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) Rao T.P., Juneja L.R., and Yokozawa T.: Green Tea Catechins against Oxidative Stress of Renal Disease, In Protective Effects of Tea on Human Health, by Jain N.K., Siddiqi M.A., and Weisburger J.H. (Ed.) 109-119, CABI International, Oxon, England, 2006.
2) Kang K.S., Yamabe N., Kim H.Y., Park J.H., and Yokozawa T.: Protective Effect of *Panax ginseng* against Diabetic Renal Damage, In Advances in Ginseng Research 2006, Oh S., and Choi K.T. (Ed.) 697-716, The Korean Society of Ginseng, Korea, 2006.
3) 姜　奇成, 山辺典子, 横澤隆子: 熱処理した薬用人参"仙参"の糖尿病ラットにおける影響. 「腎とフリーラジカルー第8集」, 玉井 浩, 柏原直樹監修, 芦田 明, 佐々木 環, 青柳一正編, 172-176, 東京医学社, 東京, 2006.
4) 横澤隆子, 山辺典子, 金 武祚: 糖尿病性腎症における(-)-epigallocatechin 3-*O*-gallate の評価. 「腎とフリーラジカルー第8集」, 玉井 浩, 柏原直樹監修, 芦田 明, 佐々木 環, 青柳一正編, 177-181, 東京医学社, 東京, 2006.
5) 東田千尋, 小松かつ子, 中村憲夫, 服部征雄: 認知症に対するコーヒーの作用. 「コーヒーの科学と機能」, 50-58, アイ・ケイコーポレーション, 東京, 2006.

◇原著論文

1) **Kang K.S., Yokozawa T., Kim H.Y., and Park J.H.: Study on the Nitric Oxide Scavenging Effects of Ginseng and Its Compounds. J. Agric. Food Chem., 54: 2558-2562, 2006.**

Abstract: In this study, an in vitro nitric oxide (·NO)-generating system was used to investigate the ·NO-scavenging effects of methanolic extracts of white ginseng (*Panax ginseng* C.A. Meyer), red ginseng, and sun ginseng and several ginsenosides and phenolic compounds. Sun ginseng extract showed the strongest activity among the three ginseng extracts. None of the ginsenosides used in this experiment showed ·NO-scavenging activity, but the phenolic compounds, such as *p*-coumaric and vanillic acids, and maltol inhibited ·NO production in a concentration-dependent manner. Moreover, maltol levels markedly increased by heat processing. Therefore, the enhanced ·NO-scavenging activity of ginseng by heat processing was closely related to phenolic acids and the increased content of maltol.

2) **Yamabe N., and Yokozawa T.: Activity of the Chinese prescription Hachimi-jio-gan against renal damage in the Otsuka Long-Evans Tokushima Fatty rat: a model of human type 2 diabetes mellitus. J. Pharm. Pharmacol., 58: 535-545, 2006.**

Abstract: Currently, in Japan, approximately 95% of patients with diabetes mellitus have non-insulin-dependent (type 2) diabetes mellitus (NIDDM), and diabetic nephropathy is a major cause of patients requiring chronic haemodialysis. A previous study showed that Hachimi-jio-gan has a protective effect in rats subjected to subtotal nephrectomy plus streptozotocin injection, a model of insulin-dependent (type 1) diabetic nephropathy. In this study, we used the Otsuka Long-Evans Tokushima Fatty (OLETF) rat, a model of human NIDDM, to investigate whether long-term administration of Hachimi-jio-gan affects glycaemic control and renal function in NIDDM. Male OLETF rats, aged 22 weeks, were divided into 4 groups of 10 and given Hachimi-jio-gan (50, 100 or 200 mg kg^{-1} daily) orally or no treatment for 32 weeks. Male Long-Evans Tokushima Otsuka (LETO) rats (n = 6) were used as non-diabetic normal controls. Hachimi-jio-gan reduced hyperglycaemia dose-dependently from 16 weeks of the administration period. Urinary protein excretion decreased significantly from an early stage, and creatinine clearance levels improved at 32 weeks. In addition, the levels of serum glycosylated protein and renal advanced glycation end-products were effectively reduced. Hachimi-jio-gan also significantly reduced the levels of thiobarbituric acid-reactive substances in renal mitochondria, although it showed only a tendency to reduce these in serum. Furthermore, long-term administration of Hachimi-jio-gan reduced renal cortical expression of proteins, such as transforming growth factor-β_1 (TGF-β_1), fibronectin, inducible nitric oxide synthase and cyclooxygenase-2. The 100- and 200-mg kg^{-1}

