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Inhibitory effects of a newly devised crude drug-preparation on intimal thickening after endothelial injury in rats

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A new preparation (PanaWang) containing eleven crude drugs was devised for self-medication to relieve subjective symptoms attendant to life-style diseases. The inhibitory effects of oral administration of PanaWang on the "accelerated atherosclerosis model" in intimal formation in rat carotid arteries after balloon endothelial denudation was examined. Administration of PanaWang 3 days before and 7 days after denudation dose-dependently suppressed the increased intimal thickening. In immunohistochemical analysis performed using a monoclonal anti-proliferating cell nuclear antigen (PCNA) antibody to stain vascular smooth muscle cells (VSMCs) in the intimal area, administration of PanaWang for 10 days reduced the proliferation of VSMCs. The inhibitory potency of PanaWang on VSMC proliferation partly contributes to its preventive effect on intimal thickening. These results indicated that, PanaWang might be useful for preventing atherosclerosis after endothelial injury resulting from long-term inappropriate life-styles.

Key words atherosclerosis, intimal thickening, vascular smooth muscle cells, balloon endothelial denudation, Panax ginseng.

Introduction

Traditional preparations containing crude drugs manufactured in Toyama have been delivered to households by traveling salesmen throughout Japan. These preparations used in the "drugs-on-deposit system (so-called Haichi-Yaku or Oki-Gusuri)" have been contributing to self-medication (preserving one's own health) and primary health care. In 2001, the industrial-governmental-university complex was organized to produce a new Haichi-Yaku to relieve various symptoms of life-style related diseases by self-medication. Based on joint studies and developments in the complex, a new preparation containing eleven crude drugs possessing anti-lipemic, anti-platelet, scavenging effects for reactive oxygen species, and so on was developed. The new preparation was named "PanaWang" in expectation that it would become a "king (Wang)" of Haichi-Yaku containing *Panax ginseng* root, a main component of the preparation.

In the course of our studies to find preventive agents against atherosclerosis from natural medicinal sources,[1] we examined here the inhibitory effects of PanaWang on intimal formation in rat carotid arteries induced by balloon endothelial denudation. The endothelial denudation model used in the present report has been considered to be an "accelerated atherosclerosis" model[2] and has been used in primary screening methods to evaluate pharmaceutical candidates.[3]

Materials and Methods

Samples and reagents. The following preparation (PanaWang) containing eleven crude drugs was used: Ginseng Radix (30% EtOH extract: 545.5), Bezoar Bovis (powder: 5), Corydalis Tuber (30% EtOH extract: 60), Cnidii Fructus (30% EtOH extract: 30), Prepared Allii Bulbus (powder: 100), Magnoliae Cortex (powder: 90), Angericae Radix (powder: 200), Paeoniae Radix (powder: 200), Cnidii Rhizoma (powder: 200), Cinnamomi Cortex (powder: 200), and Astragali Radix (powder: 200). Each figure in parentheses represents the weight (mg/day) of each component in the preparation. The common human daily dose of the preparation is 1830.5 mg. The quality standard of 11 crude drugs and HPLC profile of the preparation were described in our previous report.[4]

The sources of tranilast (reference compound), anti-PCNA monoclonal antibody (PC-10), biotinylated anti-mouse second antibody and streptavidin-conjugated peroxidase were the same as in our previous report.[5]

Animal experiments. The balloon endothelial denudation in the left carotid artery of male Wistar rats (13 weeks old, 340-360 g in body weight, Sankyo Lab. Service, Tokyo, Japan) anesthetized with pentobarbital was performed according to our previously described method.[5] PanaWang (305, 460, and 610 mg/kg/ day; 305 mg/kg is 10-fold higher than the human daily dose) was mixed in the normal diet and administered to rats (n= 8) for 3 days prior to balloon endothelial denudation and then for 7 days after the injury. Tranilast (50 mg / kg / day; 10-fold higher than the human daily dose, n = 8) suspended in 0.5% carboxymethylcellulose

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sodium salt was administered orally during the same period as the PanaWang treatment.

