

## 恒常性機能解析部門

## Division of Analysis of Homeostasis

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### ◇研究目的

漢方医学は中国医学を淵源とし、6～8世紀頃に朝鮮半島を経由してわが国にもたらされた医学体系である。それ以後、明治維新に至るまで、漢方医学は本邦の医療を担ってきた。明治以降、西洋医学を含む西洋文化は日本の伝統文化に取って代わったが、漢方医学が全く使用されなかったというわけではなく、西洋医学を学んだ医師により用いられ、現在では非常に多くの医師が漢方薬を使用している。1976年に漢方製剤が医療保険の枠組みの中に取り入れられ、2003年からは医学教育の標準的カリキュラムにも組み込まれた。

「東洋の知」は、人間存在を自然の中で生かされている心身一如の小宇宙として捉えている点で優れている。そこで恒常性機能解析分野は、東洋医学と西洋医学の異なったパラダイムを融和し、病態の個性別や個人差に基づく「個の医療」を認識した新たな治療学の形成を目指す。

### ◇研究概要

#### I) 漢方医学における病態の科学的解明

漢方医学には陰陽虚実、気血水、あるいは五臓といった独特の病態概念があり、これら諸概念の意味することを科学的に解明する。

#### II) 漢方方剤の薬理学的研究

漢方方剤は複数の生薬を組み合わせて成り立っている。一つの生薬は様々な化学成分を含んでおり、この生薬を複数組み合わせた漢方方剤は「多成分系薬物」と呼ぶことができる。この多成分系薬物である「漢方方剤」を一つの薬物単位とみなし、その効果発現の機序を免疫学的手法など、様々な西洋医学的手段を動員して明らかにする。

#### III) 漢方方剤の臨床研究

漢方医学は個々の患者の病態にきめ細かく対応出来る治療体系である。そこで、漢方治療が奏功した症例を治療経験として積み重ね、症例集積研究やプラセボを用いた無作為化試験などによるエビデンスを蓄積する。

◇原著論文

- 1) **Chino A., Sakurai H., Choo MK., Koizumi K., Shimada Y., Terasawa K., and Saiki I.: Juzentaihoto, a Kampo medicine, enhances IL-12 production by modulating Toll-like receptor 4 signaling pathways in murine peritoneal exudate macrophages. *Int. Immunopharmacol.*, 5(5): 871-882, 2005.**

**Abstract:** Juzentaihoto (TJ-48), a Kampo medicine, has been reported to affect the immune system. Although toll-like receptors (TLRs) have been identified as receptors of innate immunity, the effects of TJ-48 on TLR signaling pathways have not been thoroughly investigated. Here we evaluated the effects of TJ-48 on TLR4 signaling pathways. Peritoneal exudate macrophages (PEMs) isolated from mice orally administered TJ-48 for 11 days were stimulated with lipopolysaccharide (LPS), a ligand of TLR4, in vitro. Production of IL-12 p40 was significantly augmented in TJ-48-treated PEMs compared with that in vehicle PEMs, without affecting the surface expression of TLR4. Treatment with chemical inhibitors of NF-kappa B and p38 mitogen-activated protein kinases (MAPKs) in vitro inhibited LPS-induced IL-12 production, whereas JNK and ERK inhibitors increased IL-12 production. Immunoblotting with phosphorylation-state specific antibodies demonstrated that TJ-48 differentially affected LPS-induced phosphorylation of NF-kappa B and MAPKs. In PEMs treated with TJ-48, LPS-induced phosphorylation of p65 NF-kappa B and p38 MAPK was augmented, while that of JNK and ERK was attenuated compared with those in vehicle PEMs. These results suggest that selective modulation of the TLR4 signaling pathways by TJ-48 is involved in enhanced production of IL-12 in PEMs.