daily doses of Hachimi-jio-gan significantly reduced TGF-β_1 and fibronectin protein expression to levels below those of LETO rats. These data suggest that Hachimi-jio-gan may have a beneficial effect on the progression of diabetic nephropathy in OLETF rats by attenuating glucose toxicity and renal damage.

3) **Yoo H.H., Yokozawa T., Satoh A., Kang K.S., and Kim H.Y.: Effects of Ginseng on the Proliferation of Human Lung Fibroblasts. Am. J. Chin. Med., 34: 137-146, 2006.**

Abstract: In this study, we investigated the effects of methanolic extracts of white ginseng (*Panax ginseng* C.A. MEYER) and two kinds of heat-treated ginseng made by steaming fresh ginseng at 100 °C for 3 hours (HTG-100) or 120 °C for 3 hours (HTG-120) on the cell growth of human fibroblasts. All of the tested ginseng extracts stimulated cell growth, although the effect of HTG-120 was weaker than that of the other extracts. However, none of the ginseng extracts exhibited any effect on the growth of old cells with a population doubling level (PDL) of 48.7. Flow cytometric analysis showed that ginseng extracts raised the population of cells in G_0/G_1 phase after treatment for 24 hours, but did not exert any effect after treatment for 48 hours. These results suggest that ginsengs exert their cell growth-promoting action mainly on younger cells at an early stage of the cell cycle, and that this effect is closely associated with an increase in the population of cells in the G_0/G_1 phase.

4) **Yokozawa T., Satoh A., Nakagawa T., and Yamabe N.: Attenuating Effects of Wen-Pi-Tang Treatment in Rats with Diabetic Nephropathy. Am. J. Chin. Med., 34: 307-321, 2006.**

Abstract: Wen-pi-tang is a Chinese prescription used traditionally as a medicine to treat moderate renal failure. In this study, we used rats subjected to subtotal nephrectomy and streptozotocin injection to examine the effects of wen-pi-tang on diabetic nephropathy. Wen-pi-tang was administered at a dose of 50, 100 or 200 mg/kg body weight/day for 15 weeks. Diabetic nephropathy is one of the most serious chronic complications of diabetes mellitus, and renal dysfunction is reflected by proteinuria, decreased creatinine clearance (Ccr) and increased serum urea nitrogen and creatinine (Cr) levels. Wen-pi-tang treatment for 15 weeks resulted in significant reductions of blood glucose and serum urea nitrogen levels, while proteinuria, Ccr and serum Cr levels did not change significantly. Wen-pi-tang also lowered serum triglyceride and thiobarbituric acid-reactive substance levels in a dose-dependent manner. Furthermore, the disorders of the glucose-dependent metabolic pathway due to this pathological condition were normalized by the administration of wen-pi-tang through decreased formation of advanced glycation end-products in the kidney. Wen-pi-tang protected against the development of renal lesions, glomerular sclerosis and mesangial matrix expansion, assessed by histopathological evaluation and scoring. This study suggests that wen-pi-tang treatment could be beneficial in reducing the risk of developing diabetic nephropathy.

