

漢方診断学部門 Department of Kampo Diagnostics

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研究目的 Aims of the research projects

医療保険の薬価に収載されている漢方製剤は147種であり、また生薬は約200種である。平成9年、薬価収載の漢方製剤（いわゆるエキス剤）の全てについて「漢方医学的な病態（証）に基づいて適正に使用すること」が明記された。

証を決定できるようになるためには、基礎概念の学習とともに臨床に根ざした研修を必要とする。にもかかわらず、わが国において体系的にこれを教育する場は、医学部にも薬学部にも未だに整備されていない。

当部門は平成11年4月1日付けで、株式会社ツムラの寄付部門として設置され、本学医学部和漢診療学講座の協力の下に、全国の医師・薬剤師・医薬学生に対して、短期および長期研修コースを提供している。

漢方医学研修カリキュラムを作成するには、古典の学習にとどまらず、証をより客観的なものに育てていく必要がある。

我々は漢方方剤、生薬の薬理作用の研究および漢方医学的病態の解明を学内外諸機関と協力して行っている。

研究概要 Research projects

I) 漢方医学的病態からみた漢方方剤の薬理効果の基礎的・臨床的研究

- 1) 各種漢方方剤の指標物質濃度、及びヒトにおける血中濃度の解析
- 2) 無症候性脳血管障害に対する桂枝茯苓丸の短期および長期効果の検討
- 3) 糖尿病性腎症に対する桂枝茯苓丸の長期効果の検討
- 4) 和漢薬の抗酸化作用に関する基礎的研究

II) 病態や証を客観化するための指標を探索する基礎的・臨床的研究

- 1) 漢方医学的病態の自律神経系検査法による解析
- 2) 漢方医学的病態の品質工学的手法による解析

III) 漢方医学的病態の古典的解釈と客観的評価を統合した臨床研修プログラムの開発

- 1) 漢方医学研修による教育効果に関する検討
- 2) 傷寒論、金匱要略を中心とする古典の解釈に関する検討

◇著書 Books

- 1) 後藤博三：「虚熱」と四逆湯. 漢方診療二項の秘訣, 寺澤捷年, 花輪壽彦編, 92-93, 金原出版株式会社, 東京, 2004.
- 2) 柴原直利：補中益気湯の適応病態. 漢方診療二項の秘訣, 寺澤捷年, 花輪壽彦編, 240-241, 金原出版株式会社, 東京, 2004.
- 3) 後藤博三, 寺澤捷年：治療薬 UP-TO-DATE 2004. 漢方薬, 790-799, メディカルビュー社, 松沢佑次, 永井良三, 奥村勝彦編, 東京, 2004.
- 4) 中川孝子, 横澤隆子：糖尿病性腎症における桂枝茯苓丸の有用性. 腎とフリーラジカル第7集, 松澤直輝 青柳一正編, 128-134, 東京医学社, 東京, 2004
- 5) 中川孝子, 横澤隆子. 桂枝茯苓丸による糖尿病性腎症進展抑制作用：aminoguanidine, butylated hydroxytoluene, captopril との比較. 腎とフリーラジカル第7集, 松澤直輝 青柳一正編, 135-140, 東京医学社, 東京, 2004
- 6) 中川孝子, 横澤隆子. 温脾湯構成生薬並びに大黃・甘草成分の advanced glycation endproducts (AGEs) 形成抑制作用. 腎とフリーラジカル第7集, 松澤直輝 青柳一正編, 141-146, 東京医学社, 東京, 2004.

