J. Trad. Med. 22, 278－283, 2005

Orengedokuto inhibits neointimal formation, proliferation and migration of rat vascular smooth muscle cells *in vivo* and *in vitro*

Hwa-Jin CHUNG,[a,b] Ying LIU,[a] Ikuro MARUYAMA,[c] and Tadato TANI[*,a,d]

[a]*Deparment of Kampo-Pharmaceutics, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan.* [b]*Present address: Department of Pharmacognosy, College of Pharmacy, Ewha Womans University, 11-1 Daehyun-dong, Seodaemun-gu, Seoul, 120-750, Korea.* [c]*Department of Laboratory and Molecular Medicine, School of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan.* [d]*21st Century COE Program, University of Toyama, Sugitani 2630, Toyama 930-0194, Japan.* *(Received July 26, 2005. Accepted August 10, 2005.)*

Orengedokuto (OGT), a traditional Chinese formulation containing four crude drugs (Scutellariae Radix, Coptidis Rhizoma, Phellodendri Cortex and Gardeniae Fructus), has been used for various conditions accompanied with atherosclerotic-related disorders. Oral administration of OGT for 3 days before and 7 days after balloon injury resulted in a dose-dependent attenuation of neointimal formation and vascular smooth muscle cell (VSMC) proliferation in balloon-injured carotid arteries in cholesterol-fed rats. Furthermore, the serum collected from cholesterol-fed rats orally treated with OGT significantly reduced the migration of cultured VSMC. Thus, OGT may attenuate carotid artery neointimal formation following balloon endothelial denudation via inhibition of VSMC proliferation and migration. The inhibitory effects of OGT on neointimal formation were mediated primarily by Scutellariae Radix and Coptidis Rhizoma composed of OGT. The present results suggest that OGT may be promising candidates as preventive agents for atherosclerosis in humans.

Key words Orengedokuto, atherosclerosis, vascular smooth muscle cell, neointimal formation, proliferation, Scutellariae Radix, Kampo formulation.

Introduction

Orengedokuto (OGT, 黄連解毒湯: Huanglian-Jiedu-Tang in Chinese) is a traditional Chinese formulation (Kampo formulation in Japanese) consists of 4 crude drugs such as Scutellariae Radix, Coptidis Rhizoma, Phellodendri Cortex and Gardeniae Fructus. OGT has been used to produce relief from various conditions, including hot-flash, irritability and headache accompanied with atherosclerotic-related disorders[1] and cerebro-vascular disorder.[2] However, it is still unclear how OGT regulates the atherosclerotic lesions using *in vivo* experimental models.

The pathophysiology of atherosclerosis includes abnormal accumulation of vascular smooth muscle cell (VSMC) in response to endothelial injury,[3] a state that can be approximated using the neointimal formation model of balloon injury in the rat carotid artery.[4] The neointimal formation model has, at least in part, pathological characteristics similar to the atherosclerotic lesions in humans and is considered to be and "accelerated atherosclerosis."[5] In this model, crude drug,[6] some crude drug preparations[7,8] and Kampo formulations[9,10] have been shown to reduce progression of neointimal formation. Further, hypercholesterolemia augments various processes involved in atherogenesis, including neointimal formation.[11] Preliminarily study of 8 Kampo formulations has reported that OGT may inhibit neointimal formation in normal diet-fed rats model.[9] Thus the goal of the present study was to characterize the effects of OGT on neointimal formation and VSMC proliferation in cholesterol-fed rats. To clarify the role of 4 crude drugs conforming OGT in the expression of the efficacy, effects of 4 variant formulations, in which one of the 4 crude drugs was deprived from OGT, were also examined. Furthermore, the inhibitory effects of OGT on the migration of cultured VSMC were also evaluated by using the serum (OGT-serum) collected from cholesterol-fed rats treated orally with OGT for 10 days.

Materials and Methods

Crude drugs and chemicals. OGT was formulated using Scutellariae Radix (SCR: imported from China, 3.0 g), Coptidis Rhizoma (COR: produced in Japan, 2.0 g), Phellodendri Cortex (PHC: imported from China, 1.5 g) and Gardeniae Fructus (GAF: imported from China, 2.0 g), all of which were purchased from Tochimoto Tenkaido Co., Ltd. (Japanese Pharmacopoeia XIV standard grade; Osaka, Japan). A voucher specimen of crude drugs was established in the Department of Kampo-Pharmacutics of the Toyama Medical and Pharmaceutical University. After formulation, 8.5 grams of OGT was boiled in 600 ml of water for 40 min and then filtered and freeze-dried into powder (final weight, 2.49 ± 0.04 g) which corresponds to the common human (60 kg) daily dose.

Variant formulations of OGT were prepared by removing

*To whom correspondence should be addressed. e-mail : tanitdt@ms.toyama-mpu.ac.jp

one of the four constituents before the freeze-drying step to produce OGT without SCR (OGT-SCR: extract yield, 1.33 ± 0.02 g), OGT without COR (OGT-COR: 2.09 ± 0.03 g), OGT without PHC (OGT-PHC: 2.05 ± 0.04 g), and OGT without GAF (OGT-GAF: 1.77 ± 0.03 g) (mean ± S.D., n = 6). In order to assure consistent quality of the OGT formulations, extracts were analyzed with high-performance liquid chromatography conditions as previously described[12] (Fig. 1).

The hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitor simvastatin (SV), which was reported to inhibit VSMC proliferation in the balloon injury model,[13] was used as a positive control. SV and the other reagents, including, antiproliferating cell nuclear antigen (PCNA)-antibody (PC-10), and biotinylated anti-mouse secondary antibody streptavidin-conjugated peroxidase were used in our previous report.[10] The same chemical reagents used for Dullbecco's modified Eagle's medium (DMEM, NISSUI PHARMACEUTICAL CO., LTD), fetal bovine serum (FBS, JRH BIOSCIENCES), penicillin (GIBCO BRL) and streptomycin (GIBCO BRL) in cultured VSMC experiment were used as used in our previous report.[14]

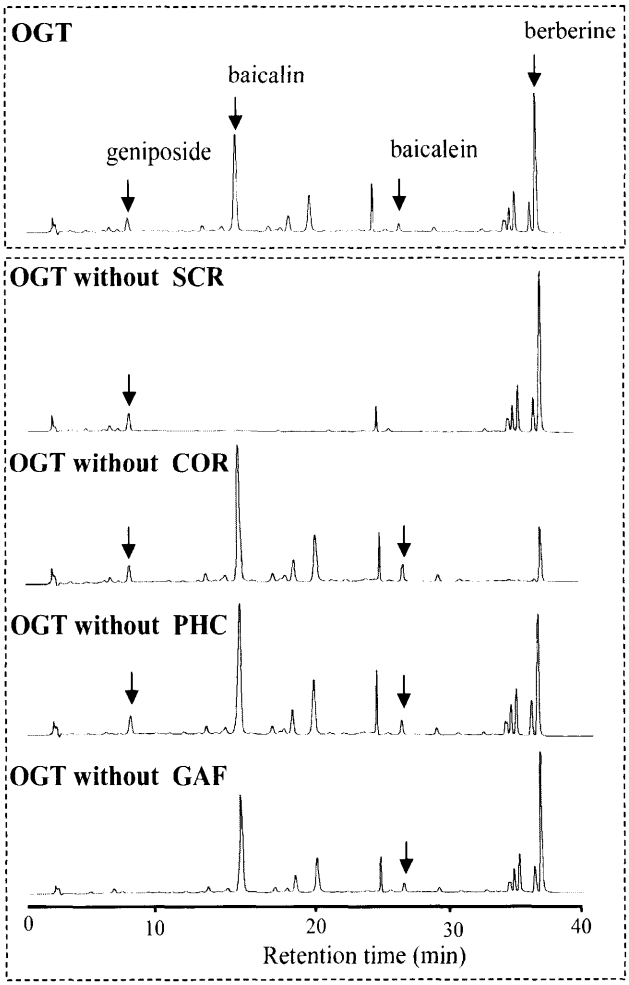

Fig. 1 High performance liquid chromatography (HPLC)-fingerprint of the methanol-soluble portion of the OGT extracts and its four variant formulations
In the methanol-soluble portion of the freeze-dried extracts of OGT, geniposide (3.94 ± 0.03 mg/g), baicalin (12.6 ± 0.1 mg/g), baicalein (0.35 ± 0.01 mg/g), and berberine (5.26 ± 0.03 mg/g) were identified by comparison with retention time and UV spectra of standard compounds purchased from Toray Co. Ltd (Japan).
HPLC analysis: JASCO HPLC system (DG 1580-54 degasser, PU-1580 pump, HG-1580-32 mixer CO-1565 column oven) with Wakosil-II 5C18 HG reversed-phase column (4.6 x 150 mm) and UV spectrometer (MD-2010, detection at 256 nm). The mobile phase was composed of A (50 mM phosphoric buffer-2% SDS-H_2O, pH 4.00) and B (A: acetonitrile= 20: 80). Linear gradient elution was performed (0 min, 90: 10; 10 min, 80: 20; 20 min, 75: 25; 28 min, 50:50; 45 min, 45:55). Chromatography was performed at 45oC with a low-rate of 1.0 mL/min.

Animal treatments. Balloon endothelial denudation was performed in the left carotid artery of pentobarbital-anesthetized rats (n= 8) as previously described.[10] A normal diet containing 1% cholesterol and OGT extract (210, 420, and 630 mg/kg/day) or one of the four variant extracts for 3 days before and then for 7 days after the injury. After this period, rats were killed and injured carotid arteries were removed for histopathologic evaluation of neointimal formation and VSMC proliferation as previously described.[10] At the time of animal sacrifice, blood samples were also obtained for measurement of total cholesterol, high-density lipoprotein (HDL)-cholesterol and low-density lipoprotein (LDL)-cholesterol using commercial assay kits according to our previous method.[10]

All animal experiments were performed in accordance the Guidelines of the Animal Care and Use Committee of Toyama Medical and Pharmaceutical University approved by the Japanese Association of Laboratory Animal Care.

