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) Yokozawa T., Yamabe N., and Kang K.S.: Safety and efficacy of Hachimi-jio-gan: a Chinese preparation to counteract oxidative damage, In Evaluation of Herbal Medicinal Products, by Houghton P., and Mukherjee P.K. (Ed.) 340-356, Pharmaceutical Press, UK, 2009.

◇原著論文

1) **Zou Y., Kim D.H., Jung K.J., Heo H.S., Kim C.H., Baik H.S., Yu B.P., Yokozawa T., and Chung H.Y.: Lysophosphatidylcholine Enhances Oxidative Stress Via the 5-Lipoxygenase Pathway in Rat Aorta During Aging. Rejuvenation Res., 12: 15-24, 2009.**

Abstract: Lysophosphatidylcholine (LPC) is a lysolipid, acting as a potent cellular mediator of various biological processes. The purpose of this study was to define the role of LPC as a possible causative factor of disrupted redox balance in aged aorta from rats. In this study, we found elevated serum LPC levels in 24-month-old rats that were correlated with the age-related increase in cytosolic phospholipase A_2 (PLA_2) activity. We also found that aortas from old rats showed increased 5-lipoxygenase (5-LO) activity. With the LPC-treated endothelial cells (YPEN-1 cells), we observed a rapid generation of reactive species, leading to enhanced oxidative stress. Our further investigations using specific 5-LO inhibitors led to the identification of a 5-LO pathway as the reactive species production source in the LPC-treated cells. Additional validation of this 5-LO pathway was made by the detection of increased leukotriene B4 generation in the LPC-treated cells. These *in vitro* data supported findings of increased expression and

activation of aortic 5-LO in old rats by LPC. Together, our data strongly suggested that LPC caused the enhancement of oxidative stress in aged aorta through reactive species generation by an activated 5-LO pathway. LPC may well be an important contributor to age-related oxidative stress in aging aorta.

2) **Sekiya M., Ichiyanagi T., Ikeshiro Y., and Yokozawa T.: The Chinese Prescription Wen-Pi-Tang Extract Delays Disease Onset in Amyotrophic Lateral Sclerosis Model Mice While Attenuating the Activation of Glial Cells in the Spinal Cord. Biol. Pharm. Bull., 32: 382-388, 2009.**

Abstract: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by the selective loss of motor neurons. There is no effective treatment or drug against ALS, and the precise mechanisms leading to the selective loss of motor neurons are still unknown. We investigated the effect of a Chinese prescription, Wen-Pi-Tang, on the ALS model mouse $SOD1^{G93A}$. Although the oral administration of Wen-Pi-Tang extract to $SOD1^{G93A}$ mice had no significant effect on body weight loss and survival time, Wen-Pi-Tang delayed disease onset. Therefore, we evaluated immunohistological changes in the spinal cord of $SOD1^{G93A}$ mice during the early disease period, and found that Wen-Pi-Tang extract inhibited neuronal loss in the lumbar segment of the spinal cord of mice. Furthermore, increased astrocytes and microglial cells, which increase prior to neuronal loss, in spinal cords were significantly reduced in the Wen-Pi-Tang treated group. Since oxidative markers, heme oxygenase-1 and inducible nitric oxide synthase, in the spinal cord were also reduced as well as the change in microglia, the administration of Wen-Pi-Tang was thought to delay disease onset by inhibiting glial cell activation.

3) **Kang K.S., Tanaka T., Cho E.J., and Yokozawa T.: Evaluation of the Peroxynitrite Scavenging Activity of Heat-Processed Ginseng. J. Med. Food, 12: 124-130, 2009.**

Abstract: To ascertain the principal active peroxynitrite ($ONOO^-$) scavenging components of heat-processed *Panax ginseng* C.A. Meyer (sun ginseng [SG]), the $ONOO^-$ scavenging activities of fractions and components of SG were compared. The results demonstrated that the $ONOO^-$ scavenging ability of SG was due to its ether fraction containing phenolic compounds. High-performance liquid chromatography analysis and $ONOO^-$ scavenging activity tests of the phenolic acids contained in SG identified vanillic acid, ferulic acid, *p*-coumaric acid, syringic acid, and maltol as the main active $ONOO^-$ scavenging components of SG. The $ONOO^-$ scavenging activities of phenolic acids and maltol were dependent on the degrees of their proton donating ability.

