細 胞 資 源 工 学

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本部門では，和漢薬資源の恒久的維持，育成を図るため，薬用生物に関する細胞工学的研究を行なうと同時に，動植物細胞の持つ遺伝情報を解析して，その薬用資源開発への応用，あるいは微生物および動物細胞を用いて生理活性物質の探索，和漢薬の薬効発現機構の解明を行なうことを目指している。本年度の主な研究テーマと成果は下記の通りである。

Ⅰ．腸内嫌気性菌によるバイオトランスフォーメーション

腸内細菌による shikonin の変換反応を検討し，生成物と各種フラビン型補酵素との関係を調べた。また，*Eubacterium* sp. A-44 感染ノトバイオートラットを作製し，経口投与後の血中サイコサポニンおよび代謝物の濃度を通常ラット，無菌ラットの場合と比較検討した。

Ⅱ．腸内細菌による薬物の代謝活性化に関与する遺伝子

Sennoside の加水分解に関与する，*Bifidobacterium* sp. 由来の酵素遺伝子を大腸菌に導入，発現させ大量の加水分解酵素を精製した。

Ⅲ．抗ウイルス薬の開発

巴豆から種々のホルボール系の化合物を単離し，その抗 HIV 作用を検討した。また，各種韓国産生薬の HIV-1 プロテアーゼ阻害作用を検討した。抗ヘルペスウイルス作用を有する蒙古産生薬を検索し，*Stephania cepharantha* に強い活性を見いだした。活性を指標に成分の単離を試み，FK-3000 を得た。この化合物は 125-250 mg/kg で，ヘルペスウイルスを皮膚に感染させたラットの帯状疱疹の進展，生存率を有意に抑制した。しかし，大量では毒性が発現した。

Ⅳ．腎疾患における病態の解明と治療薬の開発

増悪因子のフリーラジカルの関与について，ginsenoside-Rd, magnesium lithospermate B, tannin, flavonoid, 茶，大黄，黄耆，紅花，温脾湯を用い検討した。

◇著　書

1）横澤隆子，董　而博，河合悦子，玄番宗一：Magnesium lithospermate B は cisplatin による腎細胞障害を軽減する．“腎とフリーラジカル–第4集–”，遠藤　仁，下条文武監修，武田理夫，長谷川俊雄編，東京医学社，東京，1998，153–157.

2）横澤隆子，劉　中武，董　而博：腎障害における大黄牡丹皮湯の関与について．“腎とフリーラジカル –第4集–”，遠藤　仁，下条文武監修，武田理夫，長谷川俊雄編，東京医学社，東京，1998，188–191.

3）横澤隆子：生化学的アプローチ．“改訂第3版生薬学概論”，難波恒雄，津田喜典編，南江堂，東京，1998，194–201.

4）Yokozawa T.: The role of green tea tannins in renal disease. “Recent Research Developments in Agricultural & Biological Chemistry,” edited by S.G. Pandalai, Research Signpost, India, 1998, 467–483.

◇原　著

1）**El-Mekkawy S., Meselhy M. R., Nakamura N., Tezuka Y., Hattori M., Kakiuchi N., Shimotohno K., Kawahata T. and Otake T.: Anti-HIV-1 and Anti-HIV-1-Protease Substances from *Ganoderma lucidum*. *Phytochemistry*, 49 : 1651–1657, 1998.**

A new highly oxygenated triterpene named ganoderic acid α has been isolated from a methanol extract of the fruiting bodies of *Ganoderma lucidum* together with twelve known compounds. The structures of the isolated compounds were determined by spectroscopic means including 2D-NMR. Ganoderiol F and ganodermanontriol were found active as anti-HIV with an inhibitory concentration of 7.8 μg/ml for both, and ganoderic acid B, ganoderiol B, ganoderic acid C1, 3β, 5α-dihydroxy-6β-methoxyergosta-7, 22-diene, ganoderic acid α, ganoderic acid H and ganoderiol A were moderately active inhibitors against HIV-1 PR with a 50% inhibitory concentration of 0.17-0.23 mM.

2）**Ma C., Nakamura N. and Hattori M.: Saponins and *C*-Glucosyl Flavones from the Seeds of *Abrus precatorius*. *Chem. Pharm. Bull.*, 46 : 982–987, 1998.**

Two new saponins, 3-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucopyranosyl] hederagenin (named abrus-saponin I) and 3-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucopyranosyl] oleanolic acid 28-β-D-glucopyranosyl ester (abrus-saponin II), and three new flavones, 6-*C*-β-D-glucopyranosyl-4',5-dihydroxy-7,8-dimethoxyflavone (precatorin), 6-*C*-[β-D-apiofuranosyl-(1→2)-β-D-glucopyranosyl]-4',5-dihydroxy-7,8-dimethoxyflavone (precatorin II), 6-*C*-[β-D-apiofuranosyl-(1→2)-β-D-glucopyranosyl]-4',5-dihydroxy-7-methoxyflavone (precatorin III), were isolated from the seeds of *Abrus precatorius* L. together with twelve known compounds including a naturally new saponin, 3-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucopyranosyl] oleanolic acid. Their structures were determined on the basis of chemical and spectroscopic methods. In addition, the unusual NMR spectral behavior of the flavone *C*-glycosides is also discussed.

