Skeleton from *Orthosiphon stamineus*. 日本薬学会北陸支部第110回例会, 2004, 7, 金沢.

8) 門田重利, 殷　軍, 手塚康弘, Subehan, 史　麗穎, 上田純也, 松繁克道：Effect of Combination of Soft-shell Turtle "Suppon" and Essential Oil of Unicellular Chorophyte on Bone in OVX Rats. 第21回和漢医薬学会大会, 2004, 8, 富山.

9) Subehan, Tepy Usia, 岩田　宏, 渡部　烈, 門田重利, 手塚康弘：Mechanism-based Inhibition of CYP3A4 and CYP2D6 by Indonesian Medicinal Plants. 第21回和漢医薬学会大会, 2004, 8, 富山.

10) Suraj Prakash Shrestha, Suresh Awale, 手塚康弘, 上田純也, 松繁克道, 門田重利：Neoflavonoids from Nepalese propolis and their nitric oxide production inhibitory activity. 第21回和漢医薬学会大会, 2004, 8, 富山.

11) 岩田　宏, 金子哲也, Tepy Usia, 平塚　明, 渡部　烈, 手塚康弘, 門田重利：呉茱萸 (Evodiae fructus) に含まれるCYP3A4に対するmechanism-based inhibitorの同定. 第21回和漢医薬学会大会, 2004, 8, 富山.

12) Surya K. Kalauni, Suresh Awale, Arjun H. Banskota, 手塚康弘, 門田重利：New Cassane-Type Diterpenes of *Caesalpinia crista* from Myanmar. 日本生薬学会第51回年会, 2004, 9, 神戸.

13) Tepy Usia, 手塚康弘, 渡部　烈, 門田重利：CYP3A4 Inhibitory Constituents From *Piper cubeba*. 日本生薬学会第51回年会, 2004, 9, 神戸.

14) Subehan, Tepy Usia, 手塚康弘, 門田重利：Constituents of *Zingiber aromaticum* and Their CYP3A4 and CYP2D6 Inhibitory Activities. 日本生薬学会第51回年会, 2004, 9, 神戸.

◇その他　Others (*: 招待講演)

＊1) 門田重利：プロポリスの多彩な生理活性とその品質評価に関する研究. 第4回生体防御セミナーIN ぎふ (岐阜県生体防御研究会), 2004, 1, 各務原.

＊2）門田重利：Recent Studies on the Natural Medicine. 2004, 3/13, エジプト, ナショナル・リサーチセンター.
＊3）門田重利：Chemical and Biological Studies of *Taxus yunnanensis*. 2004, 3/14, エジプト, カイロ大学.
＊4）門田重利：Recent Progress in the Study of Natural Medicines. 2004, 6/23, 中国・南京大学.
＊5）門田重利：最近の天然薬物の研究. 平成16年度東海・北陸地区国立大学法人等教室系技術職員合同研修, 2004, 8/25, 富山医科薬科大学（富山）.
＊6）門田重利：Recent Studies on the Natural Medicine. 2004, 11/4, ベトナム・国立ホーチミン市大学.

◇海外調査　Field work

1）門田重利, Suresh Awale, 上田純也, Mai Thanh Thi Nguyen, Subehan：文部科学省科学研究費, 基盤研究B（2）「ベトナム, ミャンマーおよびインドネシアにおける伝統医学と天然薬物資源の調査研究」, 2004, 10/25-12/4, ベトナム, ミャンマー, インドネシア.

◇共同研究　Co-oparative researches

学内

1）宮原龍郎：富山医科薬科大学薬学部,「骨粗鬆症に有効な漢方方剤の開発研究」, 1998, 4～
2）津田正明：富山医科薬科大学薬学部,「天然薬物の高次神経機能発現を制御する活性物質の探索」, 2004, 4～

国内

1）江角浩安：国立がんセンター研究所支所,「がん生物学に基づく新しい治療法に関する研究」, 2003, 4～
2）宮川都吉：広島大学大学院・先端物質科学研究科,「酵母 Ca^{2+} シグナルによる細胞周期制御に関する総合的研究」, 2004, 4～
3）平塚　明：東京薬科大学,「和漢薬が関与する薬物相互作用に関する研究」, 2002, 4～
4）信川高寛：金沢医科大学,「紅豆杉の生物活性物質の探索」, 2004, 4～

海外

1）Dejair Message, Alfredo A. G. Fuertas：ブラジル・ヴィソーサ大学,「プロポリスの品質評価に関する研究」, 1996, 10～
2）殷　軍：中国・瀋陽薬科大学,「漢方方剤の抗骨粗鬆症活性成分に関する研究」, 2004, 10～
3）李　建新：中国・南京大学化学工学院・薬物化学研究所,「抗骨粗鬆症に有効な薬物の開発研究」, 2004, 4～
4）Tran Le Quan：ベトナム・国立ホーチミン市大学,「ベトナム産薬用植物の科学的評価に関する研究」, 2003, 4～

◇研究費取得状況　Acquisition of research funds

1）文部科学省科学研究費, 基盤研究B（2）（代表：門田重利）「ベトナム, ミャンマーおよびインドネシアにおける伝統医学と天然薬物資源の調査研究」
2）厚生労働省がん研究助成（分担：門田重利）「がん生物学に基づく新しい治療法に関する研究」
3）平成16年度研究拠点形成費補助金（COE）（フェロー：手塚康弘）「東洋の知に立脚した個の医療の創生」
4）平成16年度上原記念生命科学財団研究助成金（代表：門田重利）「漢方薬と合成医薬品の薬物相互作用」

◇研究室在籍者　Research members

学部4年生：川上つかさ，三宅克典
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大学院後期1年：史　麗穎（2004，4～）
大学院後期2年：Surya Kant Kalauni
大学院後期3年：Tepy Usia，岩田　宏，Mai Thanh Thi Nguyen
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外国人特別研究学生：李　霞（日本国際教育協会，瀋陽薬科大学大学院，2004，5/8～2005，5/7）
受託研究員：上田純也
外国人客員研究員：王　秀華（長春中医学院，2004，12/15～2005，6/15），趙　雨（長春中医学院，2004，12/15～2005，6/15）

◇学位（修士，博士）取得者　Academic degrees and theses

修士論文（2004年3月）：

Thein Zaw Linn : Chemical Constituents of the Seed Kernels of *Caesalpinia crista*

課程博士（2004年3月）：

Nhan Nguyen Trung：Chemical and Hepatoprotective Studies on the Wood of *Taxus yunnanensis*

殷　軍：Antiosteoporotic Constituents of Rhizomes of *Dioscorea spongiosa*

薬物代謝工学分野 Section of Metabolic Engineering

教　授	服部　征雄	Professor	Masao Hattori (Ph. D.)
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薬物代謝工学分野は和漢薬の薬効，毒性発現に関与する代謝系の分子生物学的研究を発展させることを設置目的とし，① 和漢薬の薬効発現に関与する腸内細菌の役割，② 薬物代謝に関する腸内細菌遺伝子の解明，③ 腎毒性物質産生機構の分子生物学的解明とその制御に関する研究を課題として取りあげ，和漢薬の薬効発現機構，生体へのレスポンスなどの基礎的研究を通じて，和漢薬の科学的評価や臨床応用をはかることを目指している。主な研究題目を以下に示す。

1．天然物のバイオトランスフォーメイション
2．和漢薬の薬効発現に関与する腸内細菌，酵素 および その遺伝子の解析
3．AIDS, C 型肝炎の予防 および 治療薬の開発
4．腎疾患，糖尿病性腎症の治療戦略
5．抗老化研究

本年度の主な研究を列挙すると：

1．*C*-配糖体マンギフェリンおよびプエラリンの C-C 結合の開裂に関与する腸内細菌を探索し *Bacteroides* 属の 2 菌種を同定した。またマンギフェリン *C*-配糖体開裂に関与する酵素を精製し，2種の蛋白よりなること，Mn^{2+} 要求性，補酵素を明らかにした。
2．台湾産樟芝菌糸体の抽出エキスおよびその分画がマウスに *Propionibacterium acnes* および LPS を投与することにより発症する劇症肝炎を顕著に抑制することを見出した。
3．中国少数民族薬物及びタイ薬用植物の C 型肝炎ウイルス由来の RNA ポリメラーゼ阻害活性を探索した。
4．腎疾患並びに糖尿病性腎症における新たな治療手段を探索するために，八味地黄丸，温脾湯，黄連，緑茶ポリフェノール，epicatechin 3-*O*-gallate，γ-aminobutyric acid を中心に検討した。
5．冠元顆粒 と ginsenoside-Rd の抗老化に及ぼす作用とその機序について検討した。

◇著書　Books

1）横澤隆子：「血管力をつければ病気は治る」，1-173，リヨン社，東京，2004.

2）中川孝子，横澤隆子：糖尿病性腎症における桂枝茯苓丸の有用性.「腎とフリーラジカル －第７集－」副島昭典，吉岡俊正監修，松澤直輝，青柳一正編，128-134，東京医学社，東京，2004.

3）中川孝子，横澤隆子：桂枝茯苓丸による糖尿病性腎症進展抑制作用 －aminoguanidine, butylated hydroxytoluene, captopril との比較－.「腎とフリーラジカル －第７集－」，副島昭典，吉岡俊正監修，松澤直輝，青柳一正編，135-140，東京医学社，東京，2004.

4）中川孝子，横澤隆子：温脾湯構成生薬ならびに大黄・甘草成分の advanced glycation end products（AGEs）形成抑制作用.「腎とフリーラジカル －第７集－」，副島昭典，吉岡俊正監修，松澤直輝，柳一正編，141-146，東京医学社，東京，2004.

◇原著　Original papers

1）**Ahn E., Akao T., Nakamura N., Komatsu K., Nishihara T., and Hattori M.: Screening of medicinal plant extracts for estrogenic activity in combination with a glycosidase treatment. *J. Trad. Med.*, 21: 81-86, 2004.**

Abstract: For the purpose of evaluating phytoestrogenic activity of medicinal plant extracts, a naringinase-pretreatment method was developed, monitoring with proliferation of MCF-7 human breast cancer cells and induction of β-galactosidase in a yeast two-hybrid assay system. Of various medicinal plant extracts examined, the extracts of *Alpinia katsumadai* (seeds), *Glycyrrhiza uralensis* (roots) and *Moghania philippinensis* (roots) showed higher estrogenic activity by pre-treatment with naringinase than the original extract themselves. The contents of liquiritigenin and isoliquiritigenin having potent estrogenic activity, appreciably increased after the naringinase treatment of the extract of *G. uralensis*. These findings suggested that orally administered crude drugs would increase their estrogenic activity, due to the hydrolysis of some glycosylated constituents by intestinal flora.

2）**Nakamura N., Hirakawa A., Gao J., Shiro M., Komatsu Y., Sheu C., and Hattori M.: Five new maleic and succinic acid derivatives from the mycelium of *Antrodia camphorata* and their cytotoxic effects on LLC tumor cell line. *J. Nat. Prod.*, 67: 46-48, 2004.**

Abstract: Five new maleic and succinic acid derivatives were isolated from the mycelium of *Antrodia camphorata*. Their structures were determined by various spectroscopic means. Maleimide derivatives **2** and **3** showed appreciable cytotoxic activity against LLC cells. (Chart 1 参照)

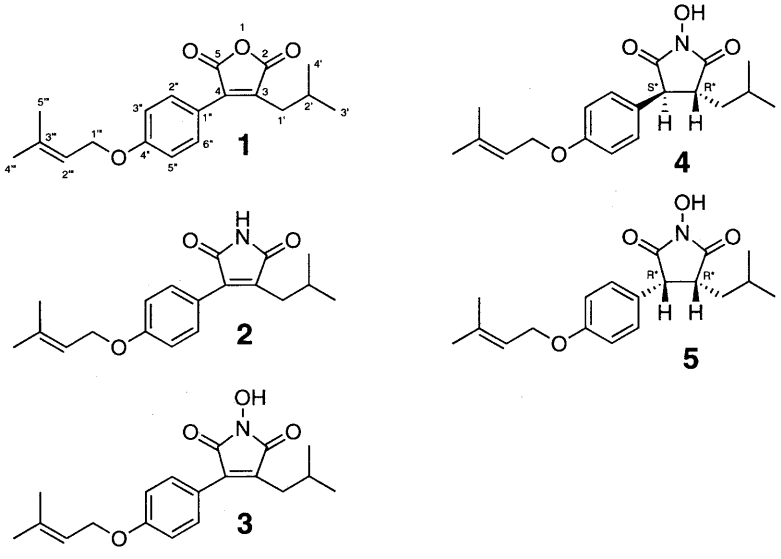
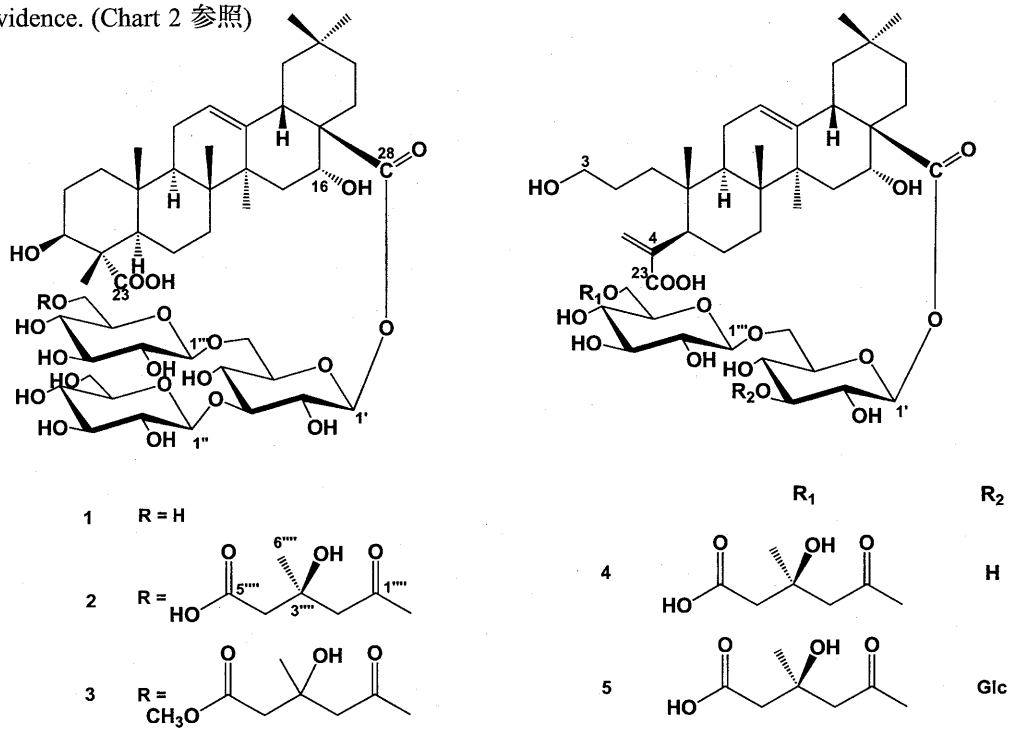


Chart 1

3）**Zhao J., Nakamura N., Hattori M., Yang X., Komatsu K., and Qiu M.: New triterpenoid saponin from the roots of *Sinocrassula asclepiadea*. *Chem. Pharm. Bull.*, 52: 230-237, 2004.**

Abstract: Five new triterpenoid monodesmosides (sinocrassulosides I-V, **1-5**) and six bisdesmosides (sinocrassulosides VI-XI, **6-11**), in which **2-11** possess different acyl groups in the glycosidic moieties, were isolated from the roots of *Sinocrassula asclepiadea* FRANCH. Sinocrassulosides VI (**4**) and V (**5**) also contained a novel A-*seco* aglycone in their structures. All of the structures were determined on the basis of spectroscopic and physicochemical evidence. (Chart 2 参照)



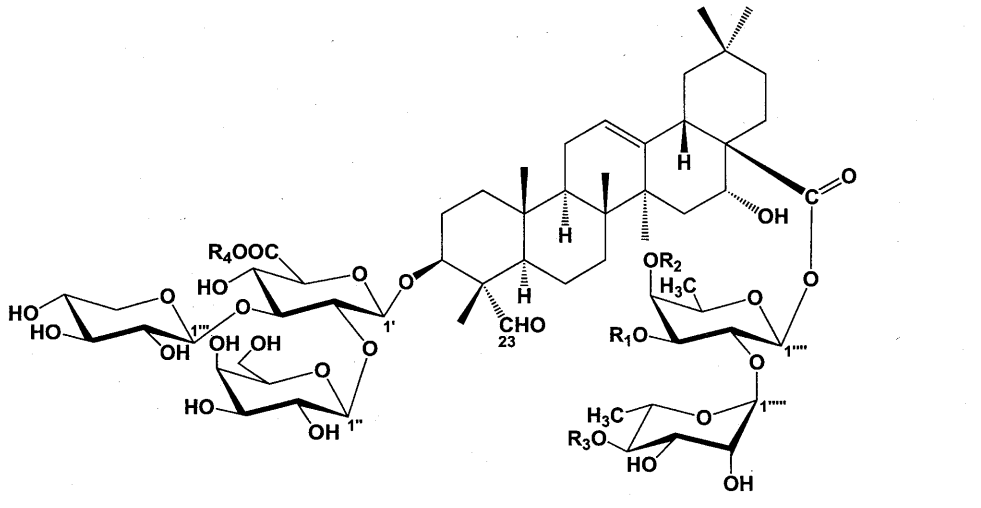


Chart 2

4) **Ahn E., Nakamura N., Akao T., Nishihara T., and Hattori M.: Estrogenic and anti-estrogenic activities of the roots of *Moghania philippinensis* and their constituents. *Biol. Pharm. Bull.*, 27: 548-553, 2004.**

Abstract: In the course of our search for natural estrogenic compounds from medicinal plants, we found that the methanolic extract from the roots of *Moghania philippinensis* (Fabaceae) showed significant effects on the proliferation of MCF-7 cells (human breast cancer) and induction of β-galactosidase activity in a yeast two-hybrid assay. Through estrogenic activity-guided fractionation, we isolated several active flavonoids including prenylated ones. The $CHCl_3$ fraction and its new constituent, 8-(1,1-dimethylallyl)genistein (**9**), appreciably increased the uterine weight in ovariectomized rats when administered orally for 14 consecutive days, in which compound **9** showed stronger estrogenic activity than genistein. Anti-estrogenic activities were also examined based on the inhibition of MCF-7 cell proliferation and β-galactosidase activity in the yeast two-hybrid assay, mediated by 17 β-estradiol. 5,7,3',4'-Tetrahydroxy-6,8-diprenylisoflavone (**6**) showed the strongest antiestrogenic activity.

5) **Zhang Y., Akao T., Nakamura N., Duan C., Hattori M., Yang X., and Liu J.: Extremly low bioavailability of magnesium lithospermate B, an active component from *Salvia miltiorrhiza*, in rat. *Planta Med.*, 70: 138-142, 2004.**

Abstract: We assessed the bioavailability of magnesium lithospermate B (MLB), a main polyphenolic component of *Salvia miltiorrhiza* and a potent antioxidant having various pharmacological activities, to evaluate its action *in vivo*. The plasma concentrations of lithospermic acid B (LSB) showed a biexponential decrease after intravenous administration of MLB to rats at doses of 4 and 20 mg/kg. The values of area under the concentration-time curve (AUC; 87.8 ± 10.9 and 1130 ± 329 μg·min/mL), total body clearance (CL_{tot}; 55.52 ± 7.07 and 23.51 ± 5.98 mL/min/kg), and distribution volume at steady state (V_{ss}; 7.60 ± 1.03 and 3.61 ± 1.16 L/kg) suggested non-linear pharmacokinetics between the two doses. After oral administration of MLB at a high dose of 100 mg/kg, The mean AUC was barely 1.26 ± 0.36 μg·min/mL. Absolute bioavailability of MLB was calculated to be 0.0002 from the AUC values after both intravenous dosing at 20 mg/kg and oral dosing at 100 mg/kg. The extremely low bioavailability was caused mainly by poor absorption from the rat gastrointestinal tract; about 65% of the dose was retained in the tract even 4 h after oral administration, and most of the dose was retained even 20 min after infusion in an *in situ* jejunal loop experiment. Urinary and biliary excretion of LSB were only 0.70% ± 0.26% and 5.10% ± 2.36%, respectively, over a 30 h time period after intravenous injection despite the large CL_{tot} and V_{ss} values, and were much less (0.010% ± 0.001% and 0.12% ± 0.04%) after oral dosing. These findings suggest that extensive metabolism, including a firstpass effect, and wide distribution of LSB besides the poor absorption contributed significantly to the extremely low systemic bioavailability.

6) **Gao J., Nakamura N., Min B., Hirakawa A., Zuo F., and Hattori M.: Quantitative determination of bitter principles in specimens of *Ganoderma lucidum* using high-performance liquid chromatography and its application to the evaluation of ganoderma products. *Chem. Pharm. Bull.*, 52: 688-695, 2004.**

Abstract: For quantitative determination of 19 triterpene constituents, including six ganoderma alcohols (**1-6**) and 13 ganoderma acids (**7-19**), in the products of *Ganoderma lucidum,* an analytical system was developed using high-performance liquid chromatography with an ODS column. The mobile phase was a linear gradient of 1% AcOH/H_2O-CH_3CN and 2% AcOH/H_2O-CH_3CN, and the elution profile was monitored at 243 and 250 nm for ganoderma alcohols and acids, respectively. The relative standard deviations of this method were less than 2.35% and 2.18% (n=5) for intraday and interday assays, and the recoveries were 90.9-100.8% and 93.4-103.9% for constituents of alcohol and acid groups, respectively, This system was applied to a quantitative determination of the constituents in 10 different products of *G. lucidum*: six usual umbrella forms of the fruiting bodies, three antlered forms of the

fruiting bodies and spores, and eight specimens from the same *G. lucidum* strain, which was parasitized on logs from different plants or different fungus beds. The analytical results indicated that the quantity and composition of these triterpenes differed appreciably among various specimens, but the relative ratio of the alcohols and acids was not significantly different when the same strain of *G. lucidum* was used.

7) **Min B., Gao J., Lee Y., Nakamura N., and Hattori M.: Chemical and biological evaluation of germinated and mature antler-shaped fruiting bodies of *Ganoderma lucidum. Natural Medicines,* 58: 91-97, 2004.**

Abstract: For the purpose of evaluating germinated (**I**) and mature (**II**) antler-shaped fruiting bodies of *Ganoderma lucidum,* aqueous extracts of both crude drugs were compared from the point of view of chemical constituents and antitumor activity. In both aqueous extracts, ganodermanontriol and ganoderic acid A were major components of *Ganoderma* alcohols and acids, respectively. However, the total lanostane-type triterpene content of **II** was 6 times greater than that of **I**. The contents and compositions of the respective triterpenes were different from each other. However, the total polysaccharide contents of aqueous extracts of **I** and **II** were not significantly different, as indicated by 13.4 and 11.7%, respectively.

The aqueous extracts of **I** and **II** showed inhibitory effects on the growth of s.c. transplanted Lewis lung carcinoma (LLC) in BDF-1 mice by intraperitoneal administration; an aqueous extract of **I** gave T/C values of 80.8 and 73.0% at doses of 100 and 500 mg/kg/d i.p., and that of **II**, T/C values of 76.5 and 61.5% at the same doses, respectively, indicating the extracts **I** and **II** were similar antitumor activity.

8) **Min B., Kwon O., Park B., Kim Y., Hattori M., Joung H., and Lee H.: Apoptosis-inducing activity of galloylglucose from *Juglans mandshurica* in human promyeloid leukemic HL-60 cells. *Nat. Prod. Sci.,* 10: 48-53, 2004.**

Abstract: Two galloyl monosaccharides, 1,2,6-trigalloylglucose (**1**, TRgG) and 1,2,3,6-tetragalloylglucose (**2**, TEgG), were isolated from the stem-bark of *Juglans mandshurica.* Two galloylglucoses showed cytotoxic effects on human promyelocytic leukemia HL-60 cells. In order to elucidate their mechanism of action, we have investigated the flow cytometric analysis after Annexin V-FITC and PI staining, caspase-3 activity, and internucleosomal DNA fragmentation in HL-60 cells. HL-60 cells treated with both compounds **1** and **2** at 150 and 100 μM, respectively, led to a morphological features of apoptosis, such as plasma membrane blebbing and cell shrinkage. TRgG (**1**) and TEgG (**2**) increased the percentage of $FITC^+$ and $FITC^+PI^+$ cell in flow cytometry after Annexin V-FITC and PI staining. The increase of apoptotic cells was preceded by the activation of caspase-3 reported to play a central role in apoptotic process and inducing internucleosomal DNA fragmentation. TEgG (**2**) showed to have stronger apoptosis inducing activity in HL-60 cell lines as compared with TRgG (**1**).

9) **Zhang Y., Akao T., Nakamura N., Hattori M., Yang X., Duan C., and Liu J.: Magnesium lithospermate B is excreted rapidly into rat bile mostly as methylated metabolites, which are potent antioxidants. *Drug Metabolism and Disposition,* 32: 752-757, 2004.**

Abstract: To elucidate the *in vivo* pharmacological activities of magnesium lithospermate B (MLB), an active constituent of Radix Salviae Miltiorrhizae, in the rat, its metabolic fate both *in vivo* and *in vitro* was investigated. High-performance liquid chromatography revealed that four major metabolites with lower polarity were excreted into bile after intravenous and oral administration of MLB. The metabolites present in combined samples of bile from rats after intravenous injection were isolated and purified by column chromatography and identified as four *meta-O*-methylated products, namely 3-monomethyl- (M1), 3,3'''-dimethyl- (M2), 3,3''-dimethyl-, and 3,3'',3''' -trimethyl-lithospermic acid B according to their spectroscopic characteristics (^1H, ^{13}C NMR, ^1H-^1H correlation spectroscopy, ^1H-detected multiple quantum coherence, and heteronuclear multiple bond coherence combined with positive ion fast atom bombardment-mass spectroscopy). After administration of MLB at an intravenous dose of 4 mg/kg or an oral

dose of 100 mg/kg, the total biliary recovery of the four metabolites after 30 h reached 95.5 ± 2.4% (with approximately 90% recovered within 2 h) or 5.5 ± 0.7%, respectively. The metabolic pathway was proposed to involve sequential formation of the four methylated metabolites. Incubation of MLB, M1, M2, or M4 in rat hepatic cytosol in the presence of S-adenosyl-L-methionine demonstrated the formation of all four metabolites, which indicated that the enzyme responsible for the biotransformation is catechol *O*-methyltransferase. MLB and its main metabolites M1 and M2 showed potent 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activities, the activity of M1 being stronger than those of caffeic acid (the monomer form of MLB) and α-tocopherol (a representative antioxidant) but weaker than that of MLB. The rapid and high biliary excretion levels of these metabolites suggested that they could undergo enterohepatic circulation in rats and that they might thereby be largely responsible for the pharmacological effects of MLB.

10) **Ma C., Nakamura N., Nawawi A., Hattori M., and Cai S.: A novel protoilludane sesquiterpene from the wood of *Xanthoceras sorbifolia*. *Chinese Chem. Lett.*, 15: 65-67, 2004.**

Abstract: A protoilludane sesquiterpene (named xanthocerapene) was isolated from the wood of *Xanthoceras sorbifolia* Bunge. Its structure, including the relative configuration was established by spectroscopic and chemical methods.

11) **Basu N. K., Kubota S., Meselhy M. R., Ciotti M., Chowdhury B., Hattori M., and Owens I. S.: Gastrointestinally distributed UDP-glucuronosyltransferase 1A10, which metabolizes estrogens and nonsteroidal anti-inflammatory drugs, depends upon phosphorylation. *J. Biol. Chem.*, 270: 28320-28329, 2004.**

Abstract: Among gastrointestinal distributed isozymes encoded at the UGT1 locus, UDP-glucuronosyltransferase 1A10 (UGT1A10) metabolizes a number of important chemicals. Similar to broad conversion of phytoestrogens (Base, N. K., Ciotti, M., Hwang, M. S., Kole, L., Mitra, P. S., Cho, J. W., and Owens, I. S. (2004) *J. Biol. Chem.* 279, 1429-1441), UGT1A10 metabolized estrogens and their derivatives, whereas UGT1A1, -1A3, -1A7, and -1A8 differentially exhibited reduced activity toward the same. UGT1A10 compared with UGT1A7, -1A8, and -1A3 generally exhibited high activity toward acidic nonsteroidal ant-inflammatory drugs and natural benzaldehyde derivatives, while UGT1A3, metabolized most efficiently aromatic transcinnamic acids known to be generated from flavonoid glycosides by microflora in the lower gastrointestinal tract. Finally UGT1A10, -1A7, -1A8, and -1A3 converted plant-based salicylic acids; methylsalicylic acid was transformed at high levels, and acetylsalicylic (aspirin) and salicylic acid were transformed at moderate to low levels. Atypically UGT1A10 transformed estrogens between pH 6 and 8 but acidic structures preferentially at pH 6.4. Furthermore evidence indicates UGT1A10 expressed in COS-1 cell depends upon phosphorylation; UGT1A10 *versus* its single, double, and triple mutants at three predicted protein kinase C phosphorylation sites incorporated [^{33}P]-orthophosphate and showed a progressive decrease with no detectable label or activity for the triple T73A/T202A/S432G-1A10 mutant. Single and double mutants revealed either null/full activity or null/additive activity, respectively. Additionally UGT1A10-expressing cultures glucuronidated 17β- [^{14}C] estradiol, whereas cultures containing null mutants at protein kinase C sites showed no estrogen conversion. Importantly UGT1A10 in cells supported 10-fold higher glucuronidation of 17β-estradiol than UGT1A1. In summary, our results suggest gastrointestinally distributed UGT1A10 is important for detoxifying estrogens/phytoestrogens and aromatic acids with complementary activity by UGT1A7, -1A8, -1A3, and/or -1A1 evidently dependent upon phosphorylation.

12) **Ahn E. M., Nakamura N., Fushimi H., Komatsu K., Batkhuu J., and Hattori M.: Constituents of the seeds of *Glycyrrhiza uralensis*. *Natural Medicines*, 58: 311, 2004.**

13) **Yokozawa T., Satoh A., and Cho E.J.: Ginsenoside-Rd attenuates oxidative damage related to aging in senescence-accelerated mice. *J. Pharm. Pharmacol.*, 56: 107-113, 2004.**

Abstract: Among the various theories of the aging process, the free radical theory, which proposes that deleterious actions of free radicals are responsible for the functional deterioration associated with aging, has received widespread attention. The theory suggests that enhancement of the antioxidative defense system to attenuate free radical-induced damage will counteract the aging process. We used senescence-accelerated mice (SAM) to investigate the relationship between aging and the antioxidative defense system and evaluated the effects of ginsenoside-Rd, the saponin from ginseng, by measuring antioxidative defense system parameters, including the glutathione (GSH)/glutathione disulfide (GSSG) redox status, antioxidative enzyme activities and level of lipid peroxidation. SAM at 11 months of age (old SAM) showed a significantly lower hepatic GSH/GSSG ratio, due to decreased GSH and increased GSSG levels, than SAM at 5 weeks of age (young SAM). However, the administration of ginsenoside-Rd at a dose of 1 or 5 mg/kg body weight/day for 30 days to 10-month-old SAM significantly increased GSH, but decreased GSSG, resulting in elevation of the GSH/GSSG ratio. In addition, ginsenoside-Rd increased the activities of glutathione peroxidase (GSH-Px) and glutathione reductase that were both significantly lower in old than young SAM. This suggests that ginsenoside-Rd could play a crucial role in enhancing the defense system through regulation of the GSH/GSSG redox status. Moreover, decreases in the superoxide dismutase (SOD) and catalase activities in old SAM compared with young SAM were also revealed, indicating that the aging process resulted in suppression of the antioxidative defense system. However, ginsenoside-Rd did not affect SOD and catalase activities. As catalase is localized in peroxisome granules and GSH-Px is present in the cytoplasm and mitochondrial matrix, the site of ginsenoside-Rd action may be the cytoplasm and mitochondrial matrix. Furthermore, the serum and liver malondialdehyde levels, indicators of lipid peroxidation, were elevated with aging, while ginsenoside-Rd inhibited lipid peroxidation. The present study indicates that the aging process leads to suppression of the antioxidative defense system and accumulation of lipid peroxidation products, while ginsenoside-Rd attenuates the oxidative damage, which may be responsible for the ntervention of GSH/GSSG redox status.