4) **Pu F., Motohashi K., Kaneko T., Tanaka Y., Manome N., Irie K., Takata J., Egashira N., Oishi R., Okamoto T., Sei Y., Yokozawa T., Mishima K., Iwasaki K., and Fujiwara M.: Neuroprotective Effects of *Kangen-karyu* on Spatial Memory Impairment in an 8-Arm Radial Maze and Neuronal Death in the Hippocampal CA1 Region Induced by Repeated Cerebral Ischemia in Rats. J. Pharmacol. Sci., 109: 424-430, 2009.**

Abstract: In the present study, we investigated the neuroprotective effects of *Kangen-karyu* (KGK) in a repeated cerebral ischemia model (2 × 10 min, 1-h interval). A 21-day pre- and post-ischemic treatment with KGK (10 – 300 mg/kg) and aspirin (5 mg/kg) improved the spatial memory impairment and neuronal death in the hippocampal CA1 region induced by repeated cerebral ischemia. However, a 7-day post-ischemic treatment with KGK did not attenuate the spatial memory impairment and neuronal death in this model. To determine the mechanism of action of KGK, we investigated the effects of a 14-day pre-ischemic treatment with KGK on cerebral blood flow in the hippocampal area of the repeated cerebral ischemia model using laser Doppler flowmetry. The 14-day pre-ischemic treatment with KGK increased the cerebral blood flow during reperfusion. These results suggest that a 21-day pre- and post-ischemic treatment with KGK can protect against brain damage caused by cerebral ischemia by increasing the cerebral blood flow in the hippocampal area.

5) **Yamabe N., Kang K.S., Park C.H., Tanaka T., and Yokozawa T.: 7-*O*-Galloyl-D-sedoheptulose Is a Novel Therapeutic Agent against Oxidative Stress and**

Advanced Glycation Endproducts in the Diabetic kidney. Biol. Pharm. Bull., 32: 657-664, 2009.

Abstract: Diabetes is the leading cause of end-stage renal failure, since glucose-dependent metabolic factors are synergistically activated within the diabetic kidney. Accordingly, in Japan, there is much debate over the health benefits of natural therapies to reduce these risk factors. In our previous study, we reported that *Cornus officinalis* SIEB. *et* ZUCC. possessed an antidiabetic effect *via* ameliorating glucose-mediated metabolic disorders as well as aminoguanidine, an inhibitor of advanced glycation endproduct (AGE) formation, with a renoprotective effect. The aim of the present study was to investigate the effect of 7-*O*-galloyl-D-sedoheptulose (GS) against diabetic oxidative stress and AGE formation. Streptozotocin-induced diabetic rats were orally administered GS for 20 d, and the changes in serum glucose levels, as well as those of body weight every 10 d were evaluated. In addition, glucose, fluorescent AGE, methylglyoxal, glycolaldehyde (GA), and immunoblotting analyses for heme oxygenase-1, receptor for AGE, N^{ϵ}-(carboxymethyl)lysine, N^{ϵ}-(carboxyethyl)lysine, and GA-pyridine were performed in the kidney at the end of the experiment. The results obtained in this study demonstrated that 20 d of treatment with GS had beneficial effects on hypoglycemic and renal metabolic abnormalities, including renal glucose, oxidative stress, and AGE formation. Together, our data help to elucidate its potential therapeutic value against diabetic kidney disease.

