複合薬物薬理学分野　Division of Medicinal Pharmacology

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◇研究目的

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

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の薬理学的解析および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) 脳血管性認知症病態モデル系における和漢薬および和漢薬成分の抗認知症作用と神経保護作用の評価ならびに作用機序の解明
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) 和漢薬を利用したうつ病態に関連する新規脳内遺伝子のクローニングと発現変化の作用解析

◇原著論文

1) **Hussein G., Nakagawa T., Goto H., Matsumoto K., Sankawa U., Watanabe H.: Astaxanthin Ameliorates Features of Metabolic Syndrome in SHR/NDmcr-cp. Life Sci. 80(6): 522-529; 2007.**

Abstract: Glucose and lipid metabolic parameters play crucial roles in metabolic syndrome and its major feature of insulin resistance. This study was designed to investigate whether dietary astaxanthin oil (ASX-O) has potential effects on metabolic syndrome features in an SHR/NDmcr-cp (cp/cp) rat model. Oral administration of ASX (50 mg/kg/day) for 22 weeks induced a significant reduction in arterial blood pressure in SHRcp. It also significantly reduced the fasting blood glucose level, homeostasis index of insulin resistance (HOMA-IR), and improved insulin sensitivity. The results also showed an improved adiponectin level, a significant increase in high-density lipoprotein cholesterol, a significant decrease in plasma levels of triglycerides, and non-esterified fatty acids. Additionally, ASX showed significant effects on the white adipose tissue by decreasing the size of the fat cells. These results suggest that ASX ameliorates insulin resistance by mechanisms involving the increase of glucose uptake, and by modulating the level of circulating lipid metabolites and adiponectin.

2) **Zhao Q., Murakami Y., Tohda M., Obi R., Shimada Y., Matsumoto K.: Chotosan, a Kampo formula, ameliorates chronic cerebral hypoperfusion-induced deficits in object recognition behaviors and in central cholinergic systems in mice. J. Pharmacol. Sci. 103(4): 360-373; 2007.**

Abstract: We previously demonstrated that the Kampo formula chotosan (CTS) ameliorated spatial cognitive impairment via central cholinergic systems in a chronic cerebral hypoperfusion (P2VO) mouse model. In this study, the object discrimination tasks were used to determine if the ameliorative effects of CTS on P2VO-induced cognitive deficits are a characteristic pharmacological profile of this formula, with the aim of clarifying the mechanisms by which CTS enhances central cholinergic function in P2VO mice. The cholinesterase inhibitor tacrine (THA) and Kampo formula saikokeishito (SKT) were used as controls. P2VO impaired object discrimination performance in the object recognition, location, and context tests. Daily administration of CTS (750 mg/kg, p.o.) and THA (2.5 mg/kg, i.p.) improved the object discrimination deficits, whereas SKT (750 mg/kg, p.o.) did not. In ex vivo assays, tacrine but not CTS or SKT inhibited cortical cholinesterase activity. P2VO reduced the mRNA expression of m3 and m5 muscarinic receptors and choline acetyltransferase but not that of other muscarinic receptor subtypes in the cerebral cortex. Daily administration of CTS and THA but not SKT reversed these expression changes. These results suggest that CTS and THA improve P2VO-induced cognitive impairment by normalizing the deficit of central cholinergic systems and that the beneficial effect on P2VO-induced cognitive deficits is a distinctive pharmacological characteristic of CTS.

3) **Li S, Wang C, Wang M, Li W, Matsumoto K, Tang Y.: Antidepressant like effects of piperine in chronic mild stress treated mice and its possible mechanisms. Life Sci. 80(15): 1373-1381; 2007.**