◇原著 Original Articles

- 1) **Cho E.J., Yokozawa T., Rhyu D.Y., Kim H.Y. and Shibahara N.: The Inhibitory Effects of 12 Medicinal Plants and Their Component Compounds on Lipid Peroxidation. Am. J. Chin. Med., 31: 907-917, 2003.**

Abstract: The antioxidative activities of 12 medicinal plants and the compounds isolated from them were investigated using the thiocyanate method to evaluate inhibitory effects on lipid peroxidation in the linoleic acid system. The peroxide levels gradually increased during incubation in the presence of linoleic acid over 3 days, and most of the plants inhibited lipid peroxidation. In particular, of the plants tested, *Cudrania tricuspidata*, *Zanthoxylum piperitum*, *Houttuynia cordata* and *Ulmus parvifolia* reduced lipid peroxidation more effectively as lipid peroxidation progressed, resulting in inhibition of about 80% relative to the control value by the 3rd day of incubation. In addition, the polyphenols isolated from the plants also showed marked and dose-dependent inhibitory effects on lipid peroxidation. The compounds with the strongest activities were 3,4-dihydroxybenzoic acid, quercetin, the quercetin glycosides quercetin-3-O-beta-D-galactoside, quercetin-3-O-alpha-L-rhamnoside, quercetin-3-O-beta-D-glucoside and quercetin-3-O-rutinoside, catechin, gallic acid, methyl gallate and rosmarinic acid isolated from *Zanthoxylum piperitum*, *Houttuynia cordata*, *Rosa rugosa* and *Cedrela sinensis*. Moreover, quercetin glycosides showed stronger activity than quercetin, suggesting that glycosylation increases the antioxidative activity of quercetin. Our results indicate that the medicinal plants and their polyphenols show promise as therapeutic agents for various disorders involving free radical reactions.

- 2) **Cho E.J., Yokozawa T., Kim H.Y., Shibahara N. and Park J.C.: Rosa rugosa attenuates diabetic oxidative stress in rats with streptozotocin-induced diabetes. Am. J. Chin. Med., 32: 487-496, 2004.**

Abstract: The effects of *Rosa rugosa* on diabetic oxidative stress were investigated using rats with streptozotocin (STZ)-induced diabetes. The diabetic rats showed less body weight gain and heavier kidney and liver weights than normal rats, while the oral administration of *Rosa rugosa* at a dose of 100 or 200 mg/kg body weight/day for 20 days attenuated the physiological changes induced by diabetes. In addition, administering *Rosa rugosa* to diabetic rats resulted in significant and dose-dependent decreases in the serum glucose and glycosylated protein levels, implying that *Rosa rugosa* improves the abnormal glucose metabolism that leads to oxidative stress. Diabetic rats had higher serum levels of superoxide and nitrite/nitrate. However, the administration of *Rosa rugosa* dose-dependently reduced

the over-production of radicals associated with diabetes, suggesting *Rosa rugosa* is a radical scavenger that would play a crucial role in protecting against diabetic oxidative stress. *Rosa rugosa* significantly and dose-dependently reduced thiobarbituric acid-reactive substance levels in serum, hepatic and renal mitochondria, implying that *Rosa rugosa* would alleviate the oxidative stress associated with diabetes by inhibiting lipid peroxidation. This study provides evidence that *Rosa rugosa* has potential as a treatment for diabetes through attenuating oxidative stress induced by the diabetic condition.

3) Nakagawa T., Yokozawa T., Sano M., Takeuchi S., Mujo Kim and Shinsuke Minamoto: Activity of (-)-epigallocatechin 3-O-gallate against oxidative stress in rats with adenine-induced renal failure. J. Agric. Food Chem., 52: 2103-2107, 2004.

Abstract: Methylguanidine (MG) is widely recognized as a strong uremic toxin. The hydroxyl radical (*OH) specifically plays an important role in the pathway of MG production from creatinine (Cr). In this study, we investigated whether oral administration of (-)-epigallocatechin 3-O-gallate (EGCg) suppresses MG production in rats with chronic renal failure after intraperitoneal Cr injection. MG production from Cr was significantly increased in rats with adenine-induced renal failure, which was more vulnerable to oxidative stress, compared with that in normal rats. However, oral administration of EGCg 30 min before and after Cr injection effectively inhibited MG production. Our findings suggest that EGCg, an excellent antioxidant from green tea, exerts protective activity in rats with chronic renal failure, resulting in suppression of Cr oxidation influenced by *OH.