6) **Jang M.H., Kim H.Y., Kang H.S., Yokozawa T., and Park J.H.: Hydroxyl Radical Scavenging Activities of Isoquinoline Alkaloids Isolated from *Coptis chinensis*. Arch. Pharm. Res., 32: 341-345, 2009.**

Abstract: The hydroxyl radical (•OH) scavenging and ferrous ion chelating activities of four isoquinoline alkaloids isolated from *Coptis chinensis* Franch were studied for the identification of their structural characteristics to scavenge •OH. The •OH was generated via Fe(II)-catalazed Fenton reaction in this study and the reliable measurement of •OH scavenging activities of isoquinoline alkaloids were achieved using electron spin resonance (ESR) spectrometry method. At the 1 mM concentration, berberrubine (85%) showed the strongest •OH scavenging activity and the next were in the decreasing order of coptisine (79%), berberine (23%), and palmatine (22%). The ferrous ion chelating effects of the alkaloids showed similar pattern with their •OH scavenging effects. These results suggest that •OH scavenging effects of the alkaloids were closely related to their ferrous ion chelating activities. In addition, metal chelating functional groups such as hydroxy group at C-9 and methylenedioxy group at C-9 and C-10 were thought to contribute to the •OH scavenging activities of the isoquinoline alkaloids.

7) **Chung S.W., Kim M.K., Chung J.H., Kim D.H., Choi J.S., Anton S., Seo A.Y., Park K.Y., Yokozawa T., Rhee S.H., Yu B.P., and Chung H.Y.: Peroxisome Proliferator-Activated Receptor Activation by a Short-Term Feeding of Zingerone in Aged Rats. J. Med. Food, 12: 345-350, 2009.**

Abstract: Peroxisome proliferator-activated receptors (PPARs), members of the nuclear hormone receptor family, are key regulators of various metabolic pathways related to lipid and glucose metabolism as well as inflammation. We examined the effect of zingerone, a major ingredient of ginger, on PPAR, hepatic nuclear factor-4 (HNF-4), and nuclear factor-κB (NF-κB) expression in 21-month-old male Sprague-Dawley rats. Two experimental groups receiving doses of either 2 or 8 mg/kg/day zingerone for 10 days were compared with young rats (6 months old) and an age-matched control group. For molecular work, the endothelial cell line YPEN-1 was used. Both the 2 and 8 mg/kg/day dose of zingerone significantly increased DNA binding activities of PPARs (2.8-fold). Expression of HNF-4 was also increased in the group receiving the 8 mg/kg/day dose. We further showed that zingerone partially prevented the age-related decline in PPAR expression. *In vitro* experiments revealed zingerone (10 μM) increased PPAR expression (2.5-fold) to a similar extent as the PPAR agonist fibrate (5 μM) and suppressed pro-inflammatory transcription factor NF-κB activity. Collectively, our findings suggest that zingerone exerts its potent anti-inflammatory action by increasing HNF-4 and PPAR activities, while suppressing NF-κB activity.

8) **Yokozawa T., Park C.H., Noh J.S., Tanaka T., and Cho E.J.: Novel action of 7-*O*-galloyl-D-sedoheptulose isolated from Corni Fructus as a hypertriglyceridaemic agent. J. Pharm. Pharmacol., 61: 653-661, 2009.**

Abstract: OBJECTIVES: We investigated the lipid-lowering activity of 7-O-galloyl-D-sedoheptulose, an active component of Corni Fructus, and related mechanisms. METHODS: Rats were fed a high-fructose diet for 6 days, followed by treatment with 7-O-galloyl-D-sedoheptulose, 5, 10 or 20 mg/kg per day, or fenofibrate (positive control). KEY FINDINGS: The high-fructose diet induced an increase in body weight, hypertriglyceridaemia, hyperglycaemia and hypertension. Administration of 7-O-galloyl-D-sedoheptulose significantly reduced the levels of triglyceride in the serum and liver (being more effective than fenofibrate) but did not lead to changes in liver weight or hepatic function, whereas fenofibrate increased the liver weight markedly. The preventive effect of 7-O-galloyl-D-sedoheptulose against the accumulation of triglyceride and cholesterol was related to the up-regulation of peroxisome proliferator-activated receptor alpha expression. CONCLUSIONS: The present study supports the role of 7-O-galloyl-D-sedoheptulose as a promising agent against hypertriglyceridaemia without hepatic side-effects.