3) **Kakuda H., Helal A. M., Nakamura N., Hattori M.: 8α-Hydroxy-11α,13-dihydrozaluzanin C. *Acta Crystallographica*, C54 : 113–114, 1998.**

In the structure of the title compound [3α,4,5,6,6α,7,8,9,9α,9β-decahydro-4,8-dihydroxy-3-methyl-6,9-bis(methylene)azuleno [4, 5β-] furan-2 (3H)-one, $C_{15}H_{20}O_4$], the secondary hydroxyl groups participate in intermolecular hydrogen bonds both as donors and acceptors (Fig.1).

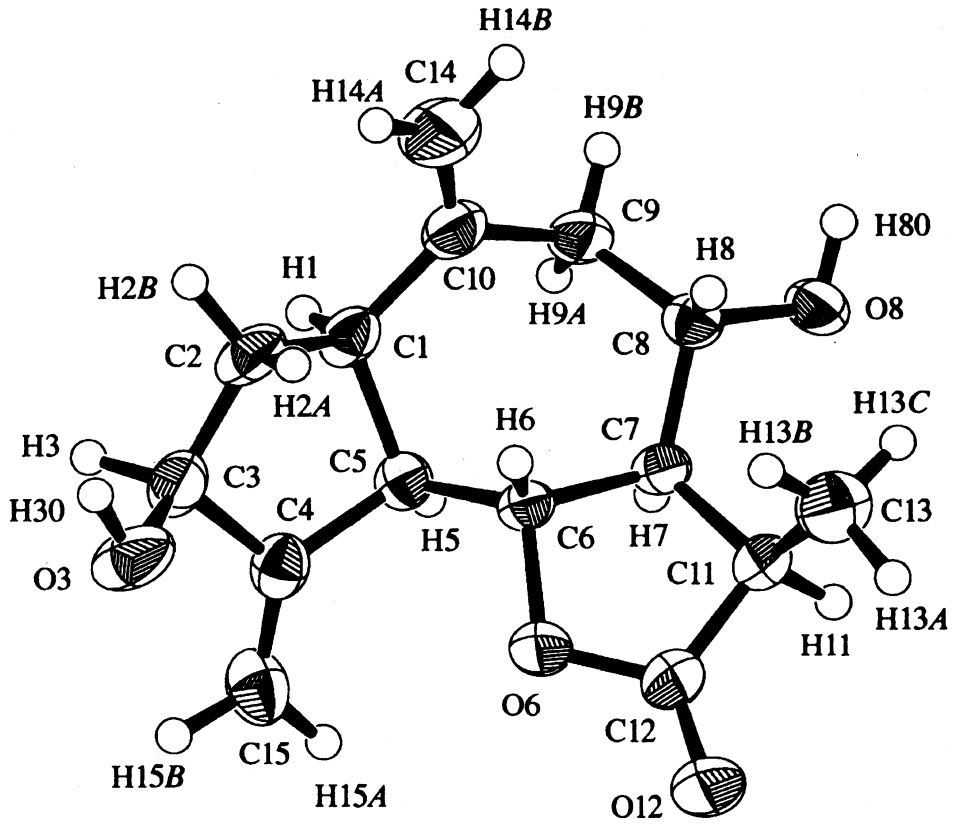


Fig. 1

4) **Meselhy M. R., Heikal O., Akao T., Hattori M., Ono H. and Sadakane C. : Disposition of Paeoniflorin and Paeonimetabolin I in Rats after Oral Administration of Toki-Shakuyaku-San (TS) and Shakuyaku-Kanzo-To (SK). *Nat. Med.*, 52 : 265–268, 1998.**

After oral administration of a traditional Chinese prescription, Toki-Shakuyaku-San (TS) at doses of 100 and 500 mg to rats, the C_{max} values of paeoniflorin (PF) in the plasma were 146.3 and 165.1 ng/ml at 60 and *ca.* 45 min (with AUC values of 14305 and 19385 ng•min/ml), whereas the C_{max} values of paeonimetabolin I (PM-I, a major metabolite of PF) were 184 and 400.3 ng/ml at 120 and 180 min (with AUC values of 98497 and 182188 ng•min/ml). When another prescription, Shakuyaku-Kanzo-To (SK) was orally given at the same doses, the C_{max} values of PF and PM-I were 128.5 and 153.5 ng/ml, and 141.7 and 726.5 ng/ml, respectively at 5 min, and at 360 and 480 min, with AUC values of PF and PM-I (48857 and 32518 ng•min/ml, and 102136 and 469305 ng•min/ml), respectively. Considerable amounts of PM-I were still detected in the rat plasma 24 hr after p.o. administration of SK. It is concluded that following p.o. administration of TS or SK, PF, one of the main constituents of

these prescriptions, was scarcely absorbed but PM-I, the intestinal bacterial metabolite, largely absorbed from the intestinal tract. Moreover, it seems likely that SK, which potentially reduces the gastric emptying rate or peristaltic movement of the intestine, takes longer time to reach the large bowel where most of PF is transformed to PM-I by intestinal bacteria.

