民族薬物研究センター　Research Center for Ethnomedicine

薬効解析部　　Division of Biofunctional Evaluation

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◇研究目的

民族薬物研究センター薬効解析部は，民族薬物の機能解析に関する研究を推進するために設置された。主な研究内容は以下のとおりであるが，わが国では高齢化や生活習慣病の増加によって疾病構造に大きな変化をきたし，漢方医学の役割や重要性が再認識されてきている。そのためにも基礎的研究を通じて各種疾患，特に慢性疾患に対する漢方薬や個々の生薬，成分の有効性を科学的に実証することが非常に重要な課題であり，これらを中心とした研究を推進している。

◇研究概要

1) 腎疾患における病態の解明と腎臓病治療薬の開発
2) 糖尿病性腎症における漢方方剤のアプローチと分子生物学的解明
3) 加齢 (老化) 及び加齢関連疾患 (いわゆる老年病) の発症機序に対するアンチエイジング産業への開拓
4) 生活習慣病分子標的因子の解明と治療薬の創出
5) 神経回路網形成および神経変性疾患（認知症，脊髄損傷，注意欠陥多動性障害）に関する基礎的研究と，それらに有効な伝統薬物の研究

◇著書

1) 山辺典子，横澤隆子：八味地黄丸の糖尿病性腎症への探索．「腎とフリーラジカル－第 9 集」，青柳一正，菱田 明監修，腎とフリーラジカル研究会企画，190-197，東京医学社，東京，2008.
2) Lee Y.A, Cho E.J., and Yokozawa T.：Ameliorative effects of proanthocyanidin on streptozotocin-induced diabetes in rats. 「腎とフリーラジカル－第 9 集」，青柳一正，菱田 明監修，腎とフリーラジカル研究会企画，198-205，東京医学社，東京，2008.
3) 横澤隆子，山辺典子，田中 隆：糖尿病における山茱萸の活性成分の探索．「腎とフリーラジカル－第 9 集」，青柳一正，菱田 明監修，腎とフリーラジカル研究会企画，209-215，東京医学社，東京，2008.
4) 金 賢柱，杉野豪俊，大久保 勉，レカ・ラジュ・ジュネジャ，横澤隆子：加齢ラット腎におけるアムラの役割．「腎とフリーラジカル－第 9 集」，青柳一正，菱田 明監修，腎とフリーラジカル研究会企画，216-223，東京医学社，東京，2008.

◇原著論文

1) **Kim H.Y., Oi Y., Kim M., and Yokozawa T.: Protective Effect of Lipoic Acid against Methylglyoxal-Induced Oxidative Stress in LLC-PK$_1$ Cells. J. Nutr. Sci. Vitaminol., 54: 99-104, 2008.**

Abstract: Methylglyoxal (MG), a reactive dicarbonyl compound, is a metabolic byproduct of glycolysis

often found at high levels in blood from diabetic patients. The effect of lipoic acid on MG-induced oxidative stress was investigated using LLC-PK_1 renal tubular epithelial cells, which are susceptible to oxidative stress. MG (500 microM) treatment induced LLC-PK_1 cell death to nearly 50% compared with non-treated control cells, but lipoic acid significantly inhibited the MG-induced cytotoxicity in a concentration-dependent manner. In addition, lipoic acid treatment dose-dependently reduced the intracellular reactive oxygen species level increased by 500 microM MG. The nitric oxide level was also increased by 500 microM MG treatment, but it was significantly inhibited by lipoic acid. Furthermore, lipoic acid treatment at 50 microM inhibited the nuclear translocation of nuclear factor-kappa B induced by MG treatment in LLC-PK_1 cells. These findings indicate that lipoic acid has potential as a therapeutic agent against the development of diabetic complications related to MG-induced oxidative stress in diabetes.

2) Kim H.Y., Jeong D.M., Jung H.J., Jung Y.J., Yokozawa T., and Choi J.S.: Hypolipidemic Effects of Sophora flavescens and Its Constituents in Poloxamer 407-Induced Hyperlipidemic and Cholesterol-Fed Rats. Biol. Pharm. Bull., 31: 73-78, 2008.