4) Nakagawa T. and Yokozawa T.: Inhibitory effects of Luobuma tea and its components against glucose-mediated protein damage. Food Chem. Toxic., 42: 975-981, 2004.

Abstract: Luobuma tea, prepared from the leaves of *Apocynum venetum* L., is a popular beverage in China. In this study, the activity of Luobuma leaf extract and its components against the formation of advanced glycation endproducts (AGEs), which are largely involved in the pathogenesis of diabetic vascular complications, was examined using the in vitro glycation reaction. Strong inhibitory activity against the formation of AGEs was shown by Luobuma aqueous extract. Following further fractionation of this extract, seven polyphenolic compounds, i.e. (+/-)-gallocatechin, (-)-epigallocatechin, (+/-)-catechin, (-)-epicatechin, epicatechin-(4beta-8)-gallocatechin, epigallocatechin-(4beta-8)-epicatechin and procyanidin B-2, were isolated by Sephadex LH-20 column chromatography. These purified compounds also exerted inhibitory activities that were more potent than the positive control, aminoguanidine. Our findings may help to explain the beneficial effects of this plant against atherosclerosis.

5) Yokozawa T., Yamabe N., Cho EJ., Nakagawa T. and Oowada S.: A study on the effects to diabetic nephropathy of Hachimi-jio-gan in rats. Nephron Exp. Nephrol., 97: e38-e48, 2004.

Abstract: Oral administration of Saiko-ka-Ryukotsu-Borei-To (SRB: a traditional Chinese formulation) has been found to prevent intimal thickening of the carotid artery after balloon endothelial denudation in cholesterol-fed rats. To clarify the mechanism of this effect, the present study investigated whether SRB inhibits vascular smooth muscle cell (VSMC) migration, which plays an important role in the development of intimal thickening after endothelial injury. The serum (SRB-serum) sampled from cholesterol-fed rats treated orally with SRB for 3 days before and 4 days after the injury dose-dependently inhibited the migration of cultured VSMCs. On the other hand, SRB extract added directly to cultured VSMCs did not inhibit the migration. It is remarkable that SRB-serum, which might contain a much lower concentration of SRB ingredients compared with SRB-extract, inhibited the cultured VSMCs migration.

The present testing system-using serum obtained from animals treated orally with traditional Chinese formulations could be a useful tool for clarifying the pharmacological efficacy of such drugs including many non-absorbable components. Furthermore, it should be useful for searching for new active compounds in serum after oral administration of traditional Chinese formulations whose active metabolites have not been identified.

6) Kim HY., Yokozawa T., Nakagawa T. and Sasaki S.: Protective effect of γ -aminobutylic acid against glycerol-induced acute renal failure in rats. Food Chem. Toxic., 42: 2009-2014, 2004.

Abstract: To investigate the effect of gamma-aminobutyric acid (GABA) on acute renal failure, we used a rat model of acute tubular necrosis induced by glycerol. After deprivation of water for 6h, the rats received an injection of 50% glycerol into the muscle of the rear limb at 10 ml/kg body weight. GABA was then administered orally to the rats (100 or 500 mg/kg body weight/day) once every 12h for 3 days. The rats with acute renal failure showed arrested body weight gain and an increase of kidney weight, whereas oral administration of GABA attenuated the physiological changes induced by acute renal failure. However, GABA administration had no significant effect on increased urine volume. Oral administration of GABA at a dose of 100 or 500 mg/kg body weight/day for 3 days significantly improved the markedly elevated levels of blood urea nitrogen and creatinine and the reduced creatinine clearance related to progression of renal failure. Moreover, the rats with acute renal failure exhibited high levels of fractional excretion of sodium (FE(Na)) due to alteration of tubule function following injection of glycerol. However, administration of GABA lowered the FE(Na) levels dose-dependently. Furthermore, urine osmolarity was markedly reduced in control rats with acute renal failure as compared with normal rats, whereas it was significantly increased by administration of GABA at a dose of 500 mg/kg body weight/day. These results indicate that GABA has potential as a therapeutic agent against the renal damage involved in acute renal failure.