14) **Yokozawa T., Rhyu D.Y., and Cho E.J.: (-)-Epicatechin 3-*O*-gallate ameliorates the damages related to peroxynitrite production by mechanisms distinct from those of other free radical inhibitors. *J. Pharm. Pharmacol.*, 56: 231-239, 2004.**

Abstract: This study was carried out to elucidate whether the protective activity of (-)-epicatechin 3-*O*-gallate (ECg) against excessive peroxynitrite ($ONOO^-$) production, is distinct from the activities of several well-known free radical inhibitors, the $ONOO^-$ inhibitors ebselen and uric acid, the superoxide anion (O_2^-) scavenger copper zinc superoxide dismutase (CuZnSOD) and the selective inducible nitric oxide synthase inhibitor L-N^6-(1-iminoethyl)lysine hydrochloride (L-NIL). To generate $ONOO^-$, male Wistar rats (n=6/group) were subjected to ischemia-reperfusion process together with lipopolysaccharide (LPS) injection. Although ECg did not scavenge the $ONOO^-$ precursors nitric oxide (NO) and O_2^-, it reduced the 3-nitrotyrosine level, a property similar to that of uric acid, but distinct from L-NIL. In addition, the elevation in myeloperoxidase activity was reversed by the administration of ECg, uric acid and SOD, but not by that of L-NIL. Furthermore, ECg was the more potent scavenger of the $ONOO^-$ decomposition product the hydroxyl radical (·OH) than any other free radical inhibitor tested. The LPS plus ischemia-reperfusion process resulted in renal dysfunction, estimated by measuring the parameters of renal functions of serum urea nitrogen and creatinine levels. However, administration of ECg ameliorated renal dysfunction more than that of the other free radical inhibitors. Moreover, ECg reduced the excessive uric acid level, while the others did not, suggesting a property of ECg distinct from the others. Furthermore, proteinuria, which was demonstrated by the low- and high-molecular weight (LMW and HMW) protein bands of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern, caused by LPS plus ischemia-reperfusion was attenuated by administration of ECg and L-NIL, after which the HMW band intensities decreased and LMW protein bands were absent Please check. This study indicates that,

in an *in vivo* model of $ONOO^-$ generation, ECg, L-NIL and uric acid exert stronger protective activity against $ONOO^-$-induced oxidative damage than SOD and ebselen, and that the mechanism whereby ECg protects against $ONOO^-$ is distinct from that of L-NIL or uric acid.

15) **Yokozawa T., Ishida A., Kashiwada Y., Cho E.J., Kim H.Y., and Ikeshiro Y.: Coptidis Rhizoma: protective effects against peroxynitrite-induced oxidative damage and elucidation of its active components. *J. Pharm. Pharmacol.*, 56: 547-556, 2004.**

Abstract: The aim of this study was to investigate the protective effects of Coptidis Rhizoma against peroxynitrite ($ONOO^-$)-induced oxidative damage and elucidate the active components of this preparation. In an *in vitro* system, Coptidis Rhizoma extract scavenged $ONOO^-$ and its precursors, nitric oxide (NO) and superoxide anion (O_2^-), and this scavenging activity was more marked for $ONOO^-$ than its precursors. In addition, against 3-morpholinosydnonimine-induced cellular damage, this extract significantly reduced cellular $ONOO^-$ formation and increased cell viability. In an *in vivo* lipopolysaccharide plus ischemia-reperfusion system that generates $ONOO^-$, the administration of Coptidis Rhizoma extract at 50 and 100 mg/kg body weight/day for 30 days exerted greater inhibition of $ONOO^-$ than NO and O_2^-, suggesting that it acts as a direct scavenger of $ONOO^-$, rather than as a scavenger of its precursors. Moreover, the suppression of the activities of the antioxidative enzymes superoxide dismutase, catalase and glutathione peroxidase was significantly attenuated by the administration of Coptidis Rhizoma extract. Furthermore, the extract ameliorated renal dysfunction judged by decreasing serum urea nitrogen and creatinine levels. To elucidate the active components of Coptidis Rhizoma extract, we evaluated and compared the effects of the phenol plus alkaloid and alkaloid fractions on $ONOO^-$-induced damage. We found that the alkaloid fraction consisting of berberine, palmatine and coptisine was the most effective at protecting against $ONOO^-$. We also confirmed that berberine (10 and 20 mg/kg body weight/day for 10 days), the main and most active alkaloid in Coptidis Rhizoma extract, was also protective, exerting NO-, O_2^-- and $ONOO^-$-scavenging activities. This study suggests that Coptidis Rhizoma could protect against $ONOO^-$-induced oxidative damage and that this effect is mainly attributable to the constituent alkaloids, especially berberine. This study is the first to demonstrate an antioxidative effect of alkaloids, including berberine, against $ONOO^-$-induced damage.

16) **Satoh A., Yokozawa T., Cho E.J., Okamoto T., and Sei Y.: Antioxidative effects related to the potential anti-aging properties of the Chinese prescription Kangen-karyu and Carthami Flos in senescence-accelerated mice. *Arch. Gerontol. Geriatr.*, 39: 69-82, 2004.**

Abstract: The popular oxidative stress theory predicts that enhancement of the antioxidative defense system to attenuate free radical-induced damage counteracts the aging process. We used senescence-accelerated mice (SAM) because SAM has been shown to suppress the antioxidative defense system and mitochondrial dysfunction induced by oxidative stress. We investigated the antioxidative effects of the Chinese prescription Kangen-karyu and its crude drug component Carthami Flos. The administration of Kangen-karyu extract at 100 mg/kg body weight/day for 10 weeks inhibited generation of nitric oxide, superoxide and the hydroxyl radical (·OH), while Carthami Flos extract showed only ·OH-scavenging activity. Diet supplemented with Kangen-karyu and Carthami Flos extracts enhanced the activities of the antioxidative enzymes superoxide dismutase in hepatic tissue and glutathione peroxidase in renal tissue, and reduced the hepatic lipid peroxidation level which increased with aging, indicating the protective action against oxidative stress by enhancing the antioxidative status. Hepatic and renal dysfunction with aging was also ameliorated by the administration of Kangen-karyu and Carthami Flos supplements. Furthermore, the observed antioxidative properties of the Chinese prescription Kangen-karyu were more evident than those of Carthami Flos. These findings suggest that the protective activity of Kangen-karyu against the oxidative tissue damages during aging may be due partly to synergistic and/or additive effects of its crude preparation. The present study strongly indicates that Kangen-karyu counteract the oxidative stress and ameliorating tissue damage possibly associated with aging in SAM.

17) **Yokozawa T., and Nakagawa T.: Inhibitory effects of Luobuma tea and its components against glucose-mediated protein damage. *Food Chem. Toxicol.*, 42: 975-981, 2004.**

Abstract: Luobuma tea, prepared from the leaves of *Apocynum venetum* L., is a popular beverage in China. In this study, the activity of Luobuma leaf extract and its components against the formation of advanced glycation endproducts (AGEs), which are largely involved in the pathogenesis of diabetic vascular complications, was examined using the *in vitro* glycation reaction. Strong inhibitory activity against the formation of AGEs was shown by Luobuma aqueous extract. Following further fractionation of this extract, seven polyphenolic compounds, i.e. (±)-gallocatechin, (-)-epigallocatechin, (±)-catechin, (-)-epicatechin, epicatechin-(4β-8)-gallocatechin, epigallocatechin-(4β-8)-epicatechin and procyanidin B-2, were isolated by Sephadex LH-20 column chromatography. These purified compounds also exerted inhibitory activities that were more potent than the positive control, aminoguanidine. Our findings may help to explain the beneficial effects of this plant against atherosclerosis.

18) **Nakagawa T., Yokozawa T., Sano M., Takeuchi S., Kim M., and Minamoto S.: Activity of (-)-Epigallocatechin 3-*O*-gallate against oxidative stress in rats with adenine-induced renal failure. *J. Agric. Food Chem.*, 52: 2103-2107, 2004.**

Abstract: Methylguanidine (MG) is widely recognized as a strong uremic toxin. The hydroxyl radical (·OH) specifically plays an important role in the pathway of MG production from creatinine (Cr). In this study, we investigated whether oral administration of (-)-epigallocatechin 3-*O*-gallate (EGCg) suppresses MG production in rats with chronic renal failure after intraperitoneal Cr injection. MG production from Cr was significantly increased in rats with adenine-induced renal failure, which was more vulnerable to oxidative stress, compared with that in normal rats. However, oral administration of EGCg 30 min before and after Cr injection effectively inhibited MG production. Our findings suggest that EGCg, an excellent antioxidant from green tea, exerts protective activity in rats with chronic renal failure, resulting in suppression of Cr oxidation influenced by ·OH.

19) **Satoh A., Yokozawa T., Tanaka T., Okamoto T., and Sei Y.: The antioxidative activity of Kangen-karyu extract delays senescence of human lung fibroblasts. *J. Trad. Med.*, 21: 87-93, 2004.**

Abstract: Replicative senescence (RS) of human diploid fibroblasts (HDFs) has become a classical model of aging and HDFs, such as WI-38 cells, display increased cellular oxidant production associated with RS. Several phenomena associated with RS are also observed in stress-induced replicative Should this be premature, as stated below? senescence (SIPS). In particular, SIPS of WI-38 cells caused by hydrogen peroxide (H_2O_2) is a useful and reasonable cellular aging model for evaluating the effects of potential anti-aging agent against oxidative stress. We used this well-established model to evaluate the anti-aging effect of Kangen-karyu, focusing on its antioxidant activity. Treatment of WI-38 cells undergoing SIPS caused by H_2O_2 with Kangen-karyu extract significantly reduced reactive oxygen species (ROS) generation and lipid peroxidation levels. In addition, the intracellular GSH levels, reflecting cellular ROS generation, were reduced by treatment with Kangen-karyu extract. These results suggest that Kangen-karyu attenuated the age-associated increase of cellular oxidative damage. Moreover, Kangen-karyu extract normalized the G_0/G_1 phase arrest and reversed the diminished cell viability resulting from exposure to H_2O_2. Furthermore, the extract prolonged the lifespan of WI-38 cells undergoing SIPS. This study suggests that Kangen-karyu may delay the aging process in cells undergoing SIPS by attenuating oxidative damage.

20) **Yokozawa T., Yamabe N., Cho E.J., Nakagawa T., and Oowada S.: A Study on the effects to diabetic nephropathy of Hachimi-jio-gan in rats. *Nephron Exp. Nephrol.*, 97: e38-e48, 2004.**

Abstract: To investigate the effects of Hachimi-jio-gan on diabetic nephropathy, we employed an animal model, rats subjected to sub-total nephrectomy followed by streptozotocin injection, and administered Hachimi-jio-gan orally at

a dose of 50, 100 or 200 mg/kg body weight/day for 15 weeks. The administration of Hachimi-jio-gan reduced dose-dependently the elevated blood glucose and urinary protein excretion levels in rats with diabetic nephropathy over the experimental period, whereas it increased creatinine clearance significantly, suggesting that Hachimi-jio-gan would prevent or delay the progression of diabetic nephropathy. In addition, the serum glycosylated protein and urea nitrogen levels were markedly elevated in rats with diabetic nephropathy compared with normal rats, and were significantly reduced by the administration of Hachimi-jio-gan, whereas Hachimi-jio-gan reversed the decrease in the serum albumin level. The serum triglyceride and total cholesterol concentrations were reduced by Hachimi-jio-gan, implying that Hachimi-jio-gan would improve the metabolic disorder of lipids caused by diabetic nephropathy. Moreover, Hachimi-jio-gan inhibited lipid peroxidation in the serum and kidney, which suggests that Hachimi-jio-gan would ameliorate oxidative stress associated with diabetic nephropathy. Furthermore, the disorders of the glucose-dependent metabolic pathway due to this pathological condition were also normalized by the administration of Hachimi-jio-gan through decreases in advanced glycation end-product formation and sorbitol levels in the kidney. Hachimi-jio-gan protected against the development of renal lesions, glomerular sclerosis, tubulointerstitial lesions, mesangial matrix expansion and arteriolar sclerosis, estimated by histopathological evaluation and scoring. This study suggests that Hachimi-jio-gan may be a novel therapeutic approach to improving diabetic nephropathy.

21) **Kitani K., Yokozawa T., and Osawa T.: Interventions in aging and age-associated pathologies by means of nutritional approaches.** ***Ann. N.Y. Acad. Sci.,*** **1019: 424-426, 2004.**

Abstract: So-called antioxidant strategies have not been shown convincingly to be effective in increasing life spans of animals. Thus, the general consensus of experimental gerontology in the last century was that the only reproducible means of prolonging survivals of animals is the calorie restriction paradigm. As a challenge against this dogma, we attempted to examine the effect of two potent antioxidants, one tetrahydrocurcumin (a biotransformed metabolite of curcumin contained in turmeric of Indian curry) and the other green tea polyphenols.

22) **Cho E.J., Yokozawa T., Kim H.Y., Shibahara N., and Park J.C.:** ***Rosa rugosa*** **attenuates diabetic oxidative stress in rats with streptozotocin-induced diabetes.** ***Am. J. Chin. Med.,*** **32: 487-496, 2004.**

Abstract: The effects of Rosa rugosa on diabetic oxidative stress were investigated using rats with streptozotocin (STZ)-induced diabetes. The diabetic rats showed less body weight gain and heavier kidney and liver weights than normal rats, while the oral administration of Rosa rugosa at a dose of 100 or 200 mg/kg body weight/day for 20 days attenuated the physiological changes induced by diabetes. In addition, giving Rosa rugosa to diabetic rats resulted in significant and dose-dependent decreases in the serum glucose and glycosylated protein levels, implying that Rosa rugosa improves the abnormal glucose metabolism that leads to oxidative stress. Moreover, the rats with STZ-induced diabetes had high serum levels of superoxide and nitrite/nitrate. However, the administration of Rosa rugosa dose-dependently reduced the overproduction of radicals associated with diabetes, suggesting Rosa rugosa is a radical scavenger that would play a crucial role in protecting against diabetic oxidative stress. Furthermore, Rosa rugosa reduced significantly and dose-dependently thiobarbituric acid-reactive substance levels of serum and hepatic and renal mitochondria, implying that Rosa rugosa would alleviate the oxidative stress associated with diabetes by inhibiting lipid peroxidation. This study provides scientific evidence that Rosa rugosa has potential as a treatment for diabetes through attenuating oxidative stress induced by the diabetic condition.

23) **Jung H.A., Chung H.Y., Yokozawa T., Kim Y.C., Hyun S.K., and Choi J.S.: Alaternin and emodin with hydroxyl radical inhibitory and/or scavenging activities and hepatoprotective activity on tacrine-induced cytotoxicity in HepG2 cells.** ***Arch. Pharm. Res.,*** **27: 947-953, 2004.**

Abstract: The antioxidative and hepatoprotective potentials of two anthraquinones, alaternin (2-hydroxyemodin)

and emodin, to scavenge and/or inhibit hydroxyl radicals generated by the Fenton reaction and to protect tacrine-induced cytotoxicity in human liver derived HepG2 cells were evaluated, respectively. The inhibitory activity on hydroxyl radical generated in a cell-free chemical system ($FeSO_4/H_2O_2$) was investigated by a fluorescence spectrophotometer using a highly fluorescent probe, 2',7'-dichlorofluorescein. The hydroxyl radical scavenging activity was determined by electron spin resonance spectroscopy using 5,5-dimethyl-1-pyrroline-N-oxide as hydroxyl radicals trapping agents. Tacrine-induced HepG2 cell toxicity was determined by a 3-[4,5-dimethylthiazole-2yl]-2,5-diphenyltertrazolium bromide assay. Although the scavenging activity of alaternin on hydroxyl radical was similar to that of emodin in dose-dependent patterns, the inhibitory activity exhibited by the former on hydroxyl radical generation was stronger than that of the latter, with IC_{50} values of 3.05 $\pm$ 0.26 μM and 13.29 $\pm$ 3.20 μM, respectively. In addition, the two anthraquinones, alaternin and emodin showed their hepatoprotective activities on tacrine-induced cytotoxicity, and the EC_{50} values were 4.02 μM and 2.37 μM, respectively. Silymarin, an antihepatotoxic agent used as a positive control exhibited the EC_{50} value of 2.00 μM. These results demonstrated that both alaternin and emodin had the simultaneous antioxidant and hepatoprotective activities.

24) **Kim H.Y., Yokozawa T., Nakagawa T., and Sasaki S.: Protective effect of γ-aminobutyric acid against glycerol-induced acute renal failure in rats. *Food Chem. Toxicol.*, 42: 2009-2014, 2004.**

Abstract: To investigate the effect of γ-aminobutyric acid (GABA) on acute renal failure, we used a rat model of acute tubular necrosis induced by glycerol. After deprivation of water for 6 h, the rats received an injection of 50% glycerol into the muscle of the rear limb at 10 ml/kg body weight. GABA was then administered orally to the rats (100 or 500 mg/kg body weight/day) once every 12 h for 3 days. The rats with acute renal failure showed arrested body weight gain and an increase of kidney weight, whereas oral administration of GABA attenuated the physiological changes induced by acute renal failure. However, GABA administration had no significant effect on increased urine volume. Oral administration of GABA at a dose of 100 or 500 mg/kg body weight/day for 3 days significantly improved the markedly elevated levels of blood urea nitrogen and creatinine and the reduced creatinine clearance related to progression of renal failure. Moreover, the rats with acute renal failure exhibited high levels of fractional excretion of sodium (FE_{Na}) due to alteration of tubule function following injection of glycerol. However, administration of GABA lowered the FE_{Na} levels dose-dependently. Furthermore, urine osmolarity was markedly reduced in control rats with acute renal failure as compared with normal rats, whereas it was significantly increased by administration of GABA at a dose of 500 mg/kg body weight/day. These results indicate that GABA has potential as a therapeutic agent against the renal damage involved in acute renal failure.

25) **Kim H.Y., Yokozawa T., Cho E.J., and Yamabe N.: Protective effects of the Chinese prescription Hachimi-jio-gan against diabetic oxidative stress. *J. Pharm. Pharmacol.*, 56: 1299-1305, 2004.**

Abstract: We used rats with streptozotocin (STZ)-induced diabetes to investigate the effects of Hachimi-jio-gan on diabetic oxidative stress. Oral administration of Hachimi-jio-gan, at a dose of 50, 100 or 200 mg/kg body weight/day, for 10 days to rats with STZ-induced diabetes resulted in significant dose-dependent decreases in serum levels of glucose and glycosylated protein, implying that Hachimi-jio-gan improves the abnormal glucose metabolism that leads to oxidative stress. Hachimi-jio-gan also showed a tendency to reduce the urine volume and significantly reduced the elevated urinary protein level. Moreover, rats with STZ-induced diabetes had high serum levels of superoxide and nitrite/nitrate. However, the administration of Hachimi-jio-gan dose-dependently reduced the overproduction of radicals associated with diabetes, suggesting the role of Hachimi-jio-gan as a radical scavenger that could protect against diabetic oxidative stress. Furthermore, thiobarbituric acid-reactive substance levels of serum, and hepatic and renal mitochondria were dose-dependently lower in the Hachimi-jio-gan-treated groups than in the

control diabetic group, which implies that Hachimi-jio-gan would alleviate the oxidative stress associated with diabetes through the inhibition of lipid peroxidation. These results indicate that Hachimi-jio-gan is a potential therapeutic agent that will reduce the damage caused by oxidative stress involved in diabetes.

26) **Yokoyama K., Shimada Y., Hori E., Nakagawa T., Takagi S., Sekiya N., Kouta K., Nishijo H., Yokozawa T., and Terasawa K.: Effects of Choto-san and hooks and stems of *Uncaria sinensis* on antioxidant enzyme activities in the gerbil brain after transient forebrain ischemia. *J. Ethnopharmacol.*, 95: 335-343, 2004.**

Abstract: Previously, we revealed that oral administrations of Choto-san, a Kampo formula, and the hooks and stems of *Uncaria sinensis* Haviland (Rubiaceae), a medicinal plant comprising Choto-san, enhanced superoxide anion and hydroxyl radical scavenging activities in the hippocampus, and prevented delayed neuronal death of pyramidal cells in the hippocampal CA1 region in a transient forebrain ischemia gerbil model. In the present study, for the purpose of clarifying whether the endogenous antioxidant enzymes contribute to these mechanisms, we investigated the effects of Choto-san extract (CSE) and *Uncaria sinensis* extract (USE) on superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities in the brain by using the same experimental model. 1.0% CSE or 3.0% USE were dissolved in water and provided to gerbils ad libitum from 7 days prior to ischemia/reperfusion (i/rp). Seven days of continuous administrations of CSE or USE without i/rp procedure enhanced CAT activity but not SOD and GSH-Px activities in both the hippocampus and cortex. CSE elevated CAT activity in the hippocampus at 7 days and in the cortex at 3 h after i/rp. USE raised CAT activity in both the hippocampus and cortex at 3 h and 7 days after i/rp. These results suggest that one of the mechanisms of the protective effects of CSE and USE against transient brain ischemia-induced neuronal damage may be their enhancing effect on CAT activity in the brain.

27) **Cho E.J., Yokozawa T., Rhee S.H., and Park K.Y.: The role of Coptidis Rhizoma extract in a renal ischemia-reperfusion model. *Phytomedicine,* 11: 576-584, 2004.**

Abstract: The role of Coptidis Rhizoma extract in ischemia-reperfusion rats was examined. The blood levels of urea nitrogen and creatinine increased significantly more in rats subjected to 24-h reperfusion than those subjected to 6-h reperfusion following 1-h ischemia, indicating functional kidney damage was more severe after the longer reperfusion time. These parameters were reduced by oral administration of Coptidis Rhizoma extract. Greater activity was exerted in rats given the extract for 30 days than for 10 days prior to ischemia-reperfusion. In addition, the serum malondialdehyde level was lower, while the glutathione/glutathione disulfide ratio and the activities of the antioxidation enzymes, superoxide dismutase and catalase, were higher in rats given Coptidis Rhizoma extract orally for 30 consecutive days prior to 1-h ischemia and 24-h reperfusion in comparison with control rats given water, indicating that Coptidis Rhizoma has a protective action against the renal dysfunction caused by the ischemia and reperfusion process. Furthermore, renal DNA of rats given Coptidis Rhizoma extract orally showed a significantly lower DNA fragmentation rate, which was dose-dependent, implying that the extract afforded the kidneys protection against oxidative stress-mediated apoptosis during the process. Our results suggest that Coptidis Rhizoma has a protective effect against renal ischemia-reperfusion injury, in that tissue damage due to oxidative stress is reduced, thus ameliorating renal function impairment.

28) **Yokozawa T., Sekiya M., Cho E.J., Kurokawa M., and Shiraki K.: Effect of Wen-Pi-Tang extract on lung damage by influenza virus infection. *Phytomedicine,* 11: 625-632, 2004.**

Abstract: The effect of Wen-Pi-Tang extract on influenza virus infection in mice was investigated. The administration of Wen-Pi-Tang extract at a dose of 100 mg/kg body weight for 8 consecutive days to influenza virus-infected mice reversed the lack of body weight gain and prevented the increase in lung weight caused by the infection in

comparison with uninfected mice, while allopurinol, a xanthine oxidase (XOD) inhibitor, did not show these effects. The serum levels of uric acid and allantoin in influenza virus-infected mice were reduced by Wen-Pi-Tang extract administration. Moreover, Wen-Pi-Tang extract reduced the uric acid level more as the dose administered, 100, 200 and 400 mg/kg body weight, increased although it exerted lower activity than allopurinol. The XOD activity of the lungs was elevated by influenza virus infection, but Wen-Pi-Tang extract administration inhibited this activity, indicating prevention of lung damage by oxygen free radicals generated by XOD. After the administration of Wen-Pi-Tang extract to influenza virus-infected mice, the lung superoxide dismutase activity was not significantly different from that of uninfected mice, whereas lung catalase activity was lower in the former than the latter, but slightly higher than that of influenza virus-infected mice, suggesting that Wen-Pi-Tang extract may prevent the generation of highly toxic hydroxyl radicals in the lung. In addition, the administration of both Wen-Pi-Tang extract and allopurinol reduced the degree of lung consolidation caused by influenza virus infection. In particular, Wen-Pi-Tang extract reduced the consolidation score in a dose-dependent manner and more markedly than allopurinol did. This study suggests that Wen-Pi-Tang extract could improve pathological conditions of the lungs induced by influenza virus infection.

29) **Yokozawa T., Kim H.Y., and Yamabe N.: Amelioration of diabetic nephropathy by dried Rehmanniae Radix (Di Huang) extract. *Am. J. Chin. Med.*, 32: 829-839, 2004.**

Abstract: The effects of dried Rehmanniae Radix (Di Huang) extract were investigated using a diabetic nephropathy model: rats given streptozotocin after nephrectomy. The results showed that this crude drug reduced the magnitudes of the increases in glucose, urea nitrogen, 5-hydroxymethylfurfural and thiobarbituric acid-reactive substance levels, with the effects being most marked in the high blood glucose group. The renal histopathological lesions, which were conspicuous in rats not given dried Rehmanniae Radix extract, were ameliorated considerably in the high blood glucose group given this extract. It appears that dried Rehmanniae Radix extract may be useful as a therapeutic agent for inhibiting the progression of diabetic nephropathy. On the basis of these results, the possible mechanisms of action of this crude drug are discussed.

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2）横澤隆子, 源　伸介, 金　武祚：糖尿病性腎症におけるエピガロカテキンガレートの有用性. 日本農芸化学会2004年会, 2004, 3, 28-31, 広島.

3）安　恩美, 中村憲夫, 赤尾光昭, 服部征雄, 西原　力：マメ科生薬千斤抜に含まれる1,1-(dimethyl)-genistein のエストロゲン作用について. 日本薬学会第124年会, 2004, 3, 29-31, 大阪.

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17) 中川孝子, 横澤隆子, 大久保 勉, ジュネジャ・レカ・ラジュ, 尾矢剛志, 柴原直利 : 緑茶ポリフェノール並びに食物繊維の糖尿病性腎症に及ぼす影響. 第21回和漢医薬学会大会, 2004, 8, 21-22, 富山.
18) 中川孝子, 横澤隆子, 後藤博三, 柴原直利, 嶋田 豊 : 自然発症糖尿病ラット (WBN/Kob) における八味地黄丸の腎保護作用. 第21回和漢医薬学会大会, 2004, 8, 21-22, 富山.
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3) 服部征雄：難波恒雄先生の御逝去を悼む. *Natural Medicine,* **58**, i-ii, 2004.

4) 服部征雄, 井出範男：霊芝清談. れいし倶楽部 **1**, 1-8, 2004.

5) 服部征雄：ヒト腸内細菌による和漢薬成分の活性化. 漢方薬・生薬研修会, 東京, 2004. 1. 25.

6) 服部征雄：Metabolic activation of natural medicines by human intestinal bacteria. Pocala Nepal, 2004. 3. 17.

7) 服部征雄：Development of anti-viral agents from natural resources. 瀋陽薬科大学, 瀋陽, 中国, 2004. 3. 24.

8) 服部征雄：Development of anti-viral agents from natural resources. 遼寧中医学院, 瀋陽, 中国, 2004. 3. 25.

9) 服部征雄：Recent progress in the researches on metabolic activation by human intestinal bacteria. 大連大学, 大連, 中国, 2004. 3. 26.

10) 服部征雄：Anti-HIV agents from natural resources. 嘉南薬理科技大学薬学院, 台南市, 台湾, 2004. 4. 30.

11) 服部征雄：ヒト腸内細菌による和漢薬成分の活性化. 漢方薬・生薬研修会, 東京, 2004. 5. 16.

12) 服部征雄 : WFWP 女子留学生日本語弁論大会富山県大会審査員, 富山市, 2004, 11, 7.
13) 横澤隆子 : 糖尿病性腎症における漢方方剤の有用性. 和漢薬 No. 616, pp.2-3, 2004.
14) 中村憲夫 : 海洋性菌類をターゲットとした防御物質で海草は身を守る？ ファルマシア（トピックス欄）Vol. 40 No. 5（トピックス欄）2004.

◇共同研究　Co-oparative researches

国内

1）下遠野邦忠（京都大学ウイルス研究所), 下遠野久美子（共立薬科大学), 垣内信子（金沢大学薬学部) :「C 型肝炎 RNA ポリメラーゼ阻害活性を指標とした抗HCV剤の開発研究」

海外

1）Ida S. Owens (National Institute of Health, USA) :「エストロゲンおよび非ステロイド抗炎症薬の代謝研究」
2）鄭　海泳（釜山大学薬学部), 青柳一正（筑波技術短期大学), 柏田良樹（新潟薬科大学), 金　賢栄（ソウル大学薬学部) :「抗酸化研究」
3）Byung Pal Yu (The University of Texas), 趙　恩珠（釜山大学生活科学部) :「老化研究」

◇研究費取得状況　Acquisition of research funds

1）日本科学協会 平成16年度笹川科学研究助成（条美智子 代表）55万円
2）「富山産ヤマブシタケに含まれる PEP 阻害物質の分離同定」富山県受託研究（服部征雄 代表）20万円.
3）「漢方薬の効果を遺伝子発現レベルで評価する系の開発」経済産業省 地域新生コンソーシアム研究開発事業（服部征雄 代表）3800万円.
4）「糖尿病性腎症に対する漢方方剤治療の基礎的検討」つくし奨学・研究基金（横澤隆子 代表）120万円.
5）「C型肝炎ウイルスレプリカーゼ及びプロテアーゼをターゲットとした抗 HCV 剤開発の試み」ウイルス肝炎研究財団（中村憲夫 代表）100万円.
6）「ヒト腸内細菌により代謝活性化される植物エストロゲン様作用物質に関する研究」富山第一銀行奨学財団（中村憲夫 代表）40万円.

◇受賞　Award s

1）服部征雄 : 5th International Congress on Natural Medicine, 大会賞, 中国瀋陽.
2）高　江静 : 5th International Congress on Natural Medicine, 青年賞, 中国瀋陽.

◇学位（修士、博士）取得者　Academic degrees and theses

薬学士：
近藤 直子：乳酸菌によるリグナン類の変換反応の検討
和田 江美子：冠元顆粒は糖尿病に有効か？

修士（薬学）：
平川 暁子：台湾産樟芝菌糸体の成分およびその生理活性
山辺 典子：八味地黄丸の糖尿病性腎症治療薬への可能性

博士（薬学）：
高　江 静：霊芝苦味成分に関する研究
安　恩 美：千斤抜のエストロゲン及び抗エストロゲン活性に関する研究

◇研究室在籍者　Research members

薬学部4年生：中村 賢一

大学院前期1年：近藤 直子, 和田 江美子, 陳　琮湜 (10月入学), 大川 美和

大学院前期2年：西畑 友尋

大学院後期1年：山辺 典子, 鄭　美和, 佐々木 澄代, 藤井　創, 姜　奇成 (10月入学), Ali Mahmoud (10月入学)

大学院後期2年：条 美智子, 韓　号峰

大学院後期3年：佐藤 亜希子, Kanjana Sangul (10月入学)

外国人客員研究員：趙　宇峰, Park Weon-Hwan, 姜　奇成, 金　英愛 (博士), 張　群, Yoo Hye-Hyun (博士), Lee Young-A, 金　賢柱 (博士), 李　建• , 李　柱相 (博士), Chaiyasut Chaiyavat (博士)

機関研究員：左　風 (博士)

COE研究員：高　江静 (博士)

研究生：Ali Mahmoud

事務補佐員：黒岩 純子

生物試験分野(10月18日まで) Division of Pharmacology
複合薬物薬理学分野(10月19日から)
Division of Medicinal Pharmacology

教 授	渡辺 裕司 (3月31日まで)	Professor	Hiroshi Watanabe (Ph.D.)
教 授	松本 欣三 (9月16日から)	Professor	Kinzo Matsumoto (Ph.D.)
助 手	東田 道久	Assistant Professor	Michihisa Tohda (Ph.D.)
助 手	村上 孝寿	Assistant Professor	Yukihisa Murakami (Ph.D.)
技術補佐員	趙 琦	Research Assistant	Qi Zhao

研究目的 Aims of the research projects

中枢神経系疾患の病態と発症機構に関する薬理学的研究を行うとともに，和漢薬をはじめ，複合成分からなる薬物の薬効に関する計量薬理学的評価，作用本体の追求および分子レベルでの作用機序の解明を目的とした研究を行っている。

研究概要 Research projects

Ⅰ）中枢神経系疾患の病態と発症機構に関する基礎研究

1）心理的ストレス反応に関わる神経機構，神経機能修飾因子とその作用分子機構の解析

2）病態モデルにおける神経伝達物質，一酸化窒素の脳内動態とそれに対する薬物作用の解析

Ⅱ）複合薬物及びその成分の中枢作用に関する神経薬理学的研究

1）脳血管性痴呆病態モデル系における和漢薬および和漢薬成分の抗痴呆作用と神経保護作用の評価

2）新規リード化合物の開発をめざした伝統薬物・民族薬の薬理作用の探索と作用機序の解析

3）受容体遺伝子発現系を用いた薬物作用と作用機序に関する電気生理学的解析

Ⅲ）遺伝子発現を指標とした薬物作用の解明と和漢薬作用に関する研究

1）慢性脳虚血により発現する脳内遺伝子のクローニングとその機能解析

2）うつ病態関連脳内遺伝子の発現変化と抗うつ薬・和漢薬の作用解析

◇原著　Original papers

1）**Mahakunakorn P., Tohda M., Murakami Y., Matsumoto K. and Watanabe H.: Antioxidant and Free Radical-Scavenging Activity of Choto-san and Its Related Constituents. Biological & Pharmaceutical Bulletin 27:38-46, 2004.**

Abstract: The antioxidant properties of Choto-san and its related constituents such as Chotoko and Choto-san without Chotoko, and phenolic compounds contained in Chotoko such as epicatechin, caffeic, acid and quercetin were evaluated. In the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay, the scavenging activity of Chotoko (IC_{50} 14.3 μg/ml) was found to be higher than that of Choto-san (IC_{50} 206.2 μg/ml) and Choto-san without Chotoko (IC_{50} 244.3 μg/ml). Epicatechin (IC_{50} 10.4 μM), caffeic acid (IC_{50} 13.8 μM), and quercetin (IC_{50} 7.1 μM) also revealed scavenging activity against DPPH radicals. Choto-san (IC_{50} 67.7 μg/ml) exhibited stronger inhibitory activity against superoxide anion formation than Choto-san without Chotoko (IC_{50} 92.4 μg/ml) but weaker activity than Chotoko (IC_{50} 18.3 μg/ml). The generation of superoxide anion was also inhibited by epicatechin (IC_{50} 175.2 μM), caffeic acid (IC_{50} 141.7 μM), and quercetin (IC_{50} 18.7 μM). In a hydroxyl radical-scavenging experiment, Choto-san (IC_{50} 2.4 mg/ml), Chotoko (IC_{50} 2.2 mg/ml), Choto-san without Chotoko (IC_{50} 2.8 mg/ml), epicatechin (IC_{50} 3.9 mM), caffeic acid (IC_{50} 3.6 mM), and quercetin (IC_{50} 1.9 mM) exhibited activity. In NG108-15 cells, when added simultaneously with H_2O_2 (500 μM), Choto-san (250 μg/ml), Chotoko (250 μg/ml), Choto-san without Chotoko (500 μg/ml), epicatechin (200 μM), caffeic acid (200 μM), and quercetin (200 μM) effectively protected cells from oxidative damage. In conclusion, the present results provide evidence that Choto-san acts as an antioxidant and cytoprotective agent against oxidative damage, which is due at least partly to the phenolic compounds contained in Chotoko.