Abstract: In this study, we investigated the hypolipidemic effects of Sophora flavescens in poloxamer 407-induced hyperlipidemic and cholesterol-fed rats. The MeOH extract and 4 fractions of S. flavescens were administered at doses of 250 and 100 mg/kg body weight, respectively, once a day for 3 d to the poloxamer 407-induced hyperlipidemic rats. Serum lipid levels such as total cholesterol (TC), triglycerides (TG), and low-density lipoprotein-cholesterol (LDL-C) were markedly elevated in the poloxamer 407-induced hyperlipidemic control rats, while lipid levels were significantly decreased in the rats administered the MeOH extract or 4 fractions of S. flavescens. In addition, serum high-density lipoprotein-cholesterol (HDL-C) was reduced in the poloxamer 407-induced hyperlipidemic control rats. However, oral administration of both the MeOH extract and 4 fractions significantly increased HDL-C levels. Of the tested fractions, the EtOAc fraction showed the strongest lipid-lowering effect, as well as a high antiatherogenic potential with atherogenic index (A.I.) values of less than 1.92. We also investigated the hypolipidemic effects of the main compounds of the EtOAc fraction, kurarinol and kuraridinol, using the hyperlipidemic and hypercholesterolemic animal models. Here, elevated TC, TG, and LDL-C levels in the poloxamer 407-induced hyperlipidemic and cholesterol-fed rats were significantly reduced after oral administration of the compounds, and HDL-C levels had a significant increase. Furthermore, A.I. values were lowered by administering kurarinol and kuraridinol. In particular, kuraridinol exhibited stronger protective activities against hyperlipidemia than kurarinol. These results suggest that S. flavescens and its constituents may be effective cholesterol-lowering agents and useful for preventing hypercholesterolemic atherosclerosis.

3) Kang K.S.*, Yamabe N., Kim H.Y., Park J.H., and Yokozawa T.: Therapeutic potential of 20(*S*)-ginsenoside Rg_3 against streptozotocin-induced diabetic renal damage in rats. Eur. J. Pharmacol., 591: 266-272, 2008.

Abstract: The inhibitors of advanced glycation endproduct and oxidative stress, as well as N-methyl-d-aspartate (NMDA) receptor antagonists have received considerable interest because of their close association with renoprotective effects. The therapeutic potential of 20(*S*)-ginsenoside Rg_3 (20(*S*)-Rg_3), isolated from Panax ginseng, against streptozotocin-induced diabetic renal damage, was investigated in this study. The diabetic rats received 5, 10, and 20 mg/kg body weight/day of 20(*S*)-Rg_3 orally via gavage for fifteen consecutive days. The physiological abnormalities such as increases in water intake and urine volume of diabetic rats were significantly decreased by the 20 mg/kg body weight of 20(*S*)-Rg_3 administration. The elevated serum glucose, glycosylated protein, and thiobarbituric acid-reactive substance levels in diabetic rats were also significantly reduced by the 20(*S*)-Rg_3 administrations. Moreover, the renal dysfunction of diabetic rats was significantly ameliorated by the 20(*S*)-Rg_3 administrations in a dose-dependent manner. These beneficial effects on diabetic renal damage were related to the inhibitory effect of 20(*S*)-Rg_3 against NMDA receptor-mediated nitrosative stress.

4) Kang K.S.*, Yamabe N., Kim H.Y., and Yokozawa T.: Role of maltol in advanced glycation end products and free radicals: in-vitro and in-vivo studies. J. Pharm. Pharmacol., 60: 445-452, 2008.