5) **Kang K.S., Kim H.Y., Pyo J.S., and Yokozawa T.: Increase in the Free Radical Scavenging Activity of Ginseng by Heat-Processing. Biol. Pharm. Bull., 29: 750-754, 2006.**

Abstract: To investigate whether or not the radical scavenging activity of ginseng is enhanced by heat processing, we evaluated the scavenging effects of white ginseng (WG), red ginseng (RG, steamed ginseng at 98-100 °C) and sun ginseng (SG, steamed ginseng at 120 °C) on nitric oxide, superoxide (O_2^-), hydroxyl (·OH) radicals and peroxynitrite ($ONOO^-$). Heat-treated ginseng (RG and SG) showed better O_2^-, $ONOO^-$ and ·OH-scavenging activities than WG. In particular, the radical scavenging activities of SG were stronger than those of RG. Furthermore, we evaluated the radical scavenging activities of maltol, salicylic acid, vanillic acid and *p*-coumaric acid, known as principal antioxidant components of ginseng, in WG, RG and SG, and also investigated their contents. Of the tested compounds, maltol, vanillic acid and *p*-coumaric acid exhibited $ONOO^-$-scavenging activity. In addition, maltol and *p*-coumaric acid showed strong ·OH-scavenging activity. Moreover, the content of maltol was remarkably increased in a temperature-dependent manner by heat processing, implying that maltol was closely related to the radical

scavenging activity of heat-processed ginseng. These findings indicate that SG may act as a free radical scavenger and protect against damage caused by oxidative stress related with these radicals.

6) **Yokozawa T., Cho E.J., Sasaki S., Satoh A., Okamoto T., and Sei Y.: The Protective Role of Chinese Prescription Kangen-karyu Extract on Diet-Induced Hypercholesterolemia in Rats. Biol. Pharm. Bull., 29: 760-765, 2006.**

Abstract: This study was carried out to investigate the protective potential of Chinese prescription Kangen-karyu, comprising six crude drugs, on coronary heart disease which is the principal cause of morbidity and mortality worldwide. The diet-induced hypercholesterolemic rat model, which shows an elevation in low density lipoprotein (LDL) cholesterol and atherosclerosis, was employed. The control rats fed a diet of 1% cholesterol and 0.5% cholic acid showed the highest cholesterol levels in serum and feces relative to those fed a normal diet, however, the rats administered Kangen-karyu extract showed reductions in these levels without changes in liver cholesterol, indicating that the reduction of serum total cholesterol by Kangen-karyu extract probably arises from an increase in cholesterol excretion. Furthermore, the administration of Kangen-karyu extract significantly prevented the elevation of serum aspartate aminotransferase and alanine aminotransferase, known as marker enzymes of liver damage. The elevated serum levels of LDL cholesterol were lowered, however, the high density lipoprotein cholesterol level was significantly elevated by Kangen-karyu extract and these were dose-dependent decreases in the atherogenic index to 15.2, 8.8 and 7.5 at oral doses of 50, 100 and 200 mg from the 19.4 control value, respectively. In addition, Kangen-karyu extract inhibited LDL oxidation in a dose-dependent manner, and the elevated level of thiobarbituric acid-reactive substances in control rats showed a decline by the administration of Kangen-karyu extract. The present study suggests that Kangen-karyu could play a protective role against hypercholesterolemia through the regulation of cholesterol levels and inhibition of lipid peroxidation.

7) **Han H.F., Nakamura N., Zuo F., Hirakawa A., Yokozawa T., and Hattori M.: Protective Effects of a Neutral Polysaccharide Isolated from the Mycelium of *Antrodia cinnamomea* on *Propionibacterium acnes* and Lipopolysaccharide Induced Hepatic Injury in Mice. Chem. Pharm. Bull., 54: 496-500, 2006.**

