J. Trad. Med. 23, 5−15, 2006

Medical and Pharmaceutical Society for WAKAN-YAKU

Review

A new formulation containing eleven crude drugs devised by the cooperative research project in Toyama

Tadato TANI,[*,a,b] Yutaka SHIMADA,[b,c] and Ikuo SAIKI[b,d]

[a]Department of Kampo-Pharmaceutics, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. [b]21st Century COE Program, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. [c]Department of Japanese Oriental medicine, Faculty of Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. and [d]Division Pathogenic Biochemistry, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. *(Accepted November 18, 2005.)*

Toyama Prefecture of Japan has promoted the production of traditional formulations containing crude drugs for self medication (Haichi-Yaku), which are designed for use by patients who have a fragile constitution. In 2001, a cooperative research project was organized to update the conventional tonic Haichi-Yaku to satisfy the requests of patients (or pre-patients) with lifestyle-related diseases due to excessive food intake. The present review describes the selection procedure for the 11 crude drugs included in a new formulation on the basis of precedents in conventional tonic formulations, traditional Chinese medical tacit knowledge and scientific pharmacological evidence. It also reports that the new formulation has pharmacological activities suitable for preventing lifestyle-related disease lesions, as planned. These results indicate that the new formulation is a promising candidate for modern-day self-medication.

In August 2005, permission for practical production of the new formulation (named PanaWang) was granted by the Ministry of Health, Labor and Welfare of Japan, and this formulation will be sold in a home-selling system from the beginning of 2006.

Key words lifestyle-related disease, self medication, atherosclerosis, Th1/Th2 balance, Kampo medicine, *Panax ginseng*.

1. Introduction

Numerous past and current studies have been conducted in order to obtain scientific support for tacit knowledge and the art of traditional medicine, especially traditional Chinese medicine (Kampo medicine in Japanese). In the current studies (Table 1), following previous chemical and pharmacological studies, clinical and biopharmaceutical studies of the efficacy and safety of traditional Chinese formulations are recently of great interest. Proteomic analysis of pathogenic alterations (証) diagnosed by Kampo medicine to establish tailor-made treatment is in progress in the 21st century COE (Center of excellence) program of Toyama Medical and Pharmaceutical University (the present University of Toyama).[1,2,3] However, much still remains to be studied in order to devise "new" formulations of crude drugs suitable for the prevention of modern diseases, such as lifestyle-related diseases. In the present report, the outline of a newly devised formulation of crude drugs for self-medication is reviewed.

Table 1. Research field of traditional Chinese medicine (Kampo medicine)

1. Succession of tacit knowledge and materials of traditional Chinese medicine	
Historical science of traditional Chinese medicine and pharmacy	
Securing and quality control of natural medicinal resources (crude drugs)	
2. Evaluation and development of traditional Chinese medicine	
Chemical analysis of active ingredients of crude drugs and formulations	
(→development of a new chemical medicines)	
(→development of quality control analysis of crude drugs)	
Pharmacological and biochemical evaluation of crude drugs and formulations	
Clinical evaluation of traditional Chinese formulations (evaluation of Kampo medical diagnostics "Sho") (→establishment of personalized medicine)	Subjects recently attracting notice
Bio-pharmaceutical evaluation of traditional Chinese formulations (ADME: absorption, distribution, metabolism and elimination) (drug-drug and drug-food interaction)	
Development of a "new" formulation including crude drugs	Promising subject

Toyama Prefecture of Japan is well-known as a center of production and commerce of traditional formulations containing crude drugs used for self-medication. Since the Edo period (17th century), various formulations produced in Toyama have been sold on credit in a home-selling system throughout Japan. The traditional formulations used in this unique "drugs-on-deposit system" have been designated Haichi-Yaku (配置薬) or Oki-Gusuri (置き薬). At present, approximately 35% of households in Japan use Haichi-Yaku for self-medication and primary health care. Although more than 50% of the national output of Haichi-Yaku was produced in Toyama (approximately 23.1 billion yen in 2004), this output is far below the previous year's level.

In 2001, a cooperative research project to devise a new "Toyama original brand of Haichi-Yaku" was organized by the pharmaceutical industries, University of Toyama, and local prefectural government in Toyama in order to promote

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

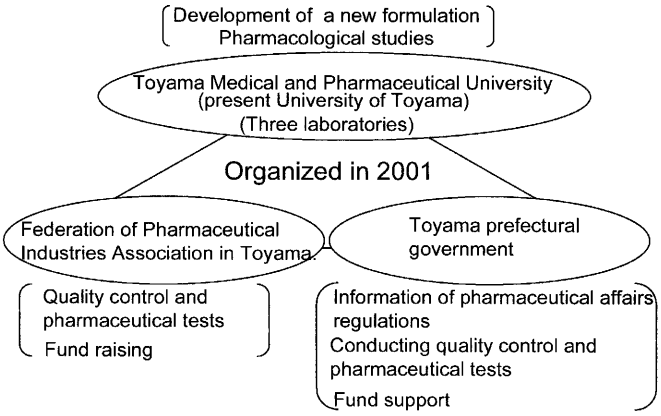

Fig. 1. Summary of the cooperative research project for "Toyama original brand Haichi-Yaku".

the Haichi-Yaku business in Toyama (Fig.1). Three laboratories of Toyama Medical and Pharmaceutical University (the present University of Toyama) took an active part in the project to form the collaborative research base among industry, Toyama Prefecture and academia, the so-called "San-Gaku-Kan-Gaku (産学官)" project. In the present review, the contents and pharmacological outline of a new formulation (trade name: PanaWang) are summarized.

2. Concept of the new formulation

In the initial discussions regarding the cooperative research project, it was decided that a new formulation should be devised within the administrative classification of the conventional tonic Haichi-Yaku (滋養強壮剤) regulated by the Ministry of Health, Labor and Welfare of Japan. Although conventional tonic Haichi-Yaku have been designed for use by customers who are infirm or have a fragile constitution, the number of these customers is decreasing, while the number of patients (or pre-patients) with so-called lifestyle-related diseases is currently increasing. Therefore, it was decided that the new formulation should be designed for the treatment of distress in either patients with a delicate and infirm constitution or those suffering from obesity and hypercholesterolemia due to excessive food intake and lack of exercise.

The symptoms and pathological lesions that can be treated with a new formulation are classified into two unique differential syndromes according to traditional Chinese medical diagnostics (Kampo diagnostics). The symptoms (**A** in Fig.2) due to infirmity or a fragile constitution are diagnosed as "deficiency syndrome (虚証)", which is the indication for treatment with tonic Kampo formulations such as Hochuekkito (補中益気湯), Juzentaihoto (十全大補湯) and Hachimijiogan (八味地黄丸), which strengthen weakened body resistance.