9) **Kim Y.J., and Yokozawa T.: Modulation of Oxidative Stress and Melanogenesis by Proanthocyanidins. Biol. Pharm. Bull., 32: 1155-1159, 2009.**

Abstract: Proanthocyanidins (PAs) are polymer chains of flavonoids known to have a high free radical scavenging capacity. However, their efficacy for use in dermatological health has not been fully explored. In the present study, we investigated the inhibitory property of PAs on melanogenesis and oxidative stress of cultured B16F10 melanoma cells (B16 cells) utilizing both oligomer and polymer PAs that were isolated from freshly crushed persimmon peel. To assess the suppressive effects of PAs against oxidative insults, lipid peroxidation, total reactive species (RS), peroxynitrite ($ONOO^-$), superoxide ($^{\cdot}O_2$), and nitric oxide ($NO^{\cdot}$) were quantitated. In addition, the reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio was measured to evaluate the cellular oxidative status. Results showed that the PAs studied had a strong inhibitory effect on the murine tyrosinase and melanin synthesis that was correlated with the modulation of oxidative stress. Thus, our present work produced evidence that in B16 cells, the anti-melanogenic capacity of PAs as shown by the inhibition of tyrosinase and melanin synthesis likely occurs through the suppression of oxidative stress by the ability of PAs to modulate total RS, $^{\cdot}O_2$, $NO^{\cdot}$, $ONOO^-$, lipid peroxidation, and redox balance.

10) **Ienaga K., Mikami H., and Yokozawa T.: First Indications Demonstrating the Preventive Effects of NZ-419, a Novel Intrinsic Antioxidant, on the Initiation and/or Progression of Chronic Renal Failure in Rats. Biol. Pharm. Bull., 32: 1204-1208, 2009.**

Abstract: The concentration of NZ-419 (5-hydroxy-1-methylimidazolidine-2,4-dione), an intrinsic antioxidant, has been shown to increase in the sera of animals and patients with chronic renal failure (CRF). This is the first report that orally administered exogenous NZ-419 prevents the initiation and/or progression of CRF in rats using an adenine-loaded model. After 24 d of adenine loading, there was a *ca.* 90% decrease in creatinine clearance (C_{Cr}) in the control rats. Treatment with NZ-419 from the beginning significantly inhibited the decrease in C_{Cr} and also the increase in serum creatinine (sCr). Bio-markers for *in vivo* hydroxyl radicals, the serum methylguanidine (sMG) level, and sMG/sCr molar ratio, not only in serum but also in the urine, kidney, liver, and muscle indicated that NZ-419 inhibited the increase in oxidative stress induced by CRF in rats. An increase of guanidinosuccinic acid, an another bio-marker of oxidative stress, was also inhibited with NZ-419.

11) **Kim H.Y., Okamoto T., and Yokozawa T.: Beneficial effects of Chinese prescription Kangen-karyu on diabetes associated with hyperlipidemia, advanced glycation endproducts,**

and oxidative stress in streptozotocin-induced diabetic rats. J. Ethnopharmacol., 124: 263-269, 2009.

Abstract: AIM OF THE STUDY: In the present study, we investigated the effects of Kangen-karyu, a traditional Chinese prescription comprising six herbs, on diabetes. MATERIALS AND METHODS: Kangen-karyu extract (50, 100, or 200mg/kg body weight) was administered to streptozotocin (STZ)-induced diabetic rats and serum and hepatic biochemical factors, and protein expressions associated with oxidative stress and advanced glycation endproduct (AGE) formation were measured. RESULTS: The oral administration of Kangen-karyu significantly ameliorated hypertriglyceridemia induced by STZ injection, while serum levels of glucose and total cholesterol were mildly affected. Kangen-karyu also markedly reduced the levels of AGEs and malondialdehyde (MDA), a lipid peroxide product used as an indicator of oxidative stress in both serum and hepatic tissue. In addition, Kangen-karyu dose-dependently lowered the expression levels of N(epsilon)-(carboxymethyl) lysine, one of the major component of AGEs closely associated with the pathogenesis of diabetes and liver cirrhosis, and receptor for AGEs, as well as the expression levels of nuclear factor-κB, inducible nitric oxide synthase, and cyclooxygenase-2 (COX-2) associated with oxidative stress. Especially, MDA levels in both serum and hepatic tissue and COX-2 expression increased by STZ were recovered by Kangen-karyu (200mg/kg body weight) to normal levels. CONCLUSIONS: Kangen-karyu showed favorable effects on hypertriglycemia, AGE formation, and oxidative stress in STZ-treated rats, suggesting beneficial effects on diabetes, diabetic hepatopathy, and liver diseases such as cirrhosis, as well as cardiovascular and cerebrovascular diseases.