Abstract: Inhibitors of advanced glycation end products (AGEs) have potential as preventive agents against diabetic complications. In-vitro AGE inhibitory activity, transition metal chelating, and free radical scavenging activity tests have been used to screen for and identify effective AGE inhibitors. In an ongoing project to elucidate AGE inhibiting active components of heat-processed ginseng, maltol was selected for more detailed investigation. Although there are several lines of evidence concerning the antioxidant activity of maltol, the in-vitro and in-vivo inhibitory effects of maltol on AGE generation have not been evaluated. In the present study, the in-vitro AGE inhibitory effects and free radical scavenging activity of maltol were investigated. In addition, the in-vivo therapeutic potential of maltol against diabetic renal damage was tested using streptozotocin (STZ)-diabetic rats. Maltol showed a stronger AGE inhibitory effect than aminoguanidine, a well known AGE inhibitor. In addition, the hydroxyl radical scavenging activity of maltol on electron spin resonance (ESR) spectrometry was slightly stronger than that of aminoguanidine. Therefore, maltol was found to have stronger in-vitro AGE inhibiting activity compared with aminoguanidine. The administration of 50 mgkg(-1) per day of maltol suppressed the elevated serum levels of glycosylated protein, renal fluorescent AGEs, carboxymethyllysine, receptors for AGEs, and nuclear factor-kappaB p65 in diabetic control rats. These beneficial effects of maltol against STZ-diabetic renal damage were thought to result from its free radical scavenging and AGE inhibitory effects.

5) Yokozawa T., Kim H.J., and Cho E.J.: Gravinol Ameliorates High-Fructose-Induced Metabolic Syndrome through Regulation of Lipid Metabolism and Proinflammatory State in Rats. J. Agric. Food Chem., 56: 5026-5032, 2008.

Abstract: Using a rat model with fructose-induced metabolic syndrome, the effect of gravinol was investigated. Male Wistar rats were fed a 65% fructose diet and administered 10 or 20 mg of gravinol/kg of body weight/day for 2 weeks. High-level fructose feeding led to hyperglycemia, hyperlipidemia, hypertri-glyceridemia, and hypertension. On the other hand, the administration of gravinol significantly lowered serum glucose and total cholesterol levels. The tail arterial blood pressure was significantly elevated with the high-fructose diet. However, rats given gravinol showed a lower blood pressure as compared with fructose-fed control rats. In addition, the triglyceride (TG) levels in serum and lipoprotein fraction were dose-dependently reduced in rats fed gravinol. The decreases of hepatic TG and total cholesterol by gravinol were responsible for the down-regulation of hepatic sterol regulatory element binding protein (SREBP)-1. However, gravinol did not affect the protein levels of hepatic peroxisome proliferator-activated receptor-alpha and SREBP-2. Moreover, gravinol administration in the fructose-fed rats markedly reduced the glycosylated protein and thiobarbituric acid-reactive substance levels in the serum and hepatic mitochondria, and it inhibited the increase of the cyclooxygenase-2 protein level as a result of the down-regulation of nuclear factor kappa B (NF-kappaB). Furthermore, the decrease of anti-apoptotic bcl-2 protein levels and the increase of pro-apoptotic bax protein levels by the high-fructose diet were reversed by gravinol. These findings suggest that fructose-induced metabolic syndrome is attenuated by gravinol administration, which is associated with the reduction of serum lipids and protection against the proinflammatory state induced by oxidative stress.

6) Kim J.Y., Kim H.S., Kang H.S., Choi J.S., Yokozawa T., and Chung H.Y.: Antioxidant Potential of Dimethyl Lithospermate Isolated from *Salvia miltiorrhiza* (Red Sage) Against Peroxynitrite. J. Med. Food, 11: 21-28, 2008.

Abstract: Peroxynitrite ($ONOO^-$) is a reactive oxidant formed from superoxide and nitric oxide that can readily oxidize cellular components, including essential protein, non-protein thiols, and DNA. $ONOO^-$ has contributed to the pathogenesis of diseases such as stroke, heart disease, Alzheimer's disease, and atherosclerosis. In this study, the ability of dimethyl lithospermate (DML), isolated from *Salvia miltiorrhiza*, to scavenge $ONOO^-$ and to protect cells against reactive species and $ONOO^-$ was

investigated. The data obtained show that DML can efficiently scavenge native $ONOO^-$ as well as $ONOO^-$ derived from the $ONOO^-$ donor 3-morpholinosydnonimine hydrochloride. Spectrophotometric analysis revealed that DML led to decreased $ONOO^-$-mediated nitration of tyrosine through electron donation. DML significantly inhibited nitration of bovine serum albumin by $ONOO^-$ in a dose-dependent manner. DML also manifested cytoprotection from cell damage induced by $ONOO^-$. The present study suggests that DML is an effective $ONOO^-$ scavenger and promotes cellular defense activity in the protection against $ONOO^-$-involved diseases.

