生物試験部門 Department of Pharmacology

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◇研究目的 Aims of the research projects

本部門では，和漢薬の新しい薬効評価法を確立するための基礎研究，和漢薬の中枢作用を定量的に評価し，その作用本体を追求する研究及びその作用機序を分子レベルで明らかにすることを目的とした研究を行っている。

◇研究概要 Research projects

Ⅰ．和漢薬の新しい薬効評価法を確立するための基礎的研究

1）脳血管性痴呆病態モデル動物の確立とそれによる和漢薬および抗痴呆薬の薬効評価
2）情動ストレス反応の不安・うつ病態モデルとしての応用と和漢薬作用
3）新規リード化合物の開発をめざした各種民族薬の薬理作用の探索と作用機構の解析

Ⅱ．中枢作用薬の神経薬理学的研究

1）心理的ストレス反応に関わる神経機構，受容体機能修飾因子，分子機序の解析
2）神経伝達物質の脳内動態および代謝回転の解析と薬物作用

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

◇著　書　Books

1）渡辺裕司：中枢神経系に作用する薬物，「Integrated Essentials 薬理学改定第4版」，粕谷豊，加藤仁，重信弘毅編，185-254，南江堂，東京，2002.

◇原著論文　Original papers

1）張紹輝，村上孝寿，東田道久，松本欣三，榊原巌，高山廣光，相見則郎，渡辺裕司：釣藤散，釣藤鉤及びそのアルカロイド成分の脳虚血予防作用:マウス水迷路学習行動を指標として．Journal of Traditional Medicines 19 : 28-36, 2002.

Abstract : It has been reported that Choto-san has an effect on treatment of vascular dementia in clinical tests. In the present study, we investigated the effect of Choto-san and its main constituent Chotoko (*Uncariae uncis cum ramulus*) on the spatial cognitive impairment induced by transient cerebral ischemia in mice in the Morris water maze performance. The spatial cognitive deficiency caused by transient cerebral ischemia in mice exhibited an increase of escape latency and a reduction of swimming time in the platform quadrant. Choto-san (750-6000 mg/kg, p.o.) and Chotoko (75-600 mg/kg, p.o.) administered one hour before the operation shortened the escape latency and increased the swimming time in the platform quadrant, showing a protective effect on the impairment induced by transient cerebral ischemia. Furthermore, we also studied the efficacy of alkaloid fraction, indole alkaloid (geissoschizine methylether) and oxindole alkaloid (rhynchophylline) of Chotoko. The alkaloid fraction (10 mg/kg, p.o.) of Chotoko and rhynchophylline (10 mg/kg, p.o) significantly improved the spatial cognitive impairment induced by transient cerebral ischemia in the Morris water maze, while the protective effect of geissoschizine methylether was weak. These results suggested that Choto-san and Chotoko have a protective effect on transient cerebral ischemia-induced spatial cognitive impairment. The beneficial effect of Chotoko is partly attributed to the oxindole alkaloid rhynchophylline.

2）Dong E., Matsumoto K. and Watanabe H.: Diazepam binding inhibitor (DBI) reduces testosterone and estradiol levels in vivo. Life Sciences 70 : 1317-1323, 2002.

Abstract : Diazepam binding inhibitor (DBI) is a putative endogenous ligand capable of binding to the central type benzodiazepine (BZD) receptor located on the $GABA_A$ receptor and the peripheral type BZD receptor on the mitochondrial outer membrane. We examined the effects of an intracerebroventricular injection of DBI on the serum levels of the gonadal hormones, testosterone and estradiol, respectively, in male and female mice. DBI (0.3-10 nmol/mouse, i.c.v.) significantly reduced the levels of both gonadal hormones in a dose-dependent manner. The decrease in the gonadal hormone levels became evident at 1 hr and lasted for at least 4 hrs after the DBI injection. The effects of DBI (3 nmol/mouse, i.c.v.) in male and female mice were completely attenuated by the coadministration of flumazenil (66 nmol/mouse), a selective antagonist for the central type BZD receptor. These results suggest that DBI acts as an endogenous modulator to regulate the levels of gonadal hormones in vivo, and that the DBI-induced decrease in gonadal hormone levels is mediated by down regulation of the GABAergic system, implicated in gonadotropin-releasing systems and/or the hypothalamic-pituitary-gonadal axis.