2）**Sumanont Y., Murakami Y., Tohda M., Vajragupta O., Matsumoto K. and Watanabe H.: Evaluation of the nitric oxide radical scavenging activity of manganese complexes of curcumin and its derivative. Biological & Pharmaceutical Bulletin 27:170-173, 2004.**

Abstract: Curcumin manganese complex (CpCpx) and diacetylcurcumin manganese complex (AcylCpCpx) were determined as to their effect on the nitric oxide radical scavenging *in vitro* method using a sodium nitroprusside generating NO system compared with their parent compound and astaxanthin, an extreme antioxidant. All compounds effectively reduced the generation of nitric oxide radicals in a dose dependent manner. They exhibited strong NO radical scavenging activity with low IC_{50} values. The IC_{50} values of curcumin, diacetylcurcumin, CpCpx and AcylCpCpx obtained are 20.39±4.10 μM, 28.76±1.48 μM, 9.79±1.50 μM and 8.09±0.99 μM, respectively. CpCpx and AcylCpCpx show greater NO radical scavenging than their parent compounds, curcumin and acetylcurcumin (AcylCp), respectively. However, the IC_{50} values of curcumin and related compounds were found to be less than astaxanthin, an extreme antioxidant, with the lower IC_{50} value of 3.42±0.50 μM.

3）**Tohda M. and Watanabe H.: Molecular cloning and characterization of a novel sequence, vof-16, with enhanced expression in permanent ischemic rat brain. Biological & Pharmaceutical Bulletin 27:1228-1235, 2004.**

Abstract: We reported previously that chronic hypoperfusion induced by permanent occlusion of the bilateral common carotid arteries (2VO) in rats caused progressive cognitive deficits and neuronal damage in the hippocampus and the white matter. These changes are similar to those observed in human dementia. Reverse transcription-polymerase chain reaction (RT-PCR) differential display was carried out to identify mRNAs encoding the intrinsic factors involved in permanent ischemia from the 2VO rat brain. Over 20 clones which showed different expression levels in 2VO and sham-operated rats were isolated. One of these, named vof-16, was markedly enhanced the expression by 2VO. The whole sequence of vof-16 mRNA was 2098 nt. The distribution of vof-16 transcripts was examined by RT-PCR and in situ hybridization. The results revealed that vof-16 was abundant in the hippocampus, the

tenia tecta, the piriform cortex and the area around the aorta. The expression levels of vof-16 in 2VO and sham-operated rat hippocampus were determined by a quantitative PCR method. The expression was abundant in the hippocampus of rats with cognitive impairment induced by 2VO. In contrast, the expression levels of vof-16 were lower in the 2VO rats with no impairment and in sham-operated rats. These results suggest that the expression levels of vof-16 may be related to the cognitive impairment induced by chronic ischemia after 2VO.

4) **Tohda M., Sukma M. and Watanabe H.: RNA editing and short variant of serotonin 2C receptor mRNA in neuronally differentiated NG108-15 cells. Journal of Pharmacological Sciences 96:164-169, 2004.**

Abstract: Two types of serotonin 2C subtype receptor mRNA, receptor-type and short variant, has been reported. The expression of the receptor-type mRNA could be detected as well as the short variant in NG108-15 cells by using a high temperature stable reverse transcriptase and the expression of the receptor-type mRNA was enhanced in drug-induced neuronal differentiated cells. The deleted sequence of the short variant include the RNA editing site by adenosine deaminase. Analysis of the sequence at the editing site revealed that the mRNA of undifferentiated cells was highly edited at sites A and B and that cytosine deaminase activity may also be involved in neuronal differentiation.

5) **Kang T.H., Murakami Y., Takayama H., Kitajima M., Aimi N., Watanabe H. and Matsumoto K.: Protective effect of rhynchophylline and isorhynchophylline on in vitro ischemia-induced neuronal damage in the hippocampus: putative neurotransmitter receptors involved in their action. Life Sciences 76:331-343, 2004.**

Abstract: Rhynchophylline and isorhynchophylline are major tetracyclic oxindole alkaloid components of *Uncaira* species, which have been long used as medicinal plants. In this study we examined the protective effects of rhynchophylline and isorhynchophylline on *in vitro* ischemia-induced neuronal damage in the hippocampus and interaction of these alkaloids with neurotransmitter receptors in a receptor expression model of *Xenopus* oocytes. *In vitro* ischemia was induced by exposing the hippocampal slices to oxygen- and D-glucose-deprived medium over 8 min. The resultant neuronal damage was elucidated as deterioration of population spike (PS) amplitudes evoked trans-synaptically by electrical stimulation of Schäffer collaterals and recorded in the CA1 area. Rhynchophylline and isorhynchophylline, as well as the N-methyl-D-aspartate (NMDA) antagonist (±)-2-amino-5-phosphono-valeric acid (APV), the muscarinic M_1 receptor antagonist pirenzepine, and the 5-HT_2 receptor antagonist ketanserin, attenuated the *in vitro* ischemia-induced neuronal damage in a concentration-dependent manner. There was no difference in the extent of protection against the neuronal damage between rhynchophylline and isorhynchophylline treatment. In *Xenopus* oocytes expressing the rat brain receptors encoded by total RNA, both rhynchophylline and isorhynchophylline reduced muscarinic receptor- and 5-HT_2 receptor-mediated current responses in a competitive manner. Together with our previous findings that rhynchophylline and isorhynchophylline have a non-competitive antagonistic effect on the NMDA-type ionotropic glutamate receptors, the present results suggest that these alkaloids exert their protective action against ischemia-induced neuronal damage by preventing NMDA, muscarinic M_1, and 5-HT_2 receptors-mediated neurotoxicity during ischemia.

6) **Tohda M., Matsumoto K., Hayashi H., Murakami Y. and Watanabe H.: DNA array analysis of gene expression changes by Choto-san in the ischemic rat brain. Journal of Traditional Medicines 21:182-186, 2004.**

Abstract: The effects of Choto-san on gene expression in the dementia model rat brain were studied using a DNA microarray system. Choto-san inhibited the expression of 181 genes that has been enhanced by permanent occlusion of the bilateral common carotid arteries (2VO). Choto-san also reverced the expression inhibition of 32 genes

induced by 2VO. These results may suggest that Choto-san, which has been therapeutically used as an antidementive drug, shows therapeutic effects through gene expression changes.

7）**Tohda M., Suwanakitch P., Jeenapongsa R., Hayashi H., Watanabe H. and Matsumoto K.: Expression changes of the mRNA of Alzheimer's disease related factors in the permanent ischemic rat brain. Biological & Pharmaceutical Bulletin 27:2021-2023, 2004.**

Abstract: The rat with permanent occlusion of the bilateral common carotid arteries (2VO) is useful model for the study of dementia. The expression changes of amyloid precursor protein (APP), secretase, α7 nicotinic acetylcholine receptor (α7NicR) and acetylcholine esterase (AChE), which are involved in Alzheimer's disease, were examined by quantitative RT/PCR in this model rat brain. The expression of APP, α7NicR and secretase were increased 4 days after 2VO. The α7NicR level at 2 days after operation already tended to increase. These result suggest that α7NicR expression was enhanced at early stage of brain ischemia. Using this model to find drugs which regulate the α7NicR expression will be useful to assay the materials with anti-dementive effect.

◇学会報告　Scientific presentation

1）森繁亮，松本欣三，東田道久，村上孝寿，高山廣光，渡邉裕司：Uncaria rhynchophyilla アルカロイド成分イソリンコフィリンの 5-HT_{2A}受容体機能抑制作用：行動薬理学的および電気生理学的検討．第77回日本薬理学会年会，2004，3/8-10，大阪．

2）東田道久，モンルディー スクマ，渡邉裕司：神経分化に伴うセロトニン 2C 受容体 mRNA editing．第77回日本薬理学会年会，2004，3/8-10，大阪．

3）Hussein G., Zhao Q., Nakamura M., Iguchi T., Goto H., Sankawa U., Watanabe H: Antihypertensive and neuroprotective effects of astaxanthin in SHR-SP. 第77回日本薬理学会年会，2004，3/8-10，大阪．

4）趙琦，村上孝寿，東田道久，渡邉裕司，松本欣三：一過性脳虚血誘発のマウス空間認知障害に対する釣藤散の予防効果における中枢コリン神経系の関与．第21回和漢医薬学会大会，2004，8/21-22，富山．

5）東田道久，マハクナコーン プラモート，松本欣三，村上孝寿，渡邉裕司：釣藤散による細胞内還元系システム活性化作用．第21回和漢医薬学会大会，2004，8/21-22，富山．

6）井口知美，趙琦，ホセイン ガージ，三川潮，中村政美，後藤博三，渡邉裕司，松本欣三：高血圧及び脳虚血性学習記憶障害に及ぼすアスタキサンチンの効果：病態モデル動物での検討．第18回日本カロテノイド研究談話会，2004，9/10-11，神戸．

7）村上孝寿，姜太炫，森繁亮，東田道久，渡邉裕司，高山廣光，松本欣三：イソリンコフィリンの *in vitro* 虚血誘発海馬神経障害およびセロトニン関連行動変化に対する効果とその作用機序．生体機能と創薬シンポジウム2004，2004，9/10-11，名古屋．

8）村上孝寿，趙琦，平尾顕三，原田恒介，東田道久，渡邉裕司，松本欣三：慢性脳虚血誘発のマウス空間認知障害に対する釣藤散の作用．第55回日本薬理学会北部会，2004，9/23-24，小樽．

9）趙琦，村上孝寿，平尾顕三，東田道久，渡邉裕司，松本欣三：一過性脳虚血誘発のマウス空間認知障害に対する釣藤散の予防効果におけるムスカリン受容体およびニコチン受容体の関与．第4回日本臨床中医薬学会学術集会，2004，11/13，東京．

10）林寿枝，東田道久，渡辺裕司，村上孝寿，松本欣三：慢性脳虚血ラットの遺伝子発現変化に及ぼす釣藤散の影響．日本薬学会北陸支部第111回例会，2004，12/5，金沢．

11）井口知美，Zhao Q.，Hussein G.，三川潮，中村政美，後藤博三，渡邉裕司，松本欣三：高血圧及び血管機能に対する藻類由来 astaxanthin の効果．日本薬学会北陸支部第111回例会，2004，12/5，金沢．

◇招待講演　Invited lectures

1）Matsumoto K. Pinna G., Watanabe H., Guidotti A., Costa.: Social isolation stress: Behavioral and Neurochemical Aspects. 5th World Congress on Stress, 2004, 6/18-19, London.

2）松本欣三：ストレス，不安，依存－行動実験のあり方と戦略．薬理学サマーセミナー2004，2004，8/30-9/1，葉山．

3）松本欣三：脳血管性痴呆病態モデル系における釣藤散の薬理作用―釣藤散の抗痴呆効果の実験薬理学的裏付け―．第25回和漢薬研究所特別セミナー，2004，10/23，富山．

◇その他　Others

1）松本欣三: 釣藤散―その効き方・その効果―．第9回和漢薬研究所夏期セミナー「ほんとうに効くのか？和漢薬！：基礎研究から最前線」，2004，8/9-11，富山．

◇共同研究　Co-oparative researches

国内

1）相見則郎，高山廣光，北島満里子：千葉大学大学院薬学研究院，「タイ薬用植物中のインドールアルカロイド類に関する創薬基礎科学研究」1994，4－

海外

1）山崎和男，笠井良次：広島大学大学院医歯薬学総合研究科，グエン・チー・スー・フォン：ベトナム薬物研究所，「ベトナム人参の薬理作用の研究」1994，4－

2）Erminio Costa, Alessandro Guidotti：アメリカ合衆国イリノイ州立大学シカゴ校精神医学研究所，「ストレス病態における神経活性ステロイドの役割」1997，4－

3）Opa Vajragupta: タイ王国マヒドン大学薬学部，「SOD mimics の脳血管性障害に対する抑制作用の研究」2001，4/1－

◇研究費取得状況　Acquisition of research funds

1）文部科学省科学研究費，基盤研究C（代表：松本欣三）「GABA 神経系機能調節およびストレス病態発現における内因性神経ステロイドの役割」 270万（2/2年目）

2）文部科学省科学研究費，21世紀中核的研究拠点形成プログラム（分担：松本欣三）「東洋の知に立脚した個の医療の創生」 200万

3）文部科学省化学研究費，萌芽研究(代表：東田道久）「和漢処方処置による脳内遺伝子発現変化に関する基礎的研究」150万(2/2年目)

4）受託研究費，（財）北陸産業活性化センター（松本欣三）地域新生コンソーシアム研究開発事業「藻類培養によるアスタキサンチンの製造及び健康補助食品の開発」 370万

◇研究室在籍者　Research members

薬学部3年生：前田幸三，水野いず美

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Ms. Arunya Sribusarakum（マヒドン大学，2004,1/9-3/4）

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Dr. Lewanich Pathama（スリナカリンウィロー大学医学部助教授，2004，9/2-11/29）

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Dr. Mayuree Tantisira（チュラロンコン大学薬学部，2004,2/15-18）
Dr. Tran Van Hien（ベトナム薬物研究所，2004, 3/1-3/30）
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Dr. Nattawut Saelim（ナレスアン大学，2004, 9/16-11/12）
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◇学位（修士，博士）取得者　Academic degrees and theses

薬学士：
江村真実：隔離飼育による情動行動の変化と脳内神経ステロイドの関連性
中西絵里香：釣藤散の構成生薬・釣藤鉤及びそのフェノール成分の抗侵害受容作用
修士(薬学)：
天野佑三子：七物降下湯の一般薬理作用及びスコポラミン誘発性学習障害に対する影響
森繁亮：釣藤鉤と含有アルカロイド成分の中枢セロトニン２受容体機能抑制作用―行動薬理学的および電気生理学的研究―
博士(薬学)：
Pramote Mahakunakorn: Study on oxindole alkaloids isolated from Uncaria species: neurotransmitter receptor-based approaches for treating and preventing neurodegenerative disorders with memory impairment

病態生化学分野　Division of Pathogenic Biochemistry

教　授	済木　育夫	Professor	Ikuo Saiki (Ph.D.)
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◇研究目的　Aims of the research projects

本分野は，病態の生化学的研究を行うとともに，和漢薬を含む種々の薬物の病態に及ぼす効果を生化学的，免疫学的，あるいは遺伝学的に研究することを目的としている。

和漢薬を中心に，構造の明らかにされた成分あるいは化合物を用いて，種々の病態に有効な薬物の探索とその作用機序を分子レベルで解明する。「証」といわれる病態変化／徴候を遺伝子工学的，免疫学的手法等を駆使してその遺伝的背景を解析し，薬物の効果発現との関連性からその科学的基盤を解明する。現在，癌，免疫，アレルギー疾患などを中心にして検討を行っている。

研究概要　Research projects

Ⅰ）がん転移機構の解明とその制御

1）がん転移に対するケモカインの作用機序解明と治療への応用

2）がん転移病態モデルの作製とその形成に関与する標的分子の探索

3）伝統薬物を中心としたがん転移の抑制物質の探索

Ⅱ）シグナル伝達分子による病態制御機構の解析

1）TAK1 活性化の分子機構

2）NF-κB のリン酸化の解析

3）自然免疫シグナルに影響を及ぼす漢方薬の探索

Ⅲ）漢方方剤テーラーメード治療法の開発

1）漢方医学の証の解明を目指した血漿プロテオミック・パターン解析

◇原著

1）**Nakamura E.S., Koizumi K. Kobayashi M. and Saiki I.: Inhibition of lymphangiogenesis-related properties of murine lymphatic endothelial cells and lymph node metastasis of lung cancer by the matrix metalloproreinase inhibitor MMI270. Cancer Sci, 95: 25-31, 2004**

Abstract: Based on a previous report on the effect of a matrix metalloproteinase (MMP) inhibitory compound, MMI270, in regulating tumor-induced angiogenesis, as well as recent findings concerning functional correlations among tumor metastasis, angiogenesis and lymphangiogenesis, we investigated the anti-metastatic efficacy of MMI270 in a murine model of lymph node metastasis of lung cancer, and analyzed whether this inhibitor could also regulate lymphangiogenesis-related properties of murine lymphatic endothelial cells (LECs) and invasive properties of Lewis lung cancer (LLC) cells. The observation that MMI270 led to a significant decrease in the weight of tumor-metastasized lymph nodes of mice led us to test its anti-lymphangiogenic and anti-invasive effects in vitro. Murine LECs were characterized by an in vitro tube formation assay, by semi-quantitative RT-PCR assay to examine the expression of mRNAs for flt-4, Flk-1, Tie-1, Tie-2, CD54/ICAM1, vWF, MMPs and uPA, and by western blotting to confirm the protein expression of flt-4 and CD31/PECAM. This is the first report on the expression of MMP-2, MMP-9 and MT1-MMP in murine LECs, as well as on the inhibition of their enzymatic activity, and of the invasive ability and tube-forming property of LECs by an MMP inhibitor. Furthermore, MMI270 was shown to strongly inhibit the activity of MMP-2 and -9 produced by LLC cells and the invasion of these cells through Matrigel. In summary, the present results indicate that MMI270, apart from its anti-tumor angiogenic application, might be useful as an anti-metastatic drug, on the basis of its downregulatation of both the lymphangiogenesis-related properties of LECs and the invasive properties of LLC cells in vitro.

2）**Teerawatanasuk N., Nakamura E.S., Koizumi K., Wangmaneerat A., Komatsu K. and Saiki I.: Anti-invasive and ant-angiogenic activities of Curucuma sp. extracts. J. Trad. Med., 21: 27-33, 2004.**

Abstract: Extracts of a herbal plant, Curcuma sp. (Zingiberaceae), were investigated for their anticancer activities. The rhizome of this plant is used in Thai folk medicine to treat cancers and to promote wound healing. In the present study, we performed preliminary bioassays to assess the anti-invasive and anti-angiogenic activities of the methanol (MeOH) and ethyl acetate (EtOAc) extracts. We found that both extracts produced moderate cytotoxic effects against murine hepatocellular carcinoma CBO140C12 cells. Interestingly, the EtOAc extract exhibited remarkable inhibitory effects on the invasion and migration of tumor cells in vitro, and on the adhesion of tumor cells to various extracellular matrix proteins. Moreover, the EtOAc extract also inhibited the formation of tube-like structures by hepatic sinusoidal endothelial (HSE) cells cultured on Matrigel-coated substrate, suggesting its anti-angiogenic activity. Altogether, our preliminary results indicate that the EtOAc extract contains active constituents that could potentially be developed into anticancer agents.

3）**Majima T., Yamada T., Tega E., Sakurai H., Saiki I. and Tani T.: Pharmaceutical evaluation of licorice before and after roasting. J. Pharm. Pharmacol., 56: 589-595, 2004.**

Abstract: Liquorice has been used for allergic-inflammatory and liver disorders in both traditional Chinese and modern medicine. In traditional Chinese formulations, it is mainly roasted liquorice that has been used rather than un-roasted liquorice. We have compared the pharmaceutical characteristics of liquorice before and after roasting to clarify the pharmaceutical significance of the roasting. Although roasted liquorice contained less glycyrrhizin (an anti-allergic component) than un-roasted liquorice, the inhibitory potency of roasted liquorice extract (200 mg x kg (-1)) on immunoglobulin E (IgE)-mediated triphasic ear swelling in mice was much greater compared with un-roasted liquorice. To search for additional active ingredients, roasted liquorice extract was subjected to gel-chromatography to give an anti-allergic fraction (Fa) of molecular weight ranging from 15000 to 200000 or more,

in which glycyrrhizin was not detected. By testing the activity of the various fractions, it was proved that the anti-allergic effect of roasted liquorice was due to glycyrrhizin, its metabolite glycyrrhetic acid, and the Fa fraction. The inhibitory potency of the Fa fraction (15 and 75 mg x kg(-1)) prepared from roasted liquorice was stronger than that prepared from un-roasted liquorice. Therefore, a pharmaceutical implication of roasting the liquorice seems to be associated with an increase in the anti-allergic property of the Fa fraction. It is notable that oral administration of the high molecular mass fraction (Fa) significantly inhibited IgE-mediated ear swelling six days after challenge at doses as low as 3, 15 or 75 mg x kg(-1).

4）**Saiki I.: Review, Kampo formulations and allergic inflammatory diseases - Efficacy for murine IgE-mediated triphasic cutaneous reaction -. J. Trad. Med., 21: 51-66, 2004.**

Abstract: We found that passive sensitization with anti-DNP IgE antibody followed by the challenge with DNFB to the mouse ear can induce the triphasic cutaneous reactions (ear swelling) of immediate phase response (IPR), late phase response (LPR) and very late phase response (vLPR), peaking at 1 h, 24 h and 8 days after the challenge, respectively. IPR was absent in mast cell-deficient mice but LPR was sufficiently observed, and vLPR was partly attenuated. LPR is a T cell-independent response, while vLPR is almost completely absent in T cell-deficient nude mice. Thus, the third phase response (vLPR) with massive infiltration of eosinophil actually represents an important inflammatory reaction mediated by T cells and partially mast cells. In this model, some Kampo formulations and synthetic anti-allergic agents inhibited the IgE-mediated triphasic cutaneous reaction. The inhibitory effects of the Kampo formulations on the triphasic cutaneous reaction were divided into several groups according to the efficacies for IPR/LPR/vLPR. For instance, the group consisting of formulations such as Tokaku-joki-to (Tao-He-Cheng-Qi-Tang, 桃核承気湯), Ji-zuso-ippo (Zhi-Tou-Chuang-Yi-Fan, 治頭瘡一方), Sho-sei-ryu-to (Xiao-Qing-Long-Tang, (小青竜湯) and Sho-saiko-to (Xiao-Chai-Hu-Tang, 小柴胡湯) significantly inhibited IPR, LPR and vLPR (i.e. +/+/+ group that showed inhibitory effects against the triphasic response), similar to the effect of prednisolone as a positive control. Oral administration of Yokukan-san (Yi-Gan-San, 抑肝散), an anti-psychosis drug in Kampo medicine, attenuated the isolation stress-exacerbated triphasic skin reactions in a dose-dependent manner, while it had almost no effect on the cutaneous reactions in the unstressed group-housed mice. On the other hand, the i.p. administration of diazepam, a classic benzodiazepine receptor agonist, suppressed the enhanced IPR and LPR in socially isolated mice, but surprisingly stimulated vLPR in both stressed and unstressed mice, differing from the efficacy of Yokukan-san. This article focuses on the anti-allergic properties of Kampo formulations and describes the effect of some Kampo formulations on IgE-mediated triphasic skin reaction in group-housed or socially isolated mice. We also discuss the mechanism of the inhibitory action and the importance of the formulation and the constituent drugs in determining the efficacy.

5）**Suntornsuk L., Koizumi K., Saitoh Y., Nakamura E.S., Kammasud N., Vajaragupta O. and Saiki I.: Anti- angiogenic effect of curcumin, curcumin ethylenediamine derivative and curcumin ethylenediamine manganese complex. J. Trad. Med., 21: 94-99, 2004.**

Abstract: We investigated the anti-angiogenic effect of curcumin, curcumin ethylenediamine derivative (curcumin ED) and curcumin ethylenediamine manganese complex (curcumin EDMn) through the inhibition of the formation of tube-like structures by human umbilical vascular endothelial cells (HUVEC). Curcumin, curcumin ED, curcumin EDMn did not show cytotoxicity to HUVEC at concentrations equal and lower than 10 μM. At the concentration of 10 μM, curcumin, curcumin ED and curcumin EDMn inhibited the tube formation by approximately 94%, 40% and 65%, respectively. These results suggest that curcumin ED and curcumin EDMn might be useful as anti-angiogenic drugs in addition to their anti-lipid peroxidase and superoxide dismutase activities as described in our previous studies.

6) **Hirabayashi Y., Yamaguchi K., Shiraishi N., Adachi Y., Saiki I. and Kitano S.: Port-site metastasis after CO_2 pneumoperitoneum: Role of adhesion molecules and prevention with antiadhesion molecules. Surg. Endosc., 18: 1113-1117, 2004.**

Abstract: BACKGROUND: Port-site metastasis is a continuing problem in laparoscopic cancer surgery. To clarify the role of adhesion molecules in the development of port-site metastasis, particularly with regard to prevention, we performed experiments in which port-site metastasis was inhibited using antibodies against extracellular matrix proteins or the active Arg-Gly-Asp (RGD) peptide after CO_2 pneumoperitoneum in a murine model. METHODS: We examined the development of port-site metastasis under the following conditions: (1) CO_2 pneumoperitoneum with or without hyaluronic acid and anti-integrin or anti-CD44 antibody and (2) CO_2 pneumoperitoneum and a RGD peptide or pseudo-RGD sequence peptide (FC-336). BALB/c mice (n = 130) were injected with 5 x 10(5) human gastric cancer cells (MKN45) and either antibody or peptide, treated with CO_2 pneumoperitoneum, and injected intraperitoneally with antibody or peptide for 5 days. Three weeks after CO_2 pneumoperitoneum, the frequency and weight of port-site metastatic tumors were determined. RESULTS: Anti-integrin antibody significantly decreased the weight of port-site metastatic tumors without hyaluronic acid (control vs anti-integrin: 8.2 +/- 7.1 vs 3.6 +/- 4.5 mg; $p < 0.05$) but not the frequency of port-site metastases. With hyaluronic acid, the frequency of port-site metastasis and the weight of port-site metastatic tumors were significantly decreased both by anti-integrin and by anti-CD44 antibody (control vs anti-integrin and anti-CD44; 95% and 8.5 +/- 7.2 mg vs 50% and 3.1 +/- 4.3 mg and 55% and 3.3 +/- 5.1 mg, respectively; $p < 0.05$). RGD peptide and FC-336 also inhibited port-site metastasis in a dose-dependent manner. CONCLUSION: Cell adhesion molecules integrin and CD44 play an important role in the development of port-site metastasis after laparoscopic cancer surgery. Intraperitoneal injection of RGD peptide or pseudo-RGD sequence peptide (FC-336) can prevent port-site metastasis.

7) **Yoshioka Y., Tsutsumi Y., Mukai Y., Shibata H., Okamoto T., Kaneda Y., Tsunoda S., Kamada H., Koizumi K., Yamamoto Y., Mu Y., Kodaira H., Sato-Kamada K., Nakagawa S. and Mayumi T.: Effective accumulation of poly(vinylpyrrolidone-co-vinyl laurate) into the spleen. J Biomed. Mater. Res. 70A: 219-223, 2004.**

Abstract: To optimize polymer-conjugated drugs as a polymeric drug delivery system, it is essential to design polymeric carriers with tissue-specific targeting capacity. Previously, we showed that polyvinylpyrrolidone (PVP) was the most suitable polymeric carrier for prolonging the blood-residency of drugs, and was one of the best parent polymers to design the polymeric carriers with targeting capacity. In this study, we synthesized some hydrophobic PVP derivatives, poly(vinylpyrrolidone-co-styrene) [poly(VP-co-S)] and poly(vinylpyrrolidone-co-vinyl laurate) [poly(VP-co-VL)], and assessed their biopharmaceutical properties after intravenous administration in mice. The elimination of hydrophobic PVP derivatives from blood was the same as PVP, and the plasma half-lives of poly(VP-co-S) were almost similar to that of poly(VP-co-VL). Poly(VP-co-VL) efficiently accumulated in the spleen, whereas poly(VP-co-S) effectively accumulated in the liver. The level of poly(VP-co-VL) in the spleen was about 20 times higher than PVP and poly(VP-co-S). These hydrophobic PVP derivatives did not show any cytotoxicity against endothelial cells in vitro. Thus, poly(VP-co-VL) may be a useful polymeric carrier for drug delivery to the spleen. This study will provide useful information to design optimal polymeric carriers with targeting capacity to the spleen and liver.

8) **Nagakawa O., Akashi T., Hayakawa Y., Junicho A., Koizumi K., Fujiuchi Y., Furuya Y., Matsuda T., Fuse H. and Saiki I.: Differential expression of integrin subunits in DU-145/AR prostate cancer cells. Oncology Rep., 12: 837-841, 2004.**

Abstract: We have established a clonal DU-145 prostate cancer cell line (DU-145/AR) stably transfected with androgen receptor cDNA. We investigated the expression of integrin subunits, adhesion to extracellular matrices, the

invasion of DU-145/AR prostate cancer cells. The expression of various integrin subunits and adhesion to various extracellular matrices in DU-145, DU-145/Neo and DU-145/AR cells were examined. The haptoinvasion and the haptotactic migration of these cells were investigated using a Transwell cell culture chamber assay. DU-145/AR cells exhibited lower expression of alpha6 and beta4 integrin subunits and higher expression of alpha2 and alpha5 than DU-145 cells. DU-145/AR cells showed significantly lower adhesion to fibronectin, laminin-1 and laminin-5 than DU-145/ Neo cells, whereas DU-145/AR cells showed higher adhesion to type I and type IV collagen. Haptoinvasion of DU-145/AR cells into Matrigel/fibronectin-coated filter was significantly reduced as compared with DU-145/Neo or DU-145 cells, but there was no significant difference between DU-145/AR and control cells in the haptotactic migration to fibronectin. Dihydrotestosterone (DHT) inhibited the invasive ability of DU-145/AR cells. These results indicate that androgen receptor may play a role in the regulation of adhesion to the extracellular matrices and invasion of prostate cancer cells through influencing the expression of specific integrin subunits.

9) **Ishihara K., Kawaguchi T., Matsuya Y., Sakurai H., Saiki I. and Nemoto H.: Synthesis and Biological Evaluation of Macrosphelide Cores, Eur. J. Org. Chem., 19: 3973-3978, 2004.**

10) **Hu J., Nakano H., Sakurai H. and Colburn N.H.: Insufficient p65 phosphorylation at S536 specifically contributes to the lack of NF-κB activation and transformation in resistant JB6 cells. Carcinogenesis, 25: 1991-2003, 2004.**

Abstract: NF-κB activation is required for TNF-alpha-induced transformation of JB6 mouse epidermal cells. Deficient activation of p65 contributes to the lack of NF-κB activation in transformation-resistant (P-) cells. We hypothesized that the differential NF-κB activation involves differential p65 phosphorylation arising from enzyme activity differences. Here we show that TNF-α induces greater ERK-dependent p65 phosphorylation at S536 in transformation sensitive (P+) cells than in P- cells. Our results establish that limited ERK content contributes to a low IκB kinase (IKKβ) level, in turn resulting in insufficient p65 phosphorylation at S536 upon TNF-alpha stimulation in P- cells. Phosphorylation of p65 at S536 appears to play a role in TNF-α-induced p65 DNA binding and recruitment of p300 to the p65 complex as well as in release of p65 bound to HDAC1 and 3. Blocking p65 phosphorylation at S536, but not at S276 or S529, abolishes p65 transactivational activity. Over-expression of p65 but not p65 phosphorylation mutant (S536A) in transformation-resistant P- cells renders these cells sensitive to TNF-α-induced transformation. Over-expression of p65 phosphorylation mimics p65-S536D or p65-S536E in P- cells and also rescues the transformation response. These findings provide direct evidence that phosphorylation of p65 at S536 is required for TNF-α-induced NF-κB activation in the JB6 transformation model. The lack of NF-κB activation seen in P- cells can be attributed to an insufficient level of p65 phosphorylation on S536 that arises from insufficient IKKβ that in turn arises from insufficient ERK. Thus, p65 phosphorylation at S536 offers a potential molecular target for cancer prevention.