- 2) **Sekiya N., Kainuma M., Hikiami H., Nakagawa T., Kouta K., Shibahara N., Shimada Y., and Terasawa K.: Oren-gedoku-to and keishi-bikuryo-gan-ryo inhibit the progression of atherosclerosis in diet-induced hypercholesterolemic rabbits. *Biol. Pharm. Bull.*, 28(2): 294-298, 2005.**

**Abstract:** In this study, we examined whether the Kampo formulas Oren-gedoku-to (OGT, Huanglian-jie-du-tang in Chinese) and Keishi-bukuryo-gan-ryo (KBG, Gui-zhi-fu-ling-wan in Chinese) could prevent the progression of atherosclerosis in cholesterol-fed rabbit, an animal model for hypercholesterolemia in vivo. Twenty-four male Japanese white rabbits (2 kg body weight) were divided into four groups. The control group was fed standard rabbit chow containing 1% cholesterol, the OGT group was fed standard rabbit chow containing 1% cholesterol and 1% OGT, the KBG group was fed standard rabbit chow containing 1% cholesterol and 1% KBG, and the vitamin E group was fed standard rabbit chow containing 1% cholesterol and vitamin E (450 mg/1000 g). All four groups were kept on these diets for 8 weeks. At the end of the experiments, the percentage of surface area of the total thoracic aorta with visible plaque was significantly reduced in the OGT and KBG groups. The serum thiobarbituric acid reactive substances of the vitamin E group showed a significantly low value compared with the control group, whereas the serum lipid peroxide levels of the OGT and KBG groups were considerably lower than that of the control groups as well as that of the vitamin E group. Furthermore, the urinary 8-hydroxydeoxyguanosine levels of the OGT and KBG groups were considerably lower than that of the vitamin E group. These results suggest that OGT and KBG prevent the progression of atheromatous plaque by creating a sounder antioxidant defense system than vitamin E.

- 3) **Sekiya N., Hikiami H., Yokoyama K., Kouta K., Sakakibara I., Shimada Y., and Terasawa K.: Inhibitory effects of *Stephania tetrandra* S. MOORE on free radical-induced lysis of rat red blood cells. *Biol. Pharm. Bull.*, 28(4): 667-670, 2005.**

**Abstract:** Crude preparations of *Stephania tetrandra* S. MOORE (ST), a traditional herbal medicine, have been used safely for arthritis and silicosis in China. In this study, we demonstrated that ST in vitro protects red blood cells from 2,2-azo-bis (2-amidinopropane) dihydrochloride (AAPH)-induced hemolysis. The

inhibitory effect was dose-dependent at concentrations of 10 to 1000 microg/ml. Moreover, tests were carried out to identify the main ingredient of ST that exerts a scavenging effect on free-radicals. Three representative alkaloids, tetrandrine, fangchinoline, and cyclanoline, isolated from ST, were found to have inhibitory activities against AAPH-induced lysis of red blood cells (RBC). Furthermore, the ingestion of 200 mg of ST extract was associated with a significant increase in free-radical scavenging effect of plasma in rats. These results suggest that ST as antioxidant inhibits AAPH-induced hemolysis of RBC both in vitro and in vivo.

- 4) Sekiya N., Hikiami H., Nakai Y., Sakakibara I., Nozaki K., Kouta K., Shimada Y., and Terasawa K.: Inhibitory effects of triterpenes isolated from Chuling (*Polyporus umbellatus* FRIES) on free radical-induced lysis of red blood cells. *Biol. Pharm. Bull.*, 28(5): 817-821, 2005.

**Abstract:** Chuling, sclerotia of *Polyporus umbellatus* FRIES, has long been used for urological disorders in traditional medicine. In this study, we demonstrated that Chuling in vitro protects red blood cells from 2,2-azo-bis(2-amidinopropane)dihydrochloride (AAPH)-induced hemolysis. The inhibitory effect was dose-dependent at concentrations of 50 to 1000 microg/ml. Moreover, tests were carried out to identify the main ingredient of Chuling with scavenging effect on free radicals. Triterpene carboxylic acids isolated from the methanol extract of Chuling, namely, polyporusterone A and polyporusterone B, were found to have inhibitory activities against AAPH-induced lysis of red blood cells. The anti-hemolytic effect was significantly stronger in polyporusterone B compared with polyporusterone A. Furthermore, the ingestion of 150 mg of Chuling was associated with a significant increase in free-radical scavenging effect of plasma in rats.