3）Kang T.H., Matsumoto K., Tohda M., Murakami Y., Takayama H., Kitajima M., Aimi N., Watanabe H.: Pteropodine and isopteropodine positively modulate the function of rat muscarinic M_1 and 5-HT_2 receptors expressed in *Xenopus* oocyte. European Journal of Pharmacology 444 : 39-45, 2002.

Abstract : Pteropodine and isopteropodine are heteroyohimbine-type oxindole alkaloid components of *Uncaria tomentosa* (Willd.) DC, a Peruvian medicinal plant known as cat's claw. In this study, the effects of these alkaloids on the function of Ca^{2+}-activated Cl^- currents evoked by stimulation of G protein-coupled muscarinic M_1 acetylcholine and 5-HT_2 receptors were studied in *Xenopus* oocytes in which rat cortex total RNA was translated.

Pteropodine and isopteropodine (1-30 μM) failed to induce membrane current by themselves. However, these alkaloids markedly enhanced the current responses evoked by both acetylcholine and 5-hydroxyhyptamine (5-HT) in a concentration-dependent and reversible manner with the maximal effects at 30 μM. Pteropodine and isopteropodine produced 2.7- and 3.3-fold increases in the acetylcholine response with EC_{50} values of 9.52 and 9.92 μM, respectively, and 2.4- and 2.5-fold increases in the 5-HT response with EC_{50} values of 13.5 and 14.5 μM, respectively. In contrast, in oocytes injected with total RNA from the rat cerebellum or spinal cord, neither alkaloid had an effect on the metabotropic current responses mediated by glutamate $receptor_{1 \text{ and } 5}$ ($mGlu_{1/5}$) receptors or ionotropic responses mediated by *N*-methyl-D-aspartate, kainic acid or glycine. Pteropodine and isopteropodine (10 μM) significantly reduced the EC_{50} values of acetylcholine and 5-HT that elicited current responses, but had no effect on the maximal current responses elicited by acetylcholine and 5-HT. On the other hand, mitraphylline, a stereoisomer of pteropodine, failed to modulate acetylcholine- and 5-HT-induced responses. These results suggest that pteropodine and isopteropodine act as positive modulators of muscarinic M_1 and 5-HT_2 receptors.

4) **Sukma M., Chaichantipyuth C., Murakami Y., Tohda M., Matsumoto K., Watanabe H.: CNS inhibitory effects of barakol, a constituent of *Cassia siamia* Lamk. Journal of Ethnopharmacology 83 : 87-94, 2002.**

Abstract : The present study determined the pharmacological profile of barakol, a major constituent of *Cassia siamea* Lamk., in rodent behavioral and neurochemical tests. Barakol reduced spontaneous locomotor activity, increased the number of sleeping animals and prolonged the thiopental-induced sleeping time, indicating a sedative effect. As for interactions between barakol and convulsants (pentylenetetrazole (PTZ), picrotoxin, bicuculline and strychnine), only a high dose (100 mg/kg, i.p.) of barakol slightly prolonged the latency of clonic convulsion induced by picrotoxin. This suggests that the sedative effect may not be induced via the GABA or glycine systems. There was no evidence of an anxiolytic effect of barakol in the plus-maze test. However, barakol (25-100 mg/kg, i.p.) could suppress methamphetamine (1 mg/kg, i.p.)-induced hyper-locomotor activity in a dose-dependent manner, indicating an effect on the dopaminergic system. In a microdialysis study, the dose of barakol (100 mg/kg) that inhibited spontaneous locomotor activity in mice did not affect the basal levels of extracellular dopamine (DA) or its metabolites in the striatum. However, pretreatment with barakol (100 mg/kg, i.p.) decreased the maximal dopamine release and dopamine turnover induced by methamphetamine (1 mg/kg, i.p.). This finding indicates that the inhibitory effect of barakol on dopamine release may account for the blocking effect of barakol on the striatum-related behavior induced by methamphetamine.