11) **Aozuka Y., Koizumi K., Saitoh Y., Ueda Y., Sakurai H. and Saiki I.: Anti-tumor angiogenesis effect of aminopeptidase inhibitor bestatin against B16-BL6 melanoma orthotopically implanted into syngeneic mice. Cancer Lett., 216: 35-42, 2004.**

Abstract: We investigated the effect of bestatin, an inhibitor of aminopeptidase N (APN)/CD13 and aminopeptidase B, on the angiogenesis induced by B16-BL6 melanoma cells. Oral administration of bestatin (100-200 mg/kg/day) was found to significantly inhibit the melanoma cell-induced angiogenesis in a mouse dorsal air sac assay. Additionally, anti-APN/CD13 mAb (WM15), which neutralizes the aminopeptidase activity in tumor cells, as well as bestatin inhibited the tube-like formation of human umbilical vein endothelial cells (HUVECs) in vitro. Furthermore, the intraperitoneal administration of bestatin (50-100 mg/kg/day) after the orthotopic implantation of B16-BL6 melanoma cells into mice reduced the number of vessels oriented towards the established primary tumor

mass on the dorsal side of mice. These findings suggest that bestatin is an active anti-angiogenic agent that may inhibit tumor angiogenesis in vivo and tube-like formation of endothelial cells in vitro through its inhibition of APN/CD13 activity.

12) **Ueda Y., Yamagishi T., Ikeya H., Hirayama N., Itokawa T., Aozuka Y., Samata K., Nakaike S., Tanaka M., Ono M. and Saiki I.: VGF1155, a novel binding antagonist of VEGF inhibits angiogenesis in vitro and in vivo. Anticancer Res., 24: 3009-3017, 2004.**

Abstract: The process of angiogenesis involves the formation of new blood vessels from established vasculature and is essential for progressive tumor growth and metastasis. Since vascular endothelial growth factor (VEGF) plays a pivotal role in tumor angiogenesis, it is reasonable to expect that antagonizing VEGF binding to its receptor may be effective in cancer therapy. Our previous study found that a novel low molecular weight compound, VGA1155, inhibited binding between radioisotope-labelled VEGF and cells overexpressing its two receptors, Flt-1 and KDR/Flk-1, that is, NIH3T3-Flt-1 and NIH3T3-KDR, respectively. In the present study, we investigated the anti-angiogenic effects of VGA1155 based on VEGF inhibition. VGA1155 inhibited VEGF-induced DNA synthesis of human umbilical vein endothelial cells (HUVEC) and human retinal endothelial cells (HREC) in a concentration-dependent manner. VGA1155 also inhibited VEGF-induced tube formation of HUVEC in vitro and tumor angiogenesis toward B16-BL6 melanoma after orthotopic implantation into the skin of the back. On the other hand, VGA 1155 did not affect the proliferation of human epidermoid carcinoma (KB) cells and mouse mammary carcinoma (MM2) cells. It also had no effect on the activity of several cytosolic kinases such as p55fyn and p56lck. These findings suggest that VGA1155 inhibits endothelial cell growth and angiogenesis by inhibiting VEGF function but not non-specific cytotoxicity. VGA1155 thus exhibits promise as an antiangiogenic or anti-tumor agent with fewer side-effects.

◇総　説

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2）小川和生，小泉桂一，櫻井宏明，済木育夫：プロテインチップを用いた東洋医学における病態（証）の科学的解明・診断支援システムの開発，ファルマシア，**40**：319-322，2004.

3）済木育夫：応用物理学会放射線分科会　テーマ講演：漢方薬の抗腫瘍効果とその作用機序，放射線，**30**：135-140，2004.

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◇学会報告（*：特別講演，シンポジウム，ワークショップ等）

＊1）Saiki I.: Effect of Kampo medicines on the IgE-mediated triphasic cutaneous reaction in mice. The MARS Nutrition Research Council, 2004. 01. 06, Singapore.

2）永川　修，小泉桂一，中村エリアネ静，小林光夫，済木育夫，布施秀樹：ヒト前立腺癌とケモカイン受容体の発現との関係，第8回北陸泌尿器科 Basic Research Meeting，2004. 02. 14，金沢

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5）土屋康紀，大西康晴，澤田成朗，吉岡伊作，済木育夫，塚田一博：Nafamost mesilate（フサン）は外科侵襲による癌転移増強を抑制する，第104回日本外科学会，2004. 04. 07-09，大阪

6）済木育夫：教育セミナー：漢方薬による癌転移抑制とその作用機序の解析，第92回日本泌尿器科学会，2004，04. 12，大阪

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10）安本和生，小泉桂一，済木育夫，高橋　豊，磨伊正義：腹膜播腫形成能を有するヒト胃癌印環細胞株における CXCR-4 発現の意義，第13回日本がん転移学会，2004. 06. 10-11，東京

11）Choo Min-Kyung，櫻井宏明，小泉桂一，済木育夫：がん細胞の TNF-α シグナル伝達系の転移能に及ぼす影響，第13回日本がん転移学会，2004. 06. 10-11，東京

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1）富山医科薬科大学医学部専門教育　講義「免疫学」，2004，01，13，富山

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◇研究費取得状況

1）文部省科学研究費，特定領域研究 C（1）（分担：済木育夫）「制癌剤スクリーニング」，（分担課題）基底膜浸潤阻害物質の検定

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3）平成16年度文部科学省科学研究費補助金若手研究B（代表：小泉桂一）「患者血清のプロテオミクス解析による漢方医学診断基準（証）の客観的評価法の構築」
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5）平成16年度 知的クラスター創成事業「とやま医薬バイオクラスター」，（代表：済木育夫）漢方方剤テーラーメード治療法の開発について
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7）文部省科学研究費，特定領域研究（2）（代表：済木育夫）「がん細胞のリンパ節転移に関与するケモカインおよび受容体の探索と分子標的治療への応用」
8）文部省科学研究費，特定領域研究（2）（代表：櫻井宏明）「NF-κB活性化シグナルにおけるp65リン酸化の果たす役割」
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大学院後期2年：Choo Min-Kyung（COE特別研究員），Pattama Singhirunnusorn，植田康嗣（社会人入学）
COE研究員：中村エリアネ静（2004.04.01～　）
受託研究員：小川和生（株ツムラ，2003. 04.～2004. 03.）
学内研究生：永川　修（富山医科薬科大学医学部・泌尿器科学，2003, 4～　）
小尾龍右（富山医科薬科大学医学部・和漢診療学，2003, 4～2004.12　）
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地野充時（富山医科薬科大学医学部・和漢診療学，2002, 4～）
吉岡伊作（富山医科薬科大学医学部・第二外科，2002, 4～）
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◇学位（修士，博士）取得者

卒業論文：
有田貴久：大腸がんの肝転移に対するフラクタルカインの抑制効果の検討
川﨑範隆：RNAi技術を用いたNF-κBリン酸化の解析
修士論文：
青塚保志：腫瘍血管新生および癌細胞の基底膜への接着に対するアミノペプチダーゼN/CD13の影響
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中村エリアネ静：Study of the effect of the matrix metalloproteinase inhibitor MMI270 on tumor

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土屋　康紀：A new pseudo-peptide of Arg-Gly-Asp (RGD) inhibits intrahepatic metastasis of orthotopically implanted murine hepatocellular carcinoma

藤内　靖喜：ヒト前立腺癌細胞株の浸潤能に対する肝細胞増殖因子（HGF）の影響

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◇人事移動

寺林昌代：非常勤職員（2003. 04. 01～2004. 03. 31）

笠井直美：非常勤職員（2004. 04. 01採用）

消化管生理学分野　Division of GastrointestinalPathophysiology

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研究目的　Aims of the research projects

消化管疾患，特に腸管免疫性疾患の病因および病態形成機序を解明し，それに基づき和漢薬等を含めた新規治療薬の創出を目指す。

研究概要　Research projects

消化管は機能性疾患が多く，不定愁訴が多岐にわたるため疾患が特定しにくい領域であり，現代医学のなかでも和漢薬治療が多く取り入れられている領域であります。このような疾患に対し，西洋医学的治療では薬理学的メカニズムの明らかな単剤を胃，小腸または大腸など部位を特定して用いる場合が多いですが，和漢薬治療では，消化管全体を生命活動の原動力となる“気”を生み出す一つのシステムと考え，西洋医学的発想にはない“消化管全体の機能を高める”ことにより不定愁訴を軽減するという統合的な考え方があります。そのためには，消化管疾患に対する十分な知識と経験が必要であり，基礎的な病態生理学的，薬理学的研究ももちろん不可欠であります。西洋薬，和漢薬にはそれぞれに特徴があり，相反させることなく両者の特長を活かして薬物治療にあたることにより，さらに治療域を広げることも重要であると考えています。

消化管生理学分野では，近年患者が急増してきている腸管免疫性疾患すなわち炎症性腸疾患(IBD)の潰瘍性大腸炎，さらに食物アレルギーを対象疾患として考えています。若年層を中心に患者が急増している潰瘍性大腸炎は，厚生労働省の特定疾患に指定されている慢性で難治性の疾患であります。腸管での免疫異常を背景とするIBDに対しては，近年の粘膜免疫学の発展を背景に，精力的な免疫学的病態研究にもかかわらず，その病因や遷延化因子などはいまだ不明であり，したがって病態を基盤とした治療法は確立されていません。また，食物アレルギーは腸管粘膜免疫機構の未熟な小児にその頻度が高く，小児の肉体的精神的発育への影響は重大であり，さらに，いわゆる「アレルギーマーチ」の引き金としても今やその解明と対策は急務でありますが，未だ充分な病態生理学的解明はなされていません。

全身免疫系では神経系さらには内分泌系との間に密接な強いクロストークが明らかにされていますが，腸管では粘膜免疫系組織が集積する粘膜固有層を中心に密な神経線維の存在が知られているにもかかわらず，粘膜免疫系，腸管神経系，消化管内分泌系などで構成される「腸管イントラネット」という統合的考え方からの研究は世界的にも始まったばかりであります。したがって，「腸管イントラネット」の破綻という観点から，腸管疾患の病因，病態に迫る研究はほとんどなされていません。

消化管生理学分野ではこの点に着目し，自律神経系・腸管神経系やリンパ球・マクロファージ・肥満細胞などの粘膜免疫系と腸管免疫性疾患の病因，病態との関連を，機能的側面のみならず形態的側面からも解明することを目的としています。特に，その病態生理的役割が明らかになりつつあるTRPV1を持つ腸管求心性知覚神経，神経系と免疫系の接点と考え始められたニコチン受容体を介するコリン性抗炎症・免疫機構およびPPARγを介する抗炎症・免疫機構などの役割の解明を目指して，遺伝子改変動物（PPARγノックアウトマウス，消化管肥満細胞のみが欠損することが見出されたPI3-キナーゼ欠損マウスなど）などを用いた新しい腸管免疫性疾患の病態モデルを確立して病態生理学的解明を行い，さらに和漢薬を含めた新規特異的治療薬を創出することを目指しています。

◇原著

1）**Ishikawa T., Nakayama S., Nakagawa T., Horiguchi K., Misawa H., Kadowaki M., Nakao A., Inoue S., Komuro T. and Takaki M.: Characterization of in vitro gutlike organ formed from mouse embryonic stem cells. American Journal Physiology-Cell Physiology, 286:1344-1352, 2004.**

Abstract: Using an embryoid body (EB) culture system, we have made a functional organlike cluster: the "gut" from embryonic stem (ES) cells (ES gut). There are many types of ES clusters, because ES cells have a pluripotent ability to develop into a wide range of cell types. Before inducing specific differentiation by exogenously added factors, we characterized comprehensive physiological and morphological properties of ES guts. Each ES gut has a hemispherical (or cystic) structure and exhibits spontaneous contractions [mean frequency: 13.5 +/- 8.8 cycles per min (cpm)]. A dense distribution of interstitial cells of Cajal (ICC) was identified by c-Kit immunoreactivity, and specific subcellular structures of ICC and smooth muscle cells were identified with electron microscopy. ICC frequently formed close contacts with the neighboring smooth muscle cells and occasionally formed gap junctions with other ICC. Widely propagating intracellular Ca(2+) concentration oscillations were generated in the ES gut from the aggregates of c-Kit immunopositive cells. Plateau potentials, possibly pacemaker potentials in ICC, and electrical slow waves were recorded for the first time. These events were nifedipine insensitive, as in the mouse gut. Our present results indicate that the rhythmic pacemaker activity generated in ICC efficiently spreads to smooth muscle cells and drives spontaneous rhythmic contractions of the ES gut. The present characterization of physiological and morphological properties of ES gut paves the way for making appropriate models to investigate the origin of rhythmicity in the gut.

2）**Ogura Y., Suruga K., Mochizuki H., Yamamoto T., Takase S. and Goda T.: Postnatal changes in gene expression of retinal dehydrogenase and retinoid receptors in liver of rats. Life Sciences, 74:1519-1528, 2004.**

Abstract: Retinoic acid (RA) plays important roles in cellular differentiation and proliferation in various tissues including the liver. To explore a possible role of RA in the postnatal development of hepatic function, we analyzed RA-generation enzyme activity and the RA-related hepatic gene expressions in the suckling and weaning rats. At 5 days after birth, retinal dehydrogenase (RALDH) activity in the liver was relatively high. Its activity decreased by 70% until day 17, and then it gradually increased to a high level by the completion of weaning period. Northern blot analysis showed that RALDH2 mRNA levels decreased in the suckling period, whereas RALDH1 mRNA levels increased in the weaning period. Retinoid X receptor alpha (RXRalpha) mRNA levels increased in the suckling period and attained to a higher level at 17 days after birth. Retinoic acid receptor alpha (RARalpha) mRNA level showed only a slight and temporary increase on day 13. The mRNA levels of hepatocyte nuclear factors (HNF-4 and HNF-1alpha) exhibited parallel increases around suckling-weaning period, and the transcript levels of albumin, a typical target gene of the hepatocyte nuclear factors, increased during the suckling-weaning transition period. Electrophoretic mobility shift assay using a putative nuclear receptor-binding element on rat HNF-1 alpha gene revealed that HNF-4 homodimer, but not RXRalpha homodimer, bound to this element. These results suggest that postnatal expressions of hepatocyte-specific genes might be up-regulated by retinoid receptors, which may be related with the alterations of RALDH expression during postnatal development in the liver.

3）**Kuramoto H., Oomori Y., Murabayashi H., Kadowaki M., Karaki S., and Kuwahara. A.: Localization of neurokinin 1 receptor (NK1R) immunoreactivity in rat esophagus. Jounal of Comparative Neurology, 478:11-21, 2004.**

Abstract: The aim of the present immunohistochemical study was to investigate the localization of neurokinin 1 receptor (NK1R) in rat esophagus and examine the relationship between NK1Rs and intrinsic cholinergic, nitrergic, or

substance P (SP) neurons. NK1R immunoreactivity (IR) was observed on the nerve cell bodies in the myenteric ganglia throughout the esophagus, but not on striated muscles and smooth muscle cells of the muscularis mucosae. The frequency of occurrence of NK1R neurons was highest in the cervical esophagus and lowest in the lower thoracic esophagus. Considerable immunoreactivity was seen on the nerve cell surfaces and was also present in the cytoplasm of cell somas and in the initial part of the axons, but not in any other nerve fibers or terminals. Dogiel type I-like morphology was observed in some of the NK1R neurons; however, the majority exhibited polymorphic morphology. Double immunolabeling indicated that a majority (77%) of the NK1R neurons were immunoreactive for choline acetyltransferase (ChAT), while a minority (23%) were immunoreactive for nitric oxide synthase (NOS)-IR. Most of the NK1R neurons (92%) were innervated by the SP nerve fibers. Triple immunolabeling indicated that 70% of the NK1R neurons were associated with intrinsic SP nerve fibers (without CGRP-IR), 59% were associated with extrinsic SP nerve fibers (with CGRP-IR), and 35% were associated with both intrinsic and extrinsic SP nerve fibers. These results suggest that SP/tachykinin released from the SP nerve fibers of intrinsic and/or extrinsic origin activates the predominantly intrinsic cholinergic neurons via NK1Rs to influence neuronal transmission or motility in rat esophagus.

4）**Kadowaki M., Kuramoto H. and Takaki M.: Combined determination with functionnal and morphological studies of origin of nerve fibers expressing transient receptor potential vanilloid 1 in the myenteric plexus of the rat jejunum. Autonomic Neuroscience-Basic & Clinical, 116:11-18, 2004.**

Abstract: The aim of this study was to determine the action of capsaicin in isolated rat intestine and the origin of nerve fibers expressing transient receptor potential vanilloid 1 (TRPV1: capsaicin receptor) in the rat jejunum by combination of functional and immunohistochemical experiments. Capsaicin (1 microM) produced a prolonged relaxation response (52. +/-15.3% of the relaxation response to papaverine, mean +/- S.D., n=27) of the isolated jejunum in the presence of atropine and guanethidine. Pretreatment with the TRPV1 antagonist, capsazepine (10 microM) and ruthenium red (3 microM) significantly reduced the relaxation response to capsaicin by 78% ($P<0.01$) and 38% ($P<0.05$), respectively. Tetrodotoxin and calcitonin gene-related peptide (CGRP)-desensitization significantly reduced the response to capsaicin by 72% ($P<0.01$) and 42% ($P<0.01$), respectively. Therefore, we investigated the distribution of TRPV1-immunoreactivity (IR) in the myenteric plexus of the rat jejunum. Using antisera raised against either the N-terminal or C-terminal domains of rat TRPV1, TRPV1-IR was present in the nerve fibers, but not in the cell bodies of myenteric neurons. These TRPV1-immunoreactive nerve fibers were running in myenteric ganglia and their interconnecting strands. Most TRPV1-immunoreactive nerve fibers showed CGRP-IR, whereas few VR1-immunoreactive nerve fibers showed substance P-IR. After chronic denervation of the extrinsic nerve supply to the jejunum, both the relaxation response to capsaicin and TRPV1-immunoreactive nerve fibers completely disappeared. These findings indicate that these TRPV1-immunoreactive nerve fibers in the rat jejunum derive from extrinsic neurons and that activation of TRPV1 produces the relaxation response in the rat jejunum, at least in part, through the release of CGRP from nerve fibers expressing TRPV1.

◇学会報告（*: 特別講演，シンポジウム，ワークショップ等）

1）山本　武，中居万寿美，瓦林真実，山口範晃，高瀬幸子，大浦昭寛，松本信助，大保稲實：後期産卵鶏のマルチトール摂取によるニワトリ卵殻及び大腿骨，脛骨のカルシウム強化．第58回日本栄養・食糧学会大会，2004，5/21-23，宮城.

2）高瀬幸子，瓦林真実，山本 武：ニワトリヒナ発達過程の肺βカロテン開裂酵素遺伝子発現とハイドロコルチゾンの関与．　第58回日本栄養・食糧学会大会，2004，5/21-23，宮城.

3）山口範晃，山本 武，高瀬幸子：ニワトリβカロテン開裂酵素遺伝子発現とハイドロコルチゾン投与の影響．　第58回日本栄養・食糧学会大会，2004，5/21-23，宮城.

4）山本 武，高瀬 幸子：ニワトリヒナ発達過程に肺βカロテン開裂酵素遺伝子発現． 第56回日本ビタミン学会，2004，5/28-29，新潟.

5）Takase S., Ogura Y., Suruga K., Mochizuki H., Yamamoto T. and Goda T.: Postnatal Changes in Gene Expression of Retinal Dehydrogenase and Retinoid Receptors in Liver of Rats. 2004 FASEB Summer Research Conferences "RETINOIDS" 2004, 6/12-17, Callaway Gardens Resort Pine Mountain, GA USA.

6）Kuramoto H. and Kadowaki M.: Immunohistochemical features of calbindin immmunoreactive neurons in the rat esophagus. 16th International Congress of International Federation of Associations of Anatomists. 2004, 8/22-27, Kyoto.

7）山口範晃，山本 武，高瀬幸子：ニワトリ小腸・肝臓のβカロテン開裂酵素：発達過程における遺伝子発現変動及び雌雄の差． 第18回カロテノイド研究談話会，2004，9/-10，神戸.

8）山口範晃，山本武，高瀬幸子：ニワトリ小腸・肝臓のβカロテン開裂酵素:発達過程における遺伝子発現とレチノ イン酸シグナリングとの関連．日本レチノイド研究会第15回学術集会，2004，11/18-19，東京.

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1）高瀬幸子，四童子好廣，山本武：小腸におけるβカロテン代謝調節のメカニズム究明． 平成13年度シーボルト大学共同教育研究報告書，2004，3.

2）高瀬幸子，山本武，四童子好廣，大浦昭寛，大保稲實，松本信助，三浦昭彦，井上昭芳：マルチトール摂取によるニワトリ卵殻のカルシウム強化に関する研究．平成14年度シーボルト大学共同教育研究報告書，2004，3.

3）藏本博史，門脇 真：食道内在神経と迷走神経副交感神経線維の関係．第1回 Enteric Nervous System 研究会，2004，10/30，京都.

4）門脇 真：腸管免疫性疾患と腸管神経系．第1回 Enteric Nervous System 研究会，2004，10/30，京都.

5）門脇 真：教官はたのし 患者に喜ばれる新薬を 北日本新聞，2004，8/30.

◇共同研究

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1）藏本博史：京都工芸繊維大学繊維学部応用生物学科細胞機能学分野，
「腸管神経系における迷走神経（副交感神経）線維の神経支配に関する研究」
「腸管知覚神経の形態学的研究」
「腸管神経系とセロトニン含有腸クロム親和性細胞及びマスト細胞のクロストークに関する神経科学的研究」
2004，5～

2）門脇 孝：東京大学大学院医学系研究科代謝栄養病態学（糖尿病・代謝内科）
「潰瘍性大腸炎の病態形成における神経型ニコチン受容体を介する抗炎症免疫機構の役割：PPARγヘテロ欠損マウスを用いた新規病態モデルでの検討」
「腸管自律神経系と腸管肥満細胞の機能形態的関係が潰瘍性大腸炎の病態形成に果たす役割：腸管肥満細胞特異的欠損マウスを用いての検討」
「食物アレルギー病態モデルによるアレルギー機序の解析：腸管肥満細胞特異的欠損マウスを用いての検討」
2004，7～

3）小安重夫：慶応義塾大学医学部免疫学
「腸管自律神経系と腸管肥満細胞の機能形態的関係が潰瘍性大腸炎の病態形成に果たす役割：腸管肥満細胞特異的欠損マウスを用いての検討」

「食物アレルギー病態モデルによるアレルギー機序の解析：腸管肥満細胞特異的欠損マウスを用いての検討」
2004，7～

◇研究費取得状況

1）平成16年度　日本学術振興会科学研究補助金　基盤研究C(2)（代表：門脇　真）「食物アレルギーと腸管免疫性疾患：特に腸管求心性知覚神経の役割について」
2）平成16年度　日本学術振興会科学研究補助金　基盤研究C(2)（分担：門脇　真）「下部食道括約筋におけるキャプサイシン感受性知覚神経の抑制反射機構」
3）平成16年度　財団法人喫煙科学研究財団（代表：門脇　真）「腸管粘膜免疫系および腸管神経系と潰瘍性大腸炎の病因，病態との関連：特にα7型ニコチン受容体を介するコリン性抗炎症・免疫機構およびPPARγを介する抗炎症・免疫機構の破綻」
4）平成16年度　富山医科薬科大学学内競争的研究補助金「戦略的経費」（代表：門脇　真）「潰瘍性大腸炎の病態形成機序における神経型ニコチン受容体を介するコリン性抗炎症免疫機構の役割：PPARγヘテロ欠損マウスを用いた新規病態モデルでの検討とそれに基づく和漢薬を中心とした治療薬探索」
5）平成16年度　富山医科薬科大学学内競争的研究補助金「戦略的経費」（代表：山本　武）「食物アレルギー性下痢誘発モデルマウスによるアレルギー機序の解析」

◇研究室在籍者

学部3年生：宇都宮菜穂，兒玉利尚

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研究目的 Aims of the research projects

植物が作り，動物の機能維持に必須な脂肪酸には二種類ある。リノール酸（n-6）は成長や生殖生理の維持に必須であるが，体内でアラキドン酸に変換されエイコサノイドと呼ばれる種々の生理活性物質（炎症メディエーター）の前駆体となっている（リノール酸カスケード）。α-リノレン酸（n-3）はエイコサペンタエン酸（EPA）やドコサヘキサエン酸（DHA）に変換され，EPAはエイコサノイド合成の前駆体として働き，DHAは脳・神経機能の維持に必須の役割をしている。n-6系とn-3系は多くの酵素，受容体の段階で競合的であり，そのバランスが身体の恒常性維持（健康と病気）に重要な因子となっている。

本研究は，摂取食品によって変わるリノール酸（n-6）系とα-リノレン酸（n-3）系のバランスが，過去半世紀に急増しているアレルギー過敏症，米国型癌，心疾患などに及ぼす影響を，長期投与の結果の解析によって評価することを目的としている(薬食一如)

研究概要　Research projects

Ⅰ）アレルギー過敏症の体質改善—基礎と臨床

リノール酸カスケードを介して作られるn-6系メディエーターは，異物（アレルゲン）の体内への侵入を防ぐ役割を果たしている。しかし，リノール酸の摂取過剰によりアラキドン酸が増えるとメディエーターが過剰に作られ，その防御反応が過剰におこって病的なアレルギー症となる。一方，α-リノレン酸系のEPAからも類似のメディエーターが作られるが，一般に作られる量は少なく活性も弱い。そして，食品を選び，n-6/n-3の比を低くすると，アレルゲン刺激で作られる炎症メディエーターの産生量と活性を低くすることができる（体質の改善）。動物実験の結果に基づき，「リノール酸を減らし，α-リノレン酸系を増やす食事療法」が，アトピー性皮膚炎の治療に有効であることを証明しつつある（共同研究）。多くの抗アレルギー薬はリノール酸カスケードを抑えて効果を発揮するので，リノール酸の多い食品の摂取を減らすという服薬指導と同時に，食物油脂の選択による体質改善が勧められる。

Ⅱ）n-6/n-3比を下げることによる米国型癌の予防—基礎と臨床

米国で先行しわが国で増えている癌（肺腺癌，大腸癌，乳癌など）に対し，リノール酸カスケードを抑えるn-6/n-3比の低い油脂，抗炎症薬，遺伝子操作が抑制的にはたらく（動物実験）。リノール酸カスケードを介して作られる過剰のプロスタグランジン，ロイコトリエンなどが炎症を持続させ，炎症細胞からの過剰の活性酸素（ROS）が遺伝子傷害をひきおこし，細胞増殖的にはたらき，発癌を促進する。また，これらメディエーターは各種転写因子を介して細胞増殖的にはたらくと理解できる。この理解に基づき，n-6/n-3比を下げることによる大腸腫瘍再発予防の介入試験を継続中である（共同研究）。

Ⅲ）紫蘇油(荏胡麻油)の開発と食用油の安全性評価

蘇子（紫蘇種子）は漢方では上品に分類され，安全性が高く，長期的に効果を示すとされている。近年工業的に使われていた紫蘇種子の油（紫蘇油）の安全性と有効性を，長期投与の結果を評価することにより示して，食用に再開発した。紫蘇油は癌の化学予防に最も有望であると評価され，脳卒中発症予防効果もある。この過程で，菜種油，オリーブ油などが脳卒中ラットの寿命を異常に短縮することが明らかとなり，その内分泌撹乱作用の本体の探索を続けている。

◇原著　Original papers

1）**Du. C., Fujii Y., Ito M., Harada M., Moriyama E., Shimada R., Ikemoto A. and Okuyama H.: Dietary polyunsaturated fatty acids suppress acute hepatitis, alter gene expression and prolong survival of Long-Evans Cinnamon rats, a model of Wilson Disease. J. Nutr. Biochem., 15(5) , 273-280, 2004.**

Abstract: In the Long-Evans Cinnamon rat, copper accumulates in the liver because of a mutation in the copper-transporting ATPase gene, and peroxidative stresses are supposed to be augmented. We examined the effects of dietary fatty acids on hepatitis, hepatic gene expression, and survival. Rats were fed a conventional, low-fat diet (CE2), a CE2 diet supplemented with 10 wt% of lard (Lar), high-linoleic soybean oil (Soy), or a mixture of docosahexaenoic acid (DHA)-rich fish oil and soybean oil (DHA/Soy). Among female rats, the mean survival times of the DHA/Soy and the Soy groups were longer by 17～20% than in the Lar and the CE2 groups. Among male rats, the survival times were much longer than in the females, but no significant difference in survival was observed among the dietary groups. Serum ceruloplasmin levels in female and male rats of all of the dietary groups were similar. Serum transaminase levels of the DHA/Soy group tended to be lower than in the CE2 group. Histological examinations revealed a marked degeneration in hepatic tissue integrity in the Lar and CE2 groups but not in the DHA/Soy group. Hepatic levels of metal-related genes, transferrin and ceruloplasmin, as well as those related to bile acid synthesis were up-regulated, and an inflammation-related gene (cyclooxygenase [COX]-2) was down-regulated in the DHA/Soy group. Some proliferation-related genes were also affected by the dietary fatty acids. These results indicate that polyunsaturated fatty acids suppress the development of acute hepatitis and prolong survival in females, regardless of whether they are of the n-6 or n-3 type, which are associated with altered gene expressions.

2）**Tokudome S., Ichikawa Y., Okuyama H., Tokudome Y., Goto C., Imaeda N., Kuriki K., Suzuki S., Shibata K., Jiang J., Wang J. and Takeda E. : The Mediterranean vs the Japanese diet. Eur. J. Clin. Nutr., 58(9), 1323, 2004.**

Abstract: Both the Mediterranean and Japanese diets are known to be healthy (Tokudome et al, 2000; Trichopoulou & Vasilopou-lou, 2000; Ferro-Luzzi et al, 2002; Serra-Majem et al, 2003). People of the Mediterranean countries enjoy a low risk of cardiovascular disease, while Japanese are famous for their longevity/health life expectancy (UN, 1998). However, there are both similarities and discrepancies in intake of foods and beverages between the two cases. The Mediterranean diet is characterized by high consumption of cereals (wheat), vegetables and fruit, fish and olive oil (Trichopou-lou & Vasilopoulou, 2000; Ferro-Luzzi et al, 2002; Serra-Majem et al, 2003). Japanese also consume large amounts of cereals (rice), vegetables and fruit, and fish, but there is much lower intake of energy and oils/fats (Tokudome et al, 2000; Health Promotion and Nutrition Division, 2003).

In a recent issue of EJCN, Dr Serra-Majem et al (2003) reported an interesting ecological finding that typical Mediterranean individuals consume high amounts of total lipids (approximately 100g/day in males and 80 g in females) and also polyunsaturated fatty acids (PUFAs) in males, and lipids (more than 40% energy) and PUFAs in both genders along with high concentrations and proportions of mono-unsaturated fatty acids (MUFAs), largely from olive oil.

In contrast, the traditional Japanese diet has been characterized by low intake of total lipids, including saturated fatty acids, MUFAs and PUFAs, particularly of n-6 PUFAs, not only absolute concentrations as well as proportions (Okuyama et al, 1997; Tokudome et al, 2000). However, the recent past has seen a change from 20% energy from lipids to 30%, whereas the ratio of n-6 PUFAs/n-3 PUFAs has shifted from 2-3 to 4-5. We assume that these changes will enhance the risk of fat-related cancers, cardiovascular disease and cerebrovascular embolisms.

Therefore, we wonder if DR Serra-Majem et al could provide information that the risk of cardiovascular disease is explained with reference to concentrations and-or percentage energy from total lipids, n-6 PUFAs and n-3 PUFAs together with its ratio. Furthermore, comments on whether the risk is modulated when the intake of vegetables and

fruit is adjusted would be welcomed because they contain antioxidant nutrients, including α-tocopherol, carotenoids, vitamin C and folic acid.

There is evidence that not only ablolute concentrations of total lipids but also the balance of fatty acids of n-6 PUFAs/n-3 PUFAs, in particular, are crucial to our health (Lands, 1995; Okuyama et al, 1997; Rose & Connolly, 1999). We propose that, even if olive oil comprises antioxidant nutrients, intake at high levels may be unhealthy. According to values for macronutrients set for the Japanese diet (Health Promotion and Nutrition Division, 2003), intake of 20-25% energy from lipids on average, with more than 50% from carbohydrates and 15-20% from proteins may be recommended for adults.