5) Yang X., Gu Z., Ma C., Hattori M. and Namba T.: A New Indole Derivative Isolated from the Root of Tuber Fleeceflower (*Polygonum multiflorum*). 中草薬, 29 : 5–11, 1998.

From the root of *Polygonum multiflorum* Thunb., a new indole derivative, named indole-3(L-α-amino-α-hydroxypropionic acid) methyl ester (XI), was isolated together with the ten known compounds, chrysophanol (I), physcione (II), emodin (III), citreorosein (IV), chrysophanol 8-*O*-β-D-glucopyranoside (V), physcione 8-*O*-β-D-glucopyranoside (VI), emodin 8-*O*-β-D-glucopyranoside (VII), torachrysone 8-*O*-β-D-glucopyranoside (VIII), 2,3,5,4'-tetrahydroxystilbene 2-*O*-β-D-glucopyranoside (IX), and methylgallate (X). Their structures were determined by spectroscopic means. These anthraquinone compounds and aloe-emodin (XII), rhein (XIII), aloe-emodin 8-*O*-β-D-glucopyranoside (XIV), chrysophanol 8-*O*-β-D-(6'-*O*-malonyl) glucopyranoiside (XV), sennoside A (XVI) and sennoside B (XVII) had no inhibitory effect against recombinant HIV-1 protease at a concentration of 100 mmol/L *in vitro*.

6) Kida H., Akao T., Meselhy M. R. and Hattori M. : Metabolism and Pharmacokinetics of Orally Administered Saikosaponin b1 in Conventional, Germ-Free and *Eubacterium* sp. A-44 Infected Gnotobiote Rats. *Biol. Pharm. Bull.*, 21 : 588–593, 1998.

The metabolic fate of saikosaponin b1 (**1**) was investigated using conventional, germ-free and *Eubacterium* sp. A-44-infected gnotobiote rats. After the oral administration of **1** to germ-free rats at a dose of 50 mg/kg, no metabolite was detected in the plasma, the cecal contents or the cumulative feces through the experiment. On the other hand, when **1** was orally given to the *Eubacterium* sp. A-44-infected gnotobiote rats, considerable amounts of its metabolites, prosaikogenin A (**2**) and saikogenin A (**3**), were detected in the rat plasma with the respective AUC_{0-10h} values of 17424 and 22260 pmol•min/ml, similar to the case of its oral administration to conventional rats (AUC_{0-10h} values of 9936 and 12414 pmol•min/ml for **2** and **3**, respectively). Furthermore, significant amounts of both metabolites were detected in the cecal contents and the cumulative feces of the gnotobiote and conventional rats, but not in those of the germ-free rats, within 10 h after the administration.

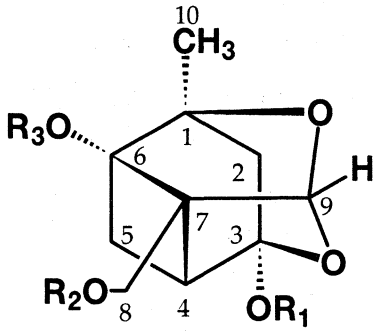
Fecal and cecal activities of hydrolyzing **1** and **2** were found in the gnotobiote and conventional rats, though there were no detectable activities in the germ-free rats. Accordingly, both hydrolyzing activities in the intestinal bacteria, such as *Eubacterium* sp. A-44, are essential for the appearance of **2** and **3** in the rat plasma and cumulative feces, since orally administered **1** was poorly absorbed from the gastrointestinal tract.

7) **Ma C., Nakamura N., Miyashiro H., Hattori M. and Shimothono K.: Inhibitory Effects of Ursolic Acid Derivatives from *Cynomorium songaricum*, and Related Triterpenes on Human Immunodeficiency Viral Protease. *Phytother. Res.*, 12: 138–142, 1998.**

From the stems of *Cynomorium songaricum* Rupr. (Cynomoriaceae), ursolic acid and its hydrogen malonate were isolated as inhibitory substances against human immunodeficiency virus (HIV-1) protease. Their 50% inhibitory concentrations (IC_{50}) were 8 μM and 6 μM, respectively. Of various dicarboxylic acid hemiesters of related triterpenes synthesized, the inhibitory activities tended to increase in the order of oxalic, malonyl, succinyl and glutaryl hemiesters of triterpenes such as ursolic acid, oleanolic acid and betulinic acid. The most potent inhibition was observed for the glutaryl hemiesters with IC_{50} of 4 μM.