7) Lee Y.A*, Cho E.J., and Yokozawa T.: Protective Effect of Persimmon (*Diospyros Kaki*) Peel Proanthocyanidin against Oxidative Damage under H_2O_2-Induced Cellular Senescence. Biol. Pharm. Bull., 31: 1265-1269, 2008.

Abstract: 8-Hydroxy-2'-deoxyguanosine (8-OHdG), one of the most abundant oxidative DNA adducts, is used as an indicator of oxidative DNA damage associated with aging. Among homologs of the silent information regulator (Sir), sirtuin 1 (SIRT1) is suggested as a regulator of the apoptotic response to DNA damage. Since it has been suggested that the aging process can be delayed by the attenuation of oxidative damage such as DNA damage or SIRT1 modulation, we focused on the protective effect against cellular oxidative damage of persimmon peel, a proanthocyanidin-rich food, in relation to its level of polymerization. We confirmed that 8-OHdG expression in TIG-1 human fibroblasts was increased by treatment with 300 microM H_2O_2 for 2 h. On the other hand, the nuclear SIRT1 level was decreased in H_2O_2-treated as compared with non-pretreated cells. However, pretreatments with polymers and oligomers led to a decrease in 8-OHdG and elevation in nuclear SIRT1 expression in a concentration-dependent manner. In particular, oligomers exerted a stronger effect. The present study supports the protective potential of proanthocyanidin from persimmon peel against oxidative damage under the aging process, and suggests that the polymerization of proanthocyanidin plays an important role in retarding aging in a cellular senescence model.

8) Lee Y.A*, Cho E.J., and Yokozawa T.: Effects of Proanthocyanidin Preparations on Hyperlipidemia and Other Biomarkers in Mouse Model of Type 2 Diabetes. J. Agric. Food Chem., 56: 7781-7789, 2008.

Abstract: The protective effect of proanthocyanidins from persimmon peel, using both oligomers and polymers, was investigated in a db/db type 2 diabetes model. Male db/db mice were divided into three groups: control (vehicle), polymer-, or oligomer- (10 mg/(kg body weight x day x p.o.)) administered mice. Age-matched nondiabetic m/m mice were used as a normal group. The administration of proanthocyanidins reduced hyperglycemia in db/db mice through a decline in the serum level of glucose and glycosylated protein. In addition, it had a strong effect on hyperlipidemia through lowering levels of triglyceride, total cholesterol, and nonesterified fatty acids. The protective effect against hyperglycemia and hyperlipidemia was greater in the groups administered the oligomeric rather than polymeric form. The increased oxidative stress in db/db mice was attenuated by the administration of oligomers through inhibiting the generation of reactive oxygen species and lipid peroxidation and elevating the reduced glutathione/oxidized glutathione ratio. On the other hand, polymers did not show such an effect. Moreover, expressions in the liver of sterol regulatory element binding protein (SREBP)-1 and SREBP-2 were downregulated by the administration of proanthocyanidins, especially the oligomeric form. Oligomers caused a slight elevation in the expression of peroxisome proliferator-activated receptors alpha. Furthermore, oligomeric proanthocyanidin regulated the expression of nuclear factor kappaB in db/db type 2 diabetes via the activation of inhibitor protein kappaB-alpha. It also attenuated the protein expressions of cyclooxygenase-2 and inducible nitric oxide synthase. This suggests that oligomers would act as a regulator in inflammatory reactions associated with oxidative stress in type 2 diabetes. The present study results suggest that proanthocyanidin administration, especially the oligomeric form, may improve

oxidative stress via the regulation of hyperlipidemia than hyperglycemia in type 2 diabetes.