3) **Tatematsu K., Hirose N., Ichikawa Y., Fujii Y., Takami A. and Okuyama H. : Nutritional evaluation of an inter-esterified perilla oil and lard in comparison with butter and margarine based on the survival of stroke-prone spontaneously hypertensive (SHRSP) rats. J. Health Sci., 50(1), 108-111, 2004.**

Abstract: Some kinds of vegetable oil and a partially-hydrogenated oil shorten the survival of the stroke-prone spontaneously hypertensive (SHRSP) rats compared with perilla seed oil, soybean oil and lard. The n-3/n-6 ratio of constituent fatty acids, phytosterol content and other factors in these oils have been proposed to affect the survival of this strain. Here, we examined the safety of a fat produced by the inter-esterification of perilla oil and lard (Perilla-Lard) on the bases of the survival of SHRSP rats. The mean survival time decreased in the order of the butter, the Perilla-Lard, the lard, the margarine and the partially-hydrogenated soybean oil (Hyd.Soy) group. The correlations between survival time and cholesterol content or phytosterol content in the diet were analyzed, and the probable health benefits of the new margarine-type fats made of animal fats and oils with high n-3/n-6 ratios were discussed.

4) **Tatematsu K., Fuma S., Satoh J., Ichikawa Y., Fujii Y. and Okuyama H. : Dietary canola oil and soybean oil fed to SHRSP rat dams differently affect the growth and survival of their male pups. J. Nutr., 134, 1347-1352, 2004.**

Abstract: Canola oil (Can), as well as some other oils, shortens the survival of SHRSP rats compared with soybean oil (Soy). Although detrimental factors other than phytosterols have not been identified, they are likely to be hydrophobic and transmissible to pups. To test this possibility, female SHRSP rats (F0) were fed a diet supplemented with Can or Soy and mated at 11 wk of age. The growth of suckling pups (F1) from the Can-fed dams was significantly retarded compared with that of pups from the Soy-fed dams. Half of the male pups (F1) were weaned to the same diet as their dams (Can-->Can and Soy-->Soy groups) and the rest were weaned to the other diet (Can-->Soy and Soy-->Can groups). The survival rate of the male pups (F1) was significantly lower in the Can-->Can group than in the Soy-->Can group, and in the Can-->Soy group than in the Soy-->Soy group, indicating that the oils fed to dams differently affected the growth and survival of pups. There were fewer pups per dam in the Can-fed dams (F0) than in the Soy-fed dams, and in the dams (F1) of the Can-->Can and Soy-->Can groups than in those of the Can-->Soy and Soy-->Soy groups. Although Can is nutritionally detrimental to SHRSP rats compared with Soy, no direct evidence has been obtained thus far relating these observations to human nutrition.

5) **Tatematsu K., Fuma S., Nagase T., Ichikawa Y., Fujii Y. and Okuyama H.: Factors other than phytosterols in some vegetable oils affect the survival of SHRSP rats. Food Chem. Toxicol., 42, 1443-1451, 2004.**

Abstract: Unusual survival-shortening activities of some vegetable oils were detected in stroke-prone spontaneously hypertensive (SHRSP) rats, and phytosterol (PS) in the oils and the tissue tocopherol status have been suggested to be the factors for the activities. Here, we re-evaluated the contribution of PS to the survival-shortening, and examined the hepatic tocopherol status. A basal diet for rodents and a test oil were mixed at a 9:1 ratio, and the diet was

given to male SHRSP rats upon weaning. The total and major PS contents of the diets and tissue lipids did not correlate with relative survival time. The free fatty acid fractions obtained by lipase and alkaline hydrolyses of canola oil (Can) and the original Can contained PS in comparable amounts but the free fatty acid fractions did not exhibit survival-shortening activities compared with the soybean oil (Soy) group. The activity was not detected in the ethyl acetate extracts of the aqueous phase after the hydrolysis. When a commercially available PS preparation was added to the Soy diet at an amount 2.8-fold higher than that in the Can diet, the mean survival time was shortened but was still significantly longer than that of the Can group. The hepatic tocopherol level was significantly higher in the Can group than in the hydrogenated Soy group and Soy group, but the former two groups exhibited a survival-shortening activity. These results indicate that factors other than PS, tocopherol status and fatty acid composition in some vegetable oils are critical for the survival-shortening activity observed in SHRSP rats.

6）**Fujii Y., Murase Y., Otake K., Yokota Y., Omoto S., Hayashi H., Okada H., Okada N., Kawai M. Okuyama H., and Imakawa K.: A potential live vector, foamy virus, directed intra-cellular expression of ovine interferon-τexhibited the resistance to HIV infection. J. Vet. Med. Sci., 66(2), 115-121, 2004.**

Abstract: Interferon-tau (IFN-tau), produced by the embryonic trophectoderm, is a member of type I IFNs required for the establishment of pregnancy in the ruminant ungulates. Although this IFN possesses antiviral activity similar to other type I IFNs, the effectiveness of IFN-tau as an antiviral agent has not been well characterized. To investigate possible antiviral effects of ovine IFN-tau (oIFN-tau), oIFN-tau-GST fusion protein was expressed in E. coli BL21, from which the purified protein isolated possessed anti-viral activity. An apathogenic human foamy virus (hFV) was then used to establish a potential recombinant live vector consisting of oIFN-tau cDNA sense (+) or antisense (-) sequence, oIFN-tau(+)/hFV or oIFN-tau(-)/hFV, respectively. Human hematopoietic and other mammalian cell lines that had been transduced with hFV vector consisting of no oIFN-tau, oIFN-tau(+)/hFV or oIFN-tau(-)/hFV construct were cultured initially for 12 days, and three of cell lines were then maintained for up to 90 days. These cells with oIFN-tau expression directed by hFV exhibited the in vitro cytopathic effect minimally. Transduced cell lines that had been cultured for 90 days were subjected to studies on human immunodeficiency virus type-1 (HIV-1) infection, which was measured with infectivity of viral particles resulted from the GFP inserted T-cell tropic HIV SF2 or macrophage tropic HIV SF162: the number of HIV-1 positive cells was reduced by the hFV driven-intra-cellular oIFN-tau expression. Since oIFN-tau/hFV transduced cells exhibited the resistance to HIV-1 infection and/or replication, oIFN-tau could be considered as one of effective antiviral agents against HIV-1. These results suggest that the hFV genome could be an effective recombinant live vector for the expression of a targeted gene in various cell types.

7）**Otake K., Omoto S., Yamamoto T., Okuyama H.,Okada H.,Okada N., Kawai M., Saksena N.K., and Fujii, .R.: HIV-1 Nef protein in the nucleus influences adipogenesis as well as viral transcription through the peroxisome proliferator-activated receptors. AIDS, 18, 189-198, 2004.**

Abstract: BACKGROUND : Although the HIV-1 Nef protein (27 kDa) localizes primarily in cytoplasm, there is considerable evidence suggesting its occasional localization in the nucleus. Nef is known to play an important role in transcriptional events and viral replication, but the actual target of Nef in the nucleus remains to be identified. OBJECTIVE: To examine the functional roles of Nef in the nucleus and its possible interactions with other unknown factors in the nucleus. METHODS: High-density microarray analysis was used to screen directly the unique functions of Nef on host gene transcription. The nuclear localization of Nef and its effects on the expression of peroxisome proliferator-activated receptors (PPAR) was examined using PPAR promoter/reporter assay and immunoblotting. A long terminal repeat/reporter assay was used to investigate the effects of Nef and PPAR on viral

transcription. RESULTS: Nef in the nucleus suppressed PPAR gamma expression and reduced fatty acid levels in human T and macrophage cell lines. Expression of Nef or PPAR suppressed viral replication; the effect of PPAR gamma or retinoid X receptor-alpha on viral replication were reduced by coexpression of Nef in MT(-)4 T cells. CONCLUSION: Nef may be involved in both viral replication and the wasting syndrome associated with AIDS.

8）**Omoto S., Brisibe E.A., Okuyama H., and Fujii Y.R. : Feline foamy virus tas protein is a DNA-binding transactivator. J. Gen. Virol., 85, 2931-2935, 2004.**

Abstract: Foamy viruses (FVs) harbour a transcriptional transactivator (Tas) and two Tas-responsive promoter regions, one in the 5' long terminal repeat (LTR) and the other an internal promoter (IP) in the envelope gene. To analyse the mechanism of transactivation of the FVs, the specificity of feline FV (FFV) Tas protein, which is more distantly related to the respective proteins of non-human primate origin, were investigated. FFV Tas has been shown specifically to activate gene expression from the cognate promoters. No cross-transactivation was noted of the prototype foamy virus and human immunodeficiency virus type 1 LTR. The putative transactivation response element of FFV Tas was mapped to the 5' LTR U3 region (approximately nt -228 to -195). FFV Tas binds to this element in addition to a previously described sequence (position -66 to -51). It is therefore concluded that FFV Tas is a DNA-binding transactivator that interacts with at least two regions in the virus LTR.

◇総　説　Review papers

1）大本真也, 伊藤真文, 奥山治美, 藤井陽一 : RNAi を用いた AIDS 治療の展望　RNA ワクチンの可能性. Molecular Medicine, 41 : pp44～49, 中山書店, 日本, 2004.

◇学会報告　Scientific presentation

1）奥山治美 : 血清コレステロール値と脂質栄養. 日本脂質栄養学会第13回大会, 2004, 9, 山形.

2）夫馬慎弥, 久井周子, 立松憲次郎, 市川裕子, 奥山治美 : 脳卒中易発症性（SHRSP）ラットに対する寿命短縮作用を軽減したキャノーラ油の調製. 日本脂質栄養学会第13回大会, 2004, 9, 山形.

3）野々垣知行, 立松憲次郎, 瀧井猛将, 林秀敏, 市川裕子, 藤井陽一, 奥山治美, 小野嵜菊夫 : 脳卒中易発症性（SHRSP）ラット脳のプリオン（PrP）分子発現に及ぼす油糧種子の影響. 日本脂質栄養学会第13回大会, 2004, 9, 山形.

4）池本敦, 堀田典子, 奥山治美 : 安静及び運動時のマウスの基礎代謝に及ぼす食事多価不飽和脂肪酸の影響. 日本脂質栄養学会第13回大会, 2004, 9, 山形.

5）立松憲次郎, 野々垣知行, 夫馬慎弥, 大平真悠子, 市川裕子, 伊藤真文, 藤井陽一, 森幸雄, 奥山治美 : 脳卒中易発症性（SHRSP）ラットにおける食用油の内分泌撹乱作用. 日本脂質栄養学会第13回大会, 2004, 9, 山形.

◇その他　Others

1）奥山治美 : 特別講演「油脂（あぶら）選びは間違っていませんか？～あぶらえ油は新方向への切り札～古川町保健センター健康セミナー, 2004, 1, 岐阜.

2）奥山治美 : 特別講演「脂質栄養の新方向と商品開発」. 岡山県食品新技術応用研究会第201回研修会, 2004, 3, 岡山.

3）奥山治美 : 特別講演「がん, 心臓病と EPA／アラキドン酸バランス」. 尾西市医師会講演会, 2004, 3, 愛知.

4）奥山治美 : 特別講演「健康長寿の油脂栄養－新方向」. NILS SEMINAR, 2004, 7, 愛知.

5）奥山治美 : 特別講演「がん・炎症性疾患予防の油脂栄養（ωバランス）」. 国際ネットワーク大学コンソーシアム, 2004, 7, 岐阜.

6）奥山治美 : 特別講演「がん, 心臓病と EPA／アラキドン酸バランス」. 枝幸医師会学術講演会, 2004,

9, 北海道.
7）奥山治美：講演「油脂（あぶら）の選び方－コレステロール説から脱却して新方向へ－」, 名古屋市立大学2004市民公開講座, 2004, 9, 名古屋.
8）奥山治美：特別講演「心疾患予防～コレステロール仮説から脂肪酸のω6/ω3バランスへ～」. 豊橋内科医会学術講演会, 2004, 11, 愛知.
9）Okuyama H.: 招待講演 High Bolld Cholesterol as a Predictor for Low Cancer Mortality and Long-evity, 3rd International Congress on the Columbus Concept, 2004, 10, Brussels.
10）奥山治美：脳を襲う種子（Seeds Invading Brain）. 名市大薬学祭講演会, 2004, 11, 名古屋.
11）奥山治美：アレルギー体質改善の油脂食品. 抗アレルギー食品開発シンポジウム2004, 2004, 12, 東京.

臨床利用分野　Division of Clinical Application

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◇研究目的　Aims of the research projects

天然薬物の臨床利用を目指して，以下のテーマについて研究している。

1）天然薬物（特に魚油中の DHA・EPA）の臨床的有効性
2）中枢神経系における自然免疫応答の制御機構の解析
3）脂肪吸収に及ぼす和漢薬の影響

◇研究概要　Research projects

Ⅰ）注意欠陥/多動性障害児における二重盲検試験により DHA が集中力をむしろ低下させる可能性があり(対照群では慣れにより検査値が向上したが，DHA 群では変化がない)，DHA は中枢性ノルアドレナリンを抑制している可能性が考えられる。また，二次的症状である攻撃性は，DHA で有意に低下した。これもノルアドレナリンの低下と関連すると思われる。

Ⅱ）マウスに LPS を投与することにより起こる摂食行動の低下がn-3系脂肪酸をあらかじめ与えておくことにより抑制されることを明らかにした。この機構を中枢神経系機能の面から解明する。

Ⅲ）LPS やサイトカインおよびその誘導剤を投与したときに起こる摂食行動の低下を中枢神経系における自然免疫応答の指標として，これに対する和漢薬の効果を評価する。

Ⅳ）食後血中中性脂質の上昇を抑える和漢薬を検索している。

◇著書　Books

1）浜崎智仁, 矢沢一良 : DHA 衝動・攻撃性を抑えうつ症状を改善する, 予防医学の権威がすすめる健康食事典週刊朝日編279-287, 朝日新聞社, 東京, 2004.

2）浜崎智仁 : N-3 系脂肪酸と行動, 機能性脂質のフロンティア, 12-18, シーエムシー出版, 東京, 2004.

3）浜崎智仁：監修「コレステロールは高いほうがいい」, マキノ出版, 東京, 2004.

◇原著　Original papers

1）**Hirayama S., Hamazaki T., and Terasawa K. : Effect of docosahexaenoic acid-containing food administration on symptoms of attention-deficit/hyperactivity disorder －a placebo-controlled double-blind study. Eur J Clin Nutr., 58: 467-473, 2004.**

Abstract: Objectives: To investigate whether docosahexaenoic acid (DHA) supplementation was able to ameliorate attention-deficit/hyperactivity disorder (AD/HD) symptoms in AD/HD children.

Design and subjects: A placebo-controlled double-blind study with 40 AD/HD (including eight AD/HD-suspected) children of 6-12y of age who were mostly without medication. Subjects of a DHA group (n=20) took active foods containing fish oil (fermented soybean milk, bread rolls and steamed bread; 3.6g DHA/week from these foods) for 2 months, whereas those of a control group (n=20) took indistinguishable control foods without fish oil. The following items were measured at the start and end of the study: (1) attention deficit, hyperactivity and impulsivity (AD/HD-related symptoms according to DSM-IV criteria); (2) aggression assessed by both parents and teachers; (3) visual perception (finding symbols out of a table); (4) visual and auditory short-term memory; (5) development of visual-motor integration; (6) continuous performance; (7) impatience.

Results: Changes in tests 1, 2, 3, 5 and 7 over time did not significantly differ between the two groups. However, visual short-term memory and errors of commission (continuous performance) significantly improved in the control group compared with the changes over time in the DHA group (P=0.02 and 0.001, respectively). Recalculation without AD/HD-suspected subjects (n=4 each group) showed similar P-values with regard to both measures.

Conclusion: DHA supplementation did not improve AD/HD-related symptoms. Treatment of ADHD with fatty acids deserves further investigation, but careful attention should be paid as to which fatty acid(s) is used.

2）**Huan M., Hamazaki K., Sun Y., Itomura M., Liu H., Kang W., Watanabe S., Terasawa K., and Hamazaki T. : Suicide attempt and n-3 fatty acid levels in red blood cells: A case control study in China. Biol Psychiatry, 56: 490-496, 2004.**

Abstract: Background: Epidemiologic studies show that low fish intake is a risk factor of suicidality; however, there are no case-control studies investigating suicide attempt risk and tissue n-3 fatty acid levels.

Methods: we recruited 100 suicide-attempt cases and another 100 control patients injured by accidents who were admitted to three hospitals affiliated with Dalian Medical University in Dalian, China. Case and control subjects were matched for age, gender, and smoking status. Those who were inebriated at the time of hospitalization were excluded. Blood was sampled immediately alter admission to a hospital. Washed red blood cells (RBCs) were obtained, and the fatty acid composition of the total RBC phospholipid fraction was analyzed by gas chromatography.

Results: Eicosapentaenoic acid (EPA) levels in RBC in the case subjects were significantly lower than those of the control subjects (.74 ± .52% vs 1.06± .62%, $p < .0001$). when the highest and lowest quartiles of EPA in RBC were compared, the odds ratios of suicide attempt was .12 in the highest quartile (95% confidence interval; .04-.36, p for trend = .0001) after adjustment for possible confounding factors.

Conclusions: our findings suggest that low n-3 fatty acid levels in tissues were a risk factor of suicide attempt. Further studies including intervention with fish oil are warranted.

3）**Watanabe S., Kanada S., Takenaka M., Hamazaki T. : Dietary n-3 fatty acids selectively attenuate LPS-induced behavioral depression in mice. Physiol Behav., 81: 605-613, 2004.**

Abstract: Systemic administration of bacterial lipopolysaccharide (LPS) induces a series of physiological and pathological alterations as well as behavioral depression in experimental animals. These alterations induced by LPS administration are known to be mediated by endogenous cytokines and arachidonate metabolites, which may be modulated by dieary n-3 fatty acids. Mice were fed a diet supplemented with n-3 or n-6 fatty acids for 4 weeks prior to LPS administration. Food-motivated behavior after intraperitoneal administration of LPS as compared with that before LPS administration was significantly depressed in the mice fed with the n-6 fatty-acid-rich diet (47% to 85% reduction; P<.05) but not significantly in the mice fed with the n-3 fatty-acid-rich diet. Depression of social exploration by intraperitoneal LPS administration in the n-3 fatty-acid rich diet group (39% reduction vs. vehicle group) was significantly less in the n-6 fatty-acid-rich diet group (76% reduction vs. vehicle group; P<.05). The behavioral depressions induced by intracerebroventricular LPS injection were not significantly different between the two dietary groups (F=.60). The elevation of serum corticosterone and the hypoglycemic response following intraperitoneal LPS administration were not significantly different between the two dietary groups (F=.57 and P=.43, respectively). We demonstrate that dietary n-3 fatty acids attenuate behavioral depression in mice peripherally administered with LPS without affecting the increase in serum corticosterone and the decrease in serum glucose concentration.

4）**Doshi M., Watanabe S., Niimoto T., Kawashima H., Ishikura Y., Kiso Y., Hamazaki T.: Effect of dietary-enrichment with n-3 polyunsaturated fatty acids (PUFA) or n-9 PUFA on arachidonate metabolism in vivo and experimentally induced inflammation in mice. Biol Pharm Bull, 27: 319-323, 2004.**

Abstract: Mice were fed a diet supplemented with palm oil (control diet), n-3 polyunsaturated fatty acids (PUFA)-, or n-9 PUFA-rich oil for 3 weeks. The n-3 PUFA-rich diet suppressed the generation of both leukotrienes (LT) and prostaglandins (PG), but the n-9 PUFA-rich diet did LT but not PGgeneration during acute inflammation. Leukocyte accumulation during acute inflammation was not different in the n-3 or n-9 PUFA-rich diet group as compared with the control group. The n-3 PUFA-rich diet but not the n-9 PUFA-rich diet suppressed Freund's adjuvant-induced granuloma formation. The n-9 PUFA-rich diet significantly attenuated galactosamine/lipopolysaccharide-induced liver injury more effectively than the n-3 PUFA-rich diet as compared with the control diet. The present study revealed the differential modification of experimentally induced inflammation in mice by dietary n-3 PUFA and n-9 PUFA, which may be due to their different effects on 5-lipoxygenease and cyclooxygenase metabolism of arachidonic acid during inflammatory processes.

◇総　説　Review papers

1）Hamazaki T.: Relationship between total cholesterol and all cause mortality: is there a need for lowering total cholesterol in the Japanese population? Current Topics in Nutraceutical Research, 2: 177-188, 2004.

2）中村典雄，大沢弘，山辺英彰，白戸研一，中村雅将，島田美智子，熊坂隆一郎，奥村謙，浜崎景，浜崎智仁，村上礼一，藤田雄：（研究報告）ループス腎炎患者における酸化ストレスマーカーに対するエイコサノイドペンタエン酸の効果について．Progress in Medicine, 24:, 175-178, 2004.

3）浜崎景，浜崎智仁：特集：エビデンスからみた機能性食品の現状3．抗動脈硬化食品（2）n-3系脂肪酸食品．栄養 評価と治療，21：47-53，2004.

◇学会報告　Scientific presentation（*: 招待講演）

＊1）浜崎智仁：脂質による行動の変化．日本農芸化学会2004年度大会シンポジウム，2004，3，広島.

2）浜崎智仁：公開講演　生活習慣病は予防から．第53回日本医学検査学会，2004，5，富山.

3）浜崎智仁：脂質栄養に関する最近の話題．第41回日本外科代謝栄養学会ランチョンセミナー，2004，7，愛媛.

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◇共同研究　Co-operative researchs

学内

1）今中常雄：薬学部教授「極長鎖脂肪酸代謝の制御に関する研究」2004.4～

国内

1）東原英二：杏林大学医学部泌尿器科学教授「前立腺癌の再発予防研究」2001.9～
2）日本油脂（株）:「ホスファチジルセリン（PS）の記憶能改善効果に関する研究」2004.7～
3）鈴木信雄：金沢大学「カルシウムの石灰化抑制剤」2004.10～

海外

1）Syafruddin, Nurpudji A Taslim：インドネシア・アイクマン研究所，ハサヌディン大学,「魚油によるマラリア予防の大規模介入試験」2002.6～
2）孫　月吉：中国・大連医科大学神経精神医学教授「交通事故と脂肪酸栄養」2004.4～
3）夏 瑢：中国・浙江中医学院助教授「中高年における血中脂肪酸と骨折」2005.1～

◇研究費取得状況　Acquisition of research funds

1）受託研究費,（株）マルハ（代表：浜崎智仁）「DAGE が喫煙者の尿中 8-HOd に及ぼす影響について」
2）平成16年度人物交流派遣事業,（財）国際文化交流事業財団（浜崎智仁）
3）厚生労働省科学研究費（分担：渡辺志朗）「数種の食用油に含まれる微量有害因子に関する研究」
4）研究拠点形成費補助金（COE プログラム）（分担：渡辺志朗）

◇研究室在籍者　Research Members

学部 3 年生：中島俊介，宮川誠一
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事務補佐員：浜谷裕子
受託研究員：浜崎景（ポリエン・プロジェクト（有）2003.4～2005.3）

◇学位（修士，博士）取得者　Academic degrees and theses

卒業研究：
　藤岡俊太郎「エイコサペンタエン酸（EPA）が血中炎症マーカーに及ぼす影響」
　山田泰広　「脳における炎症性サイトカイン発現のリボヌクレアーゼプロテクションアッセイによる評価」
修士論文（2004年 3 月）：
　岡藤文人　「脳内の極長鎖脂肪酸（VLCFA）含量に及ぼす食餌中 VLCFA 含量と cuprizone 投与の影響」
　斎藤正隆　「漢方方剤の膵リパーゼ活性および脂肪負荷後の血中トリアシルグリセロール上昇に対する作用」
　直井一久　「Zymosan 誘発腹膜炎における局所炎症反応と摂食行動障害に対するシクロオキシゲナーゼ阻害剤の影響」
課程博士（2004年 3 月）：
　ホワンミンミン「Suicide attempt and n-3 fatty acid levels in red blood cells: A case control study in China」医学博士（富山医科薬科大学）

薬効解析センター　Research Center for Ethnomedicines

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研究目的　Aims of the research projects

世界各地の民族薬物に関する資料の収集及び整理，薬効の評価及び解析並びにデータベースの構築を行い，世界の伝統薬物及び薬用植物に関する共同研究を推進する。

研究概要　Research projects

Ⅰ）伝統薬物に関するデータベース（ETHMEDmmm）の構築

和漢薬・アーユルヴェーダ薬物データベースの英語版を作成する目的で，翻訳または入力済み学術情報の整理を行った。

Ⅱ）伝統薬物の薬効の評価と解析に関する研究

1）難治性の神経疾患に対する有効性の検討とそれらの薬理作用の機序に関する研究

インド生薬 Ashwagandha から単離した 3 種の化合物の経口投与により，アミロイドβのマウス脳室内投与により誘発される空間記憶障害が改善され，その際，脳内の軸索と樹状突起の萎縮，前シナプスの減少が正常状態に戻っていることを明らかにした。活性化合物のうち withanoside IVについては，経口投与後に血清中に検出される代謝物 sominone を同定し，それが活性本体であることも明らかにした。

アミロイドβのマウス脳室内投与による空間記憶障害に対し，改善作用を有する生薬エキスを探索し，有効性が示された生薬4種からなる漢方処方を作製した。その処方について，空間記憶障害に対する作用を検討し，改善作用を明らかにした。

Ⅲ）生薬の品質評価に関する研究

1）遺伝子解析による生薬の同定法開発に関する研究

これまで同定が不可能であった修治された莪朮について，*trn*K 遺伝子領域の解析法を改良し，基源または遺伝子型を明らかにした。本法は鬱金類健康食品の基源解明にも応用可能であった。

2）生薬の基源と品質に関する研究

中国産莪朮の日本市場品には，一部 *Curcuma phaeocaulis* または *C. kwangsiensis*（gl タイプ）の単一品が認められたが，多くは混合品で，遺伝子多型が認められた。これらの精油含量はばらつきが大きく，局方の限度値未満のものも存在した。鬱金類健康食品には *C. longa* 以外の基源不明のものも存在し，curcuminoids 含量で 9 倍の差が認められた。*Curcuma* 属 7 種に由来する鬱金類生薬の，アジュバント関節炎に対する作用を検討した。文朮（*C. phaeocaulis*）メタノールエキスにより，肢の腫脹と，血清中の炎症マーカータンパク質の発現が有意に抑制された。また文朮エキスは *in vitro* 実験において COX-2 活性の抑制作用を有意に示した。

Ⅳ）世界の伝統医薬学の調査研究

漢薬の資源をアジアに探る研究の一環として，モンゴル国東部で *Ephedra* 属，*Glycyrrhiza* 属，*Astragalus* 属植物などの資源調査を行った。

◇原著　Original papers

1）**Sasaki Y., Fushimi H., and Komatsu K.: Application of Single Nucleotide Polymorphisms Analysis of *trn*K Gene to the Identification of *Curcuma* Plants. Biol. Pharm. Bull., 27: 144-146, 2004.**

Abstract: We previously found that *Curcuma* plants and drugs derived from *Curcuma longa, C. phaeocaulis, C. zedoaria,* and *C. aromatica* could be identified by the nucleotide differences at two sites and the existence of a 4-base indel on *trn*K gene. In this paper, based on species-specific nucleotide sequences, the application of a new method, single-nucleotide polymorphism (SNP) analysis was investigated to identify *Curcuma* plants more conveniently. First, three types of reverse primer were synthesized in different lengths, 34 mer, 26 mer, and 30 mer, to anneal the template DNAs from each species at sites immediately upstream from substitution positions 177 and 645, and at the site including the 4-base insertion from 728 to 731, respectively. After single-base extension reaction of these primers using fluorescent-labeled ddNTPs and PCR products of the *trn*K gene region as template, the resulting products were detected using an ABI PRISM 310 Genetic Analyzer. The electrophoretogram showed three or two peaks at different positions depending on the 27 mer, 31 mer, and 35 mer product lengths. Each peak was derived from the incorporated fluorescent-labeled ddNMPs complementary to template nucleotides at positions 645, 724, and 177, respectively. *C. phaeocaulis* showed three peaks of ddCMP, ddAMP, and ddAMP. The other three species showed two peaks derived from 27 mer and 35 mer products: peaks of ddCMP and ddAMP in *C. longa,* those of ddCMP and ddTMP in *C. zedoaria,* and those of ddTMP and ddAMP in *C. aromatica.* Thus SNP analysis to identify four *Curcuma* plants was newly developed.

2）**Zhao J., Nakamura N., Hattori M., Yang X. W., Komatsu K., and Qio M. H.: New Triterpenoid Saponins from the Roots of *Sinocrassula asclepiadea. Chem. Pharm. Bull.,* 52: 230-237, 2004.**

3）**Tohda C., Matsumoto N., Zou K., Meselhy M. R., and Komatsu K.: Aβ (25-35)-induced memory impairment, axonal atrophy and synaptic loss are ameliorated by M1, a metabolite of protopanaxadiol-type saponins. *Neuropsychopharmacology,* 29: 860-868, 2004.**

Abstract: We previously screened neurite outgrowth activities of several Ginseng drugs in human neuroblastoma, and demonstrated that protopanaxadiol (ppd)-type saponins were active constituents. Since ppd-type saponins are known to be completely metabolized to 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol (M1) by intestinal bacteria when taken orally, M1 and ginsenoside Rb_1, as a representative of ppd-type saponins, were examined for cognitive disorder. In a mouse model of Alzheimer's disease (AD) by Aβ (25-35) i.c.v. injection, impaired spatial memory was recovered by p.o. administration of ginsenoside Rb_1 or M1. Although the expression levels of phosphorylated NF-H and synaptophysin were reduced in the cerebral cortex and the hippocampus of Aβ (25-35)-injected mice, their levels in ginsenoside Rb_1- and M1-treated mice were almost completely recovered up to control levels. Potencies of the effects were not different between ginsenoside Rb_1 and M1 when given orally, suggesting that most of the ginsenoside Rb_1 may be metabolized to M1, and M1 is an active principal of ppd-type saponins for the memory improvement. In cultured rat cortical neurons, M1 showed extension activity of axons, but not dendrites. The axon-specific outgrowth was seen even when neuritic atrophy had already progressed in response to administration of A β (25-35) as well as in the normal condition. These results suggest that M1 has axonal extension activity in degenerated neurons, and improve memory disorder and synaptic loss induced by Aβ (25-35). M1 was shown to be effective in vitro and in vivo, indicating that Ginseng drugs containing ppd-type saponins may reactivate neuronal function in AD by p.o. administration.

4) **Zhu S., Fushimi H., Cai S. Q., and Komatsu K.: Species Identification from Ginseng Drugs by Multiplex Amplification Refractory Mutation System (MARMS). *Planta Med.*, 70: 189-192, 2004.**

Abstract: The multiplex amplification refractory mutation system (MARMS) was applied to the identification of 5 *Panax* species (*P. ginseng, P. japonicus, P. quinquefolius, P. notoginseng* and *P. vietnamensis*). A set of specific primers, including 2-pair primers on chloroplast *trn*K gene and nuclear 18S rRNA gene regions, respectively, was designed and synthesized for each species on the basis of species-specific sequences of the 2 genes. By using 5 sets of specific primers, in turn, PCR amplifications were performed with total DNA extracted from 5 *Panax* species as template under appropriate condition, and each resulting product was detected by agarose gel electrophoresis. The results showed that two expected fragments, one from *trn*K gene and another from 18S rRNA gene regions, were observed simultaneously only when the set of species-specific primers encountered template DNA of the corresponding species. This assay could give more reliable results for identification of not only 5 *Panax* species but also corresponding Ginseng drugs by simultaneous detection of 4-site nucleotide differences on 2 completely different genes.

5) **Yang D. Y., Fushimi H., Cai S. Q., and Komatsu K.: Molecular Analysis of *Rheum* Species Used as Rhei Rhizoma Based on Chloroplast *mat*K Gene Sequence and Its Application for Identification. *Biol. Pharm. Bull.*, 27: 375-383, 2004.**

Abstract: Rhei Rhizoma (Dahuang in Chinese) is widely known as a purgative and antiinflammatory agent. In the Japanese Pharmacopoeia, Rhei Rhizoma is prescribed for four *Rheum species, Rheum palmatum, R. tanguticum, R. officinale,* and *R. coreanum,* while the first three species are prescribed for Dahuang in the Chinese Pharmacopoeia. Due to the morphologic similarity of the aerial parts and frequent occurrence of intermediate forms, the taxonomy of this genus and the correct identification of *Rheum* species and their derivative drugs are very difficult. To resolve taxonomic problems of the genus Rheum and develop an ultimate identification method for plants and drugs, molecular analysis of the chloroplast *mat*K gene and nuclear 18S ribosomal RNA gene were performed on nine species. The sequence comparison of the *mat*K gene revealed that most species had variable sequences not only inter- but also intraspecies. However, the specimens of the same species belonged to the same subclade in the phylogenetic tree constructed based on *mat*K gene sequences, except for *R. palmatum,* in which specimens belonged to three subclades related to their production areas. The nucleotide differences at positions 587, 707, and 838 distinguished official species from others, while specific nucleotides at positions 367 and 937 became identification markers for *R. palmatum, R. tanguticum,* and *R. officinale* (or *R. coreanum*). Moreover, three groups of *R. palmatum,* each belonging to three subclades, were characterized by the nucleotides at positions 619, 769, 883, and 1061. By detecting marker nucleotides, the botanical origins of Rhei Rhizoma were determined.

