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.)
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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

薬学部4年生：林寿枝，原田恒介

大学院前期2年：井口知美，平尾顕三

大学院後期2年：Yaowared Sumanont

外国人客員研究員：

日本学術振興会・拠点大学交流事業

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

Dr. Lewanich Pathama（スリナカリンウィロー大学医学部助教授，2004，2/2-3/30）

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）

Dr. Prawpan Suwanakitch（ナレスアン大学，2004，2/2-3/31）

Mr. Do Thanh Phu（ベトナム薬物研究所，2004, 2/6-3/22）
Dr. Boonyong Tantisira（チュラロンコン大学薬学部長，2004,2/15-18）
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）
Dr. Preecha Boonchoong（ウボンラチャタニー大学，2004, 8/1-9/30）
Dr. Nattawut Saelim（ナレスアン大学，2004, 9/16-11/12）
富山伝統医学センター
Dr. Ghazi Hussein（Assistant of Khartoum University, 2001/4/1-）

◇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