5) **趙琦, 渡辺裕司, 村上孝寿, 東田道久, 松本欣三：自然発症高血圧ラットにおける釣藤散の抗高血圧作用：釣藤鉤及び石膏の役割について. Journal of Traditional Medicines 19 : 153-157, 2002.**

Abstract : To clarify a role of Chotoko (Uncaria sp.) and gypsum in the anti-hypertensive effect of a Kampo prescription Choto-san (Diao-Teng-San), we investigated effects of Choto-san except Chotoko or gypsum, Chotoko alone and gypsum alone on the blood pressure and the heart rate in spontaneously hypertensive rats (SHR). Choto-san (0.5, 1.0 and 2.0 g/kg, p.o.) produced a dose-dependent hypotensive effect, but did not affect the heart rate in SHR. It affected neither blood pressure nor heart rate in normotensive Wistar-Kyoto (WKY) rats. When Chotoko was removed from the Choto-san prescription, the hypotensive effect of Choto-san disappeared. When gypsum was taken away from the prescription, the hypotensive effect was prominently reduced as compared with the parent prescription. The extract of Chotoko alone, which was boiled for 15 min, showed hypotensive action at a dose of 200 mg/kg, p.o., while the action disappeared after boiling for 60 min. The results corresponded with a traditional hypothesis that anti-hypertensive components in Choto-san may be destroyed after boiling for over 60 min. A single administration of the extract of gypsum alone at a dose of 1 g/kg, p.o. produced a significant hypotensive effect.

These results suggest that both Chotoko and gypsum exerts an important role in the anti-hypertensive effect of Choto-san.

6) **Hai LX., Kogure T., Niizawa A., Fujinaga H., Sakakibara I., Shimada Y., Watanabe H., Teresawa K.: Suppressive effect of hochu-ekki-to on collagen induced arthritis in DBA1J mice. Journal of Rheumatology 29 : 1601-8, 2002.**

Abstract : *Objective.* To investigate the effect of hochu-ekki-to (HET) decoction on the development of collagen-induced arthritis (CIA) in mice. *Methods.* CIA was induced in male DBA/1J mice by immunization with 2 injections of bovine type II collagen (CII). HET was orally administered at different doses and with different schedules. The incidence of arthritis, arthritis index, levels of anti-CII antibody, interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α) and lymphocyte subsets were examined. *Results.* HET caused suppression of CIA development in a dose dependent fashion and exerted a suppressive effect on CIA when administered from the first CII immunization or from the onset of CIA, but not when administered for 2 weeks before CII immunization. HET inhibited the production of specific anti-CII antibody, IL-6, and TNF-α, and tended to normalize the proportions of cells in lymphocyte subsets. *Conclusion.* HET suppresses the development of CIA, and HET redistributes the population of lymphocytes in lymph node and blood and inhibits IL-6 and TNF-α secretion in CIA mice.

7) **Tohda M., Sukma M., Nomura Y., Watanabe H.: The mRNA Expression of Serotonin 2C Subtype Receptors Uncoupled With Inositol Hydrolysis in NG108-15 Cells. Japanese Journal of Pharmacology 90 : 138-44, 2002.**

Abstract : Cell culture systems seem to be useful for clarifying the cellular physiological mechanisms of serotonin 2C subtype receptors (5-HT2CR) and related drug action mechanisms. However, there are still few reports about cells that contain intrinsic 5-HT2CR. This report demonstrates by using RT/PCR that 5-HT2CR mRNA exists in splicing variant forms in NGI08-15 cells. The PCR results using a pair of primers that recognized sequences near the third intracellular loop site showed two neighboring bands at about 500 bp upon electrophoresis in acrylamide gels. The sequence analysis demonstrated that one band was the rat 5-HT2CR sequence and the other one was that of the mouse. Serotonin, however, did not enhance the inositol phosphates formation in NG108-15 cells. It has been reported that post-translational modifications of RNA, splicing and editing, occur at the site of the second intracellular loop domain in 5-HT2CR mRNA. Accordingly, a pair of primers that recognized this site were designed. The molecular size of the PCR product was shorter than that expected based on the sequence of the native 5-HT2CR. The fragment lacked the 95 nucleotides of native 5-HT2CR mRNA. This seems to be the reason why serotonin did not enhance inositol phosphates formation in NG108-15 cells.