9) Kim Y.J., Kang K.S., and Yokozawa T.: The anti-melanogenic effect of pycnogenol by its anti-oxidative actions. Food Chem. Toxicol., 46: 2466-2471, 2008.

Abstract: Pycnogenol is a natural plant extract from pine bark that contains compounds that have anti-oxidative, free-radical scavenging properties. In this work, utilizing cultured B16 melanoma cells (B16 cells), pycnogenol was investigated for its ability to inhibit tyrosinase activity and melanin biosynthesis. We also examined the anti-oxidative power of pycnogenol by measuring its suppressive effect against peroxynitrite ($ONOO^-$), superoxide ($.O_2$), nitric oxide (NO.), and hydroxyl radical (.OH)-scavenging activities using an electron spin resonance spectrometer. Results show that pycnogenol had a strong anti-tyrosinase activity and suppressed melanin biosynthesis. Further, our results showed that through its anti-oxidative properties, pycnogenol suppressed $.O_2$, NO., $ONOO^-$, and .OH in *in vitro* assays, and reactive species, $ONOO^-$, $.O_2$, and NO., while up-regulating the reduced glutathione/oxidized glutathione ratio in B16 cells. Based on the findings, we propose that pycnogenol exerts anti-melanogenic activity *via* its anti-oxidative actions.

10) Rhyu D.Y., Kang K.S., Sekiya M., Tanaka T., Park J.C., and Yokozawa T.: Active Compounds Isolated from Traditional Chinese Prescription Wen-Pi-Tang Protecting Against Peroxynitrite-Induced LLC-PK_1 Cell Damage. Am. J. Chin. Med., 36: 761-770, 2008.

Abstract: Wen-Pi-Tang, a traditional Chinese prescription, has been widely used for the treatment of patients with moderate chronic renal failure in China. Although the protective effect of Wen-Pi-Tang on peroxynitrite ($ONOO^-$)-induced renal tubular epithelial LLC-PK_1 cell damage was elucidated in our previous research, the active components of Wen-Pi-Tang have not yet been fully clarified. Therefore in the present study, we investigated the active components by using a cellular $ONOO^-$ generation system. As a result, p-coumaric acid, 4-(4'-hydroxylphenyl)-2-butanone 4'-*O*-glucopyranoside, gallic acid 3-*O*-(6'-*O*-galloyl)-beta-d-glucopyranoside, procyanidin B-1, procyanidin B-3, and (+)-catechin were isolated as active compounds inhibiting cellular $ONOO^-$ formation and cytotoxicity. In particular, the content of (+)-catechin was significantly higher than those of the other compounds, and the (+)-catechin structure was located in procyanidins B-1 and B-3. Therefore, the major bioactivity of Wen-Pi-Tang against $ONOO^-$-induced cytotoxicity in LLC-PK_1 cells was thought to be mediated by (+)-catechin. Although we cannot disregard the synergetic effect of various components in Wen-Pi-Tang, (+)-catechin is a major active compound protecting against $ONOO^-$-induced LLC-PK_1 cell damage and may be used as an index to qualify the $ONOO^-$-inhibitory activity of Wen-Pi-Tang extract.

11) Lee Y.J., Kim H.Y., Kang K.S., Lee J.G., Yokozawa T., and Park J.H.: The chemical and hydroxyl radical scavenging activity changes of ginsenoside-Rb_1 by heat processing. Bioorg. Med. Chem. Lett., 15: 4515-4520, 2008.

Abstract: The chemical and hydroxyl radical (*OH) scavenging activity changes of ginsenoside Rb_1 (Rb_1) by heat processing were investigated in this study. Rb_1 was changed into 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1, and Rg_5 by heat processing through glucosyl elimination and epimerization of carbon-20 by SN1 reaction. The glucosyl moiety, separated from Rb_1, made Maillard reaction product (MRPs) with glycine. The generations of 20(*S*)-Rg_3 and MRPs were related to the increased OH scavenging activity of Rb_1 by heat processing.