Migration of cultured VSMC. VSMC (rat thoracic aorta SMCs: A7r5, Dainippon Pharmaceutical Co., Ltd. Tokyo, Japan) were grown in DMEM supplemented with an antibiotic mixture (penicillin G: 100 unit/ml and streptomycin: 100 μg/ml) with 10% FBS and incubated at 37 ℃ in a humidified atmosphere with 5% CO_2. VSMC migration was evaluated using a two-compartment microchemotaxis chamber (Fig. 2) and as previously described.[14] Briefly, a VSMC suspension (1.5 x 10^5 cell / ml in DMEM) was placed in the upper chamber, and DMEM (600 μl) containing 5% OGT-serum (or SV-serum) was placed in the lower chamber. Three OGT-serums were collected from rats treated orally with OGT (at 210, 420 and 630 mg/kg) from 3 days before to 7 days after balloon endothelial denudation. SV-serum was collected from rats treated orally with SV for the same period as OGT administration. After 4 h of incubation, the number of VSMC that had migrated from the upper com-

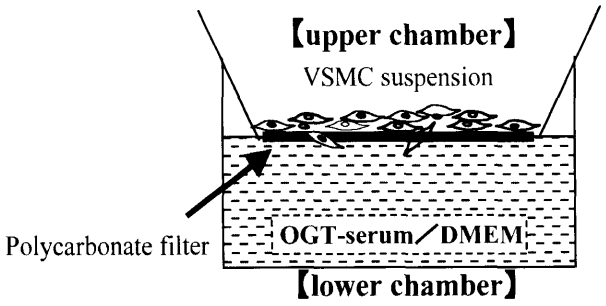

Fig. 2 Examination of migration of cultured VSMC using OGT-serum in two-compartment microchemotaxis chamber
Migration activity was calculated as the mean number of migrated cells observed (five measurements).

partment to the lower surface through the polycarbonate filter (8 μm in diameter: Nucleopore Corp.) was determined microscopically.

Statistics. Data are represented as mean ± S.D. of the indicated number (n) of experiments. Statistical comparison between different treatments was determined by one-way analysis of variation (ANOVA). Differences with probability (p) values less than 0.05 were considered statistically significant.

Results

Body weight (Table 1) and serum lipids. During the 10 days experimental period, no fatal accident was observed. Further, there was no difference in food intake and body weight (Table 1) when comparing the experimental groups, despite using super physiologic of the extracts of OGT and 4 variant formulations (15-fold higher than the common human daily dose).

The serum total cholesterol (88.8 ± 12.9 mg/dl) and LDL-cholesterol (47.3 ± 12.5 mg/dl) in the cholesterol-fed group were significantly ($p < 0.05$) increased compared with those in the normal diet-fed group (56.9 ± 7.0 and 12.4 ± 4.0 mg/dl, respectively), but serum lipid peroxides were similar when comparing the two groups. OGT administration had no effect on serum lipids (data not shown).

OGT and neointimal formation (*in vivo*) (Fig. 3). Seven days after denudation, intimal area was significantly ($p < 0.05$) higher in the cholesterol-fed control group (0.049 ± 0.007 mm^2) than in the normal diet-fed normal group (0.041 ± 0.006 mm^2). Further, luminal (0.329 ± 0.052 mm^2) and medial areas (0.124 ± 0.014 mm^2) tended to be smaller in the cholesterol-fed control group than in the normal group (0.346 ± 0.038 mm^2 and 0.131 ± 0.012 mm^2), but these differences did not reach the level of statistical significance. However, oral administration of OGT resulted in a dose-dependent attenuation of this increase of intimal area (Fig. 3) and decrease of luminal but not medial area in the cholesterol-fed rats when compared to cholesterol-fed rats treated with vehicle. Therefore as shown in Fig. 3, OGT administration resulted in a decrease in the intimal/medial layer area (I/M) ratio. SV also attenuated neointimal formation.

Variant OGT and neointimal formation (*in vivo*) (Fig. 4). As shown in Fig. 1, the profiles of four variant formulations were apparently different from original OGT profile; e.g., peaks of baicalin and baicalein in the profile of OGT without SCR disappeared, and the peak of berberine in the profile of OGT without COR was diminished when compared to the original OGT profile.

Administration of either OGT-SCR or OGT-COR had no effect on intimal area (left panel in Fig. 4) and I/M ratio (right panel in Fig. 4) in cholesterol-fed rats. By contrast, administration of either OGT-PHC or OGT-GAF resulted in attenuation of the increase in intimal area and I/M ratio, but this effect tended to be weaker than that seen with the original OGT formulation.

VSMC proliferation (*in vivo*) and migration (*in vitro*) (Fig. 5). Intimal VSMC proliferation, as assessed by the PCNA labeling index, was significantly ($p<0.05$) larger in cholesterol-fed control group (30.5 ± 3.4%) than in the normal diet-fed group (25.1 ± 2.7%).