8) **Kang T.H., Murakami Y., Matsumoto K., Takayama H., Kitajima M., Aimi N., Watanabe H.: Rhynchophylline and isorhynchophylline inhibit NMDA receptors expressed in *Xenopus* oocytes. European Journal of Pharmacology 455 : 27-34, 2002.**

Abstract : Rhynchophylline and isorhynchophylline are major tetracyclic oxindole alkaloid components of *Uncaira* species, which have been long used as medicinal plants. In this study, the effects of rhynchophylline and isorhynchophylline on the ionotropic and metabotropic glutamate receptor-mediated current responses were examined using *Xenopus* oocytes injected with total RNA prepared from rat cortices or cerebelli. Rhynchophylline and isorhynchophylline (1-100 μM) *per se* failed to induce membrane current, but these alkaloids reversibly reduced *N*-methyl-D-aspartate (NMDA)-induced current in a concentration-dependent but voltage-independent manner. The IC_{50} values of rhynchophylline and isorhynchophylline were 43.2 and 48.3 μM, respectively. Substitution of Ba^{2+} for Ca^{2+} in the recording medium did not alter the extent of rhynchophylline- and isorhynchophylline-induced suppression of NMDA currents. In contrast, neither alkaloid had an effect on the currents mediated by ionotropic kainic

acid-type and (±)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors or by the metabotropic glutamate receptor$_{1 \text{ and } 5}$ ($mGlu_{1/5}$). Rhynchophylline and isorhynchophylline (30 μM) significantly reduced the maximal current responses evoked by NMDA and glycine (a co-agonist of NMDA receptor), but had no effect on the EC_{50} values and Hill coefficients of NMDA and glycine for inducing currents. These alkaloids showed no interaction with the polyamine binding site, the Zn^{2+} site, proton site or redox modulatory site on the NMDA receptor. These results suggest that rhynchophylline and isorhynchophylline act as noncompetitive antagonists of the NMDA receptor and that this property may contribute to the neuroprotective and anticonvulsant activity of the *Uncaira* species plant extracts.

9) **Skalko-Basnet N., Tohda M., Watanabe H.: Uptake of Liposomally Entrapped Fluorescent Antisense Oligonucleotides in NG108-15 Cells: Conventional versus pH-Sensitive. Biological & Pharmaceutical Bulletin 25 : 1583-7, 2002.**

Abstract : Antisense oligodeoxynucleotides (asODN) are novel therapeutic agents designed to alter RNA metabolism, ultimately resulting in decreased production of disease-associated gene products. To investigate internalisation of liposomally delivered asODN in NG108-15 cells, a hybrid cell line of mouse neuroblastoma and rat glioma, and assure that uptake of marker corresponds to that of antisense, we compared the cellular uptake of fluorescently labelled marker (fluorescein isothiocyanate (FITC)-dextran) and antisense oligonucleotide (FITC-asODN), entrapped either in conventional soy phosphatidylcholine (SPC) liposomes or pH-sensitive liposomes (composed of dioleoylphosphatidylethanolamine and cholesteryl hemisuccinate in a molar ratio of 3 : 2). Both SPC and pH-sensitive liposomes were prepared by a modified freeze-thawing method. Entrapment efficiencies (about 20% of the original material) did not depend on the liposome compositions and fluorescent material used. Fluorescence activated cell sorting (FACS) analysis was used to quantify the association of fluorescent material with the NG108-15 cells, whereas confocal microscopy gave insight on the location of cell associated-fluorescence. Conventional liposomes failed to deliver fluorescent material into the cells, but in contrast, pH-sensitive liposomes significantly improved the uptake of both FITC-dextran and FITC-asODN, with the uptake of liposomal FITC-dextran being greater than the uptake of liposomal FITC-asODN. These results suggest that pH-sensitive liposomes can be applied as a carrier system in the delivery of genetic material into the cells.