12) Cho E.J., Okamoto T., and Yokozawa T.: Therapeutic efficacy of Kangen-karyu against H_2O_2-induced premature senescence. J. Pharm. Pharmacol., 60: 1537-1544, 2008.

Abstract: The anti-aging potential of Kangen-karyu extract was investigated using the mechanisms of the cellular aging model of stress-induced premature senescence (SIPS) in TIG-1 human fibroblasts. SIPS was induced by a sublethal dose of H_2O_2 and chronic oxidative stress with repeat treatment of low-dose H_2O_2.

Reactive oxygen species generation, lipid peroxidation, and senescence-associated beta-galactosidase activity were elevated in TIG-1 cells under SIPS induced by H_2O_2. However, Kangen-karyu extract led to significant declines in these parameters, suggesting its role in ameliorating oxidative stress-related aging. It was also observed that SIPS due to H_2O_2 treatment led to the loss of cell viability, whereas Kangen-karyu extract improved cell viability by attenuating H_2O_2-induced oxidative damage. TIG-1 cells under the condition of SIPS caused by sublethal and chronic low doses of H_2O_2 showed nuclear factor-kappaB (NF-kappaB) translocation to the nucleus from the cytosol, while Kangen-karyu extract inhibited NF-kappaB nuclear translocation, implying that Kangen-karyu extract could exert an anti-aging effect through NF-kappaB modulation. In addition, treatment with Kangen-karyu extract under H_2O_2-induced chronic oxidative stress normalized the cell cycle by reducing the number of cells in the G_0/G_1 phase and elevating the proportion of those in the S phase, indicating the role of Kangen-karyu extract in cell cycle regulation. On the other hand, Kangen-karyu extract did not exert such an effect on cell cycle regulation under acute oxidative stress induced by sublethal H_2O_2. Furthermore, treatment with Kangen-karyu extract prolonged the lifespan of TIG-1 cells under SIPS. The present study suggests that Kangen-karyu might play a therapeutic role against the aging process caused by oxidative stress.

13) Yokozawa T., Yamabe N., Kim H.Y., Kang K.S., Hur J.M., Park C.H., and Tanaka T.: Protective Effects of Morroniside Isolated from Corni Fructus against Renal Damage in Streptozotocin-Induced Diabetic Rats. Biol. Pharm. Bull., 31: 1422-1428, 2008.

Abstract: In our previous study, we reported the renoprotective effect of Hachimi-jio-gan, a Chinese traditional prescription consisting of eight medicinal plants, and also reported the effect of Corni Fructus (*Cornus officinalis* SIEB. *et* ZUCC.), a component of Hachimi-jio-gan, on diabetic nephropathy using diabetic rats. In this study, we investigated the effects of morroniside isolated from Corni Fructus on renal damage in streptozotocin-treated diabetic rats. Oral administration of morroniside at a dose of 20 or 100 mg/kg body weight/d for 20 d to diabetic rats resulted in significant decreases in increasing serum glucose and urinary protein levels. Moreover, the decreased levels of serum albumin and total protein in diabetic rats were significantly increased by morroniside administration at a dose of 100 mg/kg body weight/d. In addition, morroniside significantly reduced the elevated serum urea nitrogen level and showed a tendency to reduce creatinine clearance. Morroniside also significantly reduced the enhanced levels of serum glycosylated protein, and serum and renal thiobarbituric acid-reactive substances. Protein expressions related to the advanced glycation endproduct (AGE) level and actions, oxidative stress such as N(epsilon)-(carboxyethyl)lysine, as well as receptors for AGE and heme oxygenase-1 were increased in diabetic rats, but the levels were also significantly decreased by the administration of morroniside. This suggests that morroniside exhibits protective effects against diabetic renal damage by inhibiting hyperglycemia and oxidative stress. These results indicate that morroniside is one component partly responsible for the protective effects of Corni Fructus and Hachimi-jio-gan against diabetic renal damage.