On the other hand, the symptoms and pathological lesions (**B** in Fig.2) classified into "excess syndrome (実証)" are similar to those observed in patients with risk factors for ischemic coronary artery disease and atherosclerosis in modern medicine (the so-called deadly quartet[4]). "Excess syndrome" is the sign for treatment with Kampo formulations such as Bofutsushosan (防風通聖散), Orengedokuto (黄連解毒湯), Saikokaryukotsuboreito (柴胡加竜骨牡蛎湯) and Keishibukuryogan (桂枝茯苓丸), which eliminate the domination of pathogenic factors. We have reported the usefulness of Bofutsushosan,[5] Orengedokuto,[6,7] Saikokaryukotsuboreito[8] and Keishibukuryogan[9] in cardiovascular risk-reduction therapy.

In Kampo medical treatment, the two therapeutic methods of nourishment and elimination are usually used in coordination for actual complicated cases. Therefore, it was decided to devise a new formulation adaptable to patients with either "deficiency syndrome" or "excess syndrome".

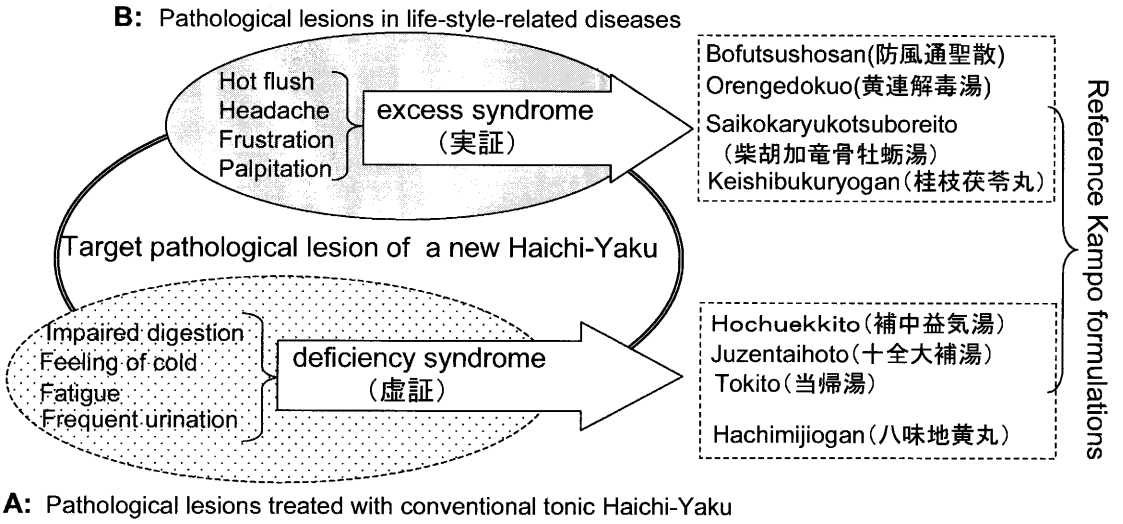

Fig. 2. Concept of the new formulation (Toyama original brand Haichi-Yaku).
The new formulation was designed to regulate either (**A**) symptoms responsive to conventional tonic Haichi-Yaku or (**B**) symptoms associated with lifestyle-related diseases. It was also designed on the basis of conventional Kampo-formulations used for excess syndrome and deficiency syndrome.

3. Selection of crude drugs for the new formulation

The crude drugs for the new formulation were selected in accordance with precedents for conventional tonic Haichi-Yaku, traditional Chinese medical tacit knowledge and scientific pharmacological evidence.

3.1. Precedents for conventional tonic Haichi-Yaku

First, from market research on the frequencies of crude drugs used in conventional tonic Haichi-Yaku, Ginseng Radix (人蔘, ginseng) and Allii Bulbus (大蒜, garlic) were selected as representative crude drugs used for conventional tonic Haichi-Yaku. Ginseng Radix and Allii Bulbus are also representative crude drugs used for "deficiency syndrome" in Kampo medicine. Since Ginseng Radix (*Panax ginseng* root) was ranked as the main component of the new formulation, it was named "PanaWang (蔘王)", which is derived from "King (Wang) of Haichi-Yaku containing Panax ginseng".

Bezoar Bovis (cattle gallbladder stones: 牛黄, Go-o in Japanese) was also chosen as a compound for PanaWang, because it is one of the representative crude drugs present in Haichi-Yaku, such as Rokushingan (六神丸), prepared in Toyama. Bezoar Bovis was also selected on the basis of reported pharmacological evidence[10] that it suppressed an "accelerated atherosclerosis model", as assessed by neointimal formation in the rat carotid artery after balloon endothelial denudation.

3.2. "Deficiency or hypofunction syndrome"

The other crude drugs included in PanaWang were selected on the basis of traditional Kampo medical knowledge (Fig. 3).

The symptoms and pathological lesions (**A** in Fig. 3) that are expected to respond to PanaWang are the impaired digestion, fatigue, palpitations and frequent urination at night found during prolonged illness, the restoration stage of some diseases and senile asthenia. These are diagnosed as "deficiency or hypofunction syndrome" caused by *Qi*-deficiency (気虚証) and *Xue*-deficiency syndrome (血虚証). In Kampo medicine, *Qi* (気) represents the physiological functions that participate in the growth and development of the human body, activate the formation and circulation of *Xue* (血: blood) and maintain body resistance against diseases. Therefore, a part of *Qi* function refers to the immune response in modern medicine. *Xue* (血) has the functions of nourishing and moistening the whole body and refers to blood function in modern medicine.

Among the crude drugs (補気薬) that invigorate and enrich *Qi*-deficiency, Ginseng Radix and Astragali Radix (黄耆) were chosen as compounds for the new formulation. *Xue*-deficiency is treated by using crude drugs that regulate and enrich *Xue* (補血薬), such as Angelicae Radix (当帰), Cnidii Rhizoma (川芎) and Paeoniae Radix (芍薬). Combinations of Ginseng Radix and Astragali Radix and of Angelicae Radix and Cnidii Rhizoma have generally been used in Kampo formulations. Furthermore, since *Qi*-deficiency often leads to increased *Xue*-deficiency, it was decided to use these five crude drugs together in the new formulation. The five crude drugs are fundamental components of Juzentaihoto (十全大補湯)[11,12,13] which is an important tonic Kampo formulation that invigorates and enriches both *Qi*- and *Xue*-deficiency and has been used for both general debility after illness and reducing the side effects of chemotherapy.[14]

Cnidii Moniieri Fructus (蛇床子) was also selected, since it is one of the crude drugs used for *Qi*-deficiency (especially Shen-deficiency: 腎虚証), a morbid state of aging