8) **Abdel-Hafez A. A., Meselhy M. R., Nakamura N., Hattori M., Watanabe H., Murakami Y., El-Gendy M. A., Mahfouz N. M. and Mohamed T. A.: Effects of Paeoniflorin Derivatives on the Scopolamine-Induced Amnesia Using a Passive Avoidance Task in Mice; Structure-Activity Relationship. *Biol. Pharm. Bull.*, 21, 1174–1179, 1998.**

Paeoniflorin (**1**) and its derivatives having a common cage-like pinane skeleton with hemiketal-acetal system, were evaluated for their effects on memory impairment induced by scopolamine in mice using a step-down type passive avoidance task. In the test session, **1** and its derivatives (Fig.2) were intraperitoneally (i.p.) administered at doses of 0.002, 0.01, 0.02 and 0.2 mmol/kg, and 30 min later (15 min before the experiment), scopolamine (1 mg/kg, i.p.) was given. These compounds showed dose-dependent attenuation in a dose range of 0.002−0.02 mmol/kg and also enhancement of scopolamine-induced decrease in step-down latency. The effects of these compounds, except that of 2',3',4',5'-*O*-tetraacetyl-3-*O*-methylpaeoniflorin (**8**), followed a bell-shaped dose response profile. 8-Debenzoyl-6-deglucosyl-3-*O*-methylpaeoniflorin (**6**) showed no significant increase in the step-down latency at all tested doses. Maximum step-down latency was obtained by 3-*O*-methylpaeoniflorin (**3**) and 2',3,3',4',5'-penta-*O*-methylpaeoniflorin (**7**) (the minimal effective dose was 0.002 mmol/kg). Relative to **3**, debenzoylation, as in 8-debenzoyl-3-*O*-methylpaeoniflorin (**4**), slightly increased the latency, while deglucosylation, as in 6-deglucosyl- 3-*O*-methylpaeoniflorin (**5**), significantly reduced the prolongation of latency. Removal of both glucose and benzoyl moieties resulted in the loss of activity as seen in **6**. These results revealed that, in addition to the cage-like pinane skeleton, the benzoyl and the glucosyl moieties are important structural elements of the paeoniflorin skeleton as its effects on scopolamine-induced amnesia.



Compound No	R_1	R_2	R_3
1	H	Bz	Glc
2	H	H	Glc
3	Me	Bz	Glc
4	Me	H	Glc
5	Me	Bz	H
6	Me	H	H
7	Me	Bz	$Glc(OMe)_4$
8	Me	Bz	$Glc(OAc)_4$
9	H	*p*-HO-Bz	Glc
10	H	Bz	6′-Bz Glc

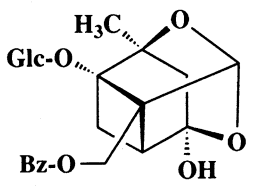
Fig. 2

9) Abdel-Hafez A. A., Meselhy M. R., Nakamura N., Hattori M., Watanabe H., Mohamed T. A., Mahfouz N. M., and El-Gendy M. A.: Potent Anticonvulsant Paeonimetabolin-I Derivatives Obtained by Incubation of Paeoniflorin and Thiol Compounds with *Lactobacillus brevis*. *Chem. Pharm. Bull.*, 46 : 1486–1487, 1998.

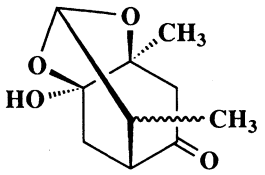
Seventeen thiopaeonimetabolin-I adducts were obtained as mixtures of diastereoisomers after incubation of paeoniflorin with *Lactobacillus brevis* in the presence of various thiols (Fig. 3). Four compounds, 8-(*n*-hexylthio)-(**8**), 8-cyclopentylthio-, 8-(*p*-tolyl)thio- and 8-benzoylthio-(**18**) paeonimetabolins, showed 100% protection against pentylenetetrazole-induced convulsions at doses of 0.125, 0.25, or 0.50 mmol/kg, relative to valproic acid (100% protection at 1.5 mmol/kg). For **8** and **18**, the principle anticonvulsant activity resided in the (*7S*)-isomers, while (*7R*)-isomers showed muscle relaxation effects.

10) Min B., Nakamura N., Miyashiro H., Bae K. and Hattori M.: Triterpenes from the Spores of *Ganoderma lucidum* and Their Inhibitory Activity against HIV-1 Protease. *Chem. Pharm. Bull.*, 46 : 1607–1612, 1998.