Abstract: Mycelia of *Antrodia cinnamomea* were extracted with chloroform and hot water. A neutral polysaccharide named *ACN2a* separated from the water extract was purified using 10% CCl_3COOH, and repeated column chromatography on HW-65 and DE-52 cellulose. Its structure was determined by chemical and spectroscopic analyses. *ACN2a* was composed of Gal, Glc, Fuc, Man and GalN (in the ratio 1:0.24:0.07:0.026:faint), in which an α-D-(1→6)-Gal linkage accounted for 73% of all linkages. The ratio of branch points was about 16% of the total residual numbers, and branches were attached to C-2 of galactosyl residues of the main chain. *ACN2a* had an average molecular weight of 12.9×10^5 Daltons, $[\alpha]_D^{25}=+115^\circ$ (c=0.44, H_2O); $[\eta]=0.0417 dl.g^{-1}$, Cp=0.2663 cal/(g· °C). The hepatoprotective effect of *ACN2a* was evaluated using a mouse model of hepatic injury that was induced by *Propionibacterium acnes* (*P. acnes*) and lipopolysaccharide (LPS). The administration of *ACN2a* (0.4, 0.8 g/kg/d, *p.o.*), significantly prevented increases in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzyme activities in mice treated with *P. acnes*-LPS, indicating hepatoprotective activity *in vivo*.

8) **Fujii H., Yokozawa T., Kim Y.A., Tohda C., and Nonaka G.: Protective Effect of Grape Seed Polyphenols against High Glucose-Induced Oxidative Stress. Biosci. Biotechnol. Biochem., 70: 2104-2111, 2006.**

Abstract: We aimed to clarify whether grape seed polyphenols (GSPs) are candidates therapeutic agents against diabetes mellitus, and to determine what degree of GSP oligomerization has the most potent efficacy. We studied the protective effects of various molecular weight GSPs (monomer, oligomer,

polymer, and oligonol) on high glucose-induced cytotoxicity. In the present study, a high concentration of glucose (30 mM) induced cytotoxicity and oxidative stress (reactive oxygen species and nitric oxide) in cultured LLC-PK_1 cells, but treatment with GSPs, especially oligomer GSPs, had potent protective effects against high glucose-induced oxidative stress. In addition, high glucose induced nuclear translocation of nuclear factor-kappa B, and increased expression of cyclooxygenase-2, inducible nitric oxide synthase, and bax, but GSP treatment inhibited them. These results indicate that GSPs have protective effects against high glucose-induced cytotoxicity, and among them, oligomer GSPs have more potent effects than other GSPs (monomer, polymer, and oligonol) on high glucose-induced renal cell damage.

9) **Kang K.S., Kim H.Y., Yamabe N., Nagai R., and Yokozawa T.: Protective Effect of Sun Ginseng against Diabetic Renal Damage. Biol. Pharm. Bull., 29: 1678-1684, 2006.**

Abstract: The effect of sun ginseng (SG, heat-processed *Panax ginseng* C.A. MEYER at 120°C) on diabetic renal damage was investigated using streptozotocin-induced diabetic rats. The diabetic rats showed loss of body weight gain, and increases in food and water intake and urine volume, while the oral administration of SG at a dose of 50 or 100 mg/kg body weight/d for 15 d attenuated water intake and urine excretion induced by diabetes. In addition, the diabetic rats given SG at a dose of 100 mg/kg body weight showed significant decreases in serum glucose, serum glycosylated protein and urinary protein levels, suggesting that SG improves the abnormal conditions that lead to oxidative stress. Furthermore, SG significantly reduced advanced glycation endproduct (AGE) formation and thiobarbituric acid-reactive substance levels elevated in the kidneys of diabetic rats. This implies that SG would alleviate the oxidative stress under diabetes through the inhibition of lipid peroxidation. SG also reduced the overexpression of cyclooxygenase-2 and inducible nitric oxide synthase in the kidney induced by hyperglycemia via deactivation the activation of nuclear factor-kappa B. Furthermore, treatment with SG decreased the levels of 3-nitrotyrosine, carboxymethyllysine and receptors for AGE which increase under diabetes. These findings indicate that oxidative stress is increased in the diabetic rat kidney and that SG can prevent renal damage associated with diabetes by attenuating the oxidative stress.