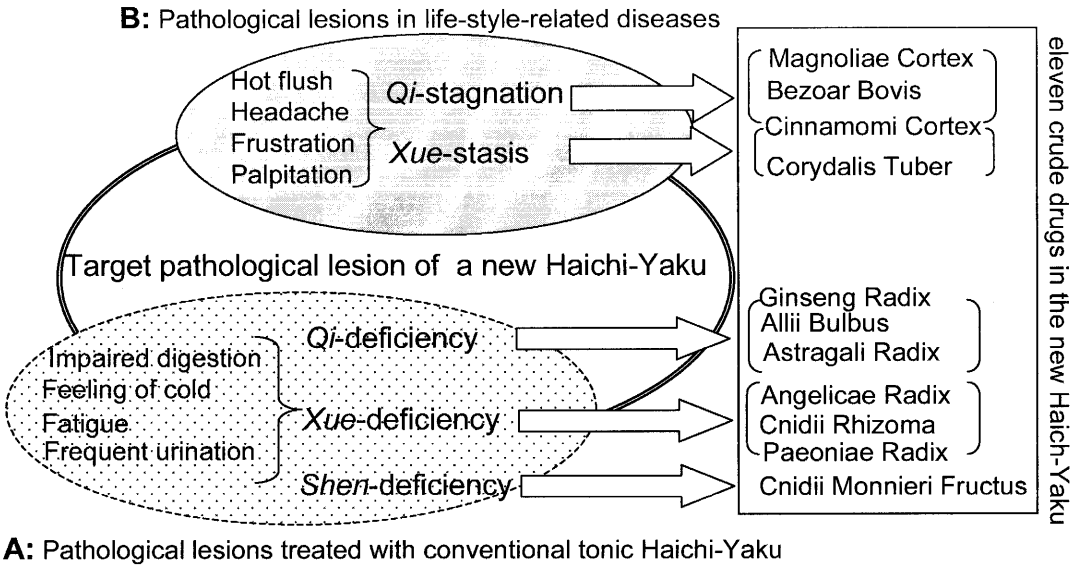

Fig. 3. Selection of crude drugs for the new formulation (Toyama original brand Haichi-Yaku).
Crude drugs were selected for the new formulation on the basis of precedents in conventional tonic formulations and Kampo-formulations, traditional Chinese medical tacit knowledge and scientific pharmacological evidence. Based on traditional Chinese medical knowledge, crude drugs that can nourish deficiencies in *Qi* and *Xue* and regulate their stagnation were selected.

manifested as fatigue, dry mouth, dizziness, lower back pain, frequent urination at night, and so on.

3.3. "Excess or hyperfunction syndrome"

The other symptoms and pathological lesions (**B** in Fig. 3) expected to respond to PanaWang are hot flushes, headaches and frustration, which are associated with so-called lifestyle-related diseases due to excessive food intake. These are diagnosed as "excess or hyperfunction syndrome" due to *Qi*-stagnation (気滞証) and *Xue*-stasis syndrome (瘀血証: Oketsu in Japanese).

Qi-stagnation is a disorder of vital energy activity manifested as local distention or pain and is treated by using crude drugs that regulate *Qi* (理気薬), such as Cinnamomi Cortex (桂皮), Magnoliae Cortex (厚朴) and Cnidii Rhizoma (川芎). These three drugs were therefore selected as compounds for PanaWang.

There is also a good pharmacological reason to select Magnoliae Cortex, since magnolol, an ingredient of Magnoliae Cortex, was shown to inhibit intimal thickening after balloon injury of the aorta in rabbits.[15] It was also reported that Cinnamomi Cortex[16] and Magnoliae Cortex[17] have inhibitory effects on platelet activation, which triggers the migration and proliferation of vascular smooth muscle cells (VSMC) in the intimal area (a pathological lesion of atherosclerosis) after endothelial cell injury.[18]

Bezoar Bovis has also been used for *Qi*-stagnation, which is caused by emotional depression and anger, and manifests as dizziness, palpitations, flushed face and irritability.

Xue-stasis syndrome is a morbid condition involving stagnation of the blood circulation or a pre-thrombosis state manifested as dark red gingiva and tongue, and is also a basic pathologic lesion of "excess syndrome". *Xue*-stasis is of special interest as one of the risk factors for ischemic coronary artery disease.[19]

Among the crude drugs for removing blood stasis and promoting blood circulation (活血薬), Corydalis Tuber (延胡索), Angelicae Radix, Cnidii Rhizoma and Cinnamomi Cortex were selected. Corydalis Tuber was also chosen on the grounds of its anti-platelet activity.[20] Since *Xue*-stasis syndrome is predominantly caused by *Qi*-stagnation, crude drugs that activate the circulation of both *Qi* and *Xue*, such as Cnidii Rhizoma (川芎) and Cinnamomi Cortex (桂皮), were chosen for use together in PanaWang.

3.4. Eleven crude drugs included in PanaWang

Therefore, a total of 11 crude drugs (Table 2) were selected as compounds for inclusion in PanaWang to regulate intermingled "deficiency syndrome" and "excess syndrome". The selection process combining Kampo medical tacit knowledge with scientific pharmacological evidence regarding crude drugs is an important aspect of Kampo pharmaceutics. The composition of the crude drugs included in PanaWang was decided based on experimental data on the effects of various pill preparations and on knowledge obtained through preparing the documents required for the production application.

Table 2. PanaWang, a new formulation
(Toyama original brand Haichi-Yaku)

PanaWang

6 pills three times a day after meals

Ginseng Radix (extract) (corresponds to 3015 mg of drug)	450 mg/day
Corydalis Tuber (extract) (corresponds to 600 mg of drug)	60 mg
Cnidii Monnieri Fructus (extract) (corresponds to 300 mg of drug)	30 mg
Prepared Allii Bulbus (Powder)	100 mg
Bezoar Bovis (Powder)	5 mg
Magnoliae Cortex (Powder)	90 mg
Angelicae Radix (Powder)	200 mg
Paeoniae Radix (Powder)	200 mg
Cnidii Rhizoma (Powder)	200 mg
Cinnamomi Cortex (Powder)	200 mg
Astragali Radix (Powder)	200 mg

166 mg／pill (6 mm diameter black pill)

4. Pharmacological effects of PanaWang

To clarify the pharmacological activities of PanaWang, the effects of PanaWang were assessed in a model animals of lifestyle-related diseases,[21] reactive oxygen species,[22] neointimal formation after endothelial injury,[23] and the balance of type I helper T cells (Th1) and Th2 cells.[24]

4.1 Effects of PanaWang on lifestyle-related disease models [21,22]

The preventive effects of PanaWang on lifestyle-related diseases models were assessed[21]. Eight-week-old male spontaneously hypertensive rats (SHR) and 30-week-old male spontaneously diabetic rats (WBN/Kob) were used in these studies. After the SHR were maintained for 1 week, they were randomly assigned to two groups. The control group received hypercholesterol chow consisting of standard chow with 4% cholesterol and 1% cholic acid, and the PanaWang group received hypercholesterol chow containing 1% (wt/wt) PanaWang for 8 weeks. Similarly, WBN/Kob rats were randomly assigned to two groups. The control group received diabetes-accelerating chow, and the PanaWang group received diabetes-accelerating chow containing 1% (wt/wt) PanaWang for 4 weeks. The rats were anesthetized and sacrificed by drawing blood from the heart. The levels of serum cholesterol, triglyceride, HDL cholesterol, lipid peroxide and fibrinogen were measured. The results showed that triglyceride and lipid peroxide decreased significantly in the PanaWang group compared to the control group of SHR (Fig. 4a), and lipid peroxide and fibrinogen decreased significantly in the PanaWang group compared to the control group of WBN/Kob (Fig. 4b).