All animal experiments and care were conducted in conformance with the Guidelines of the Animal Care and Use Committee of Toyama Medical and Pharmaceutical University approved by the Japanese Association of Laboratory Animal Care.

Histological and immunohistochemical analyses. The intimal formation in left carotid artery sections 7 days after denudation was evaluated histologically according to our previous method.[5] Proliferation of VSMC in the intimal area 7 days after denudation was also evaluated by immunohistochemical analysis using a monoclonal anti-PCNA antibody according to our previous method.[5]

Statistical analysis of data. The results are shown as the mean ± S.D. of the indicated number (n) of experiments. Statistical analysis of differences was performed by one-way and multiple analysis of variation (ANOVA). Differences were accepted as statistically significant at a p value of < 0.05.

Results and Discussion

Body weight and food intake. During the 10 days experimental period, all rats (n = 8) survived. No abnormality of body weight or food intake was recognized even when PanaWang was administered at the maximum dose (610 mg/kg; 20-fold higher than the common human daily dose) for 10 days (body weight ratio on the 10th day was 98.2 ± 1.9% of that on the first day).

Intimal formation (Fig.1). Fig.1 shows that the increase of intimal area in the denuded control group 7 days after endothelial denudation was reduced by treatment with PanaWang for 10 days. The decrease of luminal area in the denuded control group was also attenuated by the oral administration of PanaWang. The stenosis ratio, which is an index of the increase of intimal area and decrease of luminal area assessed by the equation shown in the legend of Fig.1, was dose-dependently and significantly reduced in the PanaWang treated groups. A reference compound, tranilast, which is an anti-allergic drug that is known to exert the inhibitory effects on intimal formation in animal models,[3] also significantly reduced the intimal area and stenosis ratio.

VSMC proliferation (Fig.2). The PCNA labeling index, which is an index of the number of VSMCs in growth phase in the intimal area assessed by the equation as shown in the legend of Fig.2, was dose-dependently reduced by treatment with PanaWang for 10 days. At 10-fold higher than the common human daily dose, PanaWang exerted the inhibitory effects comparable to those of tranilast. As VSMC proliferation is considered to be a major factor in the pathogenesis of intimal thickening,[6] the inhibitory effect of PanaWang on intimal formation after balloon endothelial denudation might be due to the anti-proliferation effect against VSMCs.

The intimal formation initiated by endothelial cell injury results in platelet activation and generation of reactive oxygen species, which trigger the migration and proliferation of VSMCs.[7] PanaWang contains such anti-platelet crude drugs as Ginseng Radix,[8] Bezoar Bovis,[9] Cinnamomi Cortex (cinnamic aldehyde),[10] Magnoliae Cortex (magnolol and

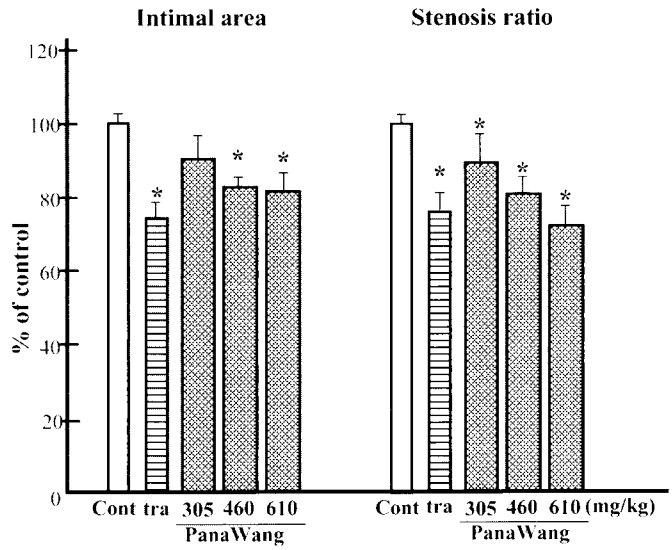

Fig. 1 Effects of PanaWang on intimal area and stenosis ratio in rat carotid artery 7 days after balloon endothelial denudation
Each value represents the percentage (mean ± S.D.) of the control value (n= 8).
Stenosis ratio (%): (intimal area) x 100 / (intimal area + luminal area).
The dose of 305 (mg/kg/day) of PanaWang is 10-fold higher than the common human daily dose.
Cont: denuded control, tra: tranilast (50 mg/kg/day; 10-fold higher than the common human daily dose).
*$p < 0.05$ significantly different from the control.