12) **Lee E.K., Chung S.W., Kim J.Y., Kim J.M., Heo H.S., Lim H.A., Kim M.K., Anton S., Yokozawa T., and Chung H.Y.: Allylmethylsulfide Down-Regulates X-Ray Irradiation-Induced Nuclear Factor-κB Signaling in C57/BL6 Mouse Kidney. J. Med. Food, 12: 542-551, 2009.**

Abstract: Allylmethylsulfide (AMS), a volatile organosulfur derivative from garlic, has been shown to have radioprotective effects in radiation-challenged cell and animal models, but the mechanism of radioprotection is not well understood. To determine the mechanism of radioprotection in an *in vivo* model, we first verified the antioxidant capacity of AMS using 2,2′-azobis(2-amidinopropane) dihydrochloride-induced human embryonic kidney 293T cells by measuring reactive oxygen species generation, reduced glutathione, protein tyrosine kinase/protein tyrosine phosphatase balance, and nuclear factor-κB (NF-κB) protein levels. We then investigated the protective effects of AMS (55 and 275 μmol/kg, intraperitoneal treatment) on 15 Gy X-ray-irradiated mouse kidney. The results showed that AMS decreased the free radical-induced lipid peroxidation in mice exposed to X-rays. Moreover, the antioxidative AMS suppressed the activation of NF-κB and its dependent genes such as vascular cell adhesion molecule-1, inducible nitric oxide synthase, and cyclooxygenase-2 through inhibition of IκBα phosphorylation and activation of IκB kinase α/β and mitogen-activated protein kinases (MAPKs). Based on these results, AMS may be a useful radioprotective agent by down-regulating the MAPKs and NF-κB signaling pathway that can be induced via X-ray irradiation.

13) **Yamabe N., Kang K.S., Hur J.M., and Yokozawa T.: Matcha, a Powdered Green Tea, Ameliorates the Progression of Renal and Hepatic Damage in Type 2 Diabetic OLETF Rats. J. Med. Food., 12: 714-721, 2009.**

Abstract: Matcha, a powdered green tea produced by grinding with a stone mill, has been popularly used in the traditional tea ceremony and foods in Japan. Matcha is well known to be richer in some nutritional elements and epigallocatechin 3-*O*-gallate than other green teas. In our previous study, epigallocatechin 3-*O*-gallate exhibited protective effects against renal damage in a rat model of diabetic nephropathy. In the present study, we investigated the preventive effects of Matcha (50, 100, or 200 mg/kg/day) on the progression of hepatic and renal damage in type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. OLETF rats were orally administered Matcha for 16 weeks, and we assessed biochemical parameters

in the serum, liver, and kidney and expression levels of major products of advanced glycation end products (AGEs), N^{ε}-(carboxylmethyl)lysine (CML) and N^{ε}-(carboxylethyl)lysine (CEL), receptor for AGE (RAGE), and sterol regulatory element binding proteins (SREBPs)-1 and -2. Serum total protein levels were significantly increased by Matcha administration, whereas the serum albumin and glycosylated protein levels as well as the renal glucose and triglyceride levels were only slightly or not at all affected. However, Matcha treatment significantly lowered the glucose, triglyceride, and total cholesterol levels in the serum and liver, renal AGE levels, and the serum thiobarbituric acid-reactive substances levels. In addition, Matcha supplementation resulted in decreases in the renal CML, CEL, and RAGE expressions as well as an increase in hepatic SREBP-2 expression, but not that of SREBP-1. These results suggest that Matcha protects against hepatic and renal damage through the suppression of renal AGE accumulation, by decreases in hepatic glucose, triglyceride, and total cholesterol levels, and by its antioxidant activities.