10) **Kang K.S., Kim H.Y., Yamabe N., and Yokozawa T.: Stereospecificity in hydroxyl radical scavenging activities of four ginsenosides produced by heat processing. Bioorg. Med. Chem. Lett., 16: 5028-5031, 2006.**

Abstract: The activity-guided fractionation of sun ginseng (SG, heat processed *Panax ginseng* C.A. MEYER at 120°C) was carried out to identify its main active hydroxyl radical (•OH) scavenging components. As a result, the *n*-BuOH fraction mainly consisting of ginsenosides showed the strongest activity. Of several ginsenosides of SG, the •OH scavenging activities of relatively high contents of 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1, and Rg_5 were compared. Rg_5 and 20(*S*)-Rg_3 showed strong •OH scavenging IC_{50} values of 0.15 and 0.44 mM, respectively, and these activities were prominently higher than each of their respective isomers. Therefore, stereospecificity exists in the •OH scavenging activities of ginsenosides produced by heat processing. Especially, the double bond at carbon-20(22) or the OH group at carbon-20 geometrically close to OH at carbon-12 is thought to increase the •OH scavenging activity of ginsenosides.

11) **Yamabe N., Yokozawa T., Oya T., and Kim M.: Therapeutic Potential of (-)-Epigallocatechin 3-*O*-Gallate on Renal Damage in Diabetic Nephropathy Model Rats. J. Pharmacol. Exp. Ther., 319: 228-236, 2006.**

Abstract: Previous investigations have demonstrated that green tea polyphenols and partially hydrolyzed guar gum as dietary fiber have antioxidative and hypolipidemic activity, respectively, supporting their reduction of risk factors in the course of diabetic nephropathy via a hypoglycemic effect and ameliorating the decline of renal function through their combined administration to rats with subtotal nephrectomy plus streptozotocin (STZ) injection. As a further study, we examined whether (-)-epigallocatechin 3-*O*-gallate

(EGCg), the main polyphenolic compound, could ameliorate the development of diabetic nephropathy. Rats with subtotal nephrectomy plus STZ injection were orally administrated EGCg at doses of 25, 50, and 100 mg/kg body weight/day. After a 50-day administration period, EGCg-treated groups showed suppressed hyperglycemia, proteinuria, and lipid peroxidation, although there were only weak effects on the levels of serum creatinine and glycosylated protein. Furthermore, EGCg reduced renal advanced glycation end-product accumulation and its related protein expression in the kidney cortex as well as associated pathological conditions. These results suggest that EGCg ameliorates glucose toxicity and renal injury, thus alleviating renal damage caused by abnormal glucose metabolism-associated oxidative stress involved in renal lesions of diabetic nephropathy.

12) **Sasaki S., Yokozawa T., Cho E.J., Oowada S., and Kim M.: Protective role of γ-aminobutyric acid against chronic renal failure in rat. J. Pharm. Pharmacol., 58: 1515-1525, 2006.**

Abstract: The protective effect of γ-aminobutyric acid (GABA) against chronic renal failure (CRF) was investigated using a remnant kidney model with 5/6 nephrectomized rats. Nephrectomy led to renal dysfunction, which was evaluated via several parameters including serum urea nitrogen, creatinine (Cr) and Cr clearance. However, the administration of GABA ameliorated renal dysfunction, and a longer administration period of GABA increased its protective effect. In addition, nephrectomized control rats showed an elevation in the fractional excretion of sodium (FE_{Na}) with an increase in urinary sodium, while GABA led to a significant decline in FE_{Na}. Moreover, nephrectomy resulted in a decrease of serum albumin and an increase of urinary protein with a change in the urinary protein pattern, whereas the rats administered GABA showed improvement in these changes associated with CRF caused by nephrectomy. This suggests that GABA would inhibit the disease progression and have a protective role against CRF. As one of the risk factors for CRF progression, hypertension was also regulated by GABA. The results also indicate that GABA may play a protective role against CRF through improvement of the serum lipid profile, with reductions in triglyceride and total cholesterol. Furthermore, nephrectomy led to renal oxidative stress with a decrease in the activity of antioxidative enzymes and elevation of lipid peroxidation. The administration of GABA attenuated oxidative stress induced by nephrectomy through an increase in superoxide dismutase and catalase, and decrease in lipid peroxidation. The histopathological lesions, including glomerular, tubular and interstitial lesions, under nephrectomy were also improved by GABA with the inhibition of fibronectin expression. This study demonstrated that GABA attenuated renal dysfunction via regulation of blood pressure and lipid profile, and it also ameliorated the oxidative stress induced by nephrectomy, suggesting the promising potential of GABA in protecting against renal failure progression.