6) **Teerawatanasuk N., Nakamura E. S., Wangma-neerat A., Komatsu K., Saiki I: Anti-invasive and anti-angiogenic activities of *Curcuma* sp. extracts. *J. Trad. Med.*, 21: 27-33, 2004.**

7) **Yang D. Y., Fushimi H., Cai S. Q., and Komatsu K.: Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) and Amplification Refractory Mutation System (ARMS) Analyses of Medicinally Used *Rheum* Species and Their Application for Identification of Rhei Rhizoma. *Biol. Pharm. Bull.*, 27: 661-669, 2004.**

Abstract: Previously, we have determined marker nucleotides on the chloroplast *mat*K gene to identify *Rheum palmatum, R. tanguticum* and *R. officinale* used as Rhei Rhizoma officially. In the present study, we further developed a convenient and efficient identification method on the basis of marker nucleotides with Amplification Refractory Mutation System analysis. On the basis of the nucleotide substitutions at positions 367 and 937 among

the three species on the *mat*K gene, at each position two kinds of reverse primers with complementary 3'-terminal nucleotides were designed. Upon PCR amplification using three sets of primers and template DNA from each species, one or two fragments (202 bp or/and 770 bp) were detected. As the resultant three fragment profiles were species-specific, the procedure enabled us to classify the botanic origins of 22 drug samples of Rhei Rhizoma.

8) **Zhu S., Zou K., Fushimi H., Cai S. Q., and Komatsu K.: Comparative Study on Triterpene Saponins of Ginseng Drugs. *Planta Med.*, 70: 666-677, 2004.**

Abstract: A comparative study on the triterpene saponins of 47 samples of Ginseng drugs derived from 12 *Panax* taxa was conducted using a reverse-phase high-performance liquid chromatography (HPLC) method. Eleven ginsenosides, which represent 4 types of typical sapogenins, were chosen as standards for quantitative determination in order to characterize the chemical constituent pattern of each Ginseng drug and investigate the relationship between genetic varieties and chemical constituent pattern. The results showed that the ginsenoside compositions in Ginseng drugs of different origins were of considerable variability. Total saponin contents varied by 10-fold from the highest drug to the lowest one. Chikusetsu-ninjin derived from *P. japonicus* (Japan) was found to have the highest content (192.80 - 296.18 mg/g) and Ginseng from *P. ginseng* to be the lowest (5.78 - 15.63 mg/g).Two main groups (I and II) suggested by phytochemical data were clearly observed; group I mainly containing dammarane saponins consisted of *P. ginseng, P. quinquefolius, P. notoginseng, P. vietnamensis* and *P. vietnamensis* var. *fuscidiscus*; and group II containing a large amount of oleanolic acid saponins was composed of *P. japonicus* (Japan), *P. zingiberensis, P. japonicus* (China), *P. japonicus* var. *angustifolius, P. japonicus* var. *major, P. japonicus* var. *bipinnatifidus* and *P. stipuleanatus.* The ratios of the subtotal of dammarane saponins to that of oleanolic acid saponins (D/O) were found to be > 1.9 and < 0.25 for groups I and II, respectively. The drug samples derived from the same botanical origin revealed similar constituent patterns, in other words, each *Panax* taxon showed its own characteristic chromatographic profile,which appeared in the specific shape of an 11-direction radar graph constructed on the basis of the result of quantitative analysis. Similarities of chemical constitution were seen among the closely phylogenetically-related taxa, including *P. ginseng* and *P. quinquefolius, P. vietnamensis* and *P. vietnamensis* var. *fuscidiscus, P. japonicus* (China) and its varieties were demonstrated, except *P. japonicus* (Japan) and *P. zingiberensis.*

9) **Zhu S., Zou K., Cai S. Q., Meselhy M. R., and Komatsu K.: Simultaneous Determination of Triterpene Saponins in Ginseng Drugs by High Performance Liquid Chromatography. *Chem. Pharm. Bull.*, 52: 995-998, 2004.**

Abstract: A HPLC method for the simultaneous determination of 11 triterpene saponins with four-type aglycones (protopanaxadiol, protopanaxatriol, ocotillol and oleanolic acid types) in Ginseng drugs was developed and validated. Using a gradient of acetonitrile and 10 mM K-phosphate buffer (pH 5.80) as the mobile phase and UV detection at 196 nm, more than 18 ginsenosides with different aglycones were separated satisfactorily within 60 min. The detection limits (signal/noise> or =3) were 0.1 g for ginsenosides Rb_1, Rc, Rd, Re and Rg_1, chikusetsusaponin III, and notoginsenoside R_2, 0.2 microg for gisenoside Ro and chikusetsusaponin IVa, 0.3 μg for chikusetsusaponin IV, and 3 μg for majonoside R_2. The calibration curve of each saponin had a correlation coefficient close to 1. Intra- and interday precisions were less than 2.1% (n = 5) and 3.3% (n = 15), respectively. The recovery rates of extraction were in the range of 96.4-102.7% for all ginsenosides. By adopting this method, the determinations of 11 ginsenosides in three Ginseng drugs derived from *Panax ginseng, Panax vietnamensis* var. *fuscidiscus* and *Panax japonicus* (Japan) were achieved.

10) **Ahn E. M., Akao T., Nakamura N., Komatsu K., Nishihara T., and Hattori M.: Screening of Medicinal Plant Extracts for Estrogenic Activity in Combination with a Glycosidase Treatment. *J. Trad. Med.*, 21: 81-86, 2004.**

11) **Long C. F., Kakiuchi N., Takahashi A., Komatsu K., Cai S. Q., and Mikage M.: Phylogenetic Analysis of the DNA Sequence of the Non-Coding Region of Nuclear Ribosomal DNA and Chloroplast of *Ephedra* Plants in China. *Planta Med.*, 70: 1080-1084, 2004.**

Abstract: Twenty-four *Ephedra* plants belonging to 8 species grown in the northern and western parts of China were phylogenetically analyzed for their non-coding DNA sequences, internal transcribed spacers (ITSs) of nuclear ribosomal DNA as well as *trn*L intron and intergenic spacers between *trn*L and *trn*F (*trn*L/ *trn*F) of the chloroplast. Based on the ITS sequences, the 8 species could be divided into 3 groups: Group 1 (*Ephedra intermedia, E. sinica, E. przewalskii*), Group 2 (*E. equisetina, E. monosperma, E. gerardiana*), and Group 3 (*E. likiangensis, E. minuta*). The species classified into Group 1 grow mainly in the north, Group 3 in the south and Group 2 in the center, suggesting their genetic and geographic relationships. A specific primer set was designed to classify the 3 groups by routine PCR. Combined analysis of ITS and *trn*L/ *trn*F differentiated the 8 *Ephedra* species.

12) **Ahn E. M., Nakamura N., Fushimi H., Komatsu K., Batkhuu J., and Hattori M.: Constituents of the seeds of *Glycyrrhiza uralensis*. *Nat. Med.*, 58: 311, 2004.**

◇総　説　Review papers

1) Komatsu K., Zhu S., and Sasaki Y.: Systematic Pharmacognostical Study on *Panax* Drugs and *Curcuma* Drugs - Phylogenetic Analysis, Molecular Authentication and Quality Evaluation -. *J. Trad. Med.*, 21: 251-270, 2004.

◇学会報告　Scientific presentation（*: 招待講演）

1) 田村隆幸，東田千尋，鄒 坤，小松かつ子： 黄耆によるAβ25-35誘発性の神経突起萎縮に対する抑制作用—基源植物の差異および修治が及ぼす影響—．日本薬学会第124年会，2004，3. 29-31，大阪.
2) 小松かつ子：フィールドワークの2つの視点—比較民族薬物学と生薬資源学，ミニシンポジウム「天然薬物のフィールドワークを考える」．日本薬学会第124年会，2004，3. 29-31，大阪.
3) Zhu S., Fushimi H., Cai S. Q., and Komatsu K.: Phylogenetic Relationship in the Genus *Panax*: inferred from Chloroplast *trn*K Gene and Nuclear 18S rRNA Gene Sequences. International Symposium on Asian Plant Diversity and Systematics, The Japanese Society for Plant Systematics, International Association of Plant Taxonomists, 2004, 7. 29-8.1, Chiba, Japan.
4) Cai S. Q., Wang X., Ma F. Y., Li J., and Komatsu K.: Studies on HPLC-Fingerprinting of Notoginseng. JSP-KSP-CCTNM Joint Seminar 2004 -International Symposium on Natural Medicines-, The Japanese Society of Pharmacognosy, 2004, 8.9-11, Kaga, Japan.
5) 橋本斎，東田千尋，小松かつ子：Aβ25-35誘発性の神経突起萎縮に対するprotopanaxadiol系サポニンの腸内細菌代謝物M1による軸索伸展作用とそのメカニズム．第21回和漢医薬学会大会，2004，8. 21-22，富山.
6) 東田千尋，畠中史幸，中山なつき，小松かつ子：NO産生系を指標とした鬱金類生薬の駆瘀血作用．第21回和漢医薬学会大会，2004，8. 21-22，富山.
7) 高橋京子，松田秀康，松永和憲，隅田昭彦，木下香葉子，小松かつ子，服部征雄，高橋幸一，東純一：動物性生薬由来成分の肝薬物代謝酵素に及ぼす影響．第21回和漢医薬学会大会，2004，8. 21-22，富山.
8) 西田裕子，高橋京子，上島悦子，小松かつ子，佐々木陽平，畠中史幸，高橋幸一，荒川行生，黒川信夫，東純一：ウコン属生薬の基源と品質：ヒト肝CYP代謝活性への影響．第21回和漢医薬学会大会，2004，8. 21-22，富山.
9) 久保山友晴，東田千尋，小松かつ子：Withanolide A, withanoside IV, withanoside VIによる神

経突起再伸展とシナプス再形成作用．日本生薬学会第51年会，2004，9. 9-10，神戸.

10) Zhu S., Zou K., Fushimi H., Cai S. Q., and Komatsu K.: Comparative study on triterpene saponins of Ginseng drugs. 日本生薬学会第51年会，2004，9. 9-10，神戸.

11) 佐々木聡子，佐々木陽平，伏見裕利，南雲清二，合田幸広，小松かつ子：日本市場に流通するガジュツの基原— *trn*K 遺伝子の塩基配列—．日本生薬学会第51年会，2004，9. 9-10，神戸.

12) 佐々木陽平，佐々木聡子，伏見裕利，南雲清二，合田幸広，小松かつ子：ガジュツ及びウコンの試験法に関する研究．日本生薬学会第51年会，2004，9.9-10，神戸.

13) 久保山友晴，東田千尋，小松かつ子：神経突起伸展及びシナプス形成を機序とする withanolide 類の空間記憶障害改善作用．第27回日本神経科学大会・第47回日本神経化学大会合同大会 Neuro 2004，2004，9. 21-23，大阪.

＊14) Komatsu K.: Recent Research on Genus *Curcuma*: Molecular Analysis, Identification and Quality Evaluation on Vasomotion Effect. The First International Conference presented by Western Pacific Regional Forum for the Harmoni- zation of Herbal Medicines, WHO/WPRO, 2004, 9.21-22, Shanghai, China.

◇その他　Others

1）東田千尋：富山県で栽培可能な生薬に関する総合的研究－新しい作用機序で抗痴呆活性を示す生薬および漢方方剤の研究．平成15年度受託研究「和漢薬・バイオテクノロジー研究」研究成果報告書，pp. 55-61, 2004.

2）服部征雄，東田千尋，小松かつ子，土屋真澄，中村憲夫：コーヒー豆のトリゴネリンと脳神経細胞．第7回「くすりと食物」シンポジウムーシーズ と ニーズー，2004，11. 19，東京.

◇共同研究　Co-operative researches

学内

1）柴原直利：富山医科薬科大学和漢薬研究所，「富山県で栽培可能な生薬に関する総合的研究」，2002～2004

海外

1）Javzan Batkhuu：国立モンゴル大学生物学部，蔡　少青：北京大学薬学院，Sitthithaworn Worapan：Srinakarinwirot 大学薬学部「漢薬の資源をアジアに探る：モンゴル及びタイ産薬用植物の調査研究」，2002～2004

◇研究費取得状況　Acquisition of research funds

1）文部省科学研究費，基盤研究(B)(2)（第3年度）（代表：小松かつ子，分担：東田千尋）「漢薬の資源をアジアに探る：モンゴル及びタイ産薬用植物の調査研究」，220万

2）(財) 田村科学技術振興財団（代表：小松かつ子，分担：東田千尋）「各種ウコン属生薬の生活習慣病予防・治療薬としての有効性評価」，30万

3）富山医科薬科大学特別経費「戦略的経費」（代表：東田千尋）「漢方処方を進化させる科学的アプローチ－痴呆を治療する処方の開発－」，35万

4）富山県受託研究「和漢薬・バイオテクノロジー研究」（分担：東田千尋）「富山県で栽培可能な生薬に関する総合的研究：新しい作用機序で抗痴呆活性を示す生薬および漢方方剤の研究」，50万

5）富山県受託研究（代表：小松かつ子，分担：東田千尋）「富山産ヤマブシタケの抗痴呆作用の検討」，40万

◇研究室在籍者　Research members（一部10月31日まで）

学部4年生：中山なつき，松山修二

大学院前期1年：市村真帆子，表　貴之，橋本　斎，劉　洪宇

大学院前期2年：佐々木聡子，杉山玲子
大学院後期3年：久保山友晴
研究機関研究員：朱　姝
技術補佐員（研究支援推進員）：幸　雅子，出口鳴美
技術補佐員：林　和子
受託研究員：石塚　修 (富山北部高校, 2004, 09.01－11.26)
外国人客員研究員：Nijsiri Ruangrungsi (Chulalongkorn University, 2004, 3. 13-3.25)
Rith Watthanachaiyingcharoen (Srinakarinwirot University, 2004, 5. 19-7.18)
Wichet Leelamanit (Mahidol University, 2004, 6. 22-7.31)
Worapan Sitthithaworn (Srinakarinwirot University, 2004, 6. 28-7.31)
Preecha Boonchoong (Ubonrachatanee University, 2004, 7. 10-8.9)
Surapong Kengtong (Chulalongkorn University, 2004, 9. 1-10. 25, 拠点大学方式学術交流事業)
Suchada Sukrong (Chulalongkorn University, 2004, 9. 28-11.11)

◇民族薬物資料館記録　Archive of Museum of Materia Medica

1）一般公開：平成16年10月30日に第7回の民族薬物資料館一般公開を実施した．予約制とし，10時，11時，14時，15時，16時からの5回に分けて各1時間，生薬の解説を加えながら館内を案内した．13:00～13:50に大阪大学大学院薬学研究科・助手の高橋京子先生による講演会「漢方薬の効き方を科学する：クスリとリスク」を行った．来館者は48名，講演会参加者は40名．

2）見学者記録（2004年4月1日 ～ 2005年3月31日）
来館者総数：874名（日本人　805名，外国人　69名）
案内総回数：124回（日本人　99回，外国人　25回）
外国人の国名（人数）：中国(20)，韓国(23)，タイ(8)，アメリカ(8)，ベトナム(5)，スリランカ(3)，不明(2)

◇民族薬物データベース記録　Record of The Data Base of Ethno-medicines in the World (ETHMEDmmm)（2004年4月1日～2005年3月31日）

アクセス数：8730件
専門検索登録者数：110名（全796名），専門検索アクセス数：2069件

漢方診断学部門 Department of Kampo Diagnostics

客員教授	柴原　直利	（医学博士）Professor Naotoshi Shibahara (M.D., Ph.D.)
客員助教授	後藤　博三	（医学博士）Associate Professor Hirozo Goto (M.D., Ph.D.)
客員助教授	酒井　伸也	（医学博士）Associate Professor Shinya Sakai (M.D., Ph.D.)
寄付研究部門教員	中川　孝子	（医学博士）Instructor Takako Nakagawa (Ph.D.)

研究目的　Aims of the research projects

医療保険の薬価に収載されている漢方製剤は147種であり，また生薬は約200種である。平成９年，薬価収載の漢方製剤（いわゆるエキス剤）の全てについて「漢方医学的な病態（証）に基づいて適正に使用すること」が明記された。

証を決定できるようになるためには，基礎概念の学習とともに臨床に根ざした研修を必要とする。にもかかわらず，わが国において体系的にこれを教育する場は，医学部にも薬学部にも未だに整備されていない。

当部門は平成11年４月１日付けで，株式会社ツムラの寄付部門として設置され，本学医学部和漢診療学講座の協力の下に，全国の医師・薬剤師・医薬学生に対して，短期および長期研修コースを提供している。

漢方医学研修カリキュラムを作成するには，古典の学習にとどまらず，証をより客観的なものに育てていく必要がある。

我々は漢方方剤，生薬の薬理作用の研究および漢方医学的病態の解明を学内外諸機関と協力して行っている。

研究概要　Research projects

Ⅰ）漢方医学的病態からみた漢方方剤の薬理効果の基礎的・臨床的研究
- １）各種漢方方剤の指標物質濃度，及びヒトにおける血中濃度の解析
- ２）無症候性脳血管障害に対する桂枝茯苓丸の短期および長期効果の検討
- ３）糖尿病性腎症に対する桂枝茯苓丸の長期効果の検討
- ４）和漢薬の抗酸化作用に関する基礎的研究

Ⅱ）病態や証を客観化するための指標を探索する基礎的・臨床的研究
- １）漢方医学的病態の自律神経系検査法による解析
- ２）漢方医学的病態の品質工学的手法による解析

Ⅲ）漢方医学的病態の古典的解釈と客観的評価を統合した臨床研修プログラムの開発
- １）漢方医学研修による教育効果に関する検討
- ２）傷寒論，金匱要略を中心とする古典の解釈に関する検討

◇著書 Books

1）後藤博三：「虚熱」と四逆湯．漢方診療二項の秘訣，寺澤捷年，花輪壽彦編，92-93，金原出版株式会社，東京，2004.

2）柴原直利：補中益気湯の適応病態．漢方診療二項の秘訣，寺澤捷年，花輪壽彦編，240-241，金原出版株式会社，東京，2004.

3）後藤博三，寺澤捷年：治療薬 UP-TO-DATE 2004．漢方薬，790-799，メディカルビュー社，松沢佑次，永井良三，奥村勝彦編，東京，2004.

4）中川孝子，横澤隆子：糖尿病性腎症における桂枝茯苓丸の有用性．腎とフリーラジカル第7集，松澤直輝 青柳一正編，128-134，東京医学社，東京，2004

5）中川孝子，横澤隆子．桂枝茯苓丸による糖尿病性腎症進展抑制作用：aminoguanidine, butylated hydroxytoluene, captoprilとの比較．腎とフリーラジカル第7集，松澤直輝 青柳一正編，135-140，東京医学社，東京，2004

6）中川孝子，横澤隆子．温脾湯構成生薬並びに大黄・甘草成分の advanced glycation endproducts（AGEs）形成抑制作用．腎とフリーラジカル第7集，松澤直輝 青柳一正編，141-146，東京医学社，東京，2004.

◇原著 Original Articles

1）**Cho EJ., Yokozawa T., Rhyu D.Y., Kim H.Y. and Shibahara N.: The Inhibitory Effects of 12 Medicinal Plants and Their Component Compounds on Lipid Peroxidation. Am. J. Chin. Med., 31: 907-917, 2003.**

Abstract: The antioxidative activities of 12 medicinal plants and the compounds isolated from them were investigated using the thiocyanate method to evaluate inhibitory effects on lipid peroxidation in the linoleic acid system. The peroxide levels gradually increased during incubation in the presence of linoleic acid over 3 days, and most of the plants inhibited lipid peroxidation. In particular, of the plants tested, Cudrania tricuspidata, Zanthoxylum piperitum, Houttuynia cordata and Ulmus parvifolia reduced lipid peroxidation more effectively as lipid peroxidation progressed, resulting in inhibition of about 80% relative to the control value by the 3rd day of incubation. In addition, the polyphenols isolated from the plants also showed marked and dose-dependent inhibitory effects on lipid peroxidation. The compounds with the strongest activities were 3,4-dihydroxylbenzoic acid, quercetin, the quercetin glycosides quercetin-3-O-beta-D-galactoside, quercetin-3-O-alpha-L-rhamnoside, quercetin-3-O-beta-D-glucoside and quercetin-3-O-rutinose, catechin, gallic acid, methyl gallate and rosamultin isolated from Zanthoxylum piperitum, Houttuynia cordata, Rosa rugosa and Cedrela sinensis. Moreover, quercetin glycosides showed stronger activity than quercetin, suggesting that glycosylation increases the antioxidative activity of quercetin. Our results indicate that the medicinal plants and their polyphenols show promise as therapeutic agents for various disorders involving free radical reactions.

2）**Cho EJ., Yokozawa T., Kim HY., Shibahara N. and Park JC.: Rosa rugosa attenuates diabetic oxidative stress in rats with streptozotocin-induced diabetes. Am. J. Chin. Med., 32: 487-496, 2004.**

Abstract: The effects of Rosa rugosa on diabetic oxidative stress were investigated using rats with streptozotocin (STZ)-induced diabetes. The diabetic rats showed less body weight gain and heavier kidney and liver weights than normal rats, while the oral administration of Rosa rugosa at a dose of 100 or 200 mg/kg body weight/day for 20 days attenuated the physiological changes induced by diabetes. In addition, administrating Rosa rugosa to diabetic rats resulted in significant and dose-dependent decreases in the serum glucose and glycosylated protein levels, implying that Rosa rugosa improves the abnormal glucose metabolism that leads to oxidative stress. Diabetic rats had higher serum levels of superoxide and nitrite/nitrate. However, the administration of Rosa rugosa dose-dependently reduced

the over-production of radicals associated with diabetes, suggesting Rosa rugosa is a radical scavenger that would play a crucial role in protecting against diabetic oxidative stress. Rosa rugosa significantly and dose-dependently reduced thiobarbituric acid-reactive substance levels in serum, hepatic and renal mitochondria, implying that Rosa rugosa would alleviate the oxidative stress associated with diabetes by inhibiting lipid peroxidation. This study provides evidence that Rosa rugosa has potential as a treatment for diabetes through attenuating oxidative stress induced by the diabetic condition.

3) **Nakagawa T., Yokozawa T., Sano M., Takeuchi S., Mujo Kim and Shinsuke Minamoto: Activity of (-)-epigallocatechin 3-O-gallate against oxidative stress in rats with adenine-induced renal failure. J. Agric. Food Chem., 52: 2103-2107, 2004.**

Abstract: Methylguanidine (MG) is widely recognized as a strong uremic toxin. The hydroxyl radical (*OH) specifically plays an important role in the pathway of MG production from creatinine (Cr). In this study, we investigated whether oral administration of (-)-epigallocatechin 3-O-gallate (EGCg) suppresses MG production in rats with chronic renal failure after intraperitoneal Cr injection. MG production from Cr was significantly increased in rats with adenine-induced renal failure, which was more vulnerable to oxidative stress, compared with that in normal rats. However, oral administration of EGCg 30 min before and after Cr injection effectively inhibited MG production. Our findings suggest that EGCg, an excellent antioxidant from green tea, exerts protective activity in rats with chronic renal failure, resulting in suppression of Cr oxidation influenced by *OH.

4) **Nakagawa T. and Yokozawa T.: Inhibitory effects of Luobuma tea and its components against glucose-mediated protein damage. Food Chem. Toxic., 42: 975-981, 2004.**

Abstract: Luobuma tea, prepared from the leaves of Apocynum venetum L., is a popular beverage in China. In this study, the activity of Luobuma leaf extract and its components against the formation of advanced glycation endproducts (AGEs), which are largely involved in the pathogenesis of diabetic vascular complications, was examined using the in vitro glycation reaction. Strong inhibitory activity against the formation of AGEs was shown by Luobuma aqueous extract. Following further fractionation of this extract, seven polyphenolic compounds, i.e. (+/-)-gallocatechin, (-)-epigallocatechin, (+/-)-catechin, (-)-epicatechin, epicatechin-(4beta-8)-gallocatechin, epigallocatechin-(4beta-8)-epicatechin and procyanidin B-2, were isolated by Sephadex LH-20 column chromatography. These purified compounds also exerted inhibitory activities that were more potent than the positive control, aminoguanidine. Our findings may help to explain the beneficial effects of this plant against atherosclerosis.

5) **Yokozawa T., Yamabe N., Cho EJ., Nakagawa T. and Oowada S.: A study on the effects to diabetic nephropathy of Hachimi-jio-gan in rats. Nephron Exp. Nephrol., 97: e38-e48, 2004.**

Abstract: Oral administration of Saiko-ka-Ryukotsu-Borei-To (SRB: a traditional Chinese formulation) has been found to prevent intimal thickening of the carotid artery after balloon endothelial denudation in cholesterol-fed rats. To clarify the mechanism of this effect, the present study investigated whether SRB inhibits vascular smooth muscle cell (VSMC) migration, which plays an important role in the development of intimal thickening after endothelial injury. The serum (SRB-serum) sampled from cholesterol-fed rats treated orally with SRB for 3 days before and 4 days after the injury dose-dependently inhibited the migration of cultured VSMCs. On the other hand, SRB extract added directly to cultured VSMCs did not inhibit the migration. It is remarkable that SRB-serum, which might contain a much lower concentration of SRB ingredients compared with SRB-extract, inhibited the cultured VSMCs migration.

The present testing system-using serum obtained from animals treated orally with traditional Chinese formulations could be a useful tool for clarifying the pharmacological efficacy of such drugs including many non-absorbable components. Furthermore, it should be useful for searching for new active compounds in serum after oral administration of traditional Chinese formulations whose active metabolites have not been identified.

6）**Kim HY., Yokozawa T., Nakagawa T. and Sasaki S.: Protective effect of γ-aminobutylic acid against glycerol-induced acute renal failure in rats. Food Chem. Toxic., 42: 2009-2014, 2004.**

Abstract: To investigate the effect of gamma-aminobutyric acid (GABA) on acute renal failure, we used a rat model of acute tubular necrosis induced by glycerol. After deprivation of water for 6h, the rats received an injection of 50% glycerol into the muscle of the rear limb at 10 ml/kg body weight. GABA was then administered orally to the rats (100 or 500 mg/kg body weight/day) once every 12h for 3 days. The rats with acute renal failure showed arrested body weight gain and an increase of kidney weight, whereas oral administration of GABA attenuated the physiological changes induced by acute renal failure. However, GABA administration had no significant effect on increased urine volume. Oral administration of GABA at a dose of 100 or 500 mg/kg body weight/day for 3 days significantly improved the markedly elevated levels of blood urea nitrogen and creatinine and the reduced creatinine clearance related to progression of renal failure. Moreover, the rats with acute renal failure exhibited high levels of fractional excretion of sodium (FE(Na)) due to alteration of tubule function following injection of glycerol. However, administration of GABA lowered the FE(Na) levels dose-dependently. Furthermore, urine osmolarity was markedly reduced in control rats with acute renal failure as compared with normal rats, whereas it was significantly increased by administration of GABA at a dose of 500 mg/kg body weight/day. These results indicate that GABA has potential as a therapeutic agent against the renal damage involved in acute renal failure.

7）**Yokoyama K., Shimada Y., Hori E., Nakagawa T., Takagi S., Sekiya N., Kouta K, Nishijo H., Yokozawa T. and Terasawa K.: Effects of Choto-san and hooks and stems of Uncaria sinensis on antioxidant enzyme activities in the gerbil brain after transient forebrain ischemia. J. Ethonopharmacol., 95: 335-343, 2004.**

Abstract: Previously, we revealed that oral administrations of Choto-san, a Kampo formula, and the hooks and stems of Uncaria sinensis Haviland (Rubiaceae), a medicinal plant comprising Choto-san, enhanced superoxide anion and hydroxyl radical scavenging activities in the hippocampus, and prevented delayed neuronal death of pyramidal cells in the hippocampal CA1 region in a transient forebrain ischemia gerbil model. In the present study, for the purpose of clarifying whether the endogenous antioxidant enzymes contribute to these mechanisms, we investigated the effects of Choto-san extract (CSE) and Uncaria sinensis extract (USE) on superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities in the brain by using the same experimental model. 1.0% CSE or 3.0% USE were dissolved in water and provided to gerbils ad libitum from 7 days prior to ischemia/reperfusion (i/rp). Seven days of continuous administrations of CSE or USE without i/rp procedure enhanced CAT activity but not SOD and GSH-Px activities in both the hippocampus and cortex. CSE elevated CAT activity in the hippocampus at 7 days and in the cortex at 3h after i/rp. USE raised CAT activity in both the hippocampus and cortex at 3 h and 7 days after i/rp. These results suggest that one of the mechanisms of the protective effects of CSE and USE against transient brain ischemia-induced neuronal damage may be their enhancing effect on CAT activity in the brain.

8）**Yokoyama K., Shimada Y., Hori E., Sekiya N., Goto H., Sakakibara I., Nishijo H., Terasawa K.: Protective effects of Choto-san and hooks and stems of Uncaria sinensis against delayed neuronal death after transient forebrain ischemia in gerbil. Phytomedicine, 11: 478-489, 2004.**

Abstract: Previously, we revealed that Choto-san (Diao-teng-san in Chinese), a Kampo formula, is effective on vascular dementia clinically, and the hooks and stems of Uncaria sinensis (Oliv.) Havil., a medicinal plant comprising Chotosan, has a neuroprotective effect in vitro. In the present study, for the purpose of clarifying their effects in vivo, we investigated whether the oral administration of Choto-san extract (CSE) or U. sinensis extract (USE) reduces delayed neuronal death following ischemia/reperfusion (i/rp) in gerbils. Transient forebrain ischemia was induced by bilateral carotid artery occlusion for 4 min, and two doses (1.0% and 3.0%) of CSE or USE were dissolved in

drinking water and provided to the gerbils ad libitum from 7 days prior to i/rp until 7 days after i/rp. It was found that 1.0% and 3.0% CSE treatments significantly reduced pyramidal cell death in the hippocampal CA1 region at 7 days post i/rp. Three percent USE treatment also inhibited pyramidal cell death significantly at 7 days after i/rp. Superoxide anion and hydroxyl radical scavenging activities of the homogenized hippocampus at 7 days after i/rp in the 1.0% CSE- and 3.0% USE-treated groups were significantly enhanced compared to those of control. Further, lipid peroxide and NO2-/NO3- levels of the homogenized hippocampus at 48h after i/rp in the 1.0% CSE- and 3.0% USE-treated groups were significantly lower than those of control. These results suggest that the oral administration of CSE or USE provides a protective effect against transient ischemia-induced delayed neuronal death by reducing oxidative damage to neurons.

9) **Goto H., Shimada Y., Sekiya N., Yang Q., Kogure T., Mantani N., Hikiami H., Shibahara N. and Terasawa K.: Effects of Keishi-bukuryo-gan on vascular function and hemorheological factors in spontaneously diabetic (WBN/kob) rats. Phytomedicine., 11:188-195, 2004.**

Abstract: Keishi-bukuryo-gan (Gui-zhi-fu-ling-wan) is a formula used for the improvement of blood circulation. Recently it has often also been used for arteriosclerosis. One of the mechanisms involved is thought to be the improvement of endothelial dysfunction, but the details are still unclear. In this study, the effect of Keishi-bukuryo-gan on vascular function and hemorheological factors in spontaneously diabetic (WBN/kob) rats was studied. Rats were given Keishi-bukuryo-gan in chow for 30 weeks. Body weight, blood glucose, endothelium-dependent/-independent relaxation, vasocontraction by free radical-induced and contractive prostanoids, triglyceride, advanced glycation endproduct, lipid peroxides, serum NO2-/NO3- and blood viscosity were measured. The results indicated that Keishi-bukuryo-gan caused a decrease in endothelium-dependent relaxation by acetylcholine to become significantly increased, and vasocontraction induced by free radicals and contractive prostanoids was significantly decreased. Furthermore, serum NO2-/NO3- and blood viscosity were significantly decreased. From these results, it was supposed that Keishi-bukuryo-gan exerted a protective effect on the endothelium. The WBN/kob rat is a useful study model for the complications of human diabetes, and Keishi-bukuryo-gan showed a protective effect against vascular injury in the susceptible rat.

10) **Yang Q., Goto H., Hikiami H., Shibahara N., Shimada Y., Terasawa K. and Tang F.: Effects of Toki-shakuyaku-san on microcirculation of bulbar conjunctiva and hemorheological factors in patients with asymptomatic cerebral infarction. J. Trad. Med., 21: 170-173, 2004.**

Abstract: In this study, the effects of Toki-shakuyaku-san on the microcirculation of bulbar conjunctiva in 11 patients with asymptomatic cerebral infarction were investigated with a video-microscopic system. After the administration of Toki-shakuyaku-san for four weeks, the flow volume rates of microcirculatory flow of the bulbar conjunctiva were increased ($p<0.05$). Hemorheological factors such as whole blood viscosity, plasma viscosity, and erythrocyte deformability were examined. Toki-shakuyaku-san improved whole blood viscosity and erythrocyte deformability ($p<0.05$), and plasma lipid peroxides decreased. These results suggested that the favorable effects of Toki-shakuyaku-san on cerebrovascular disorders take place via changes in microcirculatory flow, with the mechanisms being considered to be improvements in hemorheological factors and the anti-oxidant effect of Toki-shakuyaku-san.