14) Kim H.Y., Kang K.S., Yamabe N., and Yokozawa T.: Comparison of the Effects of Korean Ginseng and Heat-Processed Korean Ginseng on Diabetic Oxidative Stress. Am. J. Chin. Med., 36: 989-1004, 2008.

Abstract: To investigate the effects of Korean ginseng (KG, *Panax ginseng* C.A. Meyer) and heat-processed Korean ginseng (H-KG) on diabetic renal damage, we used the streptozotocin-induced diabetic rat model in this study. The diabetes-induced physiological abnormalities at early-stage were attenuated by KG or H-KG administration through reducing the blood glucose level and improving renal function. The oxidative stress-induced increases in serum and renal thiobarbituric acid-reactive substance levels were significantly reduced by KG and H-KG administrations. Moreover, the protein expressions related to oxidative stress and advanced glycation endproducts were significantly reduced in diabetic rats and/or not significantly increased compared to normal rats by KG or H-KG administration. All of these

beneficial effects of H-KG in diabetic rats were stronger than those of KG. Therefore, KG and H-KG may improve diabetic pathological conditions and prevent renal damage associated with diabetic nephropathy, and these protective effects of KG can be improved by heat-processing. This study provides scientific evidence that H-KG may be a potential therapeutic agent for pathological conditions associated with diabetic complications including diabetic nephropathy.

15) Tohda C., Naito R., and Joyashiki E.: Kihi-to, a herbal traditional medicine, improves Aβ(25-35)-induced memory impairment and losses of neurites and synapses. BMC Complementary and Alternative Medicine, 8: 49, 2008.

Abstract: Background: We previously hypothesized that achievement of recovery of brain function after the injury requires the reconstruction of neuronal networks, including neurite regeneration and synapse reformation. Kihi-to is composed of twelve crude drugs, some of which have already been shown to possess neurite extension properties in our previous studies. The effect of Kihi-to on memory deficit has not been examined. Thus, the goal of the present study is to determine the in vivo and in vitro effects of Kihi-to on memory, neurite growth and synapse reconstruction.

Methods: Effects of Kihi-to, a traditional Japanese-Chinese traditional medicine, on memory deficits and losses of neurites and synapses were examined using Alzheimer's disease model mice. Improvements of Aβ(25–35)-induced neuritic atrophy by Kihi-to and the mechanism were investigated in cultured cortical neurons.

Results: Administration of Kihi-to for consecutive 3 days resulted in marked improvements of Aβ(25–35)-induced impairments in memory acquisition, memory retention, and object recognition memory in mice. Immunohistochemical comparisons suggested that Kihi-to attenuated neuritic, synaptic and myelin losses in the cerebral cortex, hippocampus and striatum. Kihi-to also attenuated the calpain increase in the cerebral cortex and hippocampus. When Kihi-to was added to cells 4 days after Aβ(25–35) treatment, axonal and dendritic outgrowths in cultured cortical neurons were restored as demonstrated by extended lengths of phosphorylated neurofilament-H (P-NF-H) and microtubule-associated protein (MAP)2-positive neurites. Aβ(25–35)-induced cell death in cortical culture was also markedly inhibited by Kihi-to. Since NF-H, MAP2 and myelin basic protein (MBP) are substrates of calpain, and calpain is known to be involved in Aβ-induced axonal atrophy, expression levels of calpain and calpastatin were measured. Treatment with Kihi-to inhibited the Aβ(25–35)-evoked increase in the calpain level and decrease in the calpastatin level. In addition, Kihi-to inhibited Aβ(25–35)-induced calcium entry.
Conclusion: In conclusion Kihi-to clearly improved the memory impairment and losses of neurites and synapses.

16) Tohda C., Ichimura M., Bai Y., Tanaka K., Zhu S., and Komatsu K.: Inhibitory effects of Eleutherococcus senticosus extracts on amyloid β(25-35)-induced neuritic atrophy and synaptic loss. J. Pharmacol. Sci., 107: 329-339, 2008.