14) Jung H.A., Min B.S., Yokozawa T., Lee J.H., Kim Y.S., and Choi J.S.: Anti-Alzheimer and Antioxidant Activities of Coptidis Rhizoma Alkaloids. Biol. Pharm. Bull., 32: 1433-1438, 2009.

Abstract: Coptidis Rhizoma and its isolated alkaloids are reported to possess a variety of activities, including neuroprotective and antioxidant effects. Thus, the anti-Alzheimer and antioxidant effects of six protoberberine alkaloids (berberine, palmatine, jateorrhizine, epiberberine, coptisine, and groenlandicine) and one aporphine alkaloid (magnoflorine) from Coptidis Rhizoma were evaluated *via* β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) assays, along with peroxynitrite ($ONOO^-$) scavenging and total reactive oxygen species (ROS) inhibitory assays. Six protoberberine alkaloids exhibited predominant cholinesterases (ChEs) inhibitory effects with IC_{50} values ranging between 0.44—1.07 μM for AChE and 3.32—6.84 μM for BChE; only epiberberine (K_i=10.0) and groenlandicine (K_i=21.2) exerted good, non-competitive BACE1 inhibitory activities with IC_{50} values of 8.55 and 19.68 μM, respectively. In two antioxidant assays, jateorrhizine and groenlandicine exhibited significant $ONOO^-$ scavenging activities with IC_{50} values of 0.78 and 0.84 μM, respectively; coptisine and groenlandicine exhibited moderate total ROS inhibitory activities with IC_{50} values of 48.93 and 51.78 μM, respectively. These results indicate that Coptidis Rhizoma alkaloids have a strong potential of inhibition and prevention of Alzheimer's disease (AD) mainly through both ChEs and β-amyloids pathways, and additionally through antioxidant capacities. In particular, groenlandicine may be a promising anti-AD agent due to its potent inhibitory activity of both ChEs and β-amyloids formation, as well as marked $ONOO^-$ scavenging and good ROS inhibitory capacities. As a result, Coptidis Rhizoma and the alkaloids contained therein would clearly have beneficial uses in the development of therapeutic and preventive agents for AD and oxidative stress-related disease.

15) Park C.H., Yamada N., Noh J.S., Kang K.S., Tanaka T., and Yokozawa T.: The Beneficial Effects of Morroniside on the Inflammatory Response and Lipid Metabolism in the Liver of *db*/*db* Mice. Biol. Pharm. Bull., 32: 1734-1740, 2009.

Abstract: The effect of morroniside on lipid metabolism and the inflammatory response in the liver of type 2 diabetes model mice was investigated in this study. Male C57BLKS/J *db*/*db* mice were divided into the three groups: control (vehicle), morroniside 20, or 100 mg/kg body weight-treated mice. The elevated serum triglyceride and alanine aminotransferase levels as well as hepatic glucose and lipids contents in *db*/*db* mice were significantly decreased by the 8-week oral administration of morroniside in a dose-dependent manner. The generations of hepatic thiobarbituric acid-reactive substances and reactive oxygen species induced by hyperglycemia and dyslipidemia were also significantly decreased by the administration of morroniside. In addition, the barometer of an antioxidative state, the oxidized to reduced glutathione ratio, in the liver of *db*/*db* mice was markedly increased by morroniside treatment. From

protein analysis, the elevated expressions of nuclear factor-κBp65, cyclooxygenase-2, inducible nitric oxide synthase, and sterol regulatory element binding proteins (SREBP-1 and SREBP-2) were down-regulated in the liver of *db*/*db* mice. On the other hand, the administration of morroniside significantly increased hepatic peroxisome proliferator activated receptor α expression. These results suggest that morroniside would act as a regulator of hepatic inflammatory reactions and lipid metabolism in *db*/*db* mice.

