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Effects of Nanpao®, a *Kampo* Medicine, on Peripheral Blood Flow and Surface Skin Temperature in Aged Female Rats

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Abstract: The effects of long-term treatment with Nanpao, a *kampo* medicine, on cold constitution were evaluated in aged female rats. Five-month-old rats were administered Nanpao orally at doses of 0, 30, or 100 mg/kg/day. The peripheral blood flow and surface skin temperature in the hind paws were measured using a laser Doppler blood flow meter and infrared thermography, respectively. In animals treated with Nanpao, the peripheral blood flow increased dose-dependently compared to that in the control group. Moreover, the surface skin temperature after immersion in ice-cold water was higher in the Nanpao-treated groups than in the control group at all measurement times. These results suggest that Nanpao has the potential to improve cold constitution associated with decreased peripheral blood flow in women.

Key words: cold constitution, *kampo* medicine, Nanpao

Nanpao is an over the counter drug (OTC) *kampo* medicine for humans. It is a mixture of 31 Chinese crude drugs (Table 1) that exerts a nourishing and revitalizing effect. Long-term administration of Nanpao has been shown to improve age-related deterioration of some reproductive functions in aged male [12] and female rats [10]. It has also been reported to exhibit clinical effects on various age-related symptoms and to improve cold constitution in more than half of the patients treated [13].

The existence of cold constitution in women was first reported in 1956 [14], and its relation to various diseases was reported recently [1, 9]. Sadakata and Yamada [18] have suggested that cold constitution might be caused by an abnormality in the distribution of thermal receptors, which are located in the subcutaneous tissue and are responsible for temperature sensation, and by chronically reduced peripheral blood flow.

Nowadays, the majority of women suffer from cold constitution; therefore, the potential of Nanpao for improvement of cold constitution has important implications for enhancing the quality of life of women.

As part of the elucidation of the mechanism of the effects of Nanpao on cold constitution, this study was performed with a focus on improving hemodynamics and maintaining surface temperature, since Nanpao con-

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Table 1. Components and daily dose of Nanpao for human use

Linnean classification	Components Pinyin name	Amount (mg)
Cervi Parvum Cornu	Lurong	67
Canis Penis et Testis	Guanggoushen	53
Donkey Penis et Testis	Heilvshen	235
Angelicae Radix	Danggui	133
Ginseng Radix	Renshen	133
Moutan Cortex	Mudanpi	33
Hippocampus	Haima	67
Eucommiae Cortex	Duzhong	67
Asini Gelatinum	Ajiao	67
Cinnamomi Cortex	Guipi	53
Curculiginis Rhizoma	Xianmao	67
Cuscutae Semen	Tusizi	67
Psoraliae Semen	Buguzhi	67
Epimedii Herba	Yinyanghuo	133
Trigonellae Semen	Huluba	67
Morindae Radix	Bajitian	67
Cistanchis Herba	Roucongrong	67
Cynomorii Herba	Suoyang	67
Dipsaci Radix	Chuanjiduan	33
Rehmanniae Radix	Shudihuang	133
Rubi Fructus	Fupenzi	67
Aconiti Tuber	Paofuzi	67
Lycii Fructus	Gouqizi	133
Scrophulariae Radix	Xuanshen	67
Astragali Radix	Huangqi	133
Atractylodis Rhizoma	Baizhu	67
Corni Fructus	Shanzhuyu	67
Hoelen	Fuling	133
Ophiopogonis Tuber	Maimendong	67
Achyranthis Radix	Niuxi	33
Glycyrrhizae Radix	Gancao	33

tains herbs with vasodilatory effects.

In this study, the effects on cold constitution of long-term administration of Nanpao were examined quantitatively and objectively using measurements of peripheral blood flow and surface skin temperature in aged female rats.

Forty-eight female Sprague-Dawley (Crl:SD) rats were purchased from Charles River Japan, Inc., at the age of 5 months and used in this study. During the experimental period, the animals were housed individually in stainless steel wire cages (430 W × 450 D × 170 H mm) in a barrier-sustained animal room (temperature: 23 ± 1°C; relative humidity: 55 ± 5%; fresh-air ventilation: 12 changes/h; lighting: 12-h light/12-h dark cycle), and the measurements of peripheral blood flow and surface skin temperature were performed in this animal room. The animals were allowed free access to food (CRF-1, Oriental Yeast Co., Ltd., Japan) and tap water. This study was approved by the Institutional Animal Care and Use Committee of Mitsubishi Tanabe Pharma Corporation according to “Guidelines for Animal Studies”.