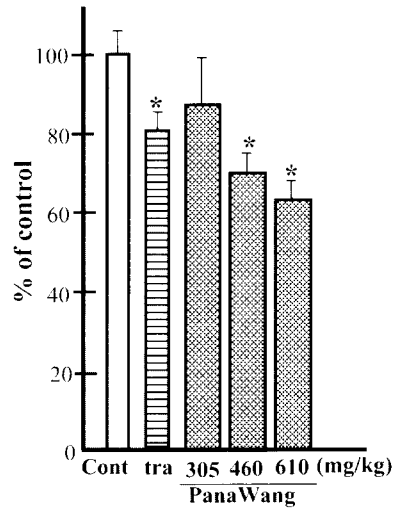

Fig. 2 Effects of PanaWang on VSMC proliferation evaluated by PCNA labeling index in rat carotid artery 7 days after balloon endothelial denudation
Each value represents the percentage (mean ± S.D.) of the control value (n= 8).
PCNA labeling index (%): (number of PCNA-positive VSMCs in intimal area) x 100 / (number of total VSMCs in intimal area).
Cont: denuded control, tra: tranilast (50 mg/kg/day).
*$p < 0.05$ significantly different from the control.

honokol),[11] and Corydalis Tuber.[12] Furthermore, it was recently reported that some components (Magnoliae Cortex, Cinnamomi Cortex, and Paeoniae Radix) of PanaWang have scavenging effects for superoxide anion.[13] Recently, magnolol isolated from Magnoliae Cortex was shown to inhibit intimal thickening after balloon injury of the aorta in rabbits.[14] These reported inhibitory effects of the crude drugs included in PanaWang against platelet functions and reactive oxygen species may, at least in part, contribute to its suppression of intimal thickening.

The aim of the present study was to examine whether intimal formation after endothelial injury is prevented by a new preparation (PanaWang) containing eleven crude drugs, which was developed to relieve subjective symptoms attendant to life-style diseases. In conclusion, the suppressive effect of PanaWang treatment for 10 days on intimal thickening after balloon endothelial denudation in rat carotid arteries may result from its inhibitory effect against VSMC proliferation. These results suggest that PanaWang is one of the promising candidates for preventing atherosclerosis resulting from long-term inappropriate lifestyles. Further studies to examine the mechanism of the effects on the migration and cell cycle of VSMCs will be necessary.

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Japanese abstract

富山県の産（富山県薬業連合会）官（富山県）学（富山医科薬科大学）の研究グループが飽食時代に適した新たな滋養強壮薬（富山オリジナルブランド配置薬）の開発を進めた。新処方の配剤生薬は従来の滋養強壮薬の効能に生活習慣病の血管病変の予防作用を付与する視点から選ばれた。文献調査や市場調査および薬理と製剤化の実験を経て11種類の生薬配合剤が創案された。新製剤（丸剤）は主薬の薬用人参の基原植物名（*Panax ginseng*）と王様（Wang）に因んでPanaWangと命名された。

本研究では10日間経口投与されたPanaWangがラット頸動脈をカテーテルで擦過した後の内膜肥厚を抑制することが明らかにされた。この内皮細胞傷害病態は加速された動脈硬化症モデルといわれている。PanaWangの作用機序は血管平滑筋細胞の増殖抑制にあることが免疫組織化学的に検証された。PanaWangの作用には配剤された11生薬の血小板凝集抑制作用（薬用人参，延胡索，牛黄など）や活性酸素種の消去作用（厚朴，桂皮，芍薬など）が関与していることが文献考証に基づいて推論された。

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