◇学会報告　Scientific Presentations

1) 中島隆太郎, 東田道久, 渡辺裕司：ラット慢性虚血脳中で早期に発現増大する新規内在性因子. 第75回日本薬理学会年会, 2002, 3/13-15, 熊本.

2) 姜太炫, 松本欣三, 高山廣光, 北島満里子, 相見則郎, 渡辺裕司：*Uncaria tomentosa* 含有アルカロイドによるセロトニン$_2$及びムスカリンM_1レセプターの修飾：*Xenopus* oocyte 遺伝子発現系での検討. 第75回日本薬理学会年会, 2002, 3/13-15, 熊本.

3) 松本欣三, Puia G., Mienville J.-M., 渡辺裕司, Costa E., Guidotti A.: 脳内アロプレグナノロン量の減少による GABA 作動性神経伝達機能の低下. 第75回日本薬理学会年会, 2002, 3/13-15, 熊本.

4) 渡辺裕司：シンポジウム「和漢薬研究の新しい展開」：漢方製剤の臨床再評価の現状. 第75回日本薬理学会年会, 2002, 3/13-15, 熊本.

5) Sukma M., Murakami Y., Tohda M., Matsumoto K., Watanabe H.: タイ薬用植物 Cassia siamensis の成分 barakol の中枢作用. 第106回日本薬学会北陸支部例会, 2002, 6/15, 富山.

6) Mahakunakorn P., Tohda M., Murakami Y., Matsumoto K. and Watanabe H.: Protective effect of Choto-san and its related constituents on hydrogen peroxide-induced cell damage in NG108-15 cells. 第19回和漢医薬学会大会, 2002, 8/31-9/1, 千葉.

7) 趙琦, 渡辺裕司, 榊原巌, 張紹輝, 村上孝寿, 東田道久, 松本欣三：釣藤鉤フェノール分画及び成分の降圧, 脳虚血予防作用. 第19回和漢医薬学会大会, 2002, 8/31-9/1, 千葉.

8）Pinna G., Matsumoto K., Dong E., Costa E., Guidotti A.: Stereoisomers and metabolites of fluoxetine (Flx) normalize neurosteroids and minimize aggression of socially isolated mice in manner unrelated to SSRI activity. Society for Neuroscience 32nd annual meeting, 2002, 11/2-7, Orland.

◇招待講演　Invited lectures

1）Watanabe H.：日本における伝統薬とその将来. 円光大学校薬学大学国際シンポジウム, 2002/1/18-19, Iksen, Korea.

2）Watanabe H.: Japanese Kampo medicine: its present and future. M.Mars International symposium on traditional medicine, 2002/7/4-5, Beijing, China.

3）Watanabe H.：きのこの和漢薬性：特徴と応用. 日本応用きのこ学会第6回大会, 2002/9/3-4, 鳥取.

4）Watanabe H.: Basic and clinical studies of the effect of a Kampo medicine on vascular dementia. The 18th International Congress of Clinical Chemistry and Laboratory Medicine, 2002/10/22-25, Kyoto.

◇その他　Others

1）松本欣三：GABA 作動性神経ステロイド allopregnanolone を介した薬理. 日本薬理学雑誌 119：59, 2002.

2）渡辺裕司：タイの薬学事情. ファルマシア 38：965-966, 2002.

3）Jeenapongsa R., Tohda M., Watanabe H.: Choto-san inhibits vof-21 mRNA expression in permanent ischaemic brain and prolongs sleeping time. 財団法人東京生化学研究会国際共同研究助成事業研究成果発表会, 2002, 12/3, 東京.