7) Yokoyama K., Shimada Y., Hori E., Nakagawa T., Takagi S., Sekiya N., Kouta K, Nishijo H., Yokozawa T. and Terasawa K.: Effects of Choto-san and hooks and stems of *Uncaria sinensis* on antioxidant enzyme activities in the gerbil brain after transient forebrain ischemia. J. Ethnopharmacol., 95: 335-343, 2004.

Abstract: Previously, we revealed that oral administrations of Choto-san, a Kampo formula, and the hooks and stems of *Uncaria sinensis* Haviland (Rubiaceae), a medicinal plant comprising Choto-san, enhanced superoxide anion and hydroxyl radical scavenging activities in the hippocampus, and prevented delayed neuronal death of pyramidal cells in the hippocampal CA1 region in a transient forebrain ischemia gerbil model. In the present study, for the purpose of clarifying whether the endogenous antioxidant enzymes contribute to these mechanisms, we investigated the effects of Choto-san extract (CSE) and *Uncaria sinensis* extract (USE) on superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities in the brain by using the same experimental model. 1.0% CSE or 3.0% USE were dissolved in water and provided to gerbils ad libitum from 7 days prior to ischemia/reperfusion (i/rp). Seven days of continuous administrations of CSE or USE without i/rp procedure enhanced CAT activity but not SOD and GSH-Px activities in both the hippocampus and cortex. CSE elevated CAT activity in the hippocampus at 7 days and in the cortex at 3h after i/rp. USE raised CAT activity in both the hippocampus and cortex at 3 h and 7 days after i/rp. These results suggest that one of the mechanisms of the protective effects of CSE and USE against transient brain ischemia-induced neuronal damage may be their enhancing effect on CAT activity in the brain.

8) Yokoyama K., Shimada Y., Hori E., Sekiya N., Goto H., Sakakibara I., Nishijo H., Terasawa K.: Protective effects of Choto-san and hooks and stems of *Uncaria sinensis* against delayed neuronal death after transient forebrain ischemia in gerbil. Phytomedicine, 11: 478-489, 2004.

Abstract: Previously, we revealed that Choto-san (Diao-teng-san in Chinese), a Kampo formula, is effective on vascular dementia clinically, and the hooks and stems of *Uncaria sinensis* (Oliv.) Havil., a medicinal plant comprising Chotosan, has a neuroprotective effect in vitro. In the present study, for the purpose of clarifying their effects in vivo, we investigated whether the oral administration of Choto-san extract (CSE) or *U. sinensis* extract (USE) reduces delayed neuronal death following ischemia/reperfusion (i/rp) in gerbils. Transient forebrain ischemia was induced by bilateral carotid artery occlusion for 4 min, and two doses (1.0% and 3.0%) of CSE or USE were dissolved in

drinking water and provided to the gerbils ad libitum from 7 days prior to i/rp until 7 days after i/rp. It was found that 1.0% and 3.0% CSE treatments significantly reduced pyramidal cell death in the hippocampal CA1 region at 7 days post i/rp. Three percent USE treatment also inhibited pyramidal cell death significantly at 7 days after i/rp. Superoxide anion and hydroxyl radical scavenging activities of the homogenized hippocampus at 7 days after i/rp in the 1.0% CSE- and 3.0% USE-treated groups were significantly enhanced compared to those of control. Further, lipid peroxide and NO₂-/NO₃- levels of the homogenized hippocampus at 48h after i/rp in the 1.0% CSE- and 3.0% USE-treated groups were significantly lower than those of control. These results suggest that the oral administration of CSE or USE provides a protective effect against transient ischemia-induced delayed neuronal death by reducing oxidative damage to neurons.