- 5) Kogure T., Itoh K., Tatsumi T., Sekiya N., Sakai S., Shimada Y., Tamura J., and Terasawa K.: The effect of Juzen-taiho-to/TJ-48 on the expression of killer-cell immunoglobulin-like receptors (CD158a/b) on peripheral lymphocytes *in vitro* experiment. *Phytomedicine*, 12(5): 327-332, 2005.

**Abstract:** Juzen-taiho-to (TJ-48), a mixture of extracts from 10 medicinal herbs, has been used traditionally to treat patients with anemia, anorexia or fatigue. It is well known that the treatment of TJ-48 result in the decrease of patient's complaints, as well as the increase of NK cytolytic activity (NK activity) although its augmentation is not clear in the other kampo formula from the clinical viewpoint. To investigate its biological activities, such as the augmentation of NK activity, we analyzed the effects of TJ-48 on the expression of killer-cell immunoglobulin-like receptors (KIRs) in vitro experiment. The peripheral lymphocytes were incubated in medium alone, or medium containing TJ-48 or interleukin-2 (IL-2) plus TJ-48 at several concentrations for 48 h. After each incubation, cells were collected and their KIRs were detected by flow cytometry using monoclonal antibodies CD158a and CD158b. TJ-48 increased the populations of CD16+CD158a+ and CD16+CD158b+ cells in a dose-dependent manner. In contrast, CD16-CD158a/b+ cells did not increase. Additionally, the extract of TJ-48 enhanced the increase of KIRs expression induced by IL-2. These actions contribute to the augmentation of NK cytolytic activity by TJ-48, and might explain, in part, its antitumor effects which has been observed in vivo.

- 6) Kainuma M., Kogure T., Sekiya N., Mitsuma T., Shimada Y., and Terasawa K.: Changes of peripheral lymphocyte population in patients with chronic hepatitis C treated with herbal medicine (Maoto) and IFN- $\beta$ . *J. Trad. Med.*, 22(2/3): 29-33, 2005.

**Abstract:** We recently reported that the herbal medicine (Maoto) might have immunomodulatory effects when used in conjunction with IFN-beta, we monitored the changes in lymphocyte populations of peripheral blood by flow-cytometry. Twenty-five patients with chronic hepatitis C were enrolled in this study. They received a daily dose of 6 million units of IFN-beta for 8 weeks. Maoto was given orally 4

times a day during the IFN-beta administration, and we monitored the changes in lymphocyte populations of peripheral blood by flow-cytometry. Six patients were sustained virological responder (SR), 10 were transient responder (TR), and 9 were nonresponders (NR). The percentage of CD16<sup>+</sup>CD56<sup>+</sup> lymphocyte populations was decreased in all groups between pretreatment and 4 weeks, but it was significantly increased in SR compared with TR and NR between 4 and 8 weeks. The percentage of HLADR<sup>+</sup>CD8<sup>+</sup> lymphocyte populations was significantly increased in SR and TR compared with NR between pretreatment and 8 weeks. Our results suggested that monitoring of changes in peripheral CD16<sup>+</sup>CD56<sup>+</sup> and HLADR<sup>+</sup>CD8<sup>+</sup> lymphocyte populations could be useful to treat chronic hepatitis C with the combination therapy of Maoto and IFN-beta.

**7) Itomura M., Hamazaki K., Sawazaki S., Kobayashi M., Terasawa K., and Watanabe S., Hamazaki T.: The effect of fish oil on physical aggression in schoolchildren—a randomized, double-blind, placebo-controlled trial. *J. Nutr. Biochem.*, 16(3): 163-171, 2005.**