Fig. 5 (left panel) shows that intimal VSMC proliferation was dose-dependently reduced by the administration of OGT (at 3 doses), resulting in a significant ($p<0.05$)

Table 1 Effect of OGT extract on body weight in cholesterol-fed rats

Drugs	3 days before denudation (%)	0 day (%)	7 days after denudation (%)
SV (0.83 mg / kg / day)	101.6 ± 1.6	101.6 ± 2.3	99.4 ± 3.3
OGT (210 mg / kg / day)	99.7 ± 1.1	100.2 ± 0.9	99.2 ± 1.8
OGT (420 mg / kg / day)	100.1 ± 1.7	100.9 ± 1.9	99.1 ± 2.0
OGT (630 mg / kg / day)	101.6 ± 1.6	101.5 ± 1.8	99.1 ± 2.4

Each value represents the % of the control body weight in the cholesterol-fed control group (denuded) and the mean ± S.D. (n =8).
OGT and SV were orally administered for 3 days before and 7 days after denudation in cholesterol-fed rats.
The SV dose (0.83 mg/kg/day) represents a dose 10-fold higher than the common human daily dose. The OGT extract doses (210, 420, 630 mg/kg/day) represent doses 5-, 10-, and 15-fold higher than the common human daily dose.

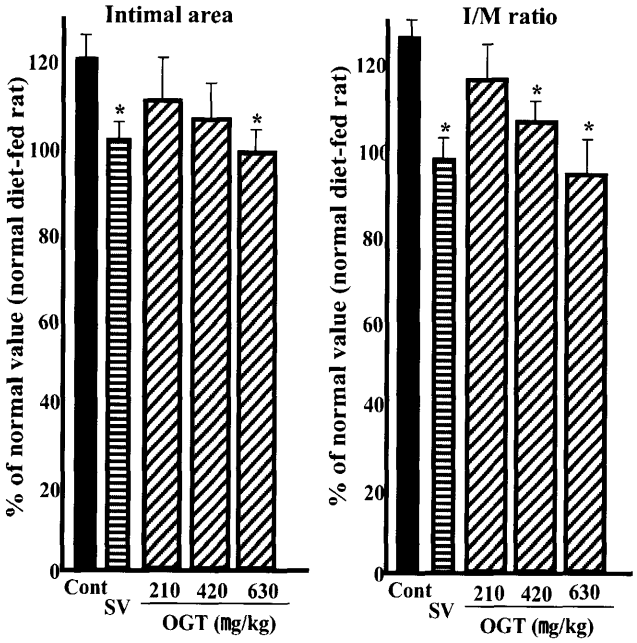

Fig. 3 Effect of OGT on neointimal formation in carotid arteries 7 days after balloon endothelial denudation in cholesterol-fed rats
Each value represents the % of the normal value (normal diet-fed rat) (mean ± S.D. : n=8).
I/M ratio: (intimal area) / (medial area).
Cont: cholesterol-fed rats denuded;
SV: Simvastatin (0.83 mg/kg/day); OGT extracts (210, 420, 630 mg/kg/day).
OGT extracts and SV were administered 3 days before and 7 days after denudation.
*Significantly different from the control group (cholesterol-fed rats denuded) at $p<0.05$.

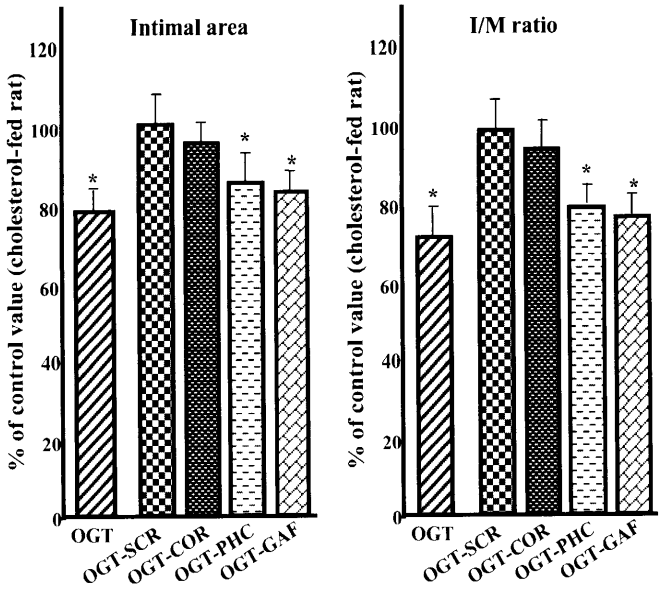

Fig. 4 Effects of four variant formulations of OGT on neointimal formation in carotid arteries 7 days after balloon endothelial denudation in cholesterol-fed rats
Each value represents the % of the control value (cholesterol-fed rats denudes) (mean ± S.D. : n=8).
OGT extracts (630 mg/kg/day); OGT-SCR (OGT without Scutellariae Radix, 440 mg/kg/day), OGT-COR (OGT without Coptidis Rhizoma, 510 mg/kg/day), OGT-PHC (OGT without Phellodendri Cortex, 520 mg/kg/day), and OGT-GAF (OGT without Gardeniae Fructus, 330 mg/kg/day),
OGT or one of the four variants of OGT extract was administered 3 days before and 7 days after denudation.
*Significantly different from the control group (cholesterol-fed rats denuded) at $p<0.05$.