Two new lanostane-type triterpenes, lucidumol A and ganoderic acid β, were isolated from the spores of *Ganoderma (G.) lucidum*, together with a new natural one and seven that were known. The structures of the new triterpenes were determined as (24*S*)-24,25-dihydroxylanost-



1



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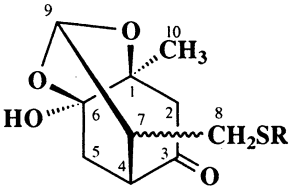

3, R =*n*-propyl
4, R = *iso*-propyl
5, R = *n*-butyl
6, R = *sec*-butyl
7, R = *iso*-butyl
8, R = *n*-hexyl
9, R = allyl
10, R = cyclopentyl
11, R = cyclohexyl
12, R = phenyl
13, R = *o*-tolyl
14, R = *m*-tolyl
15, R = *p*-tolyl
16, R = 2-naphthyl
17, R = benzyl
18, R = benzoyl
19, R = acetyl

Fig. 3

8-ene-3,7-dione and 3β,7β-dihydroxy-11,15-dioxolanosta-8,24(*E*)-dien-26-oic acid, respectively, by chemical and spectroscopic means. The quantitative analyses of 5 fruiting bodies, antlered form and spores of *G. lucidum* were performed by high performance liquid chromatography and demonstrated that ganoderic alcohol and acid contents were quite high in the spore. Of the compound isolated, ganoderic acid β, (24*S*)-lanosta-7,9(11)-diene-3β,24,25-triol (called lucidumol B), ganodermanondiol, ganodermanontriol and ganolucidic acid A showed significant anti-human immunodeficiency virus (anti-HIV)-1 protease activity with IC_{50} values of 20− 90 μM.

11) Akao T., Kida H., Hattori M., Kobashi K.: Intestinal Bacterial Hydrolysis is Required for the Appearance of Compound K in Rat Plasma after Oral Administration of Ginsenoside Rb1 from *Panax ginseng*. *J. Pharm. Pharmacol.*, 50 : 1155–1160, 1998.

Ginsenoside Rb1 from *Panax ginseng* root is transformed into compound K *via* ginsenosides Rd and F2 by intestinal bacterial flora. Among 31 defined intestinal strains from man, only *Eubacterium* sp. A-44 transformed ginsenoside Rb1 into compound K *via* ginsenoside Rd. The ginsenoside Rb1-hydrolysing enzyme isolated from *Eubacterium* sp. A-44 was identical to a previously purified geniposide-hydrolysing β-D-glucosidase.

When ginsenoside Rb1 (200 mg kg^{-1}) was administered orally to germ-free rats, neither compound K nor any other metabolite was detected in the plasma, intestinal tract or cumulative faeces 7 or 15 h after administration. Most of the ginsenoside Rb1 administered was recovered from the intestinal tract, especially the caeca, and cumulative faeces indicating poor absorption of ginsenoside Rb1. When ginsenoside Rb1 was administered orally to gnotobiote rats mono-associated with *Eubacterium* sp. A-44, a significant amount of compound K was detected in the plasma and considerable amounts were found in the caecal contents and cumulative faeces 7 and 15 h after administration. A small amount of ginsenoside Rb1 was detected in the caecal contents only 7 h after administration.

These results indicate that orally administered ginsenoside Rb1 is poorly absorbed from

the gut but that its metabolite compound K, produced by ginsenoside Rb1-hydrolysing bacteria such as *Eubacterium* sp.A-44 in the lower part of intestine, is absorbed.

12) **Min B. S., Bae K., Kim Y. H., Shimotohno K., Miyashiro H., Hattori M., Inhibitory Activities of Korean Plants on HIV-1 Protease. *Natural Product Sciences*, 4 : 241–244, 1998.**

For the development of anti-AIDS agents, thirty-seven methanol extracts of Korean plant materials were tested for their inhibitory effects on human immunodeficiency virus type-1 (HIV-1) protease. Extracts of seven plants showed more than 30% inhibitory activities on HIV-1 protease at a concentration of 100 μg/ml. The bark of *Berchemia berchemiaefolia*, the leaf of *Lindera erythrocarpa* and the whole plant of *Siegesbeckia pubescens* exhibited significant inhibititory activities on HIV-1 protease with 56.2, 50.8, and 46.6%, respectively.

13) **Kim D., Yokozawa T., Hattori M., Kadota S., Namba T.: Effects of Aqueous Extracts of *Apocynum venetum* Leaves on Hypercholesterolaemic Rats. *Phytother. Res.*, 12 : 46–48, 1998.**

The extracts of dried leaves *of Apocynum venetum* collected in different places in China (Luobuma I and II), and two processed leaves (Luobuma III and IV), were investigated on serum lipid and hepatic total cholesterol levels in cholesterol-fed rats.

The serum total cholesterol levels were significantly reduced on day 40 in rats given the roasted leaf extracts (Luobuma III and IV) with $p < 0.05$ and $p < 0.01$, respectively, when compared with a control value of cholesterol-fed rats. In the serum lipoproteins, the LDL-cholesterol levels were significantly lower in rats given each sample extract on day 40, compared with the control value, and the HDL-cholesterol levels were significantly higher than that of a control group, except for a group given Luobuma I. Furthermore, the respective atherogenic indices were significantly lower in all groups given Luobuma I to IV, compared with the control value. The hepatic total cholesterol levels were also significantly lower in all of the groups given the Luobuma extracts than that of control rats on day 40. These results suggest that the extract of *A. venetum* leaves is effective for hypercholesteraemia and the prevention of atherosclerosis.