**Abstract:** OBJECTIVES: The aim of the study was to investigate whether fish oil supplementation affected Japanese schoolchildren's behavior, with changes in aggression over time as the primary endpoint. DESIGN AND SUBJECTS: A placebo-controlled double-blind study with 166 schoolchildren 9-12 years of age was performed. The subjects of the fish oil group (n=83) took fish oil-fortified foods (bread, sausage and spaghetti). These foods were provided in amounts such that each subject in the fish oil group had an intake of 3600 mg of docosahexaenoic acid+840 mg of eicosapentaenoic acid (EPA)/week for 3 months. The rest (the controls, n=83) took control supplements. At the start and end of the study, psychological tests were performed to assess their aggression. RESULTS: Physical aggression assessed by Hostility-Aggression Questionnaire for Children in girls increased significantly (median: 13 to 15, n=42) in the control group and did not change (13 to 13, n=43) in the fish oil group with a significant intergroup difference (P=.008) with baseline as covariate. The changes in physical aggression scores over time and those of the ratio of EPA/arachidonic acid in RBC (DeltaEPA/AA) were significantly correlated in girls who agreed to blood collection (r=-.53, P=.01, n=23). On the contrary, there were no significant changes in physical aggression in boys. Aggression against others (extraggression) assessed by Picture Frustration Study did not change in the control group (median: 5 to 5) but increased significantly in the fish oil group (4 to 5) with a significant intergroup difference (P=.02) with baseline as covariate. These changes in extraggression might be explained partly by significantly lower baseline values of extraggression in the fish oil group (P=.02) than in the control group. There were no significant correlations between Deltaextraggression and DeltaEPA/AA in blood-sampled children (n=49). Impulsivity of girls assessed by parents/guardians using the diagnostic criteria for attention deficit/hyperactivity disorder of DSM-IV was reduced in the fish oil group (1 to 0) with a significant (P=.008) intergroup difference from the control group (1 to 1). There were no significant correlations between Deltaimpulsivity and DeltaEPA/AA in blood-sampled girls. In males, impulsivity reduced in both groups without any intergroup differences. CONCLUSION: There is a possibility that changes in fatty acid nutrition might affect physical aggression especially in girls.

**8) Hamazaki K., Itomura M., Huan M., Nishizawa H., Sawazaki S., Tanouchi M., Watanabe S., Hamazaki T., Terasawa K., and Yazawa K.: Effect of omega-3 fatty acid-containing phospholipids on blood catecholamine concentrations in healthy volunteers: a randomized, placebo-controlled, double-blind trial. *Nutrition.*, 21(6): 705-710, 2005.**

**Abstract:** OBJECTIVE: We previously reported that administration of fish oil rich in docosahexaenoic acid (DHA) increased the plasma ratio of epinephrine to norepinephrine (NE) at rest in young adults who were under chronic stress and that this effect was achieved mainly through depression of NE. However, not many reports have documented the effects of eicosapentaenoic acid (EPA) and DHA on blood catecholamine levels in healthy humans. Therefore, we performed another intervention study to test their

effect on catecholamines with healthy subjects under no chronic stress. **METHODS:** Twenty-one healthy young adults (15 men and 6 women) were randomly assigned to an omega-3 group (n = 9) or a control group (n = 12) in a double-blind manner. Twenty capsules of shellfish-derived lipids containing 762 mg of EPA plus DHA per day were administered to the omega-3 group for 2 mo. The controls took the same amount of placebo capsules. Fasting blood samples after a 30-min rest with a catheter in a forearm vein were obtained at the start and the end of the study for catecholamine measurements. **RESULTS:** EPA but not DHA concentrations in red blood cells significantly increased in the omega-3 group compared with the control group ( $P < 0.001$ ). Plasma NE concentrations were significantly decreased in the omega-3 group (from 1.49 +/- 0.39 nmol/L to 1.05 +/- 0.14 nmol/L) compared with the control group (from 1.12 +/- 0.24 nmol/L to 1.39 +/- 0.32 nmol/L) with analysis of covariance ( $P < 0.001$ ). The differences remained significant ( $P = 0.01$ ) even after deletion of three subjects in the omega-3 group who had the highest baseline NE values and one in the control group who had the lowest baseline NE value to nullify a significant baseline differences in NE between groups. **CONCLUSION:** This study demonstrated that EPA plus DHA supplementation lowered plasma NE concentrations in normal volunteers even at the small dose of 762 mg of EPA plus DHA per day. This effect of EPA plus DHA to lower plasma NE concentrations may be important to understand some of the effects of fish oils on diseases.