The vasomotion of the thoracic aorta was also studied by the organ bath method. The results showed that

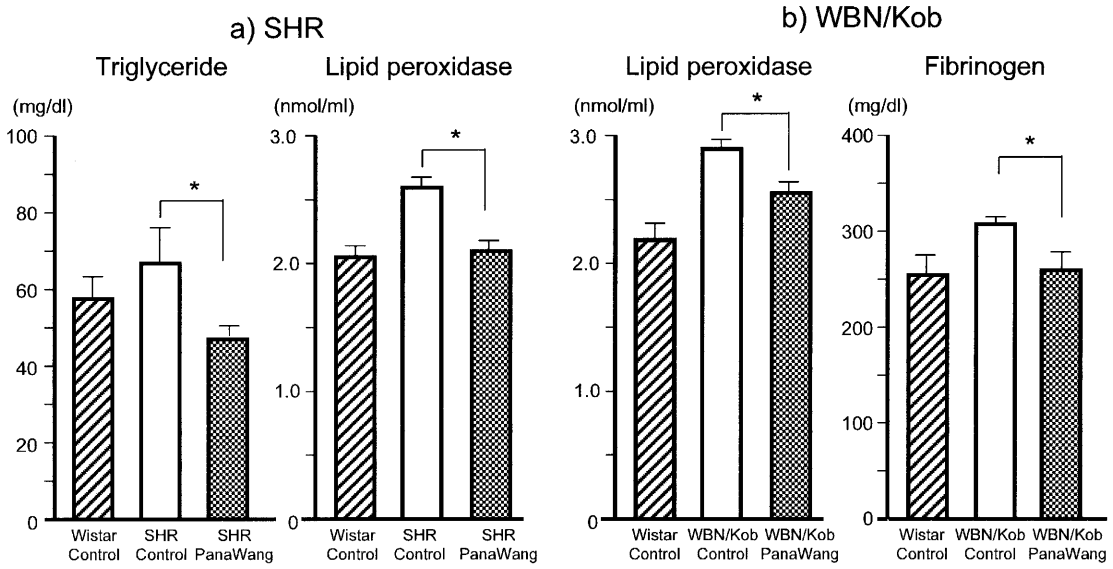

Fig. 4. Effects of PanaWang on the levels of serum triglyceride and lipid peroxidase in SHR (a), and lipid peroxidase and fibrinogen in WBN/Kob (b).
Values are mean±S.E., n=4, *p<0.05 compared with SHR or WBN/Kob control. (Modified from Ref. 21)

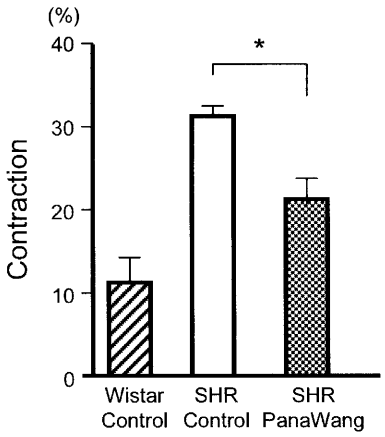

Fig. 5. Effect of PanaWang on PLA_2-induced vasocontraction.
All rat aortas had intact endothelium and had been treated with L-NAME. Contraction was expressed a percentage of the maximum contraction induced in 60 mM KCl. Values are mean±S.E., n=4, *p<0.05 compared with SHR control. (Modified from Ref. 21)

phospholipase A_2 (PLA_2)-induced contraction in the PanaWang group of SHR was significantly decreased as compared to that in the control group (Fig. 5).

The effects of PanaWang on reactive oxygen species (ROS) were also assessed.[22] The original powder of PanaWang (100g) was extracted with boiling water (500 ml) for 50 min, and then converted to freeze-dried powder

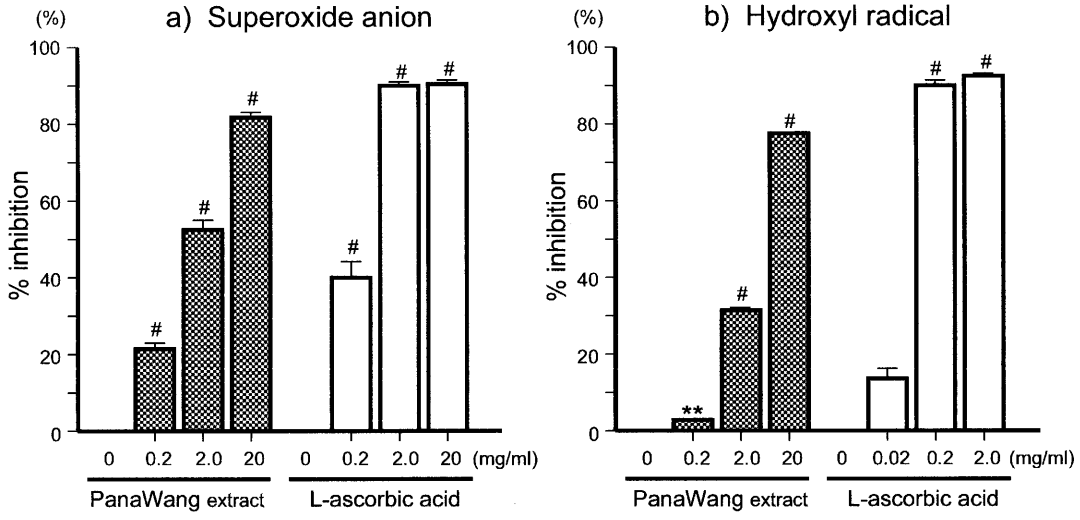

Fig. 6. Superoxide anion (a) and hydroxyl radical (b) scavenging activities of PanaWang extract and L-ascorbic acid.
Scavenging activities were assessed by the ESR technique. Values are mean±S.E., n=3, **p<0.01, #p<0.0001 compared with control (pure water). (Modified from Ref. 22)

