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、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

◇原著論文

1) **Sumanont Y., Murakami Y., Tohda M., Vajragupta O., Watanabe H., Matsumoto K.: Prevention of kainic acid-induced changes in nitric oxide level and neuronal cell damage in the rat hippocampus by manganese complexes of curcumin and diacetylcurcumin. Life Sci. 78(16): 1884-1891; 2006.**

Abstract: Curcumin is a natural antioxidant isolated from the medicinal plant *Curcuma longa* Linn. We previously reported that manganese complexes of curcumin (Cp-Mn) and diacetylcurcumin (DiAc-Cp-Mn) exhibited potent superoxide dismutase (SOD)-like activity in an in vitro assay. Nitric oxide (NO) is a free radial playing a multifaceted role in the brain and its excessive production is known to induce neurotoxicity. Here, we examined the in vivo effect of Cp-Mn and DiAc-Cp-Mn on NO levels enhanced by kainic acid (KA) and L-arginine (L-Arg) in the hippocampi of awake rats using a microdialysis technique. Injection of KA (10 mg/kg, i.p.) and L-Arg (1000 mg/kg, i.p.) significantly increased the concentration of NO and Cp-Mn and DiAc-Cp-Mn (50 mg/kg, i.p.) significantly reversed the effects of KA and L-Arg without affecting the basal NO concentration. Following KA-induced seizures, severe neuronal cell damage was observed in the CA1 and CA3 subfields of hippocampal 3 days after KA administration. Pretreatment with Cp-Mn and DiAc-Cp-Mn (50 mg/kg, i.p.) significantly attenuated KA-induced neuronal cell death in both CA1 and CA3 regions of rat hippocampus compared with vehicle control, and Cp-Mn and DiAc-Cp-Mn showed more potent neuroprotective effect than their parent compounds, curcumin and diacetylcurcumin. These results suggest that Cp-Mn and DiAc-Cp-Mn protect against KA-induced neuronal cell death by suppression of KA-induced increase in NO levels probably by their NO scavenging activity and antioxidative activity. Cp-Mn and DiAc-Cp-Mn have an advantage to be neuroprotective agents in the treatment of acute brain pathologies associated with NO-induced neurotoxicity and oxidative stress-induced neuronal damage such as epilepsy, stroke and traumatic brain injury.

2) **Pinna G., Agis-Balboa R.C, Zhubi A., Matsumoto K., Grayson D.R., Costa E., Guidotti A.: Imidazenil and diazepam increase locomotor activity in mice exposed to protracted social isolation. Proc. Natl. Acad. Sci. USA 103(11): 4275-4280; 2006.**

Abstract: In cortex and hippocampus, protracted (>4 weeks) social isolation of adult male mice alters the subunit expression of GABA type A receptors ($GABA_A$-Rs) as follows: (*i*) the mRNAs encoding $GABA_A$-R α1, α2, and γ2 subunits are decreased by approximately 50%, whereas those encoding α4 and α5 subunits are increased by approximately 100%; (*ii*) similarly, the synaptic membrane expression of the α1 subunit protein is down-regulated, and that of the α5 subunit protein is up-regulated; and (*iii*) the binding of [^{3}H]flumazenil to hippocampal synaptic membranes is decreased. Behaviorally, socially isolated (SI) mice are resistant to the sedative effects of the positive allosteric $GABA_A$-R modulators diazepam (DZP) and zolpidem. This resistance seems to be attributable to the decrease of α1-containing $GABA_A$-Rs. Paradoxically, DZP, which, unlike zolpidem, acts at α5-containing $GABA_A$-Rs, increases the locomotor activity of SI mice. Imidazenil, which fails to modulate α1-, α4-, and α6-containing $GABA_A$-Rs but is a selective positive allosteric modulator of α5-containing $GABA_A$-Rs, also increases locomotor activity in SI mice. Importantly, SI mice responded to muscimol, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3(2H)-one, and allopregnanolone similar to group-housed mice. These data suggest that a switch (a decrease in α 1 /α2 and γ2 and an increase in α4 and α5 subunits) in the composition of the heteropentameric $GABA_A$-R subunit assembly without a change in total $GABA_A$-R number occurs during social isolation. Thus, the repertoire of DZP and imidazenil actions in SI mice appears to be elicited by the allosteric modulation of $GABA_A$-Rs overexpressing α5 subunits. Benzodiazepine response mediated by α1-containing $GABA_A$-Rs is expected to be silent or reduced.