16) Park C.H., Cho E.J., and Yokozawa T.: Protection Against Hypercholesterolemia by Corni Fructus Extract and Its Related Protective Mechanism. J. Med. Food., 12: 973-981, 2009.

Abstract: To reduce cardiovascular disease (CVD) by reducing circulating cholesterol concentrations, much attention has been focused on the search for dietary interventions for hypercholesterolemia. Corni Fructus is known to exhibit several biological activities. Therefore, in the present study, its protective effect on diet-induced hypercholesterolemia was studied using a rat model fed 1% cholesterol and 0.5% cholic acid. Corni Fructus extract was administered at an oral dose of 50, 100, or 200 mg/kg of body weight/day for 10 days. The administration inhibited the elevation of both systolic and diastolic blood pressure. In addition, it lowered serum total cholesterol levels with a decrease in esterified cholesterol. Moreover, the atherogenic index was decreased in a dose-dependent manner, suggesting its protective role against CVD through regulating cholesterol and lipoprotein levels. The hepatic levels of total and free cholesterol were also reduced by Corni Fructus. This implies that free cholesterol was used for catabolism or efflux. Consequently, the levels of total cholesterol and bile acid in feces were significantly increased. Our present results also showed that the administration of Corni Fructus decreased lipid peroxidation, suggesting a protective effect against oxidative stress induced by hypercholesterolemia. Furthermore, hypercholesterolemic control rats had significantly lower expression of sterol regulatory element-binding protein (SREBP)-2 protein without any difference in SREBP-1 protein expression. On the other hand, the protein expression of SREBP-2 was significantly up-regulated by Corni Fructus. Furthermore, the protein expression of peroxisome proliferator-activated receptor α was elevated, indicating that Corni Fructus would activate fatty acid oxidation. In conclusion, Corni Fructus may protect against CVD by regulating blood pressure, cholesterol levels, and expression of proteins related to lipid metabolism.

17) Kim Y.J., Kim Y.A., and Yokozawa T.: Protection against Oxidative Stress, Inflammation, and Apoptosis of High-Glucose-Exposed Proximal Tubular Epithelial Cells by Astaxanthin. J. Agric. Food Chem., 57: 8793-8797, 2009.

Abstract: Astaxanthin is a carotenoid with powerful antioxidant properties that exists naturally in various plants, algae, and seafood. The purpose of the present study is to examine the protective action of astaxanthin against high-glucose-induced oxidative stress, inflammation, and apoptosis in proximal tubular epithelial cells (PTECs). To assess the efficacy of astaxanthin, several key markers and activities were measured, including lipid peroxidation, total reactive species (RS), superoxide ($^{\bullet}O_2$), nitric oxide ($NO^{\bullet}$), and peroxynitrite ($ONOO^-$), as well as expressions of inflammatory proteins, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), nuclear factor-kappa B (NF-κB) nuclear translocation, and levels of Bcl2/Bax protein. Results showed that astaxanthin effectively suppressed lipid peroxidation, total RS, $^{\bullet}O_2$, $NO^{\bullet}$, $ONOO^-$, iNOS and COX-2 protein levels, NF-κB nuclear translocation, and pro-apototic Bax, whereas it increased anti-apoptotic Bcl2 protein levels. On the basis of these findings, it was concluded that in PTECs, astaxanthin has a protective efficacy against several deleterious effects caused by high glucose exposure and proposed that astaxanthin should be explored further as a potential antidiabetic remedy for the treatment of diabetic nephropathy.

18) Yokozawa T., Lee Y.A., Zhao Q., Matsumoto K., and Cho E.J.: Persimmon Oligomeric Proanthocyanidins Extend Life Span of Senescence-accelerated Mice. J. Med. Food., 12:

1199-1205, 2009.