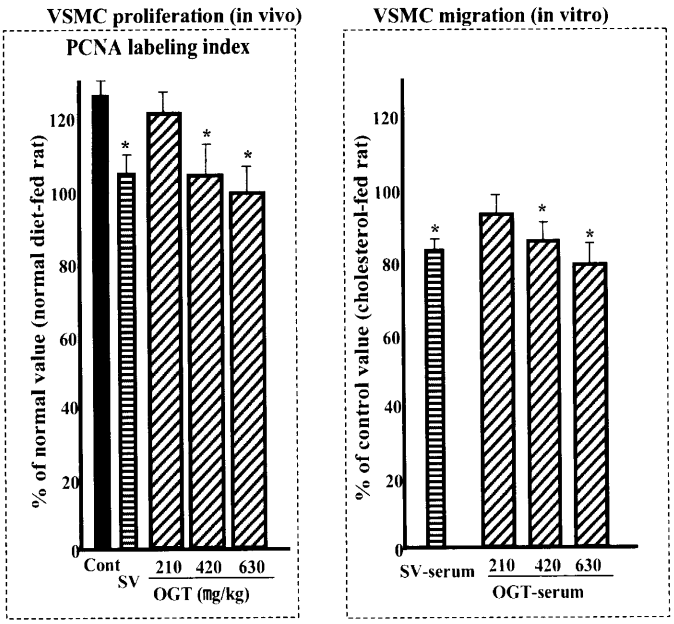

Fig. 5 Effects of OGT on VSMC proliferation (PCNA labeling index) in carotid arteries 7 days after balloon endothelial denudation in cholesterol-fed rats and OGT-serum on cultured VSMC migration
In the left panel, each value represents the % of the normal value (normal diet-fed rat denuded) (mean ± S.D. : n=8).
VSMC proliferation was examined using the PCNA labeling index (%): (number of PCNA-positive VSMCs in the intimal area) x 100 / (number of total VSMCs in the intimal area).
SV (Simvastatin: 0.83 mg/kg/day) and OGT extracts (210, 420, 630 mg/kg/day) were administered 3 days before and 7 days after denudation.
In the right panel, each value represents the % of the control value (cholesterol-fed rat denuded) (mean ± S.D. : n=8).
Migration activity was assessed according to the method described in Fig. 2.
OGT-serum 210, 420 and 630 represent the serum collected from rats treated orally with OGT (at 210, 420 and 630 mg/kg) for 10 days (from 3 days before to 7 days after balloon endothelial denudation). SV-serum was collected from rats treated orally with SV (at 0.83 mg/kg) for 10 days.
*Significantly different from the control group (cholesterol-fed rats denuded) at $p<0.05$.

decrease in the PCNA labeling index in cholesterol-fed rats at the 420 and 630 mg/kg OGT dose when compared with cholesterol-fed rats treated with vehicle. Administration of SV to cholesterol-fed rats also resulted in a decrease in intimal VSMC proliferation.

Fig. 5 (right panel) shows that two OGT-serums collected from cholesterol-fed rats orally treated with OGT (at doses 415 and 623 mg / kg of rat) significantly ($p<0.05$) reduced the migration of cultured VSMC when compared with serum collected from cholesterol-fed rats treated with vehicle. SV-serum also significantly ($p<0.05$) reduced VSMC migration.

Discussion

The present study demonstrated that OGT administered 3 days before and 7 days after endothelial cell denudation resulted in a dose-dependent reduction in neointimal formation in the rat carotid artery (Fig. 3) and in VSMC proliferation (Fig. 5 left panel). Further, OGT-serum, which was collected from rats treated orally with OGT for 10 days, reduced migration of cultured VSMC (Fig. 5 right panel). These results suggest that the ability of OGT to inhibit neointimal formation may be mediated by its effects on VSMC proliferation and migration.

It is remarkable that OGT-serum inhibited the cultured VSMC migration. This *in vitro* method of adding serum obtained from rats treated with Kampo formulations to the culture medium has been used to evaluate their pharmacological effects.[14,15,16] This method is particularly useful when investigating the pharmacological properties of Kampo formulations after absorption and metabolism, as these compounds contain many non-absorbable components. Further analytical studies of the bioactive components of OGT-serum would be of benefit.