(yield, 44.5%). Superoxide anion ($O_2^{-}\cdot$) and hydroxyl radical (HO·) scavenging activities were assessed by the electron spin resonance (ESR) technique. The results showed that PanaWang extract showed $O_2^{-}\cdot$ scavenging activity at the concentrations of 0.2 mg/ml and over, and its IC_{50} was 1.43 mg/ml (Fig. 6a). The extract showed HO· scavenging activity at the concentrations of 0.2 mg/ml and over, and its IC_{50} was 4.01 mg/ml (Fig. 6b). Peroxynitrate anion (ONO O^{-}), which was produced by the reaction of $O_2^{-}\cdot$ and nitric oxide (NO) free radical (NO·), and HO·, are known to be especially highly toxic reactive oxygen species to neuronal cells. Therefore, we studied whether PanaWang extract had a protective effect against NO donor-induced neuronal death in cultured cerebellar granule cells from rats in the MTT assay. The results showed that PanaWang extract (100 μg/ml) inhibited neuronal death induced by application of sodium nitroprusside (SNP) (30 μM) for 24 hours (Fig. 7).

From the present studies, it was clarified that PanaWang improves the plasma levels of triglyceride, lipid peroxide and fibrinogen in lifestyle-related disease models. Further, PanaWang inhibited PLA2-induced vasocontraction, so it is suggested that this drug has a protective effect on endothelial function. It is known that ROS play important roles in the progression of arteriosclerosis and disturbances of multiple organs. We showed that PanaWang has scavenging ability against ROS. Based on these results, we believe that PanaWang is a useful drug for the prevention of lifestyle-related diseases.

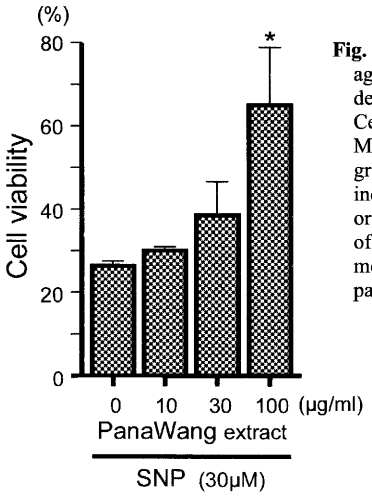

Fig. 7. Effect of PanaWang extract against SNP-induced neuronal death.
Cell viability was evaluated by the MTT assay in cultured cerebellar granule cells from rats. Cells were incubated with SNP (30μM) with or without various concentrations of PanaWang extract. Values are mean ± S.E., n=3, *p<0.05 compared with control.
(Modified from Ref. 22)

4.2. Effects of oral administration of PanaWang on an "accelerated atherosclerosis" model[23)]

The preventive effects of PanaWang on atherosclerosis were assessed by examining neointimal formation and the proliferation of vascular smooth muscle cells (VSMCs) after balloon endothelial denudation in balloon-injured carotid arteries in rats. Balloon endothelial denudation was performed in the left carotid artery of anesthetized Wistar rats (n= 8) administered a normal diet containing PanaWang (three doses, as shown in Fig. 8) for 3 days before and then

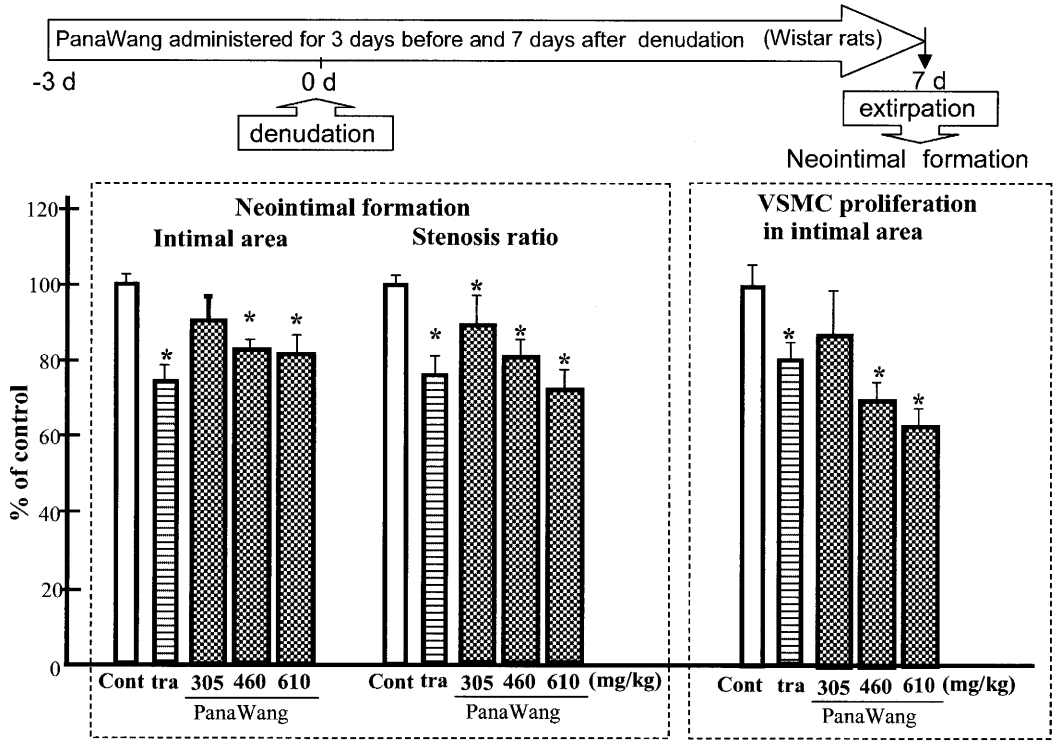

Fig. 8. Effects of PanaWang on an "accelerated atherosclerosis" model.
Each value represents the percentage (mean ± S.D.) relative the control value (n= 8). Cont: Control. Tra: tranilast, a positive control compound that was initially identified as an inhibitor of mast cell degranulation and was recently shown to inhibit VSMC proliferation in the balloon injury model. *: p<0.05 vs. the control. Stenosis ratio (%): (intimal area) x 100 / (intimal area + luminal area).
VSMC proliferation in the intimal area was assessed by the proliferating cell nuclear antigen (PCNA) labeling index, an index of the number of VSMCs in the growth phase in the intimal area evaluated using a monoclonal anti-PCNA antibody. (Modified from Ref. 23)

7 days after the injury. After this period, the rats were sacrificed and the injured carotid arteries were removed for histopathologic evaluation of intimal thickening and immunohistochemical analysis of VSMC proliferation. The present neointimal formation model in rat carotid arteries possesses, at least in part, similar pathological characteristics to atherosclerotic lesions in humans and is considered to represent "accelerated atherosclerosis".[25] Some Kampo formulations have been shown to reduce the progression of neointimal formation in this model.[5,7,8]