**9) Takagi S, Goto H, Shimada Y, Nakagomi K, Sadakane Y, Hatanaka Y, and Terasawa K.: Vasodilative effect of perillaldehyde on isolated rat aorta. *Phytomedicine.*, 12(5): 333-337, 2005.**

**Abstract:** The vasodilative effect of perillaldehyde, one of the major oil components in *Perilla frutescens* BRITTON, was studied using isolated rat aorta. Perillaldehyde at final concentrations of 0.01 to 1 mM showed dose-dependent relaxation of the aorta contracted by treatment with prostaglandin F<sub>2</sub>alpha or norepinephrine. Neither the presence of NG-nitro-L-arginine methyl ester nor removal of the aortic endothelium affected the vasodilatation, suggesting that perillaldehyde exerts a direct effect on vascular smooth muscle cells. The vasodilative effect of perillaldehyde was not inhibited by pretreatment with a beta-adrenergic receptor blocker (propranolol), an inhibitor of phosphodiesterase (theophylline), a delayed rectifier K<sup>+</sup> channel blocker (tetraethylammonium chloride), or an ATP-sensitive K<sup>+</sup> channel blocker (glibenclamide). However, perillaldehyde showed contrasting effects on vasodilatation of the aorta contracted by an influx of extracellular Ca<sup>2+</sup> - perillaldehyde caused little vasodilatation on the aorta contracted by the Ca<sup>2+</sup> ionophore A23187, while it inhibited the vasoconstriction induced by treatment with high-concentration K<sup>+</sup>, which dominantly opened the voltage-dependent Ca<sup>2+</sup> channel. These results suggest that the vasodilative effect of perillaldehyde is derived from blocking the Ca<sup>2+</sup> channels.

**10) Satoh N, Sakai S, Kogure T, Tahara E, Origasa H, Shimada Y, Kohoda K, Okubo T, and Terasawa K.: A randomized double blind placebo-controlled clinical trial of Hochuekkito, a traditional herbal medicine, in the treatment of elderly patients with weakness N of one and responder restricted design. *Phytomedicine.* 12(8): 549-554, 2005.**

**Abstract; OBJECTIVE:** To evaluate the effects of Hochuekkito, a traditional Japanese and Chinese medicine, in the treatment of elderly patients with general weakness. To devise a suitable study design for assessing the clinical effectiveness of traditional herbal medicines. **METHODS:** Fifteen elderly patients (mean +/- SD: age 78.4 +/- 7.8; m/f 3/12) participated in this study. A multicenter, prospective, randomized, double-blind, placebo-controlled study with N of one and responder restricted design was performed. After the run-in period, the patients were divided into responders and non-responders. Only responders were entered in the study, and were randomized into three groups: an active-placebo group, a placebo-active group and an active-active group. The study consisted of two 6-week terms with a 2-week washout period in between. We assessed the Short Form 36 Health Survey (SF-36) and Profile of Mood States (POMS) as an endpoint of quality of life (QOL). In addition, we assessed the biodefense status by

measuring the natural killer cytolytic activity (NK activity), IL-2 producing activity of peripheral lymphocytes, lymphocyte proliferating activity and lymphocyte cell-surface antigens. RESULTS: The physical component summary of the SF-36 analysis significantly improved in the Hochuekkito-treated group. Four components (A-H: anger-hostility, F: fatigue, T-A: tension-anxiety, C: confusion) out of six improved in the Hochuekkito-treated group in the POMS analysis. Lymphocyte proliferating activity improved in the Hochuekkito-treated group but not significantly. Concerning the surface antigens of peripheral lymphocytes, the population of CD3 positive cells and CD3CD4 double positive cells increased in the Hochuekkito-treated group. CONCLUSION: We revealed that Hochuekkito improved the QOL and immunological status of elderly patients with weakness by randomized controlled trial. Our study design might be useful for assessing the efficacy of traditional herbal medicine in the future.