Abstract: Neurons with atrophic neurites may remain alive and therefore may have the potential to regenerate even when neuronal death has occurred in some parts of the brain. This study aimed to explore effects of drugs that can facilitate the regeneration of neurites and the reconstruction of synapses even in severely damaged neurons. We investigated the effects of *Eleutherococcus senticosus* extracts on the regeneration of neurites and the reconstruction of synapses in rat cultured cortical neurons damaged by amyloid beta (Abeta)(25-35). Treatment with Abeta(25-35) (10 microM) induced axonal and dendritic atrophies and synaptic loss in cortical neurons. Subsequent treatment with the methanol extract and the water extract of *E. senticosus* (10 - 1000 ng/ml) resulted in significant axonal and dendritic regenerations and reconstruction of neuronal synapses. Co-application of the extract and Abeta(25-35) attenuated Abeta(25-35)-induced neuronal death. We investigated neurite outgrowth activities of eleutherosides B and E and isoflaxidin, which are known as major compounds in *E. senticosus*. Although eleutheroside B

protected against Abeta(25-35)-induced dendritic and axonal atrophies, the activities of eleutheroside E and isofraxidin were less than that of eleutheroside B. Although the contents of these three compounds in the water extract were less than in the methanol extract, restoring activities against neuronal damages were not different between the two extracts. In conclusion, extracts of *E. senticosus* protect against neuritic atrophy and cell death under Abeta treatment, and one of active constituents may be eleutheroside B.

◇総　説

1) 横澤隆子: 糖尿病向け漢方薬. Functional Food, 2: 23-30, 2008.
2) 東田千尋: 伝統薬物による神経変性疾患の克服 －治療薬開発と病態機序の解明に向けて－. 薬学雑誌, 128: 1159-1167, 2008.

◇学会報告　（*：特別講演，シンポジウム，ワークショップ等）

* 1) Lee Y.A, Cho E.J., Yokozawa T.: Anti-Aging Effects of Oligomeric Proanthocyanidin Isolated from Persimmon Fruits. The 12th International Conference on Oriental Medicine of Dong-Eui & Daegu Haany University, 2008, 8, 21, Busan (Invited lecture).
* 2) Tohda C. : The study of natural medicine for exploring key cascades related to neurodegenerative diseases. Educational Workshop “New Possibilities of Traditional Oriental Medicine in Neuropsychiatry” 2nd WFSBP Asia-Pasific Congress, 2008, 9, 11, Toyama (Invited lecture).
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2) 青柳一正：筑波技術大学, 田中 隆：長崎大学薬学部, 柏田良樹：新潟薬科大学薬学部, 金賢栄：ソウル大学薬学部, 永井竜児：熊本大学病態生化学, 野中源一郎：ウサイエン製薬(株)「抗酸化に関する研究」

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◇研究費取得状況

1) 文部科学省科学研究費, 基盤研究 C (代表：横澤隆子)「柿ポリフェノールオリゴマーの製造技術の確立と抗加齢機能性食品の開発」

2) 経済産業省「地域新生コンソーシアム研究開発事業」(分担：横澤隆子)「柿ポリフェノールオリゴマーを用いた抗加齢機能製品の開発」

3) つくし奨学・研究基金 (代表：横澤隆子)「糖尿病性腎症に対する漢方方剤治療の基礎的研究」「山茱萸成分の抗糖尿病作用」

4) 富山大学学長裁量経費，女性研究者リーダーシップ育成助成　(代表：東田千尋)「伝統薬物による生体機能の革新的解析および創薬戦略」

5) 知的クラスター創成事業（Ⅱ）広域化プログラム（分担：東田千尋)「天然薬物の遺伝子解析等に基づく標準化」

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卒業論文：

浦野卓矢：Icariin による記憶亢進作用に関する研究～アルツハイマー病治療薬の創薬シーズを探索するアプローチ～

長田愛子：淫羊藿（*Epimedium koreanum* の地上部）エキスによる脊髄損傷機能改善の薬理学的研究

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