14) **Yokozawa T., Liu Z.W. and Dong E. : A Study of Ginsenoside-Rd in a Renal Ischemia-Reperfusion Model. *Nephron*, 78 : 201–206, 1998.**

The effect of ginsenoside-Rd in ischemic-reperfused rats was examined. In control rats, blood and renal parameters and the activities of antioxidative enzymes in renal tissue deviated from the normal range, indicating dysfunction of the kidneys. In contrast, when ginsenoside-Rd was given orally for 30 consecutive days prior to ischemia and reperfusion, the activities of the antioxidation enzymes superoxide dismutase, catalase and glutathione peroxidase were higher, while malondialdehyde levels in serum and renal tissue were lower in the treated rats than in the controls. Decreased levels of urea nitrogen and creatinine in

serum demonstrated a protective action against the renal dysfunction caused by ischemia and recirculation. On the other hand, it was demonstrated that ginsenoside-Rd affected cultured proximal tubule cells subjected to hypoxia-reoxygenation, probably by preventing oxygen free radicals from attacking the cell membranes.

15) **Yokozawa T., Dong E., Nakagawa T., Kim D.W., Hattori M. and Nakagawa H.: Effects of Japanese Black Tea on Atherosclerotic Disorders. *Biosci. Biotechnol.* Biochem., 62 : 44–48, 1998.**

The atherogenic index was found to be significantly better in rats fed a high-cholesterol diet supplemented with black tea extract than in the ones not given the extract. It was also evident that black tea inhibited the proliferation of smooth muscle cells involved in the development and progression of atherosclerosis, and suppressed the production of oxidized low-density lipoprotein, a cause of lipid accumulation. It thus seems likely that black tea has an antiatherosclerotic action.

16) **Yokozawa T., Chen C.P. and Liu Z.W.: Effect of Traditional Chinese Prescriptions and Their Main Crude Drugs on 1,1-Diphenyl-2-picrylhydrazyl Radical. *Phytother. Res.*, 12 : 94–97, 1998.**

The inhibitory effects of 79 traditional Chinese prescriptions and 28 crude drugs on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were examined, and many demonstrated significant inhibition. The most effective crude drugs were Gallae Rhois and Rhei Rhizoma, followed in order by Cinnamomi Cortex, Ephedrae Herba, Scutellariae Radix, Perillae Herba, Paeoniae Ruber Radix and Moutan Cortex, while prescriptions composed of one or more of these crude drugs also showed strong free radical scavenging activity. These results predict that traditional Chinese medicines would be promising agents for scavenging free radicals, and for curing diseases related to free radical reactions.

17) **Yokozawa T., Dong E., Yasui T. and Muraguchi A.: Protective Effect of Wen-Pi-Tang against Apoptosis of Cultured Renal Epithelial Cells. *Phytother. Res.*, 12 : 135–137, 1998.**

In terms of the rate of DNA fragmentation and electrophoresis patterns, we demonstrated that apoptosis was induced in LLC-PK_1 cells subjected to the Fenton reaction with H_2O_2 and Fe^{2+}. In addition, Wen-Pi-Tang, which is known to inhibit the progression of renal failure in both an experimental and a clinical setting, was found to suppress this apoptosis.

18) **Kim D.W., Yokozawa T., Hattori M., Kadota S. and Namba T.: Luobuma Leaf Inhibits Oxidation of Low-density Lipoprotein in Cholesterol-fed Rats. *J. Trad. Med.*, 15 : 40–44, 1998.**

Peroxidation was found to be significantly reduced when low-density lipoprotein (LDL) isolated from the plasma of rats treated with Luobuma leaf extract orally was present in the

incubation medium in comparison with plasma from untreated rats. Of the two Luobuma extracts tested, Luobuma B extract showed considerably strong suppression of the peroxidation induced by copper. It was also evident that Luobuma extract decreased the levels of free cholesterol and LDL-cholesterol in serum, and thiobarbituric acid-reactive substance in serum and liver. From these results, it seems likely that Luobuma extract has an antiatherosclerotic action.

19) Mitsuma T., Yokozawa T., Nonaka G., Itoh T., Shimada Y. and Terasawa K.: Differences in Cathartic Action among Different Types of the Oriental Drug Rhubarb. *J. Trad. Med.*, 15 : 45–51, 1998.

Three different types of rhubarb: rhubarb A (produced in the Province of Si-Chuan (四川), China), B (cultivated and processed in Japan) and C (tablets manufactured with the processed rhizoma of *Rheum palmatum* L. from the Province of Qing-Hai (青海), China) were administered to 12 healthy male volunteers for three days each, and their quality was evaluated in terms of the number of bowel movements, bowel sounds, urinary volume and various blood chemical parameters. Upon overall assessment of these parameters, processed rhubarb, with a weaker cathartic action, is suitable for therapeutic use in patients with chronic renal failure.