Although plasma lipids lowering effects of OGT have been reported,[17] the present study demonstrated that a 10 day course of OGT had no effect on serum cholesterol levels in cholesterol-fed rats. These results suggest that the ability of OGT to reduce neointimal formation was not dependent on changes in LDL-cholesterol levels. This is consistent with studies that have demonstrated that OGT-mediated inhibition of plaque formation in cholesterol-fed rabbits by OGT occurs independent of any improvements in lipid metabolism.[18]

Omission of either Scutellariae Radix or Coptidis

Rhizoma from the OGT formulation resulted in a significant decrease in bioactivity (Fig. 4), suggesting that these two crude drugs mediate a large portion of the effect of OGT on neointimal formation. This is consistent with prior studies that have reported that two crude drugs (Scutellariae Radix and Coptidis Rhizoma)[19] and baicalein,[20] an ingredient of Scutellariae Radix, inhibit proliferation of VSMC.

OGT may prevent neointimal formation via effects on cells other than VSMC. First, activated platelets and free radical production may contribute to neointimal formation after endothelial cell injury.[21] Studies have reported that Coptidis Rhizoma and its ingredient, berberine,[22] and Scutellariae Radix and baicalein,[23] all have anti-platelet properties, furthermore, scavenging effect of Copridis Rhizoma on nitric oxide,[24] and of Scutellariae Radix[25] have free radical scavenging properties. Second, Scutellariae Radix stimulates endothelial cell proliferation and inhibits high glucose-induced endothelial cell apoptosis.[26] Third, atherosclerosis can be characterized as a state of chronic inflammatory disease[27] comprised of endothelial cell injury, generation of cytokines, and generation of reactive oxygen species.[28] Therefore, inhibitory effects of OGT on experimental inflammatory lesions,[29] cyclooxygenase-2 activity,[30] free radical production,[31] and enzymatic lipid peroxidation,[32] might contribute to its ability to inhibit neointimal formation after endothelial denudation.

Kampo formulations are administered for specific conditions or symptoms according to a unique differential diagnosis system (*yin-yang* theory). For example, in Kampo clinical practice, OGT has been used to treat patients with hot-flashes, irritation and headache,[1] which correspond to the "*yang*-syndrome" (陽証: Yo-sho in Japanese) or "heat syndrome" (熱証: Netsu-sho in Japanese). These symptoms are similar to those in patients with modern medical syndromes as "Metabolic syndrome" or "the deadly quartet."[33] Further, OGT has been used to treat "oketsu" (瘀血: *Yuxue* in Chinese), which is a manic state resulting from stagnation of the circulation or coagulation of blood. The therapeutic effect of OGT on "oketsu" may result from its ability to inhibit free radical-induced oxidation of red blood cell.[34]

Previous studies using the same denudation model have reported that the potency of the Kampo formulations used for "*yang*-syndrome" such as OGT, saikokaryukotsu-boreito (柴胡加竜骨牡蛎湯) and daijokito (大承気湯) on neointimal formation and VSMC proliferation were somewhat greater than those of the formulations used for "yin-syndrome (陰証)", such as shakanzoto (炙甘草湯) and tokito (当帰湯).[9] Since the Kampo formulations such as OGT used for "*yang*-syndrome" possess anti-inflammatory properties, these formulations may act by preventing the VSMC accumulation that typically occurs after endothelial injury.

In summary, the present study demonstrated that oral administration of OGT attenuated the increase of carotid artery neointimal formation following balloon endothelial denudation in cholesterol-fed rats via inhibition of VSMC proliferation and migration. Further, the effects of OGT on neointimal formation were mediated primarily by Scutellariae Radix and Coptidis Rhizoma. Studies to investigate whether OGT has therapeutic effects in the context of human atherosclerosis would be of benefit.