13) **Yokozawa T., Cho E.J., Okamoto T., and Sei Y.: Effects of Chinese prescription Kangen-karyu and its crude drug Tanjin on ageing process in rats. J. Pharm. Pharmacol., 58: 1591-1599, 2006.**

Abstract: The effects of the Chinese prescription Kangen-karyu and its crude drug Tanjin on the ageing process were investigated in rats. Diets supplemented with Kangen-karyu and Tanjin extracts decreased glycosylated protein levels in serum, a risk marker of ageing and ageing-related diseases. In addition, they inhibited the levels of thiobarbituric acid reactive substance in the serum and liver; Kangen-karyu in particular led to a strong decrease in hepatic mitochondrial thiobarbituric acid reactive substance. The decline in the reduced glutathione/oxidized glutathione ratio in the liver observed with ageing was ameliorated by Kangen-karyu and Tanjin, while these groups attenuated the increase in glutathione peroxidase activity of hepatic tissue against ageing. This suggests that Kangen-karyu and Tanjin regulate the glutathione redox cycle that maintains the cellular redox condition against age-related oxidative stress. Moreover, the overexpression of cytoplasmic cytochrome c observed with ageing was attenuated by Kangen-karyu and Tanjin. This provides new evidence that Kangen-karyu and Tanjin inhibit leakage

of superoxide in mitochondria and attenuate cellular oxidative damage. Furthermore, Kangen-karyu and Tanjin would maintain mitochondrial function with ageing through the regulation of related protein expression such as bax and bcl-2 proteins. In addition, Kangen-karyu reduced the expression of nuclear factor kappa B; Kangen-karyu and Tanjin did not affect the expression of inhibitor kappa B. The present study demonstrated that Kangenkaryu prevented oxidative damage and mitochondrial dysfunction with ageing. Furthermore, Kangen-karyu showed a stronger protective effect against ageing by oxidative stress than Tanjin, probably through synergistic and/or additive effects.

14) Cho E.J., Lee Y.A., Yoo H.H., and Yokozawa T.: Protective Effects of Broccoli (*Brassica oleracea*) against Oxidative Damage in Vitro and in Vivo. J. Nutr. Sci. Vitaminol., 52: 437-444, 2006.

Abstract: The antioxidative effect and protective potential against diabetes of the broccoli flower were investigated both in vitro and in a diabetic rat model. Among fractions of MeOH, CH_2Cl_2, BuOH, and H_2O, the BuOH fraction exerted the strongest inhibitory activities on 1,1-diphenyl-2-picrylhydrazyl radical, radical-induced protein oxidation, and nitric oxide generation by sodium nitroprusside. The in vitro results suggest that the BuOH fraction from the broccoli flower has a protective potential against oxidative stress. The rat model with diabetes induced by streptozotocin was employed to evaluate the protective effect of the BuOH fraction in vivo. Diabetic rats showed reduced body weight gain and heavier kidney and liver weights than normal rats, while oral administration of the BuOH fraction at an oral dose of 100 or 200 mg/kg body weight/d for 20 d attenuated the physiological changes induced by diabetes. In addition, oral administration of the BuOH fraction to diabetic rats led to significant decreases in serum glucose and glycosylated protein, while it resulted in the increase of serum albumin, implying that the BuOH fraction improves the abnormal metabolism of glucose and protein that leads to oxidative stress. Moreover, it significantly reduced thiobarbituric acid-reactive substance levels in serum, hepatic and renal mitochondria. This suggests that the BuOH fraction would alleviate the oxidative stress associated with diabetes through the inhibition of lipid peroxidation. The present study demonstrates that the BuOH fraction has an antioxidative effect in vitro and it protects against oxidative stress induced by diabetes in an in vivo model.