9) Goto H., Shimada Y., Sekiya N., Yang Q., Kogure T., Mantani N., Hikiami H., Shibahara N. and Terasawa K.: Effects of Keishi-bukuryo-gan on vascular function and hemorheological factors in spontaneously diabetic (WBN/kob) rats. *Phytomedicine*, 11:188-195, 2004.

Abstract: Keishi-bukuryo-gan (Gui-zhi-fu-ling-wan) is a formula used for the improvement of blood circulation. Recently it has often also been used for arteriosclerosis. One of the mechanisms involved is thought to be the improvement of endothelial dysfunction, but the details are still unclear. In this study, the effect of Keishi-bukuryo-gan on vascular function and hemorheological factors in spontaneously diabetic (WBN/kob) rats was studied. Rats were given Keishi-bukuryo-gan in chow for 30 weeks. Body weight, blood glucose, endothelium-dependent/-independent relaxation, vasocontraction by free radical-induced and contractive prostanoids, triglyceride, advanced glycation endproduct, lipid peroxides, serum NO₂-/NO₃- and blood viscosity were measured. The results indicated that Keishi-bukuryo-gan caused a decrease in endothelium-dependent relaxation by acetylcholine to become significantly increased, and vasocontraction induced by free radicals and contractive prostanoids was significantly decreased. Furthermore, serum NO₂-/NO₃- and blood viscosity were significantly decreased. From these results, it was supposed that Keishi-bukuryo-gan exerted a protective effect on the endothelium. The WBN/kob rat is a useful study model for the complications of human diabetes, and Keishi-bukuryo-gan showed a protective effect against vascular injury in the susceptible rat.

10) Yang Q., Goto H., Hikiami H., Shibahara N., Shimada Y., Terasawa K. and Tang F.: Effects of Toki-shakuyaku-san on microcirculation of bulbar conjunctiva and hemorheological factors in patients with asymptomatic cerebral infarction. *J. Trad. Med.*, 21: 170-173, 2004.

Abstract: In this study, the effects of Toki-shakuyaku-san on the microcirculation of bulbar conjunctiva in 11 patients with asymptomatic cerebral infarction were investigated with a video-microscopic system. After the administration of Toki-shakuyaku-san for four weeks, the flow volume rates of microcirculatory flow of the bulbar conjunctiva were increased ($p < 0.05$). Hemorheological factors such as whole blood viscosity, plasma viscosity, and erythrocyte deformability were examined. Toki-shakuyaku-san improved whole blood viscosity and erythrocyte deformability ($p < 0.05$), and plasma lipid peroxides decreased. These results suggested that the favorable effects of Toki-shakuyaku-san on cerebrovascular disorders take place via changes in microcirculatory flow, with the mechanisms being considered to be improvements in hemorheological factors and the anti-oxidant effect of Toki-shakuyaku-san.

11) Goto H., Shimada Y., Tani T., Sekiya N., Hikiami H., Sakai S., Shibahara N. and Terasawa K.: Effects of a new original formulation containing crude drugs used for self-medication. *J. Trad. Med.*, 21: 199-204, 2004.

Abstract: A new original formulation containing crude drugs used for self-medication was developed by the joint project of the Federation of Pharmaceutical Industries Association in Toyama, Toyama Prefecture (Toyama

Prefectural Institute for Pharmaceutical Research) and Toyama Medical and Pharmaceutical University. This formulation consists of 11 crude. In this study, the effect of this formulation on the model animals of life-style related disease was studied. Spontaneously hypertensive rats added to hypercholesterol diet were given this formulation in chow for 8 weeks. The results indicated that this formulation caused a decrease in vasocontraction induced by phospholipase A2. Plasma triglyceride and lipid peroxide were significantly decreased, but blood pressure was not changed. Furthermore spontaneously diabetic rats were given this formulation in chow for 4 weeks. The results indicated that this formulation caused a decrease in plasma triglyceride, lipid peroxide and fibrinogen significantly, but blood glucose was not changed. From these results, it was supposed that this formulation exerted the suppression effect of vasocontraction, improvement effect of fatty metabolism and decrease effect of fibrinogen. And this formulation is thought to be useful drug to prevent the vasocompensation based on life-style related disease.