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The effects of tea (*Camellia sinensis* L.) of three types on excessive free radicals were examined utilizing spin trapping, 1,1-diphenyl-2-picrylhydrazyl radical, lipid peroxidation, and lactate dehydrogenase leakage from cultured cells. Green tea extract presented significant antiradical effects in these four assay systems, whereas oolong tea and black tea extracts showed a rather weak protective effect against free radicals. A more potent scavenger effect using cultured cells was found with a green tea tannin mixture. Similarly to the effects of the green tea tannin mixture, (-)-epigallocatechin 3-*O*-gallate, its main ingredient, had an inhibitory effect on oxidative stress-induced apoptosis. The activities of the antioxidation enzymes in rats after subtotal nephrectomy were increased, suggesting a protective action against oxidative stress. The increased levels of uremic toxins in the blood were also reduced in rats given (-)-epigallocatechin 3-*O*-gallate. These findings indicate that (-)-epigallocatechin 3-*O*-gallate helps to inhibit the progression of renal failure by scavenging radicals.

21) Yokozawa T., Oura H. and Nishioka I.: Confirmation that Magnesium Lithospermate B Ameliorates Paraquat-Induced Injury in Cultured Renal Epithelial Cells. *Nephron*, 79 : 373–374, 1998.

Focusing attention on the clinical finding that decreased urinary excretion of paraquat is associated with aggravation of the pathological condition, we investigated the effect of

magnesium lithospermate B, a substance known to interfere with the progression of renal failure, using a cultured cell line. When magnesium lithospermate B was added to the medium at graded concentrations, LDH leakage was suppressed in proportion to an increase in the magnesium lithospermate B concentration. Leakage of MDA into the medium occurred in parallel with variation in the LDH concentration when magnesium lithospermate B was added to the medium. This finding indicates that magnesium lithospermate B may be a promising agent for use against paraquat-induced cell injury.

22) **Yokozawa T., Chen C.P., Dong E., Tanaka T., Nonaka G. and Nishioka I.: Study on the Inhibitory Effect of Tannins and Flavonoids against the 1,1-Diphenyl-2-picrylhydrazyl Radical.** ***Biochem. Pharmacol.*****, 56 : 213–222, 1998.**

Fifty-one tannins and forty-one flavonoids isolated from Oriental medicinal herbs were evaluated for their antioxidant ability with a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-generating system. The results showed that tannins and certain flavonoids are potential free-radical scavengers, and that their activity against the DPPH radical is closely associated with their chemical structure. A comparison of the two classes of compounds showed that tannins have more potential than flavonoids because almost all the tannins demonstrated significant scavenging action within a low concentration range, whereas the activity of flavonoids varied distinctively among the different compounds. An increase of galloyl groups, molecular weight, and ortho-hydroxyl structure enhanced the activity of tannins, whereas the number and position of hydroxyl groups were important features for the scavenging of free radicals by flavonoids. Moreover, it appeared that when the free hydroxyl group was methoxylated or glycosylated, the inhibitory activity was obviously decreased or even abolished.

23) **Ninomiya H., Mitsuma T., Takara M., Yokozawa T., Terasawa K. and Okuda H.: Effects of the Oriental Medical Prescription Wen-Pi-Tang in Patients Receiving Dialysis.** ***Phytomedicine*****, 5 : 245–252, 1998.**

Hemodialysis patients were treated with Wen-Pi-Tang (a type of traditional Chinese (*Kampo*)-prescription) for 8 weeks, and the changes in active oxygen production by neutrophils, superoxide dismutase (SOD) activity, methylguanidine (MG)/creatinine (Cr) ratio, blood chemistry and subjective symptoms were examined. A decrease in active oxygen production by neutrophils was observed in patients with and without phorbol myristate acetate stimulation. SOD activity and MG/Cr ratio were also reduced by the treatment. In addition, coldness of the limbs, constipation and easy fatigability were improved by Wen-Pi-Tang administration.

24) **Yokozawa T., Chen C.P., Tanaka T. and Kouno I.: Isolation from Wen-Pi-Tang of the Active Principle Possessing Antioxidation and Radical-scavenging Activities.** ***Phytomedicine*****, 5 : 367–373, 1998.**

The active principles of Wen-Pi-Tang possessing antioxidation and free radical-scavenging activities were isolated, and its properties were determined using the thiobarbituric acid-reactive substance (TBARS) procedure and a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical generating system. Significant inhibitory activities against both the formation of TBARS and generation of free radicals were shown by fractions 5, 6 and 7, which contained mainly polyphenolic compounds and phenolic glycosides as well as anthraquinone glycosides. The most active compounds were procyanidin B-2 3,3'-di-*O*-gallate, (-)-epicatechin 3-*O*-gallate, procyanidin B-2 and 1,2,6-tri-*O*- galloyl-β-glucose, the former two having been confirmed to be the most active principles of Wen-Pi-Tang in improving renal function. It is suggested that tannins make a prominent contribution to both the biological activity and pharmacological effects of Wen-Pi-Tang.