As shown in Fig. 8 (left panel), the increase in the intimal area at 7 days after endothelial denudation was reduced by treatment with PanaWang compared with that in the denuded control group. The stenosis ratio (middle panel of Fig.8), which is an index of the increase in the intimal area and decrease in the luminal area, was dose-dependently and significantly reduced in the PanaWang-treated groups. Intimal VSMC proliferation (right panel of Fig.8), which is an index of the number of VSMCs in the growth phase in the intimal area, was evaluated using a monoclonal anti-proliferating cell nuclear antigen (PCNA) antibody and found to be dose-dependently reduced by treatment with PanaWang (3 doses) for 10 days. VSMC proliferation is considered to be a major factor in the pathogenesis of neointimal formation.[26]

Furthermore, PanaWang extract was found to have protective effects against SNP-induced neuronal death in cultured cerebellar granule cells (Fig. 7). Among the crude drugs contained in PanaWang, Magnoliae Cortex and Corydalis Tuber had strong scavenging activities for superoxide anions and hydroxyl radicals.[19] These reactive oxygen species induced by mechanical disruption of endothelial cells during injury result in macrophage oxidation, which triggers the migration and proliferation of VSMCs[27] and progresses to atherosclerosis as a chronic inflammatory disease state.[28] Therefore, the inhibitory effects of the crude drugs present in PanaWang on free radical production may contribute to its ability to inhibit neointimal formation after endothelial denudation.

Thus, PanaWang may attenuate carotid artery intimal thickening following balloon endothelial denudation via inhibition of VSMC proliferation (Fig.9). Although this was a preliminary study, the results suggest that PanaWang is a promising candidate for preventing atherosclerosis resulting from long-term inappropriate lifestyles.

4.3. Effects of oral administration of PanaWang on the balance of Th1/Th2 in immune responses[24]

Crude drug prescriptions have been recognized as potentially valid by the scientific medical establishment, and their use has been increasing. Since traditional crude drug prescriptions are generally prepared from combinations of many crude drugs on the basis of oriental prescriptions and herbalogy, they may have combined effects that differ from the sum of the effects of the individual constituent crude drugs. PanaWang has been constructed based on traditional philosophy and scientific evidence, as described above.

$CD4^+$ helper T cells can be subdivided into Th1 and Th2 cells.[29] As shown in Fig. 10, Th1 cells predominantly synthesize interferon-γ (IFN-γ) and interleukin-2 (IL-2), and induce cellular immunity (*e.g.* against cancer and infection). On the other hand, Th2 cells produce IL-4, IL-5 and IL-13, and induce humoral immunity.[30] It has been demonstrated that the Th1 and Th2 types of immune response are reciprocally regulated *in vivo*.[31,32] The Th1 cytokine IFN-γ inhibits the proliferative response of Th2 cells, whereas Th2 cytokines IL-4 and IL-10 can inhibit the production of IL-2 and IFN-γ. Therefore, the balance of Th1 and Th2 cells is considered to be important for the regulation of immune functions. It has also been suggested that many diseases are partially caused by a skewed Th1 and Th2 cytokine balance.[33,34] For example, an increase in Th2 type cytokine production is observed in patients with systemic lupus erythematosus (SLE) or asthma.[35,36] Conversely, Th1 cells mediate inflammatory diseases such as graft versus host disease (GVHD).[37]

To obtain scientific evidence regarding the immunomodulating potential of PanaWang, focusing on the balance of Th1/Th2 cytokines, *ex vivo* experiments were carried out using splenocytes from normal mice orally given PanaWang

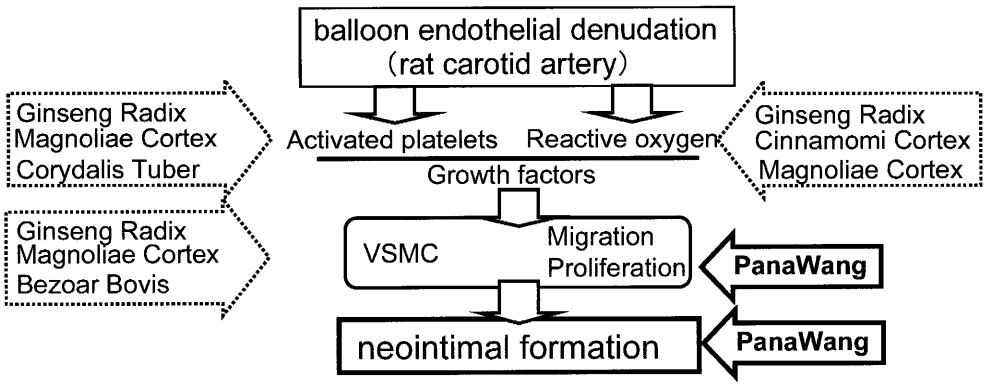

Fig. 9. Effects of PanaWang and its components on an "accelerated atherosclerosis model".
⇦ Inhibitory effects of PanaWang examined in the present experiments.
⇦ Reported inhibitory effects of crude drugs included in PanaWang. (Modified from Ref. 23)

