Effect of the herbal medicine Danggui Sini plus Wuzhuyu Shengjiang Tang on erythrocyte deformability in normal subjects: a cross-over trial


Abstract

OBJECTIVE: To show whether Danggui Sini plus Wuzhuyu Shengjiang Tang (DSWST) has any transient effect on erythrocyte deformability in normal subjects.

METHODS: A total of 25 subjects [mean age (27.8 ± 1.8) years] was enrolled in this study. The study was designed as a cross-over trial in which the subjects took part for 2 d. On the first day, blood samples were collected at baseline and 1-2 h after administration of water, whereas, on the second day, instead of water, the subjects were administered DSWST after the baseline blood sampling. The blood samples collected at baseline and after the administration water or DSWST, were examined for erythrocyte deformability.

RESULTS: The elongation index increased significantly after 2 h (P = 0.009) compared to the baseline after DSWST intake. However, after water intake, there was no significant difference observed. When comparing the percent change of erythrocyte deformability between DSWST and water, we found that after 2 h of administration, DSWST improved erythrocyte deformability significantly compared to water (P < 0.001).

CONCLUSION: DSWST has a transient effect on erythrocyte deformability in normal subjects.

INTRODUCTION

Korean herbal medicine is commonly used to treat symptoms such as coldness of hands and feet. Coldness of hands and feet can be divided into two major categories based on whether the symptoms accompany Raynaud’s phenomenon (RP), which is the change of colors in fingers when exposed to cold. The pathogenesis has not been clearly defined, but previous studies suggest that through multiple mechanisms, vascular abnormalities and decrease in microvascular circulation contribute to these conditions. Danggui Sini plus Wuzhuyu Shengjiang Tang (DSWST; Dangguisayeokgaoshuyusaenggang-tang in Korean, Tokishigyakukagoshuyushokyoko in Japanese), a compound prepared from 9 plants, is one of the most commonly used herbal medicine in these cases. DSWST was first mentioned in Shanghanlun, which was written in the Later Han era (AD 25-220). In this book, DSWST is based on the compound...
Dangguisayuk-tang, which is prepared with 7 herbs: Dazao (Fructus Jujubae), Guizhi (Ramulus Cinnamomum), Baisha (Radix Paoniae Alba), Danggui (Radix Angelicae Sinensis), Mutong (Caulis Akebiae), Gancao (Radix Glycyrrhizae) and Xixin (Herba Asari Mandshurici). Dangguisayuk-tang is described to be used on patients with complaints of cold hands and feet, and very weak pulse. DSWST, which is a combination of Dangguisayuk-tang and, Evodiae Fructus, and Zingiberis Rhizoma, has been mentioned to be used when the Dangguisayuk-tang symptoms are more severe and the coldness of the body is chronic. Traditional theories suggest that DSWST warms the interior of the body by warming the meridian and dispersing coldness. Other recent studies have shown that DSWST prevents the cold syndrome and treats dizziness with feelings of coldness. However, the actual therapeutic mechanisms are not known yet.

In rats, peripheral circulation in the tail had improved at 1 h after oral administration of DSWST. Moreover, 1 h following oral administration of DSWST, peripheral blood flow in the fingers increased significantly. Thus, by improving peripheral circulation, DSWST may have an effect on improving the symptoms related to coldness of hands and feet.

One factor that can be used to evaluate microcirculation is erythrocyte deformability. Erythrocytes have the ability to change shape under deforming forces. Increase of erythrocyte deformability helps the erythrocyte easily pass through a capillary smaller than its size, which leads to improvement in blood flow. On the other hand, decrease in erythrocyte deformability means increase in microcirculatory resistance, and can be seen not only in Raynaud’s phenomenon but also in diabetes, sepsis, sickle-cell disease, peripheral vascular diseases and peripheral neuron diseases etc.

It has been shown by some studies that, decrease of erythrocyte deformability can be seen in RP patients. Park’s study on stroke patients showed that, patients with symptoms of coldness in their hands or feet (not limited to Raynaud’s phenomenon) showed decreased erythrocyte deformability compared to the patients without these symptoms. However, the effect of DSWST, widely used for the treatment of RP patients, on erythrocyte deformability has not been shown by any study. The aim of our study was to explore the effects of DSWST on erythrocyte deformability.

MATERIALS AND METHODS

Subjects
Twenty-five healthy subjects (7 female, 18 male) between the age groups of 25 to 35 [mean age (27.8 ± 1.8) years] were enrolled in this study. Subjects with a history of cardiac disease, cerebrovascular disease, hypertension, diabetes mellitus, drug abuse, and psychiatric problems; and subjects under any other drug medication were excluded from the study. Subjects who were unable to fast for 8 h before the study were also excluded.