The female rats were stratified by body weights determined at the end of the quarantine/acclimation period and 16 females were randomly allocated to 3 groups. One group was administered 30 mg/kg/day (equivalent to the clinical daily dose for adult humans). A second group was administered 100 mg/kg/day of Nanpao; and a third group, which served as the control, was given distilled water.

Nanpao (Nanpao powder extract, Lot No.; 58025, Mitsubishi Tanabe Pharma Corp., Japan) was dissolved in distilled water just before daily administration. Nanpao or distilled water was administered to the animals by oral gavage once a day at about the same fixed time in the morning, corresponding to the clinical dosage regimen (taking the medicine within 30 min after a meal) for 5 days a week from the age of 5 months. The dosing volume was set at 5.0 ml/kg. The week in which administration began was defined as week 0 of dosing.

The peripheral blood flow volume measurement was performed using a laser Doppler blood flow meter (Omega Flow FLO-N1, Omegawave Inc., Japan). The Doppler signals are received by light-receiving fibers and recorded by a detector as frequency spectra, the mean frequency of which is proportional to the mean flow rate of erythrocytes, and the mean amplitude of which is proportional to the density of erythrocytes.

Processing of these signals can yield tissue blood flow, tissue blood volume, and blood velocity. Skin blood flow is measured continuously using this principle. Tissue blood flow was calculated using the following equation:

$$\text{Tissue blood flow} = k_1 \int \omega P(\omega)\, d\omega / l^2$$

where k_1 is a constant, ω denotes the angular frequency ($2\pi f$), $P(\omega)$ is the power spectrum of the signal, and l is the amount of light received.

The measurement was performed about 5 h after dosing at week 20 (corresponding to an age of 10 months) in all 48 animals in the control and Nanpao-treated groups. The blood flow volume was measured in the right hind paw using a non-contact probe (ST-N, Omegawave Inc.). Data were transferred to a data analysis system (Ver. 4.1, NOTOCORD-hem, Primetech Corp., Japan) and a 15-s average value representing the steady blood flow volume was measured and computed.

The surface skin temperature in the right hind paw was monitored using an infrared thermography device (Handy Thermography TH6200, NEC San-ei Instruments, Ltd., Japan). The measurement was performed about 5 h after dosing at week 33 (at the age of 13 months) in 8 females chosen at random from each of the 3 groups (24 total). Real-time thermographic images and surface temperature data from the right hind paw surface were obtained using an infrared camera before and at 3, 6, 9, and 12 min after immersion in ice-cold water. The mean surface temperature on a fixed hind paw surface was calculated using analysis software (Sat-Report, NEC Corp., Japan) at each measurement time.

Statistical analysis was carried out as described below. The homogeneity of variance was analyzed by Bartlett's test for data on the peripheral blood flow volume and surface temperature in the hind paws (level of significance: 5%). Multiple comparisons were carried out, using the parametric Dunnett method when the variance of the data was homogenous and the non-parametric Dunnett method when it was heterogeneous, to test the difference in means between the treated groups and the control group (level of significance: 5% and 1%). Moreover, the decrease rates of surface temperature at each measurement time were analyzed and compared with the surface temperature before cooling (level of significance: 5% and 1%).

In the peripheral blood flow volume measurement experiment, the peripheral blood flow volume in the right hind paw of the animals was 10.9, 11.8, and 13.0 ml/min/100 g body weight, respectively, in the control, 30, and 100 mg/kg/day groups. In the Nanpao-treated groups, the mean blood flow volume tended to increase dose-dependently compared to that of the control group (Fig. 1).

In the surface skin temperature measurement experiment, the surface skin temperature in the right hind paw of the animals was comparable in each group before immersion in ice-cold water. However, the surface skin temperature of the Nanpao-treated groups was significantly higher than that of the control group immediately after cooling, and the decrease rates of the surface skin temperature were 50.3%, 45.9%, and 40.5%, respectively, in the control, 30, and 100 mg/kg/day groups (data not shown). Moreover, the surface skin temperatures in the Nanpao-treated groups were higher than those of the control group at all measurement times, 3, 6, 9, and 12 min after cooling (Fig. 2). The thermographic images are shown in Fig. 3.