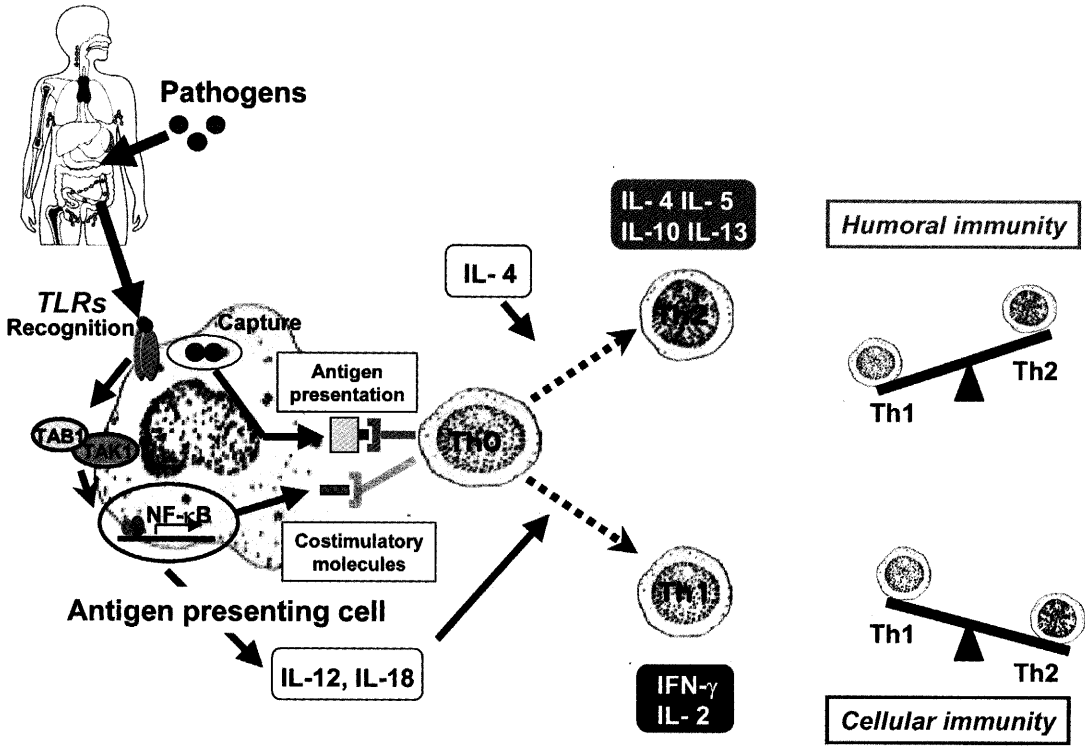

Fig. 10. Innate immunity and adaptive immunity
Phagocytosis is an important step for host defense against microbial pathogens. Innate immune cells, such as macrophages and dendritic cells, engulf pathogens by phagocytosis, and subsequently present pathogen-derived peptide antigens to naive T cells. In addition, Toll-like receptors (TLRs) recognize pathogen-derived components and induce expression of genes such as those encoding co-stimulatory molecules and inflammatory cytokines. Thus, phagocytosis-mediated antigen presentation, together with TLR-mediated expression of co-stimulatory molecules and inflammatory cytokines, instructs the development of antigen-specific adaptive immunity. Th1 cells predominantly synthesize interferon-γ (IFN-γ) and interleukin-2 (IL-2), and induce cellular immunity (*e.g.* against cancer and infection). On the other hand, Th2 cells produce IL-4, IL-5 and IL-13, and induce humoral immunity. The Th1 and Th2 types of immune response are reciprocally regulated *in vivo*. Th1 cytokine IFN-γ inhibits the proliferative response of Th2 cells, whereas Th2 cytokines IL-4 and IL-10 inhibit the production of IL-2 and IFN-γ. Therefore, the balance of Th1 and Th2 cells is considered to be important for the regulation of immune functions. Many diseases are partially caused by a skewed Th1 and Th2 cytokine balance.

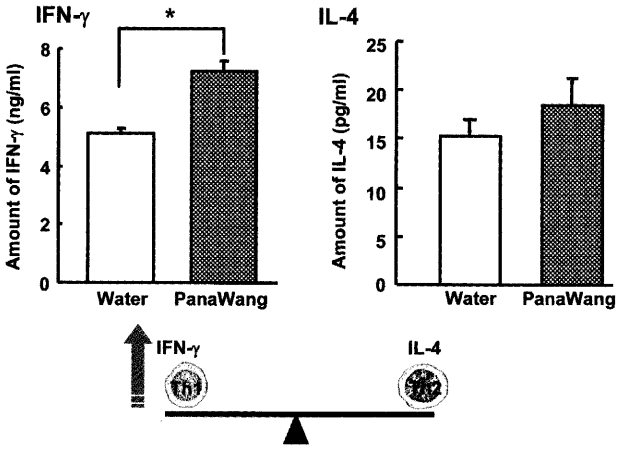

Fig. 11. Effects of PanaWang on the production of IFN-γ and IL-4 in normal splenocytes.
BALB/c mice were treated with 12.2 mg of PanaWang or water orally for 7 days. One day after the last administration, mice were sacrificed and whole splenocytes (2.5×10^6/well) suspended in RPMI medium supplemented with 10% FBS were cultured in 24-well culture plates with or without 4 ng/ml of Con A for 24 h. After the termination of the culture, the supernatants were collected and the amounts of IFN-γ and IL-4 were measured by ELISA. The results are represented by the mean ± S.D. for five mice. *, $p < 0.01$ as compared with the control using Student's two-tailed *t*-test. (Modified from Ref. 24)

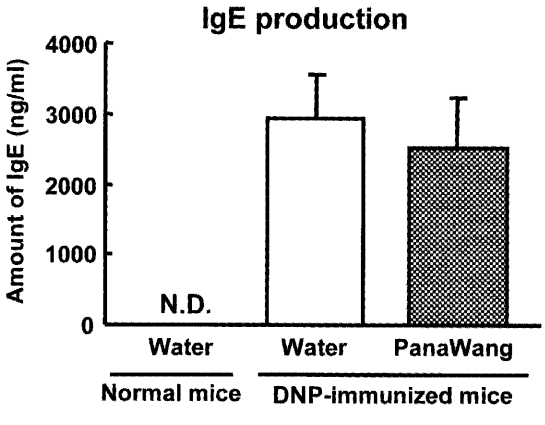

Fig. 12. Effect of PanaWang on the amount of DNP-specific IgE in serum.
BALB/c mice were intraperitoneally immunized with DNP-KLH+Alum. Boosting was carried out on day 14. Daily oral administration of PanaWang or water started on the day of priming. Sera were obtained on day 28 and the amount of DNP-specific IgE was measured by ELISA. The results are expressed as the mean ± S.D. for five BALB/c mice. N.D. means not detectable. *, $p < 0.05$. (Modified from Ref. 24)

for 7 days. Splenocytes were stimulated with the T cell mitogen Con A for 24 h, and the secretion of a Th1 cytokine (IFN-γ) and a Th2 cytokine (IL-4) was examined by ELISA. Fig. 11 shows that IFN-γ production was significantly enhanced by treatment with PanaWang for 7 days. On the other hand, IL-4 production was not affected. These results suggest that Th1 responses were up-regulated by PanaWang.

It has been shown that immunization with DNP together with Alum induces the production of antigen-specific IgE, which is a marker for Th2-mediated immune responses. To examine the effect of PanaWang on IgE production, mice were primed with DNP-KLH on day 0 and boosted on day 14. Daily oral administration of PanaWang started from the day of priming. DNP-specific IgE in serum was determined on day 28 after priming by ELISA (Fig. 12). There was no discernible difference between the PanaWang-treated group and control group in the production of IgE in the serum, suggesting that PanaWang did not affect the DNP-induced Th2 immune responses *in vivo*.

As described in Fig. 11, PanaWang enhanced Con A-induced production of IFN-γ, but not IL-4, in splenocytes of normal mice. We next investigated the effect of PanaWang on antigen-induced IFN-γ and IL-4 production in splenocytes of DNP-immunized mice. Administration of PanaWang enhanced antigen-induced IFN-γ production, whereas it did not affect IL-4 production (Fig. 13). Thus, oral administration of PanaWang leads predominantly to enhanced production of Th1 cytokine in Th2-activating mice as well as normal mice.