3) **Vajragupta O., Boonyarat C., Murakami Y., Tohda M., Matsumoto K., Olson A.J., Watanabe H.: A novel neuroprotective agent with antioxidant and nitric oxide synthase inhibitory action. Free Rad. Res. 32(2): 145-156; 2006.**

Abstract: N^{α}-vanillyl-N^{ω}-nitroarginine (N - 1) that combines the active functions of natural antioxidant and nitric oxide synthase inhibitor was developed for its neuroprotective properties. N - 1 exhibited protective effects against hydrogen peroxide-induced cell damage and the inhibitory effect on nitric oxide 'NO' production induced by calcium ionophore in NG 108-15 cells. N - 1 inhibited the constitutive NOS isolated from rat cerebellar in a greater extent than constitutive NOS from human endothelial cells. Low binding energy (-10.2 kcal/mol) obtained from docking N - 1 to nNOS supported the additional mode of action of N - 1 as an nNOS inhibitor. The in vivo neuroprotective effect on kainic acid-induced nitric oxide production and neuronal cell death in rat brain was investigated via microdialysis. Rats were injected intra-peritonially with N - 1 at 75 μmol/kg before kainic acid injection (10 mg/kg). The significant suppression effect on kainic acid-induced NO and significant increase in surviving cells were observed in the hippocampus at 40 min after the induction.

4) **Li S., Wang C., Wang M.W., Murakami Y., Matsumoto K.: Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. Pharmacol Biochem Behav. 83(2):186-193; 2006.**

Abstract: Increasing evidences indicate the concurrence and interrelationship of depression and cognitive impairments. The present study was undertaken to investigate the effects of two depressive animal models, learned helplessness (LH) and chronic mild stress (CMS), on the cognitive functions of mice in the Morris water maze task. Our results demonstrated that both LH and CMS significantly decreased the cognitive performance of stressed mice in the water maze task. The escaping latency to the platform was prolonged and the probe test percentage in the platform quadrant was reduced. These two models also increased the plasma corticosterone concentration and decreased the brain derived neurotrophic factor (BDNF) and cAMP-response element-biding protein (CREB) messenger ribonucleic acid (mRNA) levels in hippocampus, which might cause the spatial cognition deficits. Repeated treatment with antidepressant drugs, imipramine (Imi) and fluoxetine (Flu), significantly reduced the plasma corticosterone concentration and enhanced the BDNF and CREB levels. Furthermore, antidepressant treated animals showed an ameliorated cognitive performance compared with the vehicle treated stressed animals. These data suggest that both LH and CMS impair the spatial cognitive function and repeated treatment with antidepressant drugs decreases the prevalence of cognitive impairments induced by these two animal models. Those might in part be attributed to the reduced plasma corticosterone and enhanced hippocampal BDNF and CREB expressions. This study provided a better understanding of molecular mechanisms underlying interactions of depression and cognitive impairments, although animal models used in this study can mimic only some aspects of depression or cognition of human.

5) **Hussein G., Goto H., Oda S., Sankawa U., Matsumoto K., Watanabe H.: Antihypertensive potential and mechanism of action of astaxanthin: III. Antioxidant and Histopathological Effects in Spontaneous Hypertensive Rats. Biol. Pharm. Bull. 29(4): 684-688; 2006.**

Abstract: We investigated the effects of a dietary astaxanthin (ASX-O) on oxidative parameters in spontaneously hypertensive rats (SHR), by determination of the level of nitric oxide (NO) end products nitrite/nitrate (NO_2^-/NO_3^-) and lipid peroxidation in ASX-O-treated SHR. Oral administration of the ASX-O significantly reduced the plasma level of NO_2^-/NO_3^- compared to the control vehicle ($p<0.05$). The lipid peroxidation level, however, was reduced in both ASX-O- and olive oil-treated groups. We also analyzed the post-treatment effects of ASX-O on the vascular tissues by examining the changes in the aorta and coronary arteries and arterioles. The dietary ASX-O showed significant reduction in the elastin bands in the rat aorta ($p<0.05$). It also significantly decreased the [wall : lumen] aerial ratio of the

coronary arteries. These results suggest that ASX-O can modulate the oxidative condition and may improve vascular elastin and arterial wall thickness in hypertension.