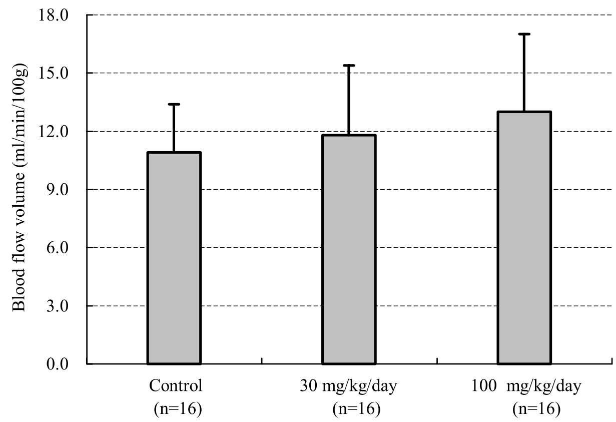

Fig. 1. Peripheral blood flow volume measured in the hind paws using a laser Doppler blood flow meter at week 20 of dosing (at the age of 10 months). Vertical lines represent SD.

Cold constitution occurs more frequently in women than in men [20], and it is believed to be caused by a disturbance in peripheral circulation involving arterial, venous, and/or microcirculation (e.g., capillaries). The balance between vascular vasodilation and contraction is precisely regulated by various mechanisms; for instance, estrogen acts not only as a direct vasodilator but also as a secondary vasodilator through the promotion of the enzyme nitric oxide synthase [17, 19]. Alterations in the levels and release pattern of the sex steroid hormone, estrogen, are known to occur as a result of reproductive aging in female mammals [2]. A precipitous loss of estrogen and alterations in progesterone secretion occur in menopausal women [2]. In addition, although sex or age differences in the peripheral blood flow volume and surface skin temperature are not well known, the responses of female rats to the hypothermic effects induced by apomorphine [15] and 5-hydroxytryptamine

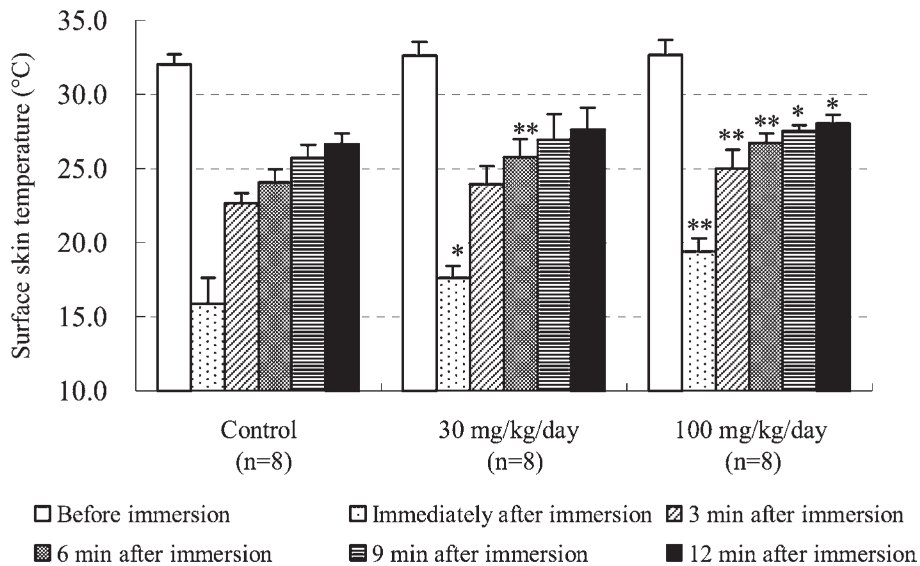

Fig. 2. Surface skin temperature in the hind paws before and after immersion in ice-cold water at week 33 of dosing (at the age of 13 months). *Significantly different from the control (P<0.05). **Significantly different from the control (P<0.01).