Acknowledgements

The authors wish to thank Drs. S. Birou and M. Miyata (1st Department of Internal Medicine, School of Medicine, Kagoshima University, Japan) for their advice on the balloon catheterization experiments. This study was supported in part by a Grant-in-Aid for the 21st Century COE Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1) Kinoshita, T.: A case of hypertension with a burning sensation of the face. *Jpn. J. Oriental Med.,* **35**, 59-63, 1984.
2) Shibahara, N., Kuwahara, Y., Katoh, M., Shimada, T., Hiyama, Y., Tosa, H., and Terasawa, K.: A trial of Kampo therapy for pain and numbness associated with cerebro-vascular disordes. *Jpn. J. Oriental Med.,* **43**, 27-36, 1992.
3) Clowes, A.W., Reidy, M.A., and Clowes, M. M.: Kinetics of cellular proliferation after arterial injury I: Smooth muscle growth in the absence of endothelium. *Lab. Invest.,* **49**, 327-333, 1983.
4) Bendeck, M.P., Conte, M., Zhang, M., Nili, N., Strauss, B.H., and Farwell, S.M.: Doxycycline modulates smooth muscle cell growth, migration, and matrix remodeling after arterial injury. *Am. J. Pathology,* **160**, 1089-1095, 2002.
5) Chen, S.J., Chen, Y.F., Miller, D.M., Li, H., Oparil, S., Mithramycin inhibits myointimal proliferation after balloon injury of the rat carotid artery in vivo. *Circulation,* **90**, 2468-2473, 1994.
6) Mizuno, M., Chung, H.J., Maruyama, I., and Tani, T.: Inhibitory effect of Bezoar Bovis on intimal formation and vascular smooth muscle cell proliferation in rat. *Am. J. Chin. Med.,* **33**, 439-447, 2005.
7) Wu, C.H., Tsai, B.R., Hsieh, W.T., Chang, G.Y., Mao, S.J., and Chang, W.C.: The preventive effects of G115 on balloon injury-induced neointima formation in rats. *Life Sciences,* **70**, 669-679, 2001.
8) Chung, H.J., Shirasaki, S., and Tani, T.: Inhibitory effect of a newly devised crude drug-preparation on intimal thickening after endothelial injury in rats. *J. Trad. Med.,* **21**, 278-280, 2004.
9) Chung, H.J., Kim, D.W., Maruyama, I., and Tani, T.: Effect of traditional Chinese formulations on rat carotid artery injured by balloon endothelial denudation. *Am. J. Chin. Med.,* **31**, 201-212, 2003.
10) Chung, H.J., Maruyama, I., and Tani, T.: Saiko-ka-Ryukotsu-Borei-To inhibits intimal thickening in carotid artery after balloon endothelial denudation in cholesterol-fed rats. *Biol. Pharm. Bull.,* **26**, 56-60, 2003.
11) Chobanian, A.V.: Single risk factor intervention may be inadequate to inhibit atherosclerosis progression when hypertension and hypercholesterolemia coexist. *Hypertension,* **18**, 130-131, 1991.
12) Okamura, N., Miki, H., Ishida, S., Ono, H., Yano, A., Tanaka, T., Ono, Y., and Yagi, A.: Simultaneous determination of baicalin, wogonoside, baicalein, wogonin, berberine, coptisine, palmatine, jateorrhizine and glycyrrhizin in Kampo medicines by ion-pair high-performance liquid chromatography. *Biol. Pharm. Bull.,* **22**, 1015-1021, 1999.
13) Indolfi, C., Cioppa, A., Stabile, E., Lorenzo, E.D., Esposito, G., Pisani, A., Leccia, A., Cavuto, L., Stingone, A.M., Chieffo, A., Capozzolo, C., and Chiariello, M.: Effects of hydroxymethyglutaryl coenzyme A reductase inhibitor simvastatin on smooth muscle cell proliferation in vitro and neointimal formation in vivo after vascular injury. *J. Am. Coll. Cardiol.,* **35**, 214-221, 2000.
14) Chung, H.J., Maruyama, I., and Tani, T.: Inhibition of vascular