6) **Li S., Murakami Y., Wang M., Maeda K., Matsumoto K.: The effects of chronic valproate and diazepam in a mouse model of posttraumatic stress disorder. Pharmacol Biochem Behav. 85(2): 324–331; 2006.**

Abstract: To better understand neurochemical and psychopharmacological aspects of post-traumatic stress disorder (PTSD), it is necessary establish an animal model of PTSD in which behavioral changes persist for a long time after the initial traumatization. The present study aimed to characterize long-term behavioral alterations in male ICR mice as an animal model of PTSD consisting of a 2-day foot shock (0.8 mA, 10 s) followed by 3 weekly situational reminders (SR), and to evaluate the effects of repeated administration of valproate and diazepam on behavioral deficits of this animal model. The results showed that the aversive procedure induced several long-term behavioral deficiencies: increased freezing behavior and anxiety level, reduced time spent in an aversive like context. Repeated treatment with valproate (100-400 mg/kg, i.p.) induced a dose-dependent reduction of these behavioral changes. In contrast, diazepam at a low dose (0.25 mg/kg) but not at a high dose (4 mg/kg) reduced the behavioral deficiencies. These results demonstrate that exposure to intense foot shock associated with repeated situational reminders elicits long-term disturbances that last about 4 weeks after the foot shock exposure. These behavioral deficits can be ameliorated by repeated administration of valproate or diazepam at some special dose ranges.

◇総　説

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2) Tohda M., Nomura M., Nomura Y.: Molecular pathopharmacology of 5-HT2C receptors and the RNA editing in the brain. J Pharmacol Sci. 100(5):427-432; 2006.

3) 村上孝寿、趙琦、松本欣三: 認知症マウスと釣藤散. 治療学 40(4):61-63; 2006.

◇学会報告　(*: 特別講演、シンポジウム、ワークショップ等)

1) Hussein Ghazi, 後藤博三, 中川孝子, 松本欣三, 三川潮, 渡邉裕司: アスタキサンチンの肥満・高血圧自然発症ラットのメタボリックシンドロームに対する改善効果. 第 79 回日本薬理学会年会, 2006, 3/8-10, 横浜.
2) Li Song, 村上孝寿, Wang Minwei , 松本欣三: 恐怖条件付けによるマウスの行動変化に対するバルプロ酸及びジアゼパム反復投与の効果. 第 79 回日本薬理学会年会, 2006, 3/8-10, 横浜..
3) Sumanont Yaowared, 村上孝寿, 東田道久, 松本欣三: ラット海馬内のカイニン酸誘発遺伝子発現変化に対するクルクミンマンガン錯体の影響. 第 79 回日本薬理学会年会, 2006, 3/8-10, 横浜.
4) Pham Thi Nguyet Hang, 東田道久, 松本欣三: セロトニン 2C 受容体 RNA editing の神経生理機能に関する研究：ラット胎児脳および成熟脳での editing と関連酵素の発現変化. 日本薬学会第 126 年会, 2006, 3/28-30, 仙台.
5) Tohda M., Monruedee S., Pham H.T.N., Matsumoto K.: RNA editing and short variant of 5-HT2C receptor in neuronal differentiated cells. Sixth IUPHAR satellite meeting on serotonin, 2006, 6/27-30, Sapporo.
6) 趙琦, 村上孝寿, 東田道久, 小尾龍右, 嶋田豊, 松本欣三: 慢性脳虚血誘発の物体認知機

能障害に対する釣藤鈎の改善作用の差異. 第 23 回和漢医薬学会大会, 2006, 8/26-27, 岐阜.

7) 小尾龍右, 東田道久, 趙琦, 村上孝寿, 嶋田豊, 松本欣三: 釣藤散の慢性脳虚血ラット脳および培養神経細胞内コロニー刺激因子-1 遺伝子発現に及ぼす影響. 第 23 回和漢医薬学会大会, 2006, 8/26-27, 岐阜.

8) 渡邉裕司, Hussein Ghazi, 後藤博三, 中川孝子, 織田しのぶ, 松本欣三, 三川潮: アスタキサンチンは動物モデルのメタボリックシンドロームを改善する. 第23回和漢医薬学会大会, 2006, 8/26-27, 岐阜.