Abstract: In this study, we investigated the antidepressant-like effect of piperine in mice exposed to chronic mild stress (CMS) procedure. Repeated administration of piperine for 14 days at the doses of 2.5, 5 and 10 mg/kg reversed the CMS-induced changes in sucrose consumption, plasma corticosterone level and open field activity. Furthermore, the decreased proliferation of hippocampal progenitor cells was ameliorated and the level of brain-derived neurotrophic factor (BDNF) in hippocampus of CMS stressed mice was up-regulated by piperine treatment in the same time course. In addition, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactic dehydrogenase (LDH) assays showed that piperine (6.25–25 μM) or fluoxetine (FLU, 1 μM) dose-dependently protected primary

cultured hippocampal neurons from the lesion induced by 10 μM corticosterone (CORT). Reverse transcription-polymerase chain reaction (RT-PCR) was used to detect the messenger ribonucleic acid (mRNA) level of BDNF in cultured neurons. Treatment with piperine (6.25–25 μM) for 72 h reversed the CORT-induced reduction of BDNF mRNA expression in cultured hippocampal neurons. In summary, up-regulation of the progenitor cell proliferation of hippocampus and cytoprotective activity might be mechanisms involved in the antidepressant-like effect of piperine, which may be closely related to the elevation of hippocampal BDNF level.

4) **Sumanont Y., Murakami Y., Tohda M., Vajragupta O., Watanabe H., Matsumoto K. : Effects of manganese complexes of curcumin and diacetylcurcumin on kainic acid-induced neurotoxic responses in the rat hippocampus. Biol. Pharm. Bull. 30(9):1732-1739; 2007.**

Abstract: This study aimed to investigate the mechanism underlying the protective effects of manganese complexes of curcumin (Cp–Mn) and diacetylcurcumin (DiAc–Cp–Mn) on kainic acid (KA)-induced excitotoxicity in the rat hippocampus. Systemic injection of KA (10 mg/kg, i.p.) caused seizures and increased the expression of neurotoxic markers, immediate early genes [c-*jun*, cyclooxygenase 2 (COX-2), brain-derived neurotrophic factor (BDNF), and heat shock protein 70 (*hsp*70)] and a delayed response gene [inducible nitric oxide synthase (*iNOS*)], which were measured at 6 and 72 h after KA injection, respectively, in the hippocampus. Pretreatment with Cp–Mn (50 mg/kg, i.p.) and DiAc–Cp–Mn (50 mg/kg, i.p.) but not with curcumin (50 mg/kg, i.p.) delayed the onset of KA-induced seizure without affecting the seizure score. KA injection induced c-Fos immunoreactivity in DG, CA1, and CA3 hippocampal regions, the expression of which peaked at 6 h after injection. Cp–Mn and DiAc–Cp–Mn treatment significantly decreased c-Fos expression elicited by KA. Moreover, Cp–Mn and DiAc–Cp–Mn administration suppressed the KA-induced expression of c-*jun*, *COX-2*, *BDNF*, and *iNOS* mRNA, whereas curcumin attenuated only *iNOS* mRNA expression. No compounds tested had an effect on KA-induced hsp70 expression. It is therefore likely that in addition to radical scavenging and SOD-like activities, the suppression of potential neuronal injury marker expression by Cp–Mn and DiAc–Cp–Mn, contributes to the neuroprotective activities of these compounds, which are superior to those of curcumin, on KA-induced excitotoxicity in the hippocampus. These results suggest the beneficial effects of Cp–Mn, and DiAc–Cp–Mn on the treatment of excitotoxicity-induced neurodegenerative diseases.

5) **Obi R., Tohda M., Zhao Q, Obi N., Hori H., Murakami Y., Goto H., Shimada Y., Ochiai H., Matsumoto K.: Chotosan enhances Macrophage colony-stimulating factor mRNA expression in the ischemic rat brain and C6Bu-1 glioma cells. Biol. Pharm. Bull. 30(12): 2250-2256; 2007.**

Abstract: Macrophage colony stimulating factor (M-CSF) is a cytokine which has been recently reported to have a neuroprotective effect on ischemic rat brain. In this study, we investigated the effect of chotosan, an oriental medicine, which has been clinically demonstrated to be effective for the treatment of vascular dementia, on MCSF gene expression in rats with permanent occlusion of bilateral common carotid arteries (P2VO) *in vivo* and in a C6Bu-1 glioma cell line *in vitro*. The expression level of M-CSF mRNA in the cerebral cortices of P2VO rats was significantly higher than that in the cerebral cortices of sham-operated animals. Repeated treatment of P2VO rats with chotosan (75 mg/kg per day) for 4 d after P2VO significantly increased the expression level of MCSF mRNA in the cortex but it had no effect on the expression of β-actin, granulocyte colony stimulating factor (G-CSF), granulocyte/macrophage colony stimulating factor (GM-CSF) mRNAs. Moreover, the present *in vitro* studies revealed that chotosan treatment (10—100 μg/ml) of C6Bu-1 glioma cells dose-dependently enhanced MCSF mRNA expression without affecting the expression of G-CSF, GM-CSF, and inducible nitric oxide synthase mRNAs. The effect of chotosan was reversed by Ro 31-8220 (1 μM), a selective protein kinase C (PKC) inhibitor, but not by H-89 (10 μM), a selective protein kinase A (PKA) inhibitor. These findings suggest that the