25) **Chung H.Y., Yokozawa T., Soung D.Y., Kye I. S., No J.K. and Baek B.S.: Peroxynitrite-scavenging Activity of Green Tea Tannin.** ***J. Agric. Food Chem.*****, 46: 4484–4486, 1998.**

Peroxynitrite formed from superoxide and nitric oxide acts as a strong reactive oxidant. However, among green tea components, catechins with a galloyl group inhibited peroxynitrite formation by 3-morpholinosydnonimine and scavenged peroxynitrite itself. Especially when compared with penicillamine as a positive control, the green tea components (-)-epigallocatechin 3-*O*-gallate and (-)-gallocatechin 3-*O*-gallate, which have two galloyl groups, showed the most potent peroxynitrite-scavenging activity, indicating that the galloyl group may contribute to this activity. A structure of flavan-3-ol linked to gallic acid may be essential for the peroxynitrite-scavenging activity.

26) **Yokozawa T., Liu Z.W. and Chen C.P.: Evaluation of Oriental Medicines Using a Cultured Renal Epithelial Cell Line, LLC-PK$_1$: Effects of Carthami Flos, Rhei Rhizoma and Astragali Radix.** ***J. Trad. Med.*****, 15 : 202–207, 1998.**

The effects of Carthami Flos, Rhei Rhizoma and Astragali Radix extract, which have been proved to ameliorate renal failure in rats with glycerol-induced renal failure, were examined in cell culture under three different culture conditions. Rhei Rhizoma extract showed the most potent effect among the three crude drugs under any of the routine, hypoxia-reoxygenation and cisplatin exposure conditions employed, suggesting that its favorable effect on proximal tubule function is due to suppression of lipid peroxidation via free radicals. In contrast, this anti-cytotoxic activity was low in Astragali Radix extract, and almost nil in Carthami Flos extract. These results indicate that Rhei Rhizoma, Astragali Radix and Carthami Flos exert their actions on different sites in the kidney.

27) **Soung D.Y., Chung H.Y. and Yokozawa T.: A Novel Action of 5-Hydroxytryptamine as a Peroxynitrite Scavenger.** ***Pharm. Pharmacol. Commun.*****, 4 : 583–586, 1998.**

Peroxynitrite is a strong oxidant formed by the reaction of a superoxide anion radical and

nitric oxide. It plays an important role in inducing apoptosis, damaging the mitochondrial respiratory chain and causing the occurrence of nitrotyrosine in cortical neurons.

We have demonstrated that 5-hydroxytryptamine (5-HT) does not inhibit peroxynitrite formation by 3-morpholinosydnonimine with the release of a superoxide anion radical and nitric oxide, however, it does markedly scavenge peroxynitrite itself.

5-HT is directly involved in the scavenging of peroxynitrite, and might play a role in retarding the development of brain disease.

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◇総　説

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◇その他

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2）服部征雄：基底膜分解酵素阻害作用を指標とした癌転移阻害剤の探索．平成9年度受託研究，和漢薬・バイオテクノロジー研究成果報告書，pp. 7–12.

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◇非常勤講師

1）服部征雄：九州大学大学院薬学研究科　大学院高度化推進講義，1998，11/12–13，福岡.

◇研究費取得状況

1）文部省科学研究費基盤研究（B）（2）（継続，服部代表）「腸内細菌による代謝活性化を利用した新しい薬物の開発」280万円。

2）文部省科学研究費萌芽的研究（継続，服部代表）「HIV由来DNA・RNAハイブリッド分解酵素をターゲットとした天然薬物の探索」50万円。

3）文部省科学研究費基盤研究（A）（1）（新規，服部分担）「新規高次神経変性疾患モデル動物・細胞の開発，神経変性機序の解析と薬効評価法の確立」50万円。

4）文部省科学研究費奨励研究(B)「実地調査をもとにした伝統薬物のデータベースの作成」（中村）18万円。

5）富山県受託研究和漢薬・バイオテクノロジー研究：癌の悪性化および転移の防止に有効な和漢薬の開発研究」（継続，服部分担）37万円。

6）財団法人ヒューマンサイエンス振興財団「HIVインテグラーゼおよび複製を制御する蛋白質を標的とする抗ウイルス剤の開発」（新規，服部分担）350万円。

7）文部省科学研究費基盤研究C「抗酸化物としての羅布麻の探索」（新規，横澤代表）180万円。

8）財団法人上原記念生命科学財団「緑茶成分(-)-Epigallocatechin 3-*O*-gallateのクレアチ

ニン酸化機構に及ぼす影響」（横澤代表）500万円。

9）薬用人参研究会「シスプラチンによる急性腎不全における薬用人参の役割」（横澤代表）100万円。

10）全日本コーヒー協会「痴呆脳に対するコーヒーの作用」（服部代表）200万円。

◇学位（修士・博士）

修士：

西本栄里：「Rhein の瀉下活性に関与するヒト腸内細菌（*Bacteroides* sp. RHEIN）について」

博士：

As'ari Nawawi：「Anti-Herpes Simplex Virus Activities of Natural Medicines, *Toona sureni* and *Stephania cepharantha*」

Ghazi Mohamed Eisa Hussein：「Inhibitory Effects of Sudanese Medicinal Plants and Their Constituents on the Proteases of Hepatitis C and Human Immunodeficiency Viruses」

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