9) 趙琦, 村上孝寿, 東田道久, 小尾龍右, 嶋田豊, 松本欣三: 慢性脳虚血誘発空間認知障害に対する釣藤鈎の改善効果の機序—アセチルコリン合成酵素及びムスカリン受容体遺伝子発現系の関与. 第 57 回日本薬理学会北部会, 2006, 9/20-21, 弘前.

* 10) 村上孝寿, 趙琦, 松本欣三: 脳血管性認知症モデルマウスにおける釣藤散の効果とその作用機序. 第 63 回日本東洋医学会甲信越支部学術総会, 2006, 10/22, 新潟.

11) Zhao Q., Murakami Y., Tohda M., Obi R., Shimada Y., Matsumoto K.: Choto-san, a Kampo formula, ameliorates spatial learning behaviour deficits and central cholinergic dysfunction caused by chronic cerebral hypoperfusion in mice. The 7th JSPS-NRCT Joint Seminar, 2006, 12/2-3, Toyama..

◇招待講演

1) 松本欣三: ストレス行動障害と神経ステロイド. 第 40 回脳の医学・生物学研究会, 2006, 3/4, 名古屋..

2) Matsumoto K., Pinna G., Guidotti A., Costa E.: Elucidation of pathophysiological roles of allopregnanolone in the brain using a mouse model of protracted social isolation. 2006CINP Asia Pacific regional meeting in Pattaya, 2006/3/14-17, Pattaya (Thailand).

3) 松本欣三: 脳血管性痴呆病態動物モデルに見る漢方薬「釣藤散」の効き目と効き方. 第 19 回フォーラム富山「創薬」研究会, 2006, 5/30, 富山.

4) Matsumoto K., Pinna G., Guidotti A., Costa.: Neuroendocrine consequences of social isolation. The 6th International Congress of Neuroendocrinology, 2006, 6/19-22, Pittsburgh (USA).

5) 松本欣三, 趙琦, 村上孝寿, 小尾龍右, 嶋田豊, 東田道久: 脳血管性認知症病態モデルにおける漢方薬「釣藤散」の薬理作用とその機序. 応用薬理シンポジウム, 2006, 9/12-13, 千葉.

6) Matsumoto K.: Gouteng-san ("Choto-san 釣藤散" in Japanese) is a "traditional medicine" but may be a "modern promising medicine" useful for treatment of vascular dementia. Workshop on traditional medicine, 2006, 10/12, Changsha (China).

7) Matsumoto K., Sumanont Y., Murakami Y., Tohda T., Vajragupta O., Watanabe H.: Neuroprotective Activities of Manganese Complexes of Curcumin and Diacetylcurcumin in A Kainic Acid-induced Neurotoxicity Model of Rats. The 7th JSPS-NRCT Joint Seminar, 2006, 12/2-3, Toyama..

8) 松本欣三, 趙琦: 漢方薬「釣藤散」－その脳血管性認知症動物モデル系における有効性と作用機序－. COE(富山大・北里大) 合同シンポジウム「天然資源からの抗感染症と病態制御へのアプローチ」－東洋の知と生命科学の融合－, 2006, 12/13, 東京.

◇共同研究

学内

1) 後藤博三：富山大学和漢医薬学総合研究所, 「Astaxanthin 含有ヘマトコッカス藻抽出物の薬理作用に関する研究」, 2001, 4/1～

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 –
2) 三川　潮： 富山県伝統医薬センター,「Astaxanthin 含有ヘマトコッカス藻抽出物の薬理作用に関する研究」, 2001, 4/1～

海外

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2) 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 -
4) Li Song: 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、萌芽的研究（代表：松本欣三）「攻撃行動制御因子の単離同定と神経生理機能に関する研究」 60 万 (2/2 年目)
2) 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」 390 万 (2/3 年目)
3) 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」 270 万 (2/3 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」 70 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」 200 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

◇研究室在籍者

薬学部 3 年生：堀仁美
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Ms. Salin Mingmalairak (文部科学省奨学金、チュラロンコン大学、2006, 10/4-)
外国人客員研究員：Dr. Ghazi Hussein (2001/4/1-)

◇学位（修士、博士）取得者

博士(薬学)：

Yaowared Sumanont: Pharmacological studies on NO scavenging and neuroprotective activities of manganese complexes of curcumin and diacetylcurcumin using a kainic acid-induced neurotoxicity model in rats.