Antigen-mediated stimulation is known to induce proliferative responses of lymphocytes. Therefore, we examined the effect of PanaWang on the antigen-induced proliferation of splenocytes. Splenocytes from immunized mice were treated with DNP for 72 h and cell proliferation was examined by the WST-1 assay. As shown in Fig. 14, Con A-stimulated proliferation of splenocytes from immunized mice was significantly enhanced by administration of PanaWang. These results indicate that oral administration of PanaWang can lead to the dominant induction of Th1-type immune responses, possibly through the induction of IFN-γ production. Since PanaWang has been shown to have a protective effect on NO-induced neuronal cell death, it is possible that down-regulation of NO function is related to the enhancement of Th1 responses by PanaWang.

Th1 cytokines induce cellular immunity by activating $CD8^+$ T cells, natural killer cells and macrophages. Th1-dominant immune responses are responsible for the induction of protection against bacterial and viral infections, and of anti-tumor effects. On the other hand, it is well accepted that allergic reactions are controlled by Th2-dominant immune responses. Therefore, enhancement of IFN-γ production by PanaWang may potentiate Th1-mediated immunity and consequently reduce Th2-mediated allergic pathogenesis.

In summary, we demonstrated that PanaWang stimulates Th1 cytokine production from splenic lymphocytes. Further studies on the production of other cytokines by lymphocytes and antigen-presenting cells such as dendritic cells will advance our understanding of the immunomodulating activity of PanaWang. In addition, we performed *ex vivo* experiments in this study; therefore, it is necessary to further clarify the enhanced Th1 responses *in vivo*, especially in some pathogenic animal models.

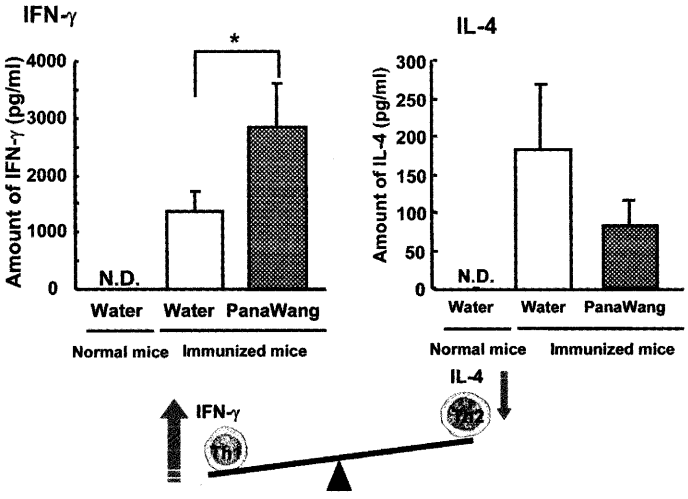

Fig. 13. Effect of PanaWang on the production of IFN-γ and IL-4 in immunized splenocytes.
Female BALB/c mice were immunized with DNP-KLH+Alum and administered PanaWang as described in Fig.12. Mice were sacrificed on day 28 and splenocytes (2.5×10^6/well) were cultured with DNP-KLH (1 μg/mL) for 72 h. The supernatants were collected and the amounts of INF-γ and IL-4 were measured by ELISA. N.D. means not detectable. The results are expressed as the mean ± S.D. for five mice. *, $p < 0.05$. (Modified from Ref. 24)

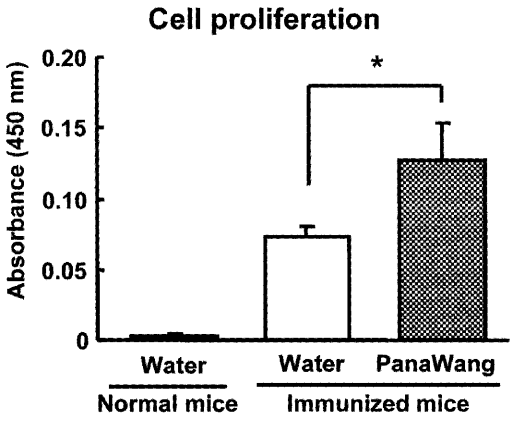

Fig. 14. Effect of PanaWang on antigen-induced proliferation of immunized splenocytes.
Female BALB/c mice were immunized with DNP-KLH+Alum and administered PanaWang as described in Fig.12. Mice were sacrificed on day 28 and splenocytes (2.5×10^6/well) were cultured with DNP-KLH (1 μg/mL) for 72 h. WST-1 solution was added 4 h before the termination of the culture. The absorbance of the culture was measured at 450 nm. The results are expressed as the mean ± S.D. for five mice. *, $p < 0.05$; **, $p < 0.01$. (Modified from Ref. 24)

5. General discussion

The aim of the present cooperative research project was to devise a new formulation of crude drugs to satisfy the requests of patients (or pre-patients) with lifestyle-related diseases due to excessive food intake. After consideration of the general principles of Kampo medical treatment, the 11 crude drugs were chosen on the basis of their effects on strengthening the body resistance (扶正) for "deficiency syndrome (虚証)" or eliminating pathogenic factors (祛邪) for "excess syndrome (実証)".

Furthermore, the pharmacological findings indicate that PanaWang may be a useful drug for the prevention of lifestyle-related diseases and may potentiate Th1-mediated immunity and consequently reduce Th2-mediated allergic pathogenesis. These results suggest that PanaWang is useful for both "deficiency syndrome" and "excess syndrome", as planned. Therefore, PanaWang, a new formulation including 11 crude drugs Table 2, may be a promising formulation suitable for modern-day self medication.

In August 2005, PanaWang received a license for practical production regulated by the Ministry of Health, Labor and Welfare of Japan, and will be sold in the home-selling system from the beginning of 2006.

Acknowledgements

This study was supported in part by the Grant for Federation of Pharmaceutical Industries Association in Toyama and by the Grant-in-Aid for the 21st Century COE Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Japanese abstract

本稿では富山県内の産（富山県薬業連合会）官（富山県）学（富山大学）プロジェクト（富山オリジナルブランド配置薬開発研究会）で開発された新たな配置薬に開発経緯と薬理作用研究の結果を概説した。

新配置薬の開発の狙いは既存の滋養強壮剤に生活習慣病の予防効果を付与することにある。新配置薬に配合する生薬は漢方医療の基本概念（虚証と実証の病態を調整する補薬と瀉薬の用法）に基づいて候補を選び，現代の薬理研究情報を加味して絞り込んだ。このようにして創案した11生薬を含む新配置薬は主薬の薬用人参（*Panax ginseng*）を含む配置薬の王様（Wang）になることを期待してパナワン（PanaWang：蔘王）と命名された。

新配置薬は血管病変の発症や進展を予防し，type I helper T cell（Th1）機能を賦活することが明らかになった。これは生活習慣病や感染症の予防やアレルギー炎症の軽減に有用であることが示唆される。新配置薬は2005年に製造承認を得て，2006年から配置薬市場で回商される。

*〒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