12) Kainuma M., Sakai S., Sekiya N., Mantani N., Ogata N., Shimada Y. and Terasawa K.: The effects of a herbal medicine (Mao-to) in patients with chronic hepatitis C after injection of Interferon- β . *Phytomedicine* 11: 5-10, 2004.

Abstract: We found that a herbal medicine (Mao-to) relieves the side effects of interferon (IFN)-beta and the combination therapy improves the biochemical response rate. However, the exact mechanism by which Mao-to is effective remains to be established. We conducted a controlled trial to clarify the effects of Mao-to. The study was carried out in 18 patients with chronic hepatitis C, and we examined subjective symptoms, body temperature and cytokines such as interleukin (IL)-beta, IL-1receptor antagonist (ra), IL-6 and TNF-alpha. Each patient received 6 million units of IFN-beta intravenously. Mao-to was given orally just before, just after, and 1 hour after IFN administration. The control study was carried out 6 months after the combination therapy of Mao-to and IFN-beta. The scores for general malaise, arthralgia and discomfort were significantly lower in the combination group than in control group. Body temperature did not significantly differ between the two groups. Plasma IL-6 level and IL-1ra were significantly elevated in the combination group compared to control ($P = 0.0057$ and 0.0003 , respectively). Mao-to did not affect plasma concentrations of IL-1beta and TNF-alpha. We considered the increment of IL-1ra caused by Mao-to is to be one of the key factors involved in reducing the flu-like symptoms accompanying IFN-beta and improving the biochemical response rate.

13) Sakai S., Ochiai H., Mantani N., Kogure T., Shimada Y. and Terasawa K.: Gene Expression in Early phase of Murine Influenza pneumonia Determined by cDNA Expression Array Technique. *Intern. J. Appl. Res. Vet. Med.*, 2: 46-51, 2004.

Abstract: BACKGROUND: Influenza virus is a worldwide health problem with significant economic consequences. To study the gene expression pattern induced by influenza virus infection, it is useful to reveal the pathogenesis of influenza virus infection; but this has not been well examined, especially in vivo study. AIMS: To assess the influence of influenza virus infection on gene expression in mice, mRNA levels in the lung and tracheal tissue 48 h after infection were investigated by cDNA array analysis. METHODS: Four-week-old outbred, specific pathogen free strain, ICR female mice were infected by intra-nasal inoculation of a virus solution under ether anesthesia. The mice were sacrificed 48 h after infection and the tracheas and lungs were removed. To determine gene expression, the membrane-based microtechnique with an Atlas cDNA expression array (mouse 1.2 array II) was performed in accordance with the manual provided. RESULTS AND CONCLUSIONS: We focused on the expression of 46 mRNAs for cell surface antigens. Of these 46 mRNAs that we examined, four (CD1d2 antigen, CD39 antigen-like 1, CD39 antigen-like 3, CD68 antigen) were up-regulated and one (CD36 antigen) was down-regulated. Although further studies are required, these data suggest that these molecules play an important role in influenza virus infection, especially the phase before specific immunity.

- 14) Sekiya N., Shimada Y., Niizawa A., Kogure T., Mantani N., Sakai S., Hikiami H. and Terasawa K.: **Suppressive Effects of *Stephania tetrandra* on the Neutrophil Function in Patients with Rheumatoid Arthritis.** *Phytother. Res.*, 18: 247-249, 2004.