Abstract: The anti-aging activities of persimmon oligomeric proanthocyanidins (POPs), reported to improve life span and behavioral characteristics associated with the aging process, were investigated using the senescence-accelerated mouse (SAM) P8, which is a good model for studies on aging-related behavioral changes as well as life span. We demonstrated that the administration of POPs extended the life span of SAMP8. In addition, POPs elevated Sirt1 expression, which is recognized as an essential factor for life span extension in the brain. On the other hand, the administration of POPs did not induce stereotypical behaviors such as rearing, jumping, and hanging from the lid of a cage, whereas food restriction increased these frequencies without a significant change in motor function. The present study suggests a promising role of POPs as anti-aging agents to extend life span, although further studies elucidating their anti-aging mechanisms acting are needed.

19) Awale S., Tohda C., Tezuka Y., Miyazaki M., Kadota S.: Protective effects of *Rosa damascena* and its active constituent on Aβ(25-35)-induced neuritic atrophy. Evid. Based Complement. Alternat. Med., Epub ahead of print, 2009.

Abstract: Dementia is a clinical syndrome characterized by multiple cognitive deficits and causes progressive neurodegeneration leading eventually to death. The incidence of dementia is increasing worldwide with the increase in ageing population. However, no effective treatment is available yet. It has been hypothesized that drugs activating neurite outgrowth might induce neuronal reconstruction and help in the recovery of brain function. Working on this hypothesis, we recently observed that the chloroform extract of the *Rosa damascena* significantly induced the neurite outgrowth activity and inhibited the Aβ (25-35)-induced atrophy and cell death. Further workup led the isolation of a very long polyunsaturated fatty acid having molecular formula C(37)H(64)O(2) as an active constituent. The structure of this compound was established by extensive analysis of fragmentations observed in EI-MS mode. The isolated compound protected Aβ(25-35)-induced atrophy and displayed strong neurite outgrowth activity. The length of dendrite in the cells treated with this compound were comparable to those of nerve growth factor (NGF) treated cells.

20) Matsuya Y., Yamakawa Y., Tohda C., Teshigawara K., Yamada M. and Nemoto H.: Synthesis of Sominone and Its Derivatives Based on RCM Strategy: Discovery of A Novel Anti-Alzheimer's Disease Medicine Candidate "Denosomin". Org. Lett., 11: 3970-3973, 2009.

Abstract: Synthesis of sominone was achieved starting from dehydroepiandrosterone on the basis of an RCM strategy for the construction of a delta-lactone side chain. This synthetic protocol was applied for the synthesis of several analogous derivatives including 1-deoxy-24-norsominone (denosomin), which was revealed to exhibit notable bioactivities for new antidementia chemotherapy, exceeding the original natural compound sominone.

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single i.p. injection of sominone into normal mice, they could better memorize scenery information than control mice. Sixty minutes after sominone injection, RET phosphorylation was increased, particularly in the hippocampus of mice. After the memory tests, the densities of axons and dendrites were increased in the hippocampus by sominone administration. CONCLUSIONS AND IMPLICATIONS: Sominone could reinforce the morphological plasticity of neurons by activation of the RET pathway and thus enhance memory. Sominone, a compound with low molecular weight, may be a GDNF-independent stimulator of the RET pathway and/or a novel modulator of RET signalling.

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◇共同研究

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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, 金 賢栄：Jinju National University, Park Jeong-Hill：ソウル大学薬学部, 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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5) 門脇 真：富山大学,「PI3 kinase の神経変性疾患への関与に関する研究」
6) John Mielke：University of Waterloo,「Sominone のシナプス可塑性促進作用の研究」

◇研究費取得状況

1) (財) 浦上食品・食文化振興財団研究助成 (分担：横澤隆子)「スパイスの経口投与による温熱作用増感効果：未病のバイオマーカーとしての AGEs の変化」
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」
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5) 武田科学振興財団　薬学系研究奨励 (薬学) (代表：東田千尋)「伝統薬物研究から展開する神経創薬戦略－アルツハイマー病と脊髄損傷を克服する画期的な治療薬開発の実現に向かう研究－」
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修士論文：

城屋敷枝里：Sominone の神経回路網形成を基盤とする記憶への作用と分子メカニズムに関する研究