**11) Sekiya N, Shimada Y, Shintani T, Tahara E, Kouta K, Shibahara N, and Terasawa K.: Reduction of perception of chronic fatigue in an observational study of patients receiving 12 weeks of kampo therapy. *J. Altern. Complem. Med.*, 11(5): 895-901, 2005.**

**Abstract:** Objective: The aim of this study was to observe the influence of Kampo therapy on latent chronic fatigue of patients with chronic diseases. Subjects: One hundred and seventy-three (173) consecutive patients with chronic diseases came to our department for the first time. Design: This was a prospective study. Patients were divided into two groups: a chronic fatigue group (CFG) and a nonchronic fatigue group (NCFG). Based on Kampo diagnosis, both groups were prescribed Kampo formulae as an extract or decoction for 12 weeks. Outcome Measures: By using questionnaires, patients were assessed concerning their physical and mental types of fatigue, their sleep situation, and their attitude toward work or housekeeping, both before and after 12 weeks of treatment, according to Kampo diagnosis. Results: The mental fatigue, physical fatigue, and sleep scores of both groups, and the work score of CFG, were decreased. The rate of reduction of the fatigue score was significantly greater in CFG than in NCFG. The factor responsible for this difference in fatigue score was physical fatigue. Conclusions: A reduction of the perception of chronic fatigue was observed in patients receiving 12 weeks of Kampo therapy.

◇ 総 説

- 1) 寺澤捷年：随証治療・その将来の展望. 日本東洋医学雑誌, 56(6), 879-892, 2005.
- 2) Sekiya N., Shimada Y., Terasawa K.: Kampo (Japanese Oriental) Medicine and Atherosclerosis. Commentary for International Atherosclerosis Society Website.

◇ 症例報告 Case reports

- 1) 関矢信康, 引網宏彰, 後藤博三, 柴原直利, 嶋田 豊, 寺澤捷年：梔子柏皮湯が奏功したパニック障害の3症例. 日本東洋医学雑誌, 56(1), 97-101, 2005.
- 2) 引網宏彰, 関矢信康, 古田一史, 嶋田 豊, 寺澤捷年：強直性脊椎炎に対する烏頭剤の使用経験. 日本東洋医学雑誌, 56(2), 281-286, 2005.
- 3) 小尾龍右, 鉄村 進, 平崎能郎, 木村豪雄, 古田一史, 三瀧忠道, 嶋田 豊, 寺澤捷年：早期胃癌に対する幽門側胃切除後の食思不振に香蘇散合六君子湯が奏功した一例. 日本東洋医学雑誌, 56(4), 577-583, 2005.
- 4) 中田真司, 小林 豊, 貝沼茂三郎, 嶋田 豊, 寺澤捷年：補中益気湯合桂枝茯苓丸がB型慢性肝炎に有効であった2症例. 日本東洋医学雑誌, 56(4), 585-590, 2005.
- 5) 関矢信康, 地野充時, 大野賢二, 寺澤捷年：臍疝痛を主訴とする起立性調節障害に対する安中散の応用. 漢方の臨床, 52(9), 1313-1317, 2005.

◇学会報告 (\*: 特別講演、シンポジウム、ワークショップ等)

- 1) 寺澤捷年：随証治療・その将来の展望. 第 56 回日本東洋医学会学術総会, 2005, 5, 富山.
- 2) 関矢信康, 後藤博三, 嶋田 豊, 寺澤捷年：九味檳榔湯の新たな使用目標に関する一考察. 第 56 回日本東洋医学会学術総会, 2005, 5, 富山.
- 3) 関矢信康, 寺澤捷年：動脈硬化と和漢薬—基礎から臨床—. 桂枝茯苓丸の抗酸化作用. 第 22 回和漢医薬学会大会, 2005, 8, 東京.
- 4) 寺澤捷年: 高齢者医療と漢方. 日本内科学会学術集会 第 33 回内科学の展望「高齢化時代の内科学」, 2005, 11, 島根.