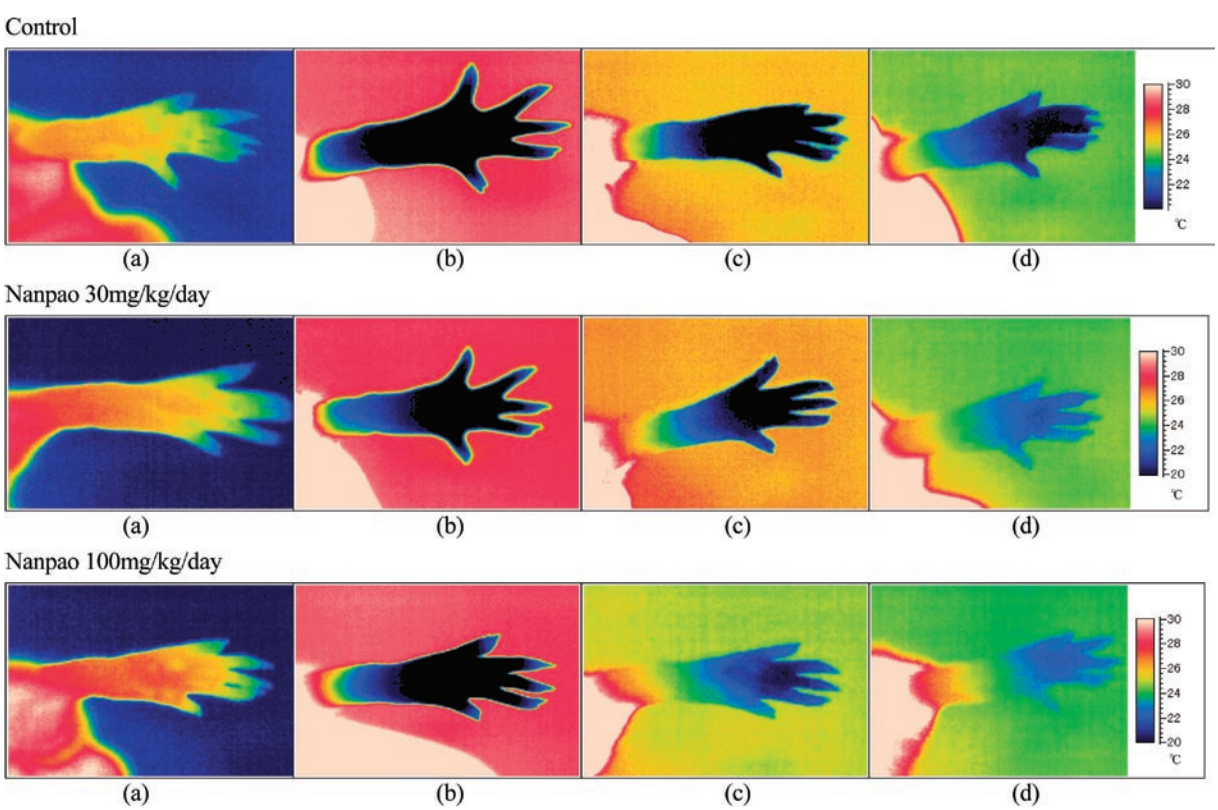

Fig. 3. Thermograph of hind paw before and after immersion in ice-cold water at week 33 of dosing (at the age of 13 months). (a) Thermograph of hind paw before immersion. (b) Thermograph of hind paw immediately after immersion. (c) Thermograph of hind paw 6 min after immersion. (d) Thermograph of hind paw 12 min after immersion.

[3] are greater than those occurring in males. Therefore, aged female rats as used in this study are considered to be an appropriate model for evaluating cold constitution. The results suggest that long-term administration of Nanpao may protect against diminished peripheral circulatory function, accelerate recovery from disturbances in peripheral blood flow produced by cooling, and improve the surface skin temperature in aged female rats.

Some of the constituent herbs of Nanpao, such as *Angelicae radix*, *Cinnamomi cortex*, and *Rehmanniae radix*, are known to have pharmacological actions on peripheral vasodilation, as evidenced by increased blood flow and elevated skin temperature in the tails of rats and mice [7, 8, 11, 16, 21]. Furthermore, aqueous extracts of *Astragali radix* and *Ginseng radix* induce nitric oxide generation in cultured rat vascular smooth muscle cells [4, 5], and 50% ethanolic extracts of *Rehmanniae radix* increase the amount of the blood flow in the veins and peripheral circulation [16]. Moreover, *Danggui buxue tang*, which contains only *Astragali radix* and *Angelicae radix*, induces estrogen-driven promoter activity *in vitro* and stimulates blood flow *in vivo* [6]. Taking account of these findings, the inhibitory effect of Nanpao on decreased skin surface temperature after cooling may be mainly due to increasing peripheral blood flow volume by additive or synergistic pharmacologic effects of the various herbs contained in Nanpao.

The results of the present study strongly support the clinical effects of Nanpao on cold constitution [13], and also suggest that Nanpao has the potential to produce improvement of peripheral blood flow and subsequent surface skin temperature.

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