upregulatory effect of chotosan on M-CSF mRNA expression involves PKC and may play an important role in the antivascular dementia action of this formula.

6) **Chung M., Nakamura N., Tohda M., Hattori M.: Effects of Tokishakuyakusan on hypothalamic pituitary adenylate cyclase-activating polypeptide (PACAP) and PACAP type I receptor (PAC1) expression in hypophysectomized and ovariectomized rats. J. Trad. Med. 24: 24-30; 2007.**

7) **Chung M., Tohda M. Hattori M.: Effects of Tokishakuyakusan on the ovary in hypophysectomized rats. J. Trad. Med. 24: 31-38, 2007.**

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2) 松本欣三:東洋医学と生命科学の融合を目指して 脳血管性認知症病態モデルにおける和漢薬作用. Biophilia 3(2): 64-67; 2007.

3) 趙琦, 村上孝壽, 小尾龍右, 嶋田豊, 松本欣三:血圧と認知機能への漢方の効能. 血圧 14(5):185-190; 2007.

◇著　書

1) 松本欣三訳：ファーマコセラピー第 65 章「薬物関連障害：アルコール，ニコチンおよびカフェイン」Doering P.L.著, Dipiro J.T. 他編, 百瀬弥寿徳訳者代表, 1573-1594, ブレーン出版, 東京, 2007.

2) 東田道久訳：ファーマコセラピー第 68 章「双極性障害」Frankhauser M.P. and Freeman M.P. 著, Dipiro J.T. 他編, 百瀬弥寿徳訳者代表, 1655-1690, ブレーン出版, 東京, 2007.

◇学会報告　(*: 特別講演，シンポジウム，ワークショップ等)

1) 小尾龍右, 趙琦, 松本欣三, 村上孝寿, 嶋田豊, 東田道久: 釣藤散による colony stimulating factor-1 mRNA 発現変化: 脳虚血および C6 グリオーマによる検討. 第 80 回日本薬理学会年会, 2007, 3/8-10, 名古屋.

2) 村上孝寿, 東田道久, 松本欣三: 学習性無力マウスの回避行動障害に対する desipramine および fluoxetine 反復投与の効果. 日本薬学会第 127 年会, 2007, 3/28-30, 富山.

3) Pham Thi Nguyet Hang, 東田道久, 松本欣三: 成熟・胎児脳および初代培養神経細胞中でのセロトニン 2C 受容体 RNA editing の経日的変化. 日本薬学会第 127 年会, 2007, 3/28-30, 富山.

* 4) 松本欣三:和漢薬標準化の内と外 和漢医薬学研究の国際化と和漢薬標準化 その意義と課題. 第 24 回和漢医薬学会大会, 2007, 9/8-9, 富山.

5) 趙琦, 村上孝寿, 榊原巌, 松本欣三: 脳卒中易発症自然高血圧ラットの高血圧病態及び脳内ムスカリン受容体遺伝子発現に対する中国湖北省産及び広東省産菊花の影響. 第 24 回和漢医薬学会大会, 2007, 9/8-9, 富山.

6) 東田道久: 細胞内 cAMP 濃度に依存した釣藤散による M-CSF mRNA 発現の両方向性調節. 第 24 回和漢医薬学会大会, 2007, 9/8-9, 富山.

7) 東田道久，松本欣三: 補中益気湯長期間処置による NG108-15 細胞の遺伝子発現変化. 第24回和漢医薬学会大会, 2007, 9/8-9, 富山.

8) 東田道久, Pham Thi Nguyet Hang, Monrudee Sukma, 松本欣三: セロトニン 2C 受容体 mRNA short variant の経日的発現量変化と RNA editing との関連性. 第 58 回日本薬理学会北部会, 2007, 9/29, 札幌.