11) **Goto H., Shimada Y., Tani T., Sekiya N., Hikiami H., Sakai S., Shibahara N. and Terasawa K.: Effects of a new original formulation containing crude drugs used for self-medication. J. Trad. Med., 21: 199-204, 2004.**

Abstract: A new original formulation containing crude drugs used for self-medication was developed by the joint project of the Federation of Pharmaceutical Inductries Association in Toyama, Toyama Prefecture (Toyama

Prefectural Institute for Pharmaceutical Research) and Toyama Medical and Pharmaceutical University. This formulation was consists of 11 crude. In this study, the effect of this formulation on the model animals of life-style related disease was studied. Spontaneously hypertensive rats added to hypercholesterol diet were given this formulation in chow for 8 weeks. The results indicated that this formulation caused a decrease in vasocontraction induced by phospholipase A2. Plasma triglyceride and lipid peroxide were significantly decreased, but blood pressure was not changed. Furthermore spontaneously diabetic rats were given this formulation in chow for 4 weeks. The results indicated that this formulation caused a decrease in plasma triglyceride, lipid peroxide and fibrinogen significantly, but blood glucose was not changed. From these results, it was supposed that this formulation exerted the suppression effect of vasocontraction, improvement effect of fatty metabolism and decrease effect of fibrinogen. And this formulation is thought to be useful drug to prevent the vasocomplication based on life-style related disease.

12) **Kainuma M., Sakai S., Sekiya N., Mantani N., Ogata N., Shimada Y. and Terasawa K.: The effects of a herbal medicine (Mao-to) in patients with chronic hepatitis C after injection of Interferon-ß. Phytomedicine 11: 5-10, 2004.**

Abstract: We found that a herbal medicine (Mao-to) relieves the side effects of interferon (IFN)-beta and the combination therapy improves the biochemical response rate. However, the exact mechanism by which Mao-to is effective remains to be established. We conducted a controlled trial to clarify the effects of Mao-to. The study was carried out in 18 patients with chronic hepatitis C, and we examined subjective symptoms, body temperature and cytokines such as interleukin (IL)-beta, IL-1receptor antagonist (ra), IL-6 and TNF-alpha. Each patient received 6 million units of IFN-beta intravenously. Mao-to was given orally just before, just after, and 1 hour after IFN administration. The control study was carried out 6 months after the combination therapy of Mao-to and IFN-beta. The scores for general malaise, arthralgia and discomfort were significantly lower in the combination group than in control group. Body temperature did not significantly differ between the two groups. Plasma IL-6 level and IL-1ra were significantly elevated in the combination group compared to control (P = 0.0057 and 0.0003, respectively). Mao-to did not affect plasma concentrations of IL-1beta and TNF-alpha. We considered the increment of IL-1ra caused by Mao-to is to be one of the key factors involved in reducing the flu-like symptoms accompanying IFN-beta and improving the biochemical response rate.

13) **Sakai S., Ochiai H., Mantani N., Kogure T., Shimada Y. and Terasawa K.: Gene Expression in Early phase of Murine Influenza pneumonia Determined by cDNA Expression Array Technique. Intern. J. Appl. Res. Vet. Med., 2: 46-51, 2004.**

Abstract: BACKGROUND: Influenza virus is a worldwide health problem with significant economic consequences. To study the gene expression pattern induced by influenza virus infection, it is useful to reveal the pathogenesis of influenza virus infection; but this has not been well examined, especially in vivo study. AIMS: To assess the influence of influenza virus infection on gene expression in mice, mRNA levels in the lung and tracheal tissue 48 h after infection were investigated by cDNA array analysis. METHODS: Four-week-old outbred, specific pathogen free strain, ICR female mice were infected by intra-nasal inoculation of a virus solution under ether anesthesia. The mice were sacrificed 48 h after infection and the tracheas and lungs were removed. To determine gene expression, the membrane-based microtechnique with an Atlas cDNA expression array (mouse 1.2 array II) was performed in accordance with the manual provided. RESULTS AND CONCLUSIONS: We focused on the expression of 46 mRNAs for cell surface antigens. Of these 46 mRNAs that we examined, four (CD1d2 antigen, CD39 antigen-like 1, CD39 antigen-like 3, CD68 antigen) were up-regulated and one (CD36 antigen) was down-regulated. Although further studies are required, these data suggest that these molecules play an important role in influenza virus infection, especially the phase before specific immunity.

14) **Sekiya N., Shimada Y., Niizawa A., Kogure T., Mantani N., Sakai S., Hikiami H. and Terasawa K.: Suppressive Effects of Stephania tetrandra on the Neutrophil Function in Patients with Rheumatoid Arthritis. Phytother. Res., 18: 247-249, 2004.**

Abstract: Crude preparations of Stephania tetrandra (ST), a traditional herbal medicine, have been used safely for arthritis and silicosis in China. The concentration of granulocyte elastase - alpha 1 protease inhibitor complex in plasma is enhanced in inflammatory processes, e.g. in septicaemia and rheumatoid arthritis (RA), being an expression of granulocyte activation during inflammatory response. It has previously been reported that ST showed beneficial and immunomodulatory effects in the treatment of relatively mild RA. After the administration of ST for 12 weeks, the proportion of granulocytes and the granulocyte count in peripheral blood decreased significantly. The lipid peroxide and human granulocyte elastase levels of stored plasma declined significantly. Furthermore, both the leukocyte/elastase ratio and granulocyte/elastase ratio increased significantly. The findings of this study suggest that the suppressive effect of ST administration on excessive granulocyte activation resulted in the improvement of inflammation with rheumatoid arthritis.

15) **Shimada Y., Yokoyama K., Goto H., Sekiya N., Mantani N., Tahara E., Hikiami H. and Terasawa K.: Protective effect of Keishi-bukuryo-gan and its constituent medicinal plants against nitric oxide donor-induced neuronal death in cultured cerebellar granule cells. Phytomedicine. 11: 404-410, 2004.**

Abstract: Keishi-bukuryo-gan (Gui-Zhi-Fu-Ling-Wan) (KBG) is a traditional Chinese/Japanese medical (Kampo) formulation that has been administered to patients with "Oketsu" (blood stagnation) syndrome. In the process of neuronal cell death induced by brain ischemia, excessive generation of nitric oxide (NO) free radicals is implicated in the neurotoxicity. In the present study, we examined the protective effects of KBG and its constituent medicinal plants against NO donors, sodium nitroprusside (SNP) and 2,2'-(hydroxynitrosohydrazino)bis-ethanamine (NOC18)-induced neuronal death in cultured rat cerebellar granule cells (CGCs). MTT assay showed cell viability to be significantly increased by the addition of KBG extract (KBGE) (100 microg/ml), Cinnamomi Cortex extract (CCE) (3, 10 and 30 microg/ml), Paeoniae Radix extract (PRE) (100 microg/ml) and Moutan Cortex extract (MCE) (10 and 30 microg/ml) compared with exposure to SNP (30 microM, 24 h) only. Also, cell viability was significantly increased by the addition of KBGE (100 and 300 microg/ml), CCE (30 and 100 microg/ml), PRE (100 and 300 microg/ml) and MCE (30 and 100 microg/ml) compared with exposure to NOC 18 (100 microM, 48 h) only. Persicae Semen extract and Hoelen extract did not protect against NO donor-induced neuronal death. These results suggest that KBG has protective effect against NO-mediated neuronal death in cultured CGCs and that it is derived from Cinnamomi Cortex, Paeoniae Radix and Moutan Cortex.

16) **Kogure T., Sato N., Tahara E., Sakai S., Shimada Y., Ochiai H., Origasa H. and Terasawa K.: Assessment of the effects of traditional herbal medicines on elderly patients with weakness using a self-controlled trial. Geriatr. Gerontol. Int., 4: 169-174, 2004.**

Abstract: Background: The objectives of this study were to evaluate the effects of traditional herbal medicine on elderly patients with weakness, and to devise a suitable study design for assessing the clinical effectiveness of traditional herbal medicines. Methods: Twenty-one elderly patients with weakness (mean age, 78.2±7.5; male : female, 8 : 13) were studied using a self-controlled design with a run-in period. The observation term was 3 months, and quality of life (short form-36 and profile of mood status) were adopted as evaluation endpoints. In addition, natural killing activity and surface antigens (CD19, CD3, CD4, CD16, CD56, CD158a, CD158b) on lymphocytes obtained from peripheral blood were analyzed to evaluate patients' immune status. Results: EK-41 (Hochu-ekki-to), EK-48 (Juzen-taiho-to) and EK-98 (Ogi-kenchu-to) were administered to 10, 10 and one patients, respectively. There were no dropouts due to side-effects. Results of the short form-36 were significantly improved after 3 months, with

the patients in the EK-48 group showing greater improvement than those in the EK-41 group. Each component of the profile of mood status was improved by the treatment, and the improvement of V, D and F was especially significant. D and A-H were considerably improved in the patients of the EK-48 group. In contrast, the improvement of D and T-A was most marked in the EK-41 group. Some augmentation of NK activity was observed after 3 months, but the effect was not significant. Although neither the CD4/8 ratio nor the percentages of CD3+, CD19+ or CD16+ CD158a + cells was changed significantly, the percentage of CD56+, CD56+ CD16+ and CD16+ CD158b + cells were significantly increased. Conclusion: A preliminary clinical trial for elderly patients with weakness was carried out to assess the efficacy of traditional medicines, resulting in evidence of their clinical and immunomodulating effects, although this evidence had some limitations. In addition, we obtained some insights into the elements of designing studies suitable for assessing the clinical efficacy of traditional herbal medicines.

17) **Imanishi N., Mantani N., Sakai S., Sato M., Katada Y., Ueda K., Terasawa K. Ochiai H.: Inducible activity of Ginger Rhizome (Zingiber offifinale Rosc.) on the mRNA expression of macrophage-inducible nitric oxide (NO) synthase and NO production in a macrophage cell line, RAW264.7 cells. Am. J. Chin. Med., 32: 727-735, 2004.**

Abstract: We have investigated the effect of Zingiber offifinale Rosc. (ZOR) on macrophage-inducible nitric oxide (NO) synthase (macNOS) mRNA expression and NO production in RAW264.7 cells, a murine macrophage cell line; 100 microg/ml ZOR can induce macNOS mRNA expression, but induction effects at a dose below 10 microg/ml were weak or negligible. Kinetic studies showed that macNOS mRNA can be detected from 4 hours to 24 hours after dosing, with a peak at 8 hours. In accordance with the induction of macNOS mRNA expression, NO concentrations increased from 3.4 microM at 2 hours to almost 150 microM at 24 hours, reflecting a longer period of macNOS mRNA expression. The activity of ZOR can be considered to contribute, at least in part, to the beneficial effects of ZOR through the macNOS-mediated activation of the biodefense mechanism.

18) **Akao T., Sakashita Y., Hanada M., Goto H., Shimada Y., and Terasawa K. : Enteric excretion of Baicalein, a flavone of scutellariae radeix, via glucuronidation in rat: Involvement of multidrug resistance- associated protein 2. Pharmaceutical Research, 21: 2120-2126, 2004.**

Abstract: PURPOSE: Baicalin (BG) and its aglycone, baicalein (B), are strong antioxidants and have various pharmacological actions. The purpose of this study was to evaluate efflux of BG from rat intestinal mucosal cell following glucuronidation of B absorbed after oral administration of B. METHODS: The absorption and excretion of BG and B were evaluated in rats using the in situ jejunal loop technique and in vitro jejunal everted sac experiments. BG and B levels were determined by high-performance liquid chromatography with electro-chemical detection to ensure selectivity and high sensitivity. RESULTS: A large amount (30.4% recovery) of BG, but no B, was detected in the intestinal lumens of germ-free rats 4 h after oral administration of B (12.1 mg/kg), in comparison with a substantial recovery (55.1%) of unabsorbed BG 4 h after its administration. During the in situ rat jejunal loop absorption experiment, B disappeared rapidly, and 8% of the lost B was excreted into the loop as BG 20 min after infusing 0.1 mM B. In an in vitro absorption experiment using everted rat jejunal sac, BG also appeared outside the sac, accompanied by the disappearance of B from the outer (mucosal) side. However, very little of B was transferred to the inner (serosal) side of the sac, and only a trace of BG was detected inside the sac. Thus, in both the loop and the everted sac systems, the efflux of BG from the mucosal surface was saturated with the concentration of B added. Moreover, the efflux rate of BG in the everted jejunal sac from Eisai hyperbilirubinemic rat (EHBR) was significantly lower by 56.4% than that from Sprague-Dawley rat. CONCLUSIONS: These results indicate that, in rat, a large proportion of any B absorbed is retained, transformed into BG within the intestinal mucosal cells, and coordinately excreted through multidrug resistance-associated protein 2 (MRP2) into the intestinal lumen..

19) **Sekiya N., Goto H., Kouta K., Tani T., and Shimada Y.: Evaluation of Toyama original brand formulation about scavenging effect for reactive oxygen species and protective activity on nitric oxide donor-induced neuronal death. J. Trad. Med., 21: 287-293, 2004..**

Abstract: The present study was performed to evaluate Toyama original brand formulation A and B about the scavenging activity for superoxide anion and hydroxyl radical by using electron spin resonance method and protective activity against nitric oxide (NO) donor-induced neuronal death in cultured cerebellar granule cells. As a result, both formulation A and B showed strong radical scavenging effects. It appeared that Corydalis turtschaninovii Besser forma yanhuso Y.H.CHOU et C.C.HSU and Magnolia obovata THUNBERG which were not contained in prescription B, had strong scavenging activities for superoxide anion and hydroxyl radical with the analysis for constituents of the formulations. Furthermore, both formulation A and B had protective effects against NO donor-induced neuronal death in cultured cerebeller granule cells. The protective effect of formulation A was somewhat stronger than that of formulation B. Corydalis turtschaninovii and Magnolia obovata also had protective activities against NO-mediated neuronal death. From these findings, Toyama original brand formulation A may be more useful than formulation B for maintaining and improving health.

◇総　説 Reviews

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2) 関矢信康, 引網宏彰, 古田一史, 小尾龍右, 後藤博三, 柴原直利, 嶋田　豊, 寺澤捷年：清暑益気湯が奏功した気管支喘息の3症例. 日本東洋医学雑誌, 55: 811-815, 2004.

3) 森　昭憲, 関矢信康, 堀江延和, 引網宏彰, 後藤博三, 嶋田　豊, 寺澤捷年：関節リウマチの増悪に対し、桂姜棗草黄辛附湯が速やかに効果を示した一例. 日本東洋医学会雑誌, 55: 469-472, 2004.

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6) 貝沼茂三郎　酒井伸也　嶋田　豊　寺澤捷年：IFNβと麻黄湯の併用によるインターフェロンリバウンド療法を行った1例. 肝臓, 45：303-307, 2004.

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29) 樋口康子，加藤 敦，足立伊佐雄，木津治久，引網宏彰，中川孝子，後藤博三：乾姜に含まれるアルドースレダクターゼ阻害物質について．日本生薬学会第51回年会，2004，9，神戸.
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3）柴原直利：症例検討・漢方医学教育の実際．漢方医学カンファレンス2003(Ⅳ)，2004，3，東京.
4）柴原直利：下肢冷感に対する漢方治療・パネルディスカッション．第1回血栓血管勉強会，2004，3，富山
5）柴原直利：症例検討．富山漢方カンファレンス，2004，5，富山.
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23）柴原直利：疼痛に対する漢方治療・症例検討．漢方医学セミナー IN 神戸，2004，11，神戸．

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◇共同研究　Co-operative researches

1．学内

1）嶋田　豊：富山医科薬科大学和漢診療学講座，「漢方医学の臨床研修プログラムの開発」，1999，4～

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2．国内

1）矢野　宏：東京電気大学客員教授，「品質工学手法を用いた漢方医学の病態解析」，2002，4～

◇非常勤講師　Part-time lecturer

1）柴原直利：富山医科薬科大学，「和漢医薬学入門」，2004.4.30.

2）柴原直利：岡山大学，「東洋医学」，2004.6.7.

3）柴原直利：福井大学，「東洋医学」，2004.7.24.

4）柴原直利：弘前大学，「東洋医学」，2004.9.13.

5）柴原直利：富山医科薬科大学，「東洋医学概論」，2004.10.14.～

6）中川孝子：富山福祉短期大学，「家政学実習Ⅱ（食生活領域）」，2004.10.14.～

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◇研究費取得状況　Acquisition of research funds

1）和漢薬・バイオテクノロジー研究「富山県で栽培可能な生薬に関する総合的研究」（新規，柴原代表，後藤分担）250万

◇研究室在籍者Research members

1．長期研修生

1）堀江　延和（医師，千葉県，2004，1～3）

2）楊　文潔　（医師，中国，2004，1）

3）大野　賢二（薬学院生，富山県，2004，1～3）

4）清水　久夫（薬学院生，富山県，2004，1～3）

5）西畑　友尋（薬学院生，富山県，2004，1～3）

6）酒本　忠幸（医師，千葉県，2004，4～12）

7）木村　真梨（鍼灸師，大阪府，2004，4～12）

8）池田　知枝美（薬学院生，熊本県，2004，9）

9）徳永　紘子（薬学生，熊本県，2004，9）

2．短期研修生

1）大川原　健（医学生，長野県，2004，1.13～1.30）

2）渡邊　長子（医師，千葉県，2004，2.23～2.27）

3）早苗　弘喜（医学生，福岡県，2004，2.23～2.27）

4）末吉　弘尚（医学生，大阪府，2004，3.1～3.5）

5）木村　卓二（医学生，鳥取県，2004，3.15～3.19）

6）仲地　紀勝（医師，岩手県，2004，3.15～3.19）

7）阿部　優作（医学生，青森県，2004，3.15～3.26）

8）竹野　良平（医師，東京都，2004，3.22～3.30）

9）荒川　友恵（薬剤師，愛知県，2004，4.12～4.16）

10）木村　紀遵（医学生，大阪府，2004，4.12～4.16）

11）大杉　友香（薬剤師，東京都，2004，4.19～4.23）

12）井上　隆弥（医師，大阪府，2004，5.10～5.14）
13）宮坂　英　（医師，広島県，2004，5.10～5.21）
14）山本　佳乃子（医師，埼玉県，2004，5.24～5.28
15）宮坂　史路（医師，北海道，2004，6.28～7.2）
16）遠藤　志織（医学生，静岡県，2004，8.2～8.6）
17）小林　希衣（薬剤師，東京都，2004，8.23～8.27）
18）土屋　寧子（医学生，兵庫県，2004，8.23～8.27）
19）近藤　匠巳（医学生，兵庫県，2004，8.23～8.27）
20）松本　祐二（医師，島根県，2004，10.25～10.29）

和漢薬製剤開発部門　Department of Kampo-pharmaceutics

教　授　谿　　忠人　Professor　　Tadato Tani (Ph.D.)
助　手　何　　菊秀　Assistant Professor　　Ju-Xiu He (Ph. D.)

研究目的　Aims of the research projects

和漢薬製剤開発部門は，富山県と県内の薬業界からの寄付部門として2004年７月に開設された。本部門は富山県の薬業（とくに配置薬産業）を支援する実用研究（Kampo-pharmaceutics）と人材育成を目指している。さらに漢方医療の経験知を継承検証しこれに現代科学の客観知を加味して漢方医療情報研究（Kampo-informatics）を行い，県民の健康福祉にも貢献する。

これらの研究と教育は富山医科薬科大学21世紀 COE プログラム「東洋の知に立脚した個の医療の創生」の基盤研究と連携して遂行される。

研究概要　Research projects

Ⅰ）新和漢薬製剤（とくに配置薬）の開発支援研究（Kampo-pharmaceutics）
　１）漢方薬材研究：
　　和漢薬製剤原料生薬の資源科学研究　【原著論文：2, 6；総説：1】
　２）漢方薬剤研究：
　　新和漢薬製剤の開発と評価研究　【原著論文：3, 4, 7, 8】
　　新和漢薬処方を考案する医薬史学的基礎研究【原著論文：1】

Ⅱ）既存の漢方製剤の評価研究　【原著論文：5】

Ⅲ）漢方医療情報研究（Kampo-informatics）
　１）漢方医療情報の蒐集と評価と公表　【著書：1, 2；その他論説：3】
　２）漢方医薬学の教育啓蒙活動　【その他講演：10－24】

なお，2004年１月から６月までの谿と何の研究業績は，以前の所属（漢方薬学分野）で実施されたものであるが，この和漢薬製剤開発部門の項に記載する。

◇著書 Books

1）(分担執筆) 谿　忠人： 漢方薬総論：薬局・薬店における漢方製剤の使い方.「OTC ハンドブック」堀美智子（監修）学術情報流通センター，東京，2004年，pp.1035-1039.

2）谿　忠人（著）:「漢方処方ガイドー疾患別漢方処方の使い分けー」.（改訂第 3 版），株式会社マディソン，東京，2004.

◇原著論文 Original papers

1）**府和隆子，片貝真寿美，小曽戸 洋，谿 忠人：『内外傷弁惑論』における内傷治療の用薬規範．和漢医薬学雑誌，21 (2) : 100–106 (2004).**

Abstract: Nei-Wai-Shang-Bian-Huo-Lun (Naigaisho-benwaku-ron in Japanese) written in the 13 th century is a traditional Chinese medical formulary discussing differentiation on endogenous and exogenous diseases. The endogenous diseases (Nei-Shang in Chinese and Naisho in Japanese) manifested as dyspepsia, anorexia, short breath and fatigue are morbid conditions of deficiency of *pi*-and *wei qi* (Hi-I-Ki-Kyo in Japanese), which is correspondent to decline in digestive function. For curing the deficiency of *pi*- and *wei-qi* caused by intemperance in eating and drinking, overwork, and excessive emotional changes, the formulary was recommended Bu-Zhong-Yi-Qi-Tang Hochu-Ekki-To in Japanese), in which 4 drugs (Astragali, Glycyrrhizae and Ginseng Radices, and Atractilodes Rhizome) act as a principle drugs replenishing *qi*, which means the functions (vital energy) of various organs of the body. The use of two drugs (Cimicifugae and Bupleuri Radices) in the formulation, which is used for morbid condition of muscle and loosening organs as prolapsed uterus, is a noteworthy theory in the formulary. Furthermore, the use of the drugs with sweet in taste and cold in nature used in the formulation Shang-Mai-San (Sho-Myaku-San in Japanese), which is used for syndrome of dry cough with short breath and palpitation to improve the heat syndrome induced by deficiency of *yin* (In-Kyo in Japanese), is also characteristic of the formulary.

2）**Majima T., Yamada T., Tega E., Sakurai H., Saiki I., Tani T.: Pharmaceutical evaluation of liquorice before and after roasting. *J. Pharm. Pharmacol.*, 56 (5): 589-595 (2004)**

Abstract: Liquorice has been used for allergic-inflammatory and liver disorders in both traditional Chinese and modern medicine. In traditional Chinese formulations, roasted liquorice has been mainly used than un-roasted liquorice. In the present study, pharmaceutical characteristics of liquorice before and after roasting were compared to clarify the pharmaceutical significance of the roasting. Although roasted liquorice contained less glycyrrhizin (GL, an anti-allergic component) than un-roasted liquorice, the inhibitory potency of roasted liquorice extract (200 mg kg^{-1}) on IgE-mediated triphasic ear swelling in mice was much greater than that of un-roasted liquorice. In order to search for additional active ingredients, roasted liquorice extract was subjected to gel-chromatography to give an anti-allergic fraction (Fa) of molecular weight ranging from 15,000 to 200,000 or more, in which GL was not detected. By testing the activity of the various fractions, it was proved that the anti-allergic effect of roasted liquorice was due to GL, its metabolite glycyrrhetic acid (GA), and the Fa fraction. The inhibitory potency of the Fa fraction (15 and 75 mg kg^{-1}) prepared from roasted liquorice was stronger than that prepared from un-roasted liquorice. Therefore, a pharmaceutical implication of the roasting of liquorice seems to be associated with increased anti-allergic property of the Fa fraction. It is also notable that oral administration of the high molecular mass fraction (Fa) significantly inhibited IgE-mediated ear swelling 6 days after challenge at doses as low as 3, 15 or 75 mg kg^{-1}.

3）**Baba T., Nishino T., Tani T.: Citri Unshiu Pericarpium prolongs mean residence time of guaiacol after oral administration of wood creosote pill to rats. *J. Trad. Med.*, 21(3):137-142 (2004)**

Abstract: In Japan, wood creosote pills containing four herbal drugs have been used to treat food poisoning and

diarrhea. It was previously reported that among the four herbal drugs used, Citri Unshiu Pericarpium (CUP2, Chinpi in Japanese) plays an important role in sustaining the dissolution of the active constituents of wood creosote (guaiacol) from the pill. To clarify the pharmaceutical role of CUP2 in this pill, pharmacokinetic interactions between CUP2 and guaiacol were examined after oral administration of a wood creosote pill containing four herbal drugs (P4R) to rats. The mean residence time (*MRT*) of guaiacol in the P4R-treated rats was significantly longer than that of the rats treated with a variant pill (P4R with a reduced amount or without CUP2). There were no significant differences in the area under the mean concentration versus time curve from zero to 5 h ($AUC_{0\text{-}5\,h}$) between the two groups. The prolongation effect of CUP2 on the *MRT* of guaiacol was thought to be partly due to the mean dissolution time (*MDT*) of guaiacol from the pill. Since a long *MRT* and *MDT* are indexes of the duration effects of drugs, CUP2 might be a good adjuvant for prolonging anti-diarrhea effects after oral administrations of wood creosote pills.

4）後藤博三, 嶋田　豊, 谿　忠人, 関矢信康, 引網宏彰, 酒井伸也, 柴原直利, 寺澤捷年：富山オリジナルブランド配置薬の生活習慣病モデル動物に対する効果. 和漢医薬学雑誌, 21 (4): 199–204 (2004).

Abstract: A new original formulation containing crude drugs used for self-medication was developed by the joint project of the Federation of Pharmaceutical Inductries Association in Toyama, Toyama Prefecture (Toyama Prefectural Institute for Pharmaceutical Research) and Toyama Medical and Pharmaceutical University. This formulation was consists of 11 crude drugs. In this study, the effect of the formulation on the model animals of life-style related disease was studied. Spomtaneously hypertensive rats added to hypercholesterol diet were given this formulation in chow for 8 weeks. The results indicated that this formulation caused a decrease in vasocontraction induced by phopholipase A2. Plasma triglyceride and lipid peroxide were significantly decreased, but blood pressure was not changed. Furthermore spontaneously diabetic rats were given this formulation in chow for 4 weeks. The results indicated that this formulation caused a decrease in plasma triglyceride, lipid peroxide and fibrinogen significantly, but blood glucose was not changed. From these results, it was supposed that this formulation exerted the suppression effect of vasocontraction, improvement effect of fatty metabolism and decrease effect of fibrinogen. And this formulation is thought to be useful drug to prevent the vasocomplication based on life-style related disease.

5）**Chung H.-J., Maruyama I., Tani T.: Inhibition of vascular smooth muscle cell migration by serum from rats treated orally with Saiko-ka-Ryukotsu-Borei-To, a traditional Chinese formulation. *J. Pharm. Pharmacol.*, 56 (10): 1323-1326 (2004)**

Abstract: Oral administration of Saiko-ka-Ryukotsu-Borei-To (SRB: a traditional Chinese formulation) has been found to prevent intimal thickening of the carotid artery after balloon endothelial denudation in cholesterol-fed rats. To clarify the mechanism of this effect, the present study investigated whether SRB inhibits vascular smooth muscle cell (VSMC) migration, which plays an important role in the development of intimal thickening after endothelial injury. The serum (SRB-serum) sampled from cholesterol-fed rats treated orally with SRB for 3 days before and 4 days after the injury dose-dependently inhibited the migration of cultured VSMCs. On the other hand, SRB extract added directly to cultured VSMCs did not inhibit the migration. It is remarkable that SRB-serum, which might contain a much lower concentration of SRB ingredients compared with SRB-extract, inhibited the cultured VSMCs migration.

The present testing system-using serum obtained from animals treated orally with traditional Chinese formulations could be a useful tool for clarifying the pharmacological efficacy of such drugs including many non-absorbable components. Furthermore, it should be useful for searching for new active compounds in serum after oral administration of traditional Chinese formulations whose active metabolites have not been identified.

6）**Yokota Y., Suzuki H., Tani T.: Discrimination of genuine and alternative bear bile preparations by principal component analysis. *J. Trad. Med.*, 21(5): 231-236 (2004).**

Abstract: Bear bile preparations (Fel Ursi and Yu-tan in Japanese) has been traditionally prepared from gallbladder bile of wild bear and used for the treatment of gastric and hepatobiliary disorders. Due to the decline of wild bear resources, bear bile preparations might be adulterated with cattle or pig bile to fulfill the market demand. In order to define and confirm the quality of bear bile preparations we established the principal component analysis (PCA) based on eight HPLC-peak area data for the discrimination of genuine bear bile preparations from alternative (false and mixed) ones. By the present PCA method, the genuine bear bile was clearly discriminated from cattle, pig and/-or bile mixture. Furthermore out of 173 samples of commercial bear bile preparations collected from 1975 to 2002 in Japan, 48 samples of false and mixed bile preparations were found. The present PCA method based on eight HPLC-peak area data made it possible to discriminate between genuine bear bile preparations and alternative ones

7）**Chung H.-J., Shirasaki S., Tani T.: Inhibitory effects of a newly devised crude drug-formulation on intimal thickening after endothelial injury in rats. *J. Trad. Med.*, 21 (6): 278-280 (2004)**

Abstract: A new preparation (PanaWang) containing eleven crude drugs was devised for self-medication to relieve subjective symptoms attendant to life-style diseases. The inhibitory effects of oral administration of PanaWang on the "accelerated atherosclerosis model" in intimal formation in rat carotid arteries after balloon endothelial denudation was examined. Administration of PanaWang 3 days before and 7 days after denudation dose-dependently suppressed the increased intimal thickening. In immunohistochemical analysis performed using a monoclonal anti-proliferating cell nuclear antigen (PCNA) antibody to stain vascular smooth muscle cells (VSMCs) in the intimal area, administration of PanaWang for 10 days reduced the proliferation of VSMCs. The inhibitory potency of PanaWang on VSMC proliferation partly contributes to its preventive effect on intimal thickening. These results indicated that, PanaWang might be useful for preventing atherosclerosis after endothelial injury resulting from long-term inappropriate life-styles.

8）**関矢信康、後藤博三, 谿　忠人、嶋田　豊：新開発の富山オリジナルブランド配置薬処方の活性酸素種消去活性および神経細胞保護作用の検討. 和漢医薬学雑誌, 21 (6) : 287-293 (2004).**

Abstract: The present study was performed to evaluate Toyama original brand formulation A and B about the scavenging activity for superoxide anion and hydroxyl radical by using electron spin resonance method and protective activity against nitric oxide (NO) donor-induced neuronal death in cultured cerebellar granule cells. As a result, both formulation A and B showed strong radical scavenging effects. It appeared that *Corydalis turtschaninovii* Besser forma yanhuso Y.H.Chou et C.C.Hsu and *Magnolia obovata* Thunberug which were not contained in prescription B, had strong scavenging activities for superoxide anion and hydroxyl radical with the analysis for constituents of the formulations. Furthermore, both formulation A and B had protective effects against NO donor--induced neuronal death in cultured cerebellar granule cells. The protective effect of formulation A was somewhat stronger than that of formulation B. *Corydalis turtschaninovii* and *Magnolia obovata* also had protective activities against NO-mediated neuronal death. From these findings, Toyama original brand formulation A may be more useful than formulation B for maintaining and improving health.

◇**総説**　Review articles

1）谿　忠人：中国で栽培した *Glycyrrhiza uralensis* 根の評価～ 薬用甘草資源の確保と中国の砂漠化防止を目指して ～Minophagen Medical Review, 49(2): 49-60 (2004).

◇学会報告　Scientific presentation

1．特別講演，国際学会，シンポジストなど

1）谿　忠人：（特別講演）芍薬甘草湯と西洋薬の相互作用－腸内細菌の代謝活性と活性代謝物の吸収動態変動を中心にして－．日本東洋医学会北陸支部，2004.7.11．福井．

2）He J-X., Goto E., Akao T., Tani T.: Alterations of intestinal metabolism of paeoniflorin and pharmacokinetics of its metabolite paeonimetabolin-I from Shaoyao-Gancao-Tang by some co-administered synthetic drugs. 5th International Congress on Natural Medicine, 2004. 9,4-5. Shenyang (China).