◇共同研究　Co-operative researches

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-

2）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-

3）Erminio Costa, Alessandro Guidotti：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-

4）Opa Vajragupta：タイ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，萌芽的研究（代表：渡辺裕司）「老年痴呆モデルラットの対光縮瞳反射に関する研究」 50万（2/2年目）

2）文部科学省科学研究費，基盤研究Ｂ（２）（代表：渡辺裕司）「慢性脳循環障害モデル動物の白質および神経細胞変性発症機序の解析」 380万（2/2年目）

3）文部科学省科学研究費，基盤研究Ｂ（２）（代表：東田道久）「慢性虚血ラット脳中で発現変化する新規単離因子の生理機能・発現制御機構の解明」 180万（2/2年目）

4）受託研究費，小野薬品工業（渡辺裕司）「一過性脳虚血マウスの空間学習行動に対する poly（ADP-ribose)-synthase 阻害薬の影響の研究」 250万

5）平成14年度科学技術振興調整経費（渡辺裕司）「脳血管性痴呆，高血圧などの生活習慣病を予防・治療する漢方方剤及び生薬類の研究」1,232万

6）平成14年度富山県受託研究費，和漢薬・バイオテクノロジー研究（渡辺裕司）「免疫系・血液血管系に作用する家庭薬や薬食同源食品の開発」 50万

◇研究室在籍者　Research Members

学部3年生：江村真実，中西絵里香
学部4年生：井口知美，平尾顕三
大学院前期1年：天野佑三子，神野雄一，森繁亮
大学院後期2年：Pramote Mahakunakorn
大学院後期3年：Kang Tai-Hyun
Monrudee Sukma
技術補佐員：趙琦（2001, 4-）
中村政美（2002, 4-）
外国人客員研究員：
相婷博士（富山伝統医学センター研究員，2001, 4/1-2003/3/31）
Dr. Ghazi Hussein（富山伝統医学センター研究員，2001/4/1-2003/3/31）
○日本学術振興会・拠点大学交流事業
Dr. Surachai Unchern（チュラロンコン大学準教授，2002, 1/8-3/7）
Dr. Nguyen Thi Thu Huong（ベトナム薬物研究所ホーチミン副支所長，2002, 2/1-3/31）
Dr. Rattima Jeenapongsa（ナレスアン大学講師，2002, 2/14-3/16）
Dr. Nguyen Thi van Thai（ベトナム薬物研究所研究員，2002, 3/1-31）
Dr. Boonyong Tantisira（チュラロンコン大学薬学部長，2002, 3/2-15）
Dr. Yuvadee Wongkrajang（マヒドン大学，2002, 5/6-20）
Dr. Aroonsri Priprem（マハサラカム大学健康科学部長，2002, 5/6-20）
Dr. Bungorn Sripanidkulchai（コンケン大学，2002, 5/6-20）
Dr. Vimon Tantishaiyakul（プリンスオブソンクラ大学薬学部長，2002, 5/6-20）
Ms. Yaowared Sumanont（コンケン大学講師，2002, 6/1-8/30）
○富山医科薬科大学国際交流基金
Dr. Penchom Peungvicha（マヒドン大学，2002,5/6-6/5）
○東京生化学研究会外国人研究者招聘奨学金
Dr. Rattima Jeenapongsa（ナレスアン大学講師，2002, 4/1-）
外国人特別研究学生
Mr. Luis Miguel Melendez Queija（松下国際財団，ペルー薬剤師，2001, 4/5-2002, 5/2）
Ms. Chantana Boonyarat（日本国際教育協会及びタイ王立教育協会，マヒドン大学大学院，2002, 4/26-2003/3/31）
Ms. Onrawee Khongsombat（日本国際教育協会，チュラロンコン大学大学院，2002, 5/26-2003/4/30）
Ms. Yaowared Sumanont（文部科学省奨学金，コンケン大学講師，10/1-）

◇学位取得者　Academic degrees and Thesis

修士：2002年3月
中島隆太郎　両側総頸動脈永久結紮ラット脳に発現する新規遺伝子vof-21の研究
博士：2002年3月
張紹輝　痴呆モデルマウスの学習行動障害に対する四物湯及び釣藤散の作用に関する研究