* 9) 松本欣三: 脳血管性認知症動物モデルで捉えた和漢薬の有効性と作用機構. 富山大学 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」シンポジウム, 2007, 11/16, 富山.

10) 趙琦,小尾龍右, 東田道久, 堀仁美, 村上孝寿，後藤博三, 嶋田豊, 松本欣三: 釣藤散のマクロファージ刺激因子遺伝子発現に及ぼす効果: ラット虚血脳及び C6Bu-1 グリオーマ細胞での検討. 富山大学 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」シンポジウム, 2007, 11/16, 富山.

◇招待講演

1) Matsumoto K., Zhao Q., Murakami Y., Tohda M., Obi R., Shimada Y.: Neuropharmacological evidence for availability of a Kampo medicine Chotosan in clinical treatment of vascular dementia: From behavior to molecular aspect. Joint Symposium: Evidence-based Approach to Traditional Medicine and Modern Medicine, 2007, 6/26, Beijing

2) Matsumoto K., Zhao Q.: Neuropharmacological evidence for availability of a Kampo medicine Chotosan in clinical treatment of vascular dementia. The 33rd Congress on Science and Technology of Thailand, 2007, 10/18-20, Nakhon Si Thammarat (Thailand).

3) 松本欣三，趙琦，村上孝寿, 東田道久，小尾龍右，嶋田豊: 脳血管性認知症治療における漢方薬・釣藤散の有用性：その実験薬理学的証拠と作用機序. 第 7 回日本臨床中医薬学会学術大会　日中シンポジウム I　基礎系, 2007, 12/8, 東京.

◇その他

1) Matsumoto K.: Chotosan, a “traditional Chinese Japanese (Kampo) medicine”, may be a “modern promising drug” for vascular dementia treatment: Evidence from neuropharmacological studies. Seminar in National Institute of Materia Medica, 2007, 11/28, Hanoi.

◇共同研究

研究所内

1) 服部征雄：薬物代謝工学，「体内女性ホルモンに与える和漢薬の影響に関する研究」, 2005, 4/1～

学内

1) 嶋田豊：富山大学大学院医学薬学研究部,「慢性脳虚血ラット脳における遺伝子発現に及ぼす釣藤散の作用に関する研究」, 2001, 4/1～

2) 常山幸一：富山大学大学院医学薬学研究部,「漢方薬の抗認知症効果を担う脳内メディエータに関する研究」, 2007, 4/1～

国内

1) 渡邉裕司, 三川　潮：富山県伝統医薬センター,「天然薬物の薬効と品質の評価に関する研究」, 2001, 4/1～

海外

1) Erminio Costa, Alessandro Guidotti：米国イリノイ州立大学シカゴ校精神医学研究所,「ス

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2) 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 -

3) Li Song: 中国瀋陽薬科大学, 「ストレス誘発の情動障害及び学習記憶障害に関する神経薬理学的研究」, 2005, 2/16-

◇研究費取得状況

1) 文部科学省科学研究費, 基盤研究 B (代表：松本欣三)「漢方薬の薬効を利用した脳血管性痴呆治療標的分子の探索・同定とその生理機能解析」 290 万 (3/3 年目)

2) 文部科学省科学研究費, 基盤研究 B (代表：東田道久)「和漢薬をプローブとした生体内機能分子の同定と生理機能・病態変化の解析」 210 万 (3/3 年目)

3) 文部科学省科学研究費, 萌芽研究 (代表：東田道久)「脳の知的機能に関与するかもしれない新規単離因子 Vof-21 の分子生理学的基礎研」 80 万 (1/3 年目)

4) 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」

5) 富山県, 和漢薬・バイオテクノロジー研究費, (代表：松本欣三)「神経精神性障害に対する薬理学的及び分子生物学的解析と応用に関する基礎研究」 65 万

6) 小野薬品工業株式会社 (代表：松本欣三)「モデル動物を用いた脳血管性障害発症関連因子に関する研究」 152 万

7) 富山県, 新世紀産業, (代表：松本欣三)「漢方方剤テーラーメード治療法の開発 (天然薬物の薬効と品質の評価に関する研究)」 200 万

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