Abstract: Crude preparations of *Stephania tetrandra* (ST), a traditional herbal medicine, have been used safely for arthritis and silicosis in China. The concentration of granulocyte elastase - alpha 1 protease inhibitor complex in plasma is enhanced in inflammatory processes, e.g. in septicaemia and rheumatoid arthritis (RA), being an expression of granulocyte activation during inflammatory response. It has previously been reported that ST showed beneficial and immunomodulatory effects in the treatment of relatively mild RA. After the administration of ST for 12 weeks, the proportion of granulocytes and the granulocyte count in peripheral blood decreased significantly. The lipid peroxide and human granulocyte elastase levels of stored plasma declined significantly. Furthermore, both the leukocyte/elastase ratio and granulocyte/elastase ratio increased significantly. The findings of this study suggest that the suppressive effect of ST administration on excessive granulocyte activation resulted in the improvement of inflammation with rheumatoid arthritis.

- 15) Shimada Y., Yokoyama K., Goto H., Sekiya N., Mantani N., Tahara E., Hikiami H. and Terasawa K.: **Protective effect of Keishi-bukuryo-gan and its constituent medicinal plants against nitric oxide donor-induced neuronal death in cultured cerebellar granule cells.** *Phytomedicine*. 11: 404-410, 2004.

Abstract: Keishi-bukuryo-gan (Gui-Zhi-Fu-Ling-Wan) (KBG) is a traditional Chinese/Japanese medical (Kampo) formulation that has been administered to patients with "Oketsu" (blood stagnation) syndrome. In the process of neuronal cell death induced by brain ischemia, excessive generation of nitric oxide (NO) free radicals is implicated in the neurotoxicity. In the present study, we examined the protective effects of KBG and its constituent medicinal plants against NO donors, sodium nitroprusside (SNP) and 2,2'-(hydroxynitrosohydrazino)bis-ethanamine (NOC18)-induced neuronal death in cultured rat cerebellar granule cells (CGCs). MTT assay showed cell viability to be significantly increased by the addition of KBG extract (KBGE) (100 microg/ml), Cinnamomi Cortex extract (CCE) (3, 10 and 30 microg/ml), Paeoniae Radix extract (PRE) (100 microg/ml) and Moutan Cortex extract (MCE) (10 and 30 microg/ml) compared with exposure to SNP (30 microM, 24 h) only. Also, cell viability was significantly increased by the addition of KBGE (100 and 300 microg/ml), CCE (30 and 100 microg/ml), PRE (100 and 300 microg/ml) and MCE (30 and 100 microg/ml) compared with exposure to NOC 18 (100 microM, 48 h) only. Persicae Semen extract and Hoelen extract did not protect against NO donor-induced neuronal death. These results suggest that KBG has protective effect against NO-mediated neuronal death in cultured CGCs and that it is derived from Cinnamomi Cortex, Paeoniae Radix and Moutan Cortex.

- 16) Kogure T., Sato N., Tahara E., Sakai S., Shimada Y., Ochiai H., Origasa H. and Terasawa K.: **Assessment of the effects of traditional herbal medicines on elderly patients with weakness using a self-controlled trial.** *Geriatr. Gerontol. Int.*, 4: 169-174, 2004.

Abstract: Background: The objectives of this study were to evaluate the effects of traditional herbal medicine on elderly patients with weakness, and to devise a suitable study design for assessing the clinical effectiveness of traditional herbal medicines. Methods: Twenty-one elderly patients with weakness (mean age, 78.2 ± 7.5 ; male : female, 8 : 13) were studied using a self-controlled design with a run-in period. The observation term was 3 months, and quality of life (short form-36 and profile of mood status) were adopted as evaluation endpoints. In addition, natural killing activity and surface antigens (CD19, CD3, CD4, CD16, CD56, CD158a, CD158b) on lymphocytes obtained from peripheral blood were analyzed to evaluate patients' immune status. Results: EK-41 (Hochu-ekki-to), EK-48 (Juzen-taiho-to) and EK-98 (Ogi-kenchu-to) were administered to 10, 10 and one patients, respectively. There were no dropouts due to side-effects. Results of the short form-36 were significantly improved after 3 months, with