15) Tohda C., Tamura T., Matsuyama S., and Komatsu K.: Promotion of axonal maturation and inhibition of dementia by *Astragalus mongholicus*. Br. J. Pharmacol., 149: 532-541, 2006.

Abstract: BACKGROUND AND PURPOSE: 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. EXPERIMENTAL APPROACH: We investigated the effects of extracts of *Astragalus mongholicus* on the cognitive defect in mice caused by injection with the amyloid peptide Aβ(25-35). We also examined the effect of the extract on the regeneration of neurites and the reconstruction of synapses in cultured neurons damaged by Aβ (25-35). KEY RESULTS: *A. mongholicus* extract (1 g kg(-1) day(-1) for 15 days, p.o.) reversed Aβ (25-35)-induced memory loss and prevented the loss of axons and synapses in the cerebral cortex and hippocampus in mice. Treatment with Aβ (25-35) (10 μM) induced axonal atrophy and synaptic loss in cultured rat cortical neurons. Subsequent treatment with *A. mongholicus* extract (100 μg/ml) resulted in significant axonal regeneration, reconstruction of neuronal synapses, and prevention of Aβ (25-35)-induced neuronal death. Similar extracts of *A. membranaceus* had no effect on axonal atrophy, synaptic loss, or neuronal death. The major known components of the extracts (astragalosides I, II, and IV) reduced neurodegeneration, but the activity of the extracts did not correlate with their content of these three astragalosides. CONCLUSION AND IMPLICATIONS: *A. mongholicus* is an important candidate for the treatment of memory disorders and the main active constituents may not be the known

astragalosides.

16) **Naito R., and Tohda C.: Characterization of anti-neurodegenerative effects of *Polygala tenuifolia* in Aβ(25-35)-treated cortical neurons. Biol. Pharm. Bull., 29: 1892-1896, 2006.**

Abstract: Although *Polygala tenuifolia* WILLD (PT) was classically mentioned as an anti-dementia drug in Chinese and Japanese traditional medicine, basic research showed only enhancement of the cholinergic function. In Alzheimer's disease, neuritic atrophy and synaptic loss occur prior to neuronal death event, and may be the first trigger of the memory impairment. Therefore, we studied effects of *Polygala tenuifolia* WILLD (PT) on Aβ (25-35)-induced neuronal damage using rat cortical neurons for characterization of activities of PT under Abeta-induced neuronal damage. Treatment with the water extract of PT enhanced axonal length dose-dependently after Aβ (25-35)-induced axonal atrophy. However, dendritic atrophy and synaptic loss induced by Aβ (25-35) were not recovered by treatment with PT extract. In contrast, Aβ (25-35)-induced cell damage was completely inhibited by PT extract. By characterization of PT effects on neuronal morphological plasticity and cell damage, usefulness as well as an insufficiency of PT as an anti-dementia drug was clarified.

17) **Kuboyama T., Tohda C., and Komatsu K.: Withanoside IV and its active metabolite, sominone, attenuate Aβ(25-35)-induced neurodegeneration. Eur. J. Neurosci., 23: 1417-1427, 2006.**

Abstract: At the present, medication of dementia is limited to symptomatic treatments such as the use of cholinesterase inhibitors. To cure dementia completely, that is regaining neuronal function, reconstruction of neuronal networks is necessary. Therefore, we have been exploring antidementia drugs based on reconstructing neuronal networks in the damaged brain and found that withanoside IV (a constituent of Ashwagandha; the root of *Withania somnifera*) induced neurite outgrowth in cultured rat cortical neurons. Oral administration of withanoside IV (10 μmol/kg/day) significantly improved memory deficits in Aβ (25-35)-injected (25 nmol, i.c.v.) mice and prevented loss of axons, dendrites, and synapses. Sominone, an aglycone of withanoside IV, was identified as the main metabolite after oral administration of withanoside IV. Sominone (1 μM) induced axonal and dendritic regeneration and synaptic reconstruction significantly in cultured rat cortical neurons damaged by 10 μM Aβ(25-35). These data suggest that orally administrated withanoside IV may ameliorate neuronal dysfunction in Alzheimer's disease and that the active principle after metabolism is sominone.