smooth muscle cell migration by serum from rats treated orally with Saiko-ka-Ryukotsu-Borei-To, a traditional Chinese formulation. *J. Pharm. Pharmacol.,* **56**, 1323-1326, 2004.
15) Umeda, M., Amagaya, S., and Ogihara, Y.: Effects of certain herbal medicines on the biotransformation of arachidonic acid: a new pharmacological testing method using serum. *J. Ethnopharmacol.,* **23**, 91-98, 1988.
16) Yamamoto, K., Zhang, J., Yamamoto, M., Yamamoto, N., Kanai, M., Aoyagi, M., and Zhang B.: The diverse effects of a Chinese medicine, QingNaoYiZhiFang, on the proliferation of human arterial smooth muscle cells and endothelial cells. *Biol. Pharm. Bull.,* **27**, 1010-1013, 2004.
17) Sekiya, N., Kogure, T., Kita, T., Kasahara, Y., Sakahibara, I., Goto, H., Shibahara, N., Shimada, Y., and Terasawa, K.: Reduction of plasma triglyceride level and enhancement of plasma albumin concentration by Oren-gedoku-to administration. *Phytomedicine,* **9**, 455-460, 2003.
18) Sekiya, N., Kainuma, M., Hikiami, H., Nakagawa, T., Kouta, K., Shibahara, N., Shimada, Y., and Terasawa, K.: Oren-gedoku-to and Keishi-bukuryo-gan-ryo inhibit the progression of atherosclerosis in diet-induced hypercholesterolemic rabbits. *Biol. Pharm. Bull.,* **28**, 294-298, 2005.
19) Yokozawa, T., Houri, T., Hattori, M., Oura, H., Kawamura, K., Yamahara H., and Nunoura, Y.: Effects on the proliferation of smooth muscle cells of oriental medical prescriptions used for the treatment of arteriosclerosis. *Natural Medicine,* **50**, 9-13, 1996.
20) Huang, H.C., Wang, H.R., and Hsieh, L.M.: Antiproliferative effects of baicalein, a flavonoid from a Chinese herb, on vascular smooth muscle cell. *Eur. J. Pharmacol.,* **251**, 91-93, 1994.
21) Wilentz, J.R., Sanborn, T.A., Haudenschild, C.C., Valeri, C.R., Ryan, T.J., and Faxon, D.P.: Platelet accumulation in experimental angioplasty: time course and relation to vascular injury. *Circulation* **75**, 636-642, 1987.
22) Harrori, T., Furuta, K., Nagao, T., Nagamatsu, T., Ito, M., and Suzuki, Y.: Studies on the antinephritic effects of plant components (4): Redution of protein excretion by berberine and coptisine in rats with original-type anti-GBM nephrits. *Japan. J. Pharmacol.,* **59**, 159-169, 1992.
23) Kubo, M., Matsuda, H., Tani, T., Arichi, S., Kimura, Y., and Okuda, H.: Studies on Scutellariae Radix. XII. Anti-thrombic actions of various flavonoids from Scutellariae radix. *Chem. Pharm. Bull.,* **33**, 2411-2415, 1985.
24) Yokozawa, T., Chen, C.P., and Tanaka, T.: Direct scavenging of nitric oxide by traditional crude drugs. *Phytomedicine.* **6**, 453-463, 2000.
25) Gao, Z., Huang, K., Yang, X., and Xu, H.: Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of *Scutellaria baicalensis* Georgi. *Biochim. Biophys. Acta,* **1472**, 643-650, 1999.
26) Suh, K.S., Nam, Y.H., Ahn, Y.M., Kim, N.J., Park, C.Y., Koh, G., Oh, S., Woo, J. T., Kim, S.W., Kim, J.W., and Kim, Y.S.: Effect of Scutellariae Radix extract on the high glucose-induced apoptosis in cultured vascular endothelial cells. *Biol. Pharm. Bull.,* **26**, 1629-1632, 2003.
27) Ross, R.: Atherosclerosis－An inflammatory disease. *N. Engl. J. Med.,* **340**, 115-126, 1999.
28) Libby, P.: Molecular bases of the acute coronary syndromes. *Circulation,* **91**, 2844-2850, 1995.
29) Mizukawa, H., Yoshida, K., Honmura, A., Uchiyama, Y., Kaku, H., Nakajima, S., and Haruki, E.: The effect of Orengedokuto on experimentally-inflamed rats. *Am. J. Chin. Med.,* **21**, 71-78, 1993.
30) Fukutake, M., Miura, N., Yamamoto, M., Fukuda, K., Iijima, O., Ishikawa, H., Kubo, M., Okada, M., Komatsu, Y., Sasaki, H., Wakabayashi, K., Ishige A., and Amagaya, S.: Suppressive effect of the herbal medicine Oren-gedoku-to on cyclooxygenase-2 activity and azoxymrthane-induced aberrant crypt foci development in rats. *Cancer Letters,* **157**, 9-14, 2000.
31) Fushitani, S., Tsuchiya, K., Minakuchi, K., Takasugi, M., and Murakami, K.: Studies on attenuation of post-ischemic brain injury by Kampo medicines. -Inhibitory effects of free radical production. I. *Yakugaku Zasshi,* **114**, 388-394, 1994.
32) Hayashi, T., Ohta, Y., Inagaki, S., and Harada, N.: Inhibitory action of Oren-getoku-to extract on enzymatic lipid peroxidation in rat liver microsome. *Biol. Pharm. Bull.,* **24**, 1165-1170, 2001.
33) Kaplan, N.M.: The deadly quartet. upper-body obesity, glucose intolerance, hypertriglyceridemia and hypertension. *Arch Internal Med.,* **149**, 1514-1520, 1989.
34) Sekiya, N., Shibahara, N., Sakakibara, I., Hattori, N., Goto, H., and Terasawa, K.: Inhibitory effects of Orengedokuto (HuanglianJieDu-Tang) on free radical-induced lysis of human red blood cells. *Phytother. Res.,* **17**, 147-151, 2003.

Japanese abstract

ヒトの動脈硬化病変には内皮細胞傷害に続く血管内皮細胞の遊走と増殖が含まれている。ラットの頸動脈を擦過した後の内膜肥厚（neointimal formation）を評価する実験は，この過程を10日間で再現できるので“accelerated atherosclerosis” modelといわれている。コレステロールを負荷すると内膜肥厚は増大する。今回この病態モデルにおいて，黄連解毒湯（OGT）はラットにおける内膜肥厚と血管内皮細胞の増殖を用量依存的に抑制した（in vivo）。さらに黄連解毒湯を10日間経口投与した後のラット血清が血管内皮細胞の遊走を抑制することも明らかになった（in vitro）。なおこの実験ではLDL-cholesterolなど血清脂質の改善作用は認められなかったので，黄連解毒湯の内膜肥厚は血管内皮細胞の増殖と遊走の抑制にあると考えられる。

動脈硬化病変は内皮細胞傷害後のサイトカインや酸化ストレスが関与する慢性の炎症性病変であることから，黄連解毒湯やその配剤生薬の抗炎症作用や抗酸化作用に関する文献に基づいて考察した。黄連解毒湯の作用機序の解析は今後の課題であるが，今回の実験結果は黄連解毒湯が動脈硬化病変の一部を担う内膜肥厚を抑制し生活習慣病に伴う血管病変を予防する可能性を示唆している。

*〒930-0194　富山市杉谷2630
富山大学和漢医薬学総合研究所 和漢薬製剤開発部門　谿　忠人