the patients in the EK-48 group showing greater improvement than those in the EK-41 group. Each component of the profile of mood status was improved by the treatment, and the improvement of V, D and F was especially significant. D and A-H were considerably improved in the patients of the EK-48 group. In contrast, the improvement of D and T-A was most marked in the EK-41 group. Some augmentation of NK activity was observed after 3 months, but the effect was not significant. Although neither the CD4/8 ratio nor the percentages of CD3+, CD19+ or CD16+ CD158a + cells was changed significantly, the percentage of CD56+, CD56+ CD16+ and CD16+ CD158b + cells were significantly increased. Conclusion: A preliminary clinical trial for elderly patients with weakness was carried out to assess the efficacy of traditional medicines, resulting in evidence of their clinical and immunomodulating effects, although this evidence had some limitations. In addition, we obtained some insights into the elements of designing studies suitable for assessing the clinical efficacy of traditional herbal medicines.

17) Imanishi N., Mantani N., Sakai S., Sato M., Katada Y., Ueda K., Terasawa K. Ochiai H.: Inducible activity of Ginger Rhizome (*Zingiber officinale* Rosc.) on the mRNA expression of macrophage-inducible nitric oxide (NO) synthase and NO production in a macrophage cell line, RAW264.7 cells. *Am. J. Chin. Med.*, 32: 727-735, 2004.

Abstract: We have investigated the effect of *Zingiber officinale* Rosc. (ZOR) on macrophage-inducible nitric oxide (NO) synthase (macNOS) mRNA expression and NO production in RAW264.7 cells, a murine macrophage cell line; 100 microg/ml ZOR can induce macNOS mRNA expression, but induction effects at a dose below 10 microg/ml were weak or negligible. Kinetic studies showed that macNOS mRNA can be detected from 4 hours to 24 hours after dosing, with a peak at 8 hours. In accordance with the induction of macNOS mRNA expression, NO concentrations increased from 3.4 microM at 2 hours to almost 150 microM at 24 hours, reflecting a longer period of macNOS mRNA expression. The activity of ZOR can be considered to contribute, at least in part, to the beneficial effects of ZOR through the macNOS-mediated activation of the biodefense mechanism.

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◇共同研究 Co-operative researches

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- 1) 嶋田 豊：富山医科薬科大学和漢診療学講座，「漢方医学の臨床研修プログラムの開発」，1999，4～
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2. 国内

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◇非常勤講師 Part-time lecturer

- 1) 柴原直利：富山医科薬科大学，「和漢医薬学入門」，2004.4.30.
- 2) 柴原直利：岡山大学，「東洋医学」，2004.6.7.
- 3) 柴原直利：福井大学，「東洋医学」，2004.7.24.
- 4) 柴原直利：弘前大学，「東洋医学」，2004.9.13.
- 5) 柴原直利：富山医科薬科大学，「東洋医学概論」，2004.10.14.～
- 6) 中川孝子：富山福祉短期大学，「家政学実習Ⅱ（食生活領域）」，2004.10.14.～
- 7) 中川孝子：富山福祉短期大学，「家政学概論Ⅰ（食生活領域）」，2004.10.14.～

◇研究費取得状況 Acquisition of research funds

- 1) 和漢薬・バイオテクノロジー研究「富山県で栽培可能な生薬に関する総合的研究」（新規，柴原代表，後藤分担）250万

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- 12) 井上 隆弥 (医師, 大阪府, 2004, 5.10~5.14)
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- 15) 宮坂 史路 (医師, 北海道, 2004, 6.28~7.2)
- 16) 遠藤 志織 (医学生, 静岡県, 2004, 8.2~8.6)
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- 20) 松本 祐二 (医師, 島根県, 2004, 10.25~10.29)