3）谿　忠人：（シンポジウム：薬学・医学教育改革における生薬学の将来）漢方医療薬学からみた生薬学の研究と教育．日本生薬学会第51回年会，2004.9.9，神戸．

4）谿　忠人：（シンポジウム：予防薬学－食と健康－）飽食の時代に適した予防的和漢薬製剤の開発．第6回地域研究交流フォーラム「21世紀の薬箱」，2004.10.8，大阪．

2．一般報告

5）Rauchensteiner F., Matsumura Y., Yamamoto Y., Yamaji S., Tani T.: Development of environmental friendly analysis of *Glycyrrhiza* species from Europe and China by capillary zone electrophoresis (CZE) The 124th Annual Meeting of Pharmaceutical Society of Japan. 2004.3.29-31, Osaka.

6）佐藤祐司，何　菊秀，谿　忠人，赤尾光昭：芍薬甘草湯の鎮痛鎮痙作用－甘草成分グリシクマリンによる腸管収縮抑制－．日本薬学会第124回年会，2004.3.29-31，大阪．

7）佐藤祐司，後藤博三，嶋田　豊，谿　忠人，井上正浩，赤尾光昭：甘草成分グリシクマリンのラット血管収縮抑制作用：内皮異存性，非依存性弛緩．第21回和漢医薬学会大会，2004.8.21-22，富山．

8）永井秀昌，山本　豊，Rauchensteiner F.，佐藤祐司，赤尾光昭，谿　忠人：中国内蒙古で栽培した *Glycyrrhiza uralensis* 根と薬用甘草との腸管収縮抑制作用の比較．第21回和漢医薬学会大会，2004.8.21-22，富山．【若手優秀発表賞受賞】

9）後藤恵美，何　菊秀，赤尾光昭，谿　忠人：下痢病態における芍薬甘草湯成分の腸内細菌による代謝および血中動態変動．第21回和漢医薬学会大会，2004.8.21-22，富山．

10）片貝真寿美，谿　忠人：『千金方』傷寒方（第九・十巻）における用薬法．第21回和漢医薬学会大会，2004.8.21-22，富山．

11）大野賢二，鄭　和珍，谿　忠人：防風通聖散の血管内皮細胞擦過傷害後の内膜肥厚抑制作用．第21回和漢医薬学会大会，2004.8.21-22，富山．

12）Chung H-J., Liu Y., Maruyama I., Tani T.: Serum obtained from cholesterol-fed rats orally treated with Oren-gedoku-to inhibits migration of vascular smooth muscle cells. 第21回和漢医薬学会大会，2004.8.21-22，富山．

13）谿　忠人，白崎聖子，鄭　和珍：富山オリジナルブランド配置薬の創案と血管内皮細胞擦過傷害後の内膜肥厚と血管平滑筋細胞の増殖抑制作用．第21回和漢医薬学会大会，2004.8.21-22，富山．

14）後藤博三，嶋田　豊，谿　忠人，関矢信康，引網宏彰，酒井伸也，柴原直利，寺澤捷年：富山オリジナルブランド配置薬の生活習慣病モデル動物に対する効果．第21回和漢医薬学会大会，2004.8.21-22，富山．

15）関矢信康，嶋田　豊，後藤博三，古田一史，谿　忠人：富山オリジナルブランド処方A，Bの活性酸素種消去活性および神経細胞保護作用の検討．第21回和漢医薬学会大会，2004.8.21-22，富山．

16）所　崇，谿　忠人，北島　勲：関節リウマチに薬効を示す漢方方剤の探索と転写因子活性調節機能の解明．第21回和漢医薬学会大会，2004.8.21-22，富山．

17）谿　忠人：漢方医療薬学の諸問題－腸内細菌の配糖体代謝に着目した薬物相互作用を中心にして－．平成16年度科学技術振興調整費「漢方有効性の検証方法の確立と応用展開」報告会（主催：鹿児島大学医学部臨床検査医学），2004.12.17．鹿児島．

◇その他（漢方医薬の Health and Medical Information 活動）：

1．論説

1）谿　忠人：漢方医療薬学（漢方薬材学と漢方薬剤学）．和漢薬，No.615：1-2（2004）．

2）谿　忠人：巻頭言－言葉の限界と有用性－．漢方の臨床，51(7)：873（2004）．

3）谿　忠人，国重敦子，山形和子，大石蕗子，堀美智子：実践的問題解決塾23．配糖体の吸収（1．「アグリコン」て，何？　2．この抗生物質，漢方薬と一緒にのんでもいいですか？）．調剤と情報，10 (8)：1089-1098（2004）

4）Rauchensteiner Florian: Kampo－How the Japanese update traditional medicine. *Viennese Ethnomedicine Newsletter,* 7(1): 24-30 (2004).

2．新聞など

5）谿　忠人：富山オリジナルブランド配置薬（パナワン）開発の経過（記事）．薬日新聞．2004.1.14.

6）谿　忠人：産官学で配置用の滋養薬（記事）．北陸中日新聞．2004.2.16.

7）谿　忠人：富山オリジナルブランド配置薬開発研究（富山県薬業配置部会連合会定例部会長会の講演記録）．薬日新聞．2004.3.3, 3.10

8）谿　忠人：配置向け和漢薬の開発（記事）．北日本新聞．2004.4.26.

9）谿　忠人：貝原益軒『養生訓』に学ぶ．CiC 市民健康講座（主催：財団法人富山県観光物産センター）（記事）．北日本新聞．2004.5.16.

3．講義・講演・ラジオ

1）配置販売員研修と富山オリジナルブランド配置薬の広報

10）谿　忠人：富山オリジナルブランド配置薬開発研究の概要．富山県薬業配置部会連合会記念講演会（主催：富山県薬業連合会）．2004.1.9．富山．

11）谿　忠人：富山オリジナルブランド配置薬の狙い．富山県薬業配置部会連合会幹部会及び青年配置員の合同研修会（主催　富山市商工労働部薬業物産課）．2004.1.13．富山．

12）谿　忠人：富山オリジナルブランド医薬品「パナワン」について．第38回富山県医薬品配置薬業者大会（主催：富山県薬業連合会），2004.8.18．富山．

13）富山医科薬科大学：富山オリジナルブランド配置薬開発（ポスター展示）．NEAR2004・中国（主催：富山県／ジェトロ／NEAR2004中国実行委員会）．2004.9.7-8．富山．

14）富山医科薬科大学：富山オリジナルブランド配置薬開発（ポスター展示）．ほくほくFGビジネスフォーラム（主催：ほくほくFG）．2004.9.13．富山．

15）谿　忠人：富山オリジナルブランド医薬品「パナワン」について．水橋薬業会10周年記念講演会（主催：富山県水橋薬業会），2004.10.15．富山．

16）富山医科薬科大学：富山県の産官学が連携した富山オリジナルブランド配置薬開発（ポスター展示）．産官学ビジネスショウ（主催：名古屋大学研究協力・国際部），2004.11.17-19．名古屋．

17）谿　忠人：富山オリジナルブランド配置薬（PanaWang）研究の概要と寄付部門「和漢薬製剤開発研究部門」の開設経緯．和漢薬・バイオテクノロジー成果発表会（主催：富山県厚生部），2004.12.2．富山．

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18）谿　忠人：漢方薬文化を知り感じる．放送大学面接授業．2004.4.17-18．富山県立大学・富山医科薬科大学

19）谿　忠人：貝原益軒『養生訓』に学ぶ．CiC 市民健康講座（主催：財団法人富山県観光物産センター），2004.5.15．富山．

20）谿　忠人：配糖体について．ラジオ NIKKEI．2004.6.19．

21）谿　忠人：縄文人が飽食すると……？（二毛作で人生を過ごす漢方医療の知恵）．富山県民生涯学習カレッジ（主催：富山地区生涯学習団体協議会），2004.10.1．富山．

22）谿　忠人：二毛作人生を過ごすための養生と漢方医療の知恵．（主催：年をとらないための生活講

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24) 谿　忠人：冷えと痛みと漢方薬. 市民公開講座（主催：大阪漢方医学振興財団), 2004.12.4. 大阪.

◇共同研究

1．学内

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2）嶋田　豊博士：医学部•和漢診療学講座教授「富山オリジナルブランド配置薬開発研究」2001.4～

3）北島　勲博士：医学部・臨床検査医学教授「冷えと痛みを軽減する漢方方剤の評価研究」2002.4～

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3．海外

1）蔡　少青博士，王　旋博士：北京大学葯学院教授（中国）「栽培生薬と野生生薬の判別と同質性」1999.6～

2）鄭　和珍博士：梨花女子大学校薬学部生薬学教室（韓国）・博士研究員「血管平滑筋細胞に及ぼす漢方薬の研究」2003.4～

◇学外活動・非常勤講師等

1）谿　忠人：（財）大阪漢方医学振興財団（理事）(1998.3～現在に至る)

2）谿　忠人：放送大学学園非常勤講師（2004.4.1～2005.3.31)

◇学会役員等

1）谿　忠人：和漢医薬学会 理事（2000.4～2004.3)（J. Trad. Med. 編集委員長：2004.4.～現在に至る）

2）谿　忠人：日本生薬学会 評議員（2002.4～2004.3)

3）谿　忠人：東亜医学協会 評議員（2003.4～現在に至る）

◇研究費取得状況

1）平成16年度21世紀 COE プログラム「東洋の知に立脚した個の医療の創生」(分担：谿　忠人)「基盤研究：地球環境に配慮した薬用資源の開発と漢方薬学的評価」

2）富山県薬業連合会共同研究（代表：谿　忠人)「平成16年度富山オリジナルブランド配置薬開発研究」

3）平成16年度富山県受託研究「和漢薬・バイオテクノロジー研究」(分担：谿　忠人)「冷えと痛みに対する和漢薬の探索」

◇研究室在籍者（2004年7月発足時点：職員２名＋院生３名＋学生２名＋JSPS ポスドク１名）

大学院薬学研究科前期２年：大野賢二，後藤恵美，永井秀昌

薬学部４年生：岡崎瑞希　卒業論文：Capillary electrophoresis 法を用いた日本産防已と中国産青風藤の比較

真々田和矢　卒業論文：『傷寒論』から特定生薬の用薬規範を探る～和漢薬製剤の創案を目指した医薬史学的研究～

３年生（2004/10から）長澤美佳，行政貴裕

外国人客員研究員：Florian Rauchensteiner（日本学術振興会特別研究員）（2002.7～2004.7）

：何　菊秀（2004.4～2004.6）

受託研究員：馬場達也（大幸薬品）（2003.10～2004.3）

協力研究員：横田洋一，村上守一（富山県薬事研究所）（2004.8.1～2005.3.31）森元康夫，範本文哲（カネボウ漢方ヘルスケア研究所）（2004.8.1～2005.3.31）

◇学位（修士・博士）取得者

2004.3　課程博士（薬学）

何　菊秀　Influences of co-administered synthetic drugs on bioavailability of glycyrrhizin and paeoniflorin from Shaoyao-Gancao-Tang and proposal of an appropriate medication regimen to alleviate the problems

2004.3　修士（薬学）

金子真利亜　挿し木栽培したコガネバナ根の capillary electrophoresis 法による評価

並木香奈　潰瘍性大腸炎モデルラットに有用な漢方方剤の探索

庭野友理　牛黄の評価と代替品の開発：血管平滑筋細胞への作用を中心に

劉　穎　Oren-Gedoku-To inhibits intimal thickening of carotid artery in cholesterol-fed rats: The role of its crude drugs in expression of the efficacy

◇研究室来訪者

１）2004.5.14：丸山征郎教授（鹿児島大学医学部・臨床検査医学）「動脈硬化を予防する漢方方剤の研究」打ち合わせ

２）2004.8.20-25：鄭　和珍博士（梨花女子大学校薬学部，韓国）「血管平滑筋細胞機能に及ぼす漢方方剤の研究」打ち合わせ

研究所の活動記録

2004年 和漢薬研究所活動記録

1月13日(火)　第247回研究所セミナー

Harold Schmitz 博士 (Director of Science, Mars Incorporated, USA)
Theobroma cacao: A surprising source of natural products with vascular biology activity.
Howard-Yana Shapiro 博士 (Director of Plant Science, Master Foods, USA)
Recent advances in the understanding of Theobroma cacao genetics and agroecology.

1月23日(金)　第3回拠点大学交流事業連絡協議会

(於：名鉄トヤマホテル；担当：生物試験分野)

2月13日(金)　第248回研究所セミナー

岡部　進博士（京都薬科大学応用薬理学教室教授）
抗潰瘍薬の開発の歴史：貝殻からプロトンポンプ阻害薬まで

2月24日(火)　第249回研究所セミナー

Kun Zou（鄒 坤）博士（薬効解析センター・客員教授）
Saponins from Cortex Albiziae
Unnikrishman Payyappallimana 博士（薬効解析センター・客員助教授）
Databases on traditional medicine and malaria management in Ayurveda

3月23日(火)　第250回研究所セミナー

奥山治美博士（和漢薬研究所客員教授，名古屋市立大学大学院薬学研究科教授）
食用油，植物ステロールと内分泌撹乱作用

3月30日(火)　モンゴル国立大学生物学部(モンゴル)との部局間交流協定締結

4月7日(水)　第251回研究所セミナー

Amanda J. Steward 博士 (Plant Products and Human Nutrituion Group, University of Glasgow)
Phenolics in Green Tea: Human ilestomy study of absorption and excretion

6月15日(火)　第252回研究所セミナー

門脇　真博士（和漢薬研究所消化管生理学分野・教授）
消化器病に対する最適治療薬をめざして

7月16日(金)　第253回研究所セミナー

井上純一郎博士（東京大学医科学研究所、癌・細胞増殖大部門・教授）
TNF 受容体ファミリーのよるシグナル伝達— 分子メカニズムと生理機能 —

7月22日(木)　第254回研究所セミナー

安保　徹 博士（新潟大学大学院・医歯学総合研究科免疫学・医動物学分野教授）
白血球の自律神経支配　-東洋医学との深い関係-

8月9日(月)～11日(水)　第9回和漢薬研究所夏期セミナー
（共催：富山医科薬科大学21世紀COEプログラム，担当：複合薬物薬理学分野）

「ほんとうに効くのか？和漢薬！　基礎研究から最前線」

9月3日(金)　遼寧中医学院薬学院・中薬研究所(中国)との部局間交流協定締結

9月13日(月)　第255回研究所セミナー

笹又理央博士（山之内製薬・創薬研究部薬理研究所応用薬理研究室・室長）
医薬品が世に出るまで－臨床試験・承認申請を中心に－

10月22日(金)　第256回研究所セミナー

高橋京子博士（大阪大学大学院薬学研究科臨床薬効解析学分野助手，薬効解析センター研究協力員）
Pharmaceutical Serviceの質の向上～OTC薬配合生薬を中心に～

10月23日(土)　第25回和漢薬研究所特別セミナー
（共催：富山医科薬科大学21世紀COEプログラム，担当：複合薬物薬理学分野）

和漢薬の薬理学的実証性と研究の新展開―個の医療の創生をめざして－

10月30日(土)　民族薬物資料館一般公開（担当：薬効解析センター）

講演：高橋京子博士（大阪大学大学院薬学研究科助手）「漢方の効き方を科学する：クスリとリスク」

11月8日(月)　第257回研究所セミナー

小山晴己氏（文部科学省学術機関課・課長補佐）
附置研究所を取り巻く情勢の変化について

11月19日(金)　第258回研究所セミナー

Goodenowe Dayan博士，Yamazaki Yasuyo博士（Phenomenome Discoveries Inc., Canada）
非標的メタボローム解析研究の新展開

12月4日(土)　21世紀COE国際シンポジウム（主催21世紀COEプログラム，和漢薬研究所共催）

「薬用資源の保全とその有効利用」

12月21日(火)　南京大学化学化工学院(中国)との部局間交流協定締結

12月22日(水)　伝統医学活性化財団(インド)との部局間交流協定締結

研究所主催のセミナー・シンポジウム・公開講座など

第９回和漢薬研究所夏期セミナー

第１日目：８月９日（月）

歓迎の挨拶　和漢薬研究所長　服部征雄

漢方基礎講義：

１）漢方３大古典と中医学と日本漢方
　　谿　忠人（和漢薬製剤開発部門）

２）ほんとうに効く薬を探すー漢方薬から，本草書から，フィールドから
　　木村孟淳（日本薬科大学）

漢方臨床基礎講義：

１）漢方の診断
　　柴原直利（漢方診断学部門）

２）漢方薬の有効性評価の新しい試み
　　酒井伸也（漢方診断学部門）

和漢薬談義：「漢方」
　　寺澤捷年（富山医科薬科大学医学部）

自由討論・談話会

研究所研究室紹介

第２日目：８月10日（火）

講義実証編１：和漢薬の成り立ちー「釣藤散」を例にして

１）釣藤散の臨床と研究
　　嶋田　豊（富山医科薬科大学医学部和漢診療学）

２）釣藤散―その効き方・その効果
　　松本欣三（生物試験分野）

講義実証編２：和漢薬の有効性を考える

１）駆瘀血薬の臨床
　　後藤博三（漢方診断学部門）

２）モデル動物を用いた駆瘀血剤の基礎研究：作用に特異性はあるのか
　　鳥居塚和生，伊田喜光（昭和大学薬学部生薬・植物薬品化学）

体験実習

１）「気血水」診断法　柴原直利（漢方診断学部門）
２）生薬方剤の鑑定　小松かつ子（薬効解析センター）
３）丸薬作り　山路誠一（漢方薬学分野）

和漢薬談義：「鍼灸の世界」
　　津田昌樹　先生（夢恵堂）

自由討論・談話会

第３日目：８月11日（水）

特別講義：「ここまでわかった漢方薬の効果」
　　済木育夫（病態生化学分野）

まとめ・自由討論

午後　希望者は，民族薬物資料館および和漢薬研究所研究室訪問

第21回和漢医薬学会大会

日時　平成16年8月21—22日
場所　富山国際会議場
後援　富山県，富山市，21世紀COEプログラム推進委員会，財団法人 富山県高等教育振興財団，国立大学法人 富山医科薬科大学・和漢薬研究所
大会テーマ　『自然の摂理と天然の恵みを巧みに利用した全人医療の確立をめざして』

和漢医薬学会大会は2年に一度富山に巡ってくる慣習になっており，今回は服部征雄が大会長を仰せつかった。伝統医学の持つ優れた人間味を取りあげ，上記のテーマを設定した。シンポジウムは「自然の摂理」,「天然の恵み」,「全人医療」のキーワードで組み立ててみた。また，多くの学会が肥大化し，一般発表がポスター形式に移行せざるを得ない状況下，若手発表者に口頭発表の機会をもっと与えるべきとの判断から本大会では口頭発表を復活し，口頭，ポスター両発表形式で35歳以下の若手優秀発表者を表彰することにした。口頭の場合は受け持たれた二人の座長に優秀発表者を推薦してもらい，ポスター発表の場合は和漢薬研究所を中心とした助手以上のスタッフに審査をお願いした。大会の懇親会の席上，優秀発表者の授賞式を行う関係上，優秀発表賞にエントリーを希望する方々の発表を初日に設定した。賞状には大会のポスターに使われた立山連峰が入ったカラフルなデザインが採用された。また，副賞には富山への交通費程度の金一封が手渡された。口頭発表から4人，ポスター発表から5人が選ばれたが，偏ることの無い妥当な選出であったと思う。総演題数122（口頭発表44；ポスター発表78）の内，優秀賞にエントリーした数は51であり42%が若手発表者であったことになる。

その他，オール・ジャパン コンソーシアム形成を取りあげ和漢薬研究のネットワークに関して多くの先生方から提案してもらった。特別講演は天外伺朗氏，岸本忠三元大阪大学総長にお願いした。天外氏はソニーのロボット分野の最高責任者であるが，医療分野にも造詣が深くこの種の学会としては極めて異例な特別講演者であり，市民の参加も多かった。

市民講座は同じ会場で，食と健康を取りあげたが大変好評であった。大会参加者総数は517名であり富山開催としてはまずまずのできであったと考えている。以下，本大会の主なプログラムを列記する。

特別講演Ⅰ：ホロトロピック・センター構想　天外 伺朗
特別講演Ⅱ：IL-6；分子生物学と医学　岸本 忠三
大会長講演：最近のヒト腸内細菌による代謝研究　服部 征雄
学会賞受賞講演：基礎研究による和漢薬の薬効解明　山田 陽城
学会奨励賞受賞講演：
　肝機能に対する小柴胡湯の影響に関する薬剤疫学的研究　赤瀬 朋秀
　新たな甘草資源の開発（栽培甘草の実用化）*Glycyrrhiza uralensis* 根の栽培研究と薬材規格と薬剤特性評価　山本 豊
　和漢薬治療中の関節リュウマチ患者221名の上部消化管内視鏡所見の検討　酒井 伸也
シンポジウムⅠ：和漢薬資源の今昔　-温故知新-
シンポジウムⅡ：オール・ジャパン コンソーシアム形成について
シンポジウムⅢ：自然治癒力を考える
シンポジウムⅣ：糖尿病性腎症治療戦略　-基礎から臨床まで-
ランチョンセミナーⅠ：Prokinetics（消化管機能改善）としての六君子湯の位置付け
ランチョンセミナーⅡ：麻黄附子細辛湯の構成生薬について
市民公開講座　テーマ：食と健康
　抗酸化食品による生活習慣病予防　大澤 俊彦
　フリーラジカル・活性酸素と疾患　吉川 敏一

（文責 服部 征雄）

21世紀 COE 国際シンポジウム

主　催　　21世紀 COE プログラム（国立大学法人富山医科薬科大学）
共　催　　日本生薬学会関西支部，富山医科薬科大学・和漢薬研究所
日　時　　平成16年12月 4 日（土）9:30〜18:00
場　所　　富山県民会館304号室（富山市新総曲輪 4-18）
シンポジウムテーマ　「薬用資源の保全とその有効利用」
主　旨　　21世紀 COE（卓越した拠点）プログラム「東洋の知に立脚した個の医療の創生」の一環として国際シンポジウムを実施

富山医科薬科大学 COE プログラムの課題名は「東洋の知に立脚した個の医療の創生」である。このプログラムの構成は13人のコア・メンバーと10人のフェローである。「東洋の知」の内容は広汎であり，哲学，伝統的薬物，伝統的医療技術などを包含している。

教育研究組織は大きく 2 群に分けられている。その第一群は臨床・基礎研究グループで，糖尿病性網膜症，アトピー性皮膚炎，更年期障害および関節リュウマチを対象疾患としている。西洋医学的に同一の疾患と認識されるそれぞれの病態も，漢方医学的な見地からは亜群に分類される。これを地球儀に喩えると，経度と緯度の関係である。そこで，この経度と緯度の交差する地点の特徴を患者血液のプロテオーム解析によって明らかにすることを主要な研究手段としている。

第 2 グループは基盤研究として，ユーラシア大陸東部の薬用資源の探索とそのデータ・ベースの構築と公開，生薬成分の腸内細菌による代謝，漢方方剤の薬理作用に取り組んでいる。またプロテオーム解析で得られた情報から，病理学的特性を検出し，あるいはノックアウト，ノックイン動物を作製して経度と緯度の交差地点の特徴を分子生物学的に解明するなどの教育研究を行っている。

以上のように本プログラムは西洋医学と東洋医学の異なったパラダイムを融和し，「個」を認識した新たな治療学を形成しようとするものであるが，今回の国際シンポジウムでは第 2 グループの活動を一層推進するために企画されたものである。現在直面している薬用資源の諸問題や薬用資源の有効利用に関して，各国の状況が明らかになり，今後の国際的に連携した取り組みの方向が明らかになることを期待している。

プログラム

講　演

演題 1 ：中国におけるマオウとカンゾウの資源の現状およびその利用と保護対策
　　蔡　　少青　博士（北京大学薬学院・教授）

演題 2 ：GACPと薬用資源の利用
　　木内　文之　博士（国立医薬品食品衛生研究所　筑波薬用植物栽培試験場 場長）

演題 3 ：天然資源を素材とした健康飲料の開発
　　服部　征雄　博士　（富山医科薬科大学・和漢薬研究所教授）

演題 4 ：韓国における天然薬物資源とGAP政策の問題点
　　陸　　昌洙　博士（慶熙大学校・薬学大学・名誉教授）

演題 5 ：モンゴル産薬用植物の現状と将来性
　　J. Batkhuu 博士（国立モンゴル大学生物学部　助教授）

演題 6 ：ジンセノシドを多量に含有するタラの木の有効利用
　　中村　憲夫　博士（富山医科薬科大学・和漢薬研究所　助手）

演題 7 ：延胡索の品質評価について
　　王　　峥涛　博士（上海中医薬大学　教授）

演題 8 ：遺伝子解析を応用した健康食品および生薬の評価
　　小松かつ子　博士（富山医科薬科大学・和漢薬研究所　教授）

総合討論

第25回和漢薬研究所特別セミナー

和漢薬研究所特別セミナーは，和漢医薬学研究の発展をめざして昭和56年（1981）に第１回を全国規模で開催して以来，今年で25回目の開催を迎えるに至った。

今回は，平成15年度文部科学省「21世紀 COE プログラム」に採択された本学の研究教育拠点形成事業「東洋の知に立脚した個の医療の創生」（拠点リーダー　寺澤捷年教授）との共催で，「和漢薬の薬理学的実証性と研究の新展開—個の医療の創生をめざして—」をテーマに研究所特別セミナーを企画した。和漢医薬学は元来，「個の医療」の側面を持っているが，これを客観的に利用するうえで，疾患に対する和漢薬処方の根拠を示していくことが求められている。そこで本セミナーでは，①多成分系薬剤としての和漢薬の薬能・薬効が，臨床研究においてどの程度具体的証拠として明らかにされつつあるのか，また前臨床研究ではどのような裏付けがとられ，どこまで和漢薬の作用原理・治療原理に迫りつつあるのかという実証的成果を各シンポジストの先生方よりお話し頂いた。またこれらの②和漢薬の薬理学的実証性を踏まえて，近年急速な進展を遂げている生命情報科学的方法論を導入し，「個の医療」としての和漢薬学に科学的根拠を与えようとする和漢薬先端研究の現況を紹介していただいた。

民族薬物資料館　一般公開

民族薬物資料館の一般公開は今年で７回目を数える。例年どおり，一日５回，各回１時間，館内を案内しながら，漢方薬，和漢薬，健康食品原料，インド薬物などについて解説し，また民族薬物データベースの使い方を説明した。今年は特に，正倉院に納められている生薬と同類の生薬の展示並びにその説明に力を入れた。また，大学院生らによる研究内容のポスター発表を行った。予約者は24名であったが，実際の来館者は48名を数え，真剣に話を聞かれていた。講演会には，大阪大学大学院薬学研究科の高橋京子先生をお招きし，「漢方薬の効き方を科学する：クスリとリスク」と題してご講演いただいた。会場に集まった約40名の参加者は，上手な和漢薬の使い方を勉強しようと熱心に耳を傾けていた。

公開日：2004年10月30日（大学祭期間中）

１．一般公開をどのように知りましたか？

①新聞	1
②ポスター	8
③ホームページ	3
④学園祭	11
⑤人に聞いた	5
⑥案内状	5
⑦その他	3

２．説明はどうでしたか？（複数回答 可）

①わかりやすかった	30
②わかりにくかった	0
③もっと詳しく知りたい	3
④もっと簡単な説明がいい	1
⑤難しかった	0

⑥その他

- とても丁寧な説明だったのでよくわかった
- 多元的説明でよかった

３．生薬にどのような興味をお持ちですか？

- 薬効　2
- 和漢薬の授業で興味を持った　2
- 生薬＝くすり　を明確に出来た
- 植物の利用
- 栽培
- 副作用が少ない
- 効き目が緩い
- 一部診療に使っている
- 多くの生薬があることがわかった
- 食卓にのるものにどのような効果があるのかを知り，生活に生かしたい
- あまり害を受けずに体質改善できそう
- 具体的にどのように服用するべきかを知りたい
- 富山経済の起爆剤と成り得るかどうか
- 21世紀の日本は和漢薬の時代だと思う
- 良いお薬を広くPR出来たらいい
- 動物生薬
- 効能、基源
- 自分の飲んでいる薬の名もあり興味をひかれた
- 薬の作用等がわかり、よかった
- 市販の生薬，漢方薬，健康食品，サプリメント等の効果
- 医食同源
- 対症療法ではなく普段の食事で摂れないか

４．資料館一般公開に参加され一言感想をお書き下さい

- 詳しい（親切な）説明でよかった　3
- いろいろな薬に驚いた　3
- せんぶりは苦かった　2
- 非常に興味深く拝見した　2
- 熊がいた
- すごい数にびっくり
- 毎年参加しているが何度来ても楽しい
- 普段何気なく使っている薬の使用方法、原材料などがどのようなものか現物をみて驚き感心が深まった
- 大切な資料が地震発生時に壊れないようにしてほしい
- 大変参考になった
- 有益なお話でよかった
- 貴重なものが見られてよかった
- また是非来たい
- 知識がないがとても楽しく見学できた
- 学問は面白い
- 随分整備され管理が大変と思う
- 保存、管理も整っていて感心した
- いろいろな地域に多くの生薬があることを知り勉強になった
- 世界の珍しい生薬等を見ることが出来よかった
- 今年は熊出現が多発，熊も人間も動物，生きたいものだ

和漢薬研究所の部局学術交流協定

和漢薬研究所は2004年にモンゴル（モンゴル国立大学生物学部），中国（遼寧中医学院薬学院・中薬研究所および 南京大学化学化工学院），インド（伝統医学活性化財団）と部局間学術交流協定を締結した。

従来の研究機関と合わせて８ヶ国（韓国，ブラジル，エジプト，ネパール，タイ，中国，モンゴル，インド）15研究機関と交流協定を締結し，天然薬物の研究交流（学生交流，研究者交流，共同研究，学術情報交換）を推進している。

国	機　　関
韓　　国	ソウル大学天然薬物化学研究所，圓光大学薬用資源研究センター，東國大学韓医学研究所
ブラジル	サンパウロ大学薬学部
エジプト	カイロ大学薬学部
ネパール	トリブバン大学理工学研究所，ポカラ大学
タ　　イ	シラパコーン大学薬学部，コンケン大学薬学部
中　　国	南京中医薬大学薬学院，大連理工大学化工学院，南京大学化学化工学院，遼寧中医学院薬学院・中薬研究所
モンゴル	モンゴル国立大学生物学部
イ ン ド	伝統医学活性化財団

The 21st century COE (Center of Excellence) Program "Advanced approach to personalized medicine based on oriental philosophy"

(東洋の知に立脚した個の医療の創生)

The 21st century COE (Center of Excellence) Program is the grant to form a global research base in specialized and high potential fields supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan (文部科学省). In 2003, from Toyama Medical and Pharmaceutical University, the research titled "Advanced approach to personalized medicine based on oriental philosophy" was selected for COE program.

Institute of Natural Medicine (和漢薬研究所) and Graduate School of Medicine (especially Department of Japanese Oriental Medicine: 和漢診療学講座) are positioned as a COE in Japan for the research and education of traditional medical and pharmaceutical sciences with foreign universities and research institutes.

Project Leader: Katsutoshi Terasawa (Professor, School of Medicine)

Project Members: (Institute of Natural Medicine) Masao Hattori, Ikuo Saiki, Katsuko Komatsu, Kinzo Matsumoto, Tadato Tani; (Graduate School of Medicine) Seiji Hayasaka, Ichiro Kato, Sigeru Saito, Yutaka Shimada, Masahiko Toyoda, Koichi Tsuneyama; (Graduate School of Pharmaceutical Sciences) Yasushi Kuraishi

Project Summary:

1. **Clinical study for diagnosis and molecular basis of personalized medicine based on oriental philosophy**

   Holistic patterns of symptom and individual pathogenic alterations, so called "Sho (証)" diagnosed by Kampo medicine (漢方医学), are investigated by proteomics using plasma of patients with different disease states. It is one of the strategies to study "Oketsu (瘀血)", a state of insufficient blood circulation and blood stasis resulting in autoimmune, allergic inflammatory and thrombopoietic diseases, for the purpose of understanding and establishing the personalized medicine.

2. **Basic study on pathogenesis and molecular mechanism of various diseases to achieve the personalized medicine.**

   It is important to investigate the expression of genes associated with various diseases including rheumatoid arthritis and atopic dermatitis, in order to clarify the scientific basis of Kamp medicine. The production of knock-in and knock-out animals leads to a specific characterization of the constitution (responder/non-responder) for Kampo medicines. Biochemical and molecular studies of "Oketsu" are performed based on the characters such as blood rheology and NO responsiveness. It is necessary to introduce the bio-informatics for clinical study on time-relapse state of diseases.

3. **Basic study on traditional medicines (ethnomedicines):**

   Sources, qualities, use and effects of traditional medicinal resources in the world are examined. The database on traditional drugs and their formulations is constructed.

The goals of the project of Toyama Medical and Pharmaceutical University COE program are to construct personalized medical treatment based on both western and oriental philosophy, to maintain useful traditional medical resources and to use them efficiently.

和漢薬研究所年報　第31巻

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