18) **Tohda C., Nakayama N., Hatanaka F., and Komatsu K.: Comparison of anti-inflammatory activities of six *Curcuma* rhizomes: a possible curcuminoid-independent pathway mediated by *Curcuma phaeocaulis* extract. Evidence-based Complementary and Alternative Medicine, 3: 255-260, 2006.**

Abstract: We aimed to compare the anti-inflammatory activities of six species of *Curcuma* drugs using adjuvant arthritis model mice. When orally administered 1 day before the injection of adjuvant, the methanol extract of *Curcuma phaeocaulis* significantly inhibited paw swelling and the serum haptoglobin concentration in adjuvant arthritis mice. Also when orally administered 1 day after the injection of adjuvant, the methanol extract of *Curcuma phaeocaulis* significantly inhibited paw swelling. Other *Curcuma* species (*Curcuma longa, Curcuma wenyujin, Curcuma kwangsiensis, Curcuma zedoaria* and *Curcuma aromatica*) had no significant inhibitory effects on adjuvant-induced paw swelling. Cyclooxygenase (COX)-2 activity was significantly inhibited by the methanol extract of *C. phaeocaulis*. Curcuminoids' (curcumin, bis-demethoxycurcumin and demethoxycurcumin) were rich in *C. longa*, but less in *C. phaeocaulis* and *C. aromatica*, not in *C. wenyujin, C. kwangsiensis* and *C. zedoaria*, suggesting that curcuminoids' contents do not relate to inhibition of arthritis swelling. Therefore, *C. phaeocaulis* may be a useful drug among *Curcuma* species for acute inflammation, and the active constituents of *C.*

phaeocaulis are not curcuminoids.

19) **Tohda C., Hashimoto I., Kuboyama T., and Komatsu K.: Metabolite 1 of protopanaxadiol-type saponins, an axonal regenerative factor, stimulates teneurin-2 linked by PI3-kinase cascade. Neuropsychopharmacology, 31: 1158-1164, 2006.**

Abstract: We previously showed that 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol (M1), a metabolite of protopanaxadiol-type ginseng saponins by intestinal bacteria had axonal extension activity in degenerated neurons, and improved memory disorder and synaptic loss induced by an active fragment of amyloid beta, Aβ (25-35). It is unknown how M1 shows these effects in neurons. To clarify the signal transduction mechanism of M1-induced axonal extension, phosphorylated proteins by M1 stimulation were identified because most cellular signal pathways are regulated by phosphorylation/dephosphorylation. The combination of immunoprecipitation and MALDI-TOF-MS revealed that teneurin-2 and mPar3 were specifically phosphorylated by M1 stimulation. Because mPar3 is known as an axonal specifying molecule and to be regulated by phosphatidylinositol 3-kinase (PI3-kinase), the involvement of teneurin-2 and PI3-kinase in the M1 signal was studied. In teneurin-2-deficient cortical neurons, M1-induced axonal extension and PI3-kinase activation were significantly inhibited. In addition, treatment with PI3-kinase inhibitor also reduced M1-induced axonal extension. These results suggest that M1 induces axonal outgrowth through the teneurin-2-PI3-kinase cascade.

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3) 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」

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5) 倉知正佳：富山大学、「統合失調症に関する研究」

◇研究費取得状況

1) 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」

2) 科学技術振興機構（分担：横澤隆子）「血管障害性生活習慣病に対する予防食品の開発研究」

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」

4) 文部科学省研究費補助金、基盤研究 C　（代表：東田千尋）　「新規の抗痴呆薬となる withanolide 類を用いたシナプス形成機序の解明」

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藤井　創：低分子化ポリフェノールの効率的製造法、構造解析および抗酸化能について