Preparation of Danggui Sini plus Wuzhuyu Shengjiang Tang (DSWST)

The Department of Preliminary Pharmaceutical Preparation of Kyung Hee University Korean Medical Center (KHUKMC) prepared DSWST as dry extract granules, 3 g per pouch. Although the original DSWST in Shang Han Lun is composed of 9 herbs, the DSWST used in this study is composed of 8 herbs: Dazao (Fructus Jujubae) 5 g, Ganjiang (Rhizoma Zingiberis) 4 g, Danggui (Radix Angelicae Sinensis) 3 g, Guizhi (Ramulus Cinnamomum) 3 g, Baisha (Radix Paoniae Alba) 3 g, Wuzhuyu (Fructus Evodiae Rutaecarpae) 2 g, Gancao (Radix Glycyrrhizae) 2 g and Xixin (Herba Asari Mandshurici) 2 g. Akebiae Caulis was excluded due to the minor toxicities of the plant. These herbs were first cut into small pieces. The herb mixture was then extracted in a reflux condenser for 3 h with hot water. The solution was filtered through filter paper and concentrated using a spray drying process. The dry extract was granulated using 3 binders, lactose, polyvinylpyrrolidone, dextrin and ethanol. The ethanol was evaporated after binding.

Study design

This study was designed as a cross-over trial, where subjects participated over a period of 2 d. On both days, an 8-h fasting blood sample was taken from the subjects at 8 o’ clock in the morning. After first sampling, subjects were given 150 mL of water on the first day followed by second sampling after 1-2 h. However, on the second day, instead of water, subjects were administered DSWST orally, and the blood sample was taken after same duration.

Measurement of erythrocyte deformability

The primary outcome of this study is the elongation index (EI) of erythrocyte deformability. The EI is defined as (L - W) / (L + W), where L is the major axis and W is the minor axis of the erythrocyte ellipse at the shear stress of 3 Pa. To measure the EI, a Rheoscan-D microfluidic ektacytometer (Rheo Meditech, Seoul, Republic of Korea) was used. One drop of blood was obtained from each subject’s fingertip (6.0 μL) and was mixed with 0.5 mL of a highly viscous PVP solution (31 mPa) in phosphate-buffered saline (0.14 mM). A 0.5 mL aliquot of this mixture was placed in the test chamber of a disposable kit, which included a microchannel (Rheo Meditech). Differential pressure drove the mixture through the microchannel (0.2 mm × 4 mm × 40 mm), and laser beam (wavelength, 635 nm) from a 1.5 mW laser diode passed through the diluted mixture during the flow. The diffraction...
pattern of the moving erythrocytes at multiple shear stresses was projected onto a screen, and the images were captured by a CCD-video camera every 0.5 s. The images were analyzed using an ellipse-fitting computer program. The average shear stress ranged from 0 to 30 Pa, and the EI was calculated at 3 Pa. The microchannel was discarded after each measurement.

Statistical analysis
The SPSS 12.0 (SPSS for Windows, Version 12.0. Chicago, SPSS Inc. Chicago, IL, USA) was used for statistical analysis. Data were summarized as mean ± standard deviation or mean ± standard error of mean. The changes in erythrocyte deformability from baseline to post-treatment in each intervention were compared by repeated measures analysis of variance (ANOVA). To compare the percent change of erythrocyte deformability between the DSWST intake period and the water intake period, paired t-test was performed. A value of $P < 0.05$ was considered significant.

RESULTS
After DSWST intake, the elongation index (EI) of erythrocyte deformability increased after 1 and 2 h, whereas it showed a tendency to decrease after water intake (Table 1). Compared to baseline, the difference was significant after 2 h after DSWST intake ($P = 0.009$) (Figure 1A). However, EI showed no significance compared to baseline after water intake. When comparing the percent change of EI between DSWST and water intake, we found that after 2 h, DSWST improved erythrocyte deformability significantly compared to water ($P < 0.001$, Figure 1B).

<table>
<thead>
<tr>
<th>Time</th>
<th>DSWST</th>
<th>Control</th>
</tr>
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<tbody>
<tr>
<td>Baseline</td>
<td>0.327 ± 0.014</td>
<td>0.333 ± 0.016</td>
</tr>
<tr>
<td>After 1 h</td>
<td>0.330 ± 0.014</td>
<td>0.330 ± 0.016</td>
</tr>
<tr>
<td>After 2 h</td>
<td>0.334 ± 0.014</td>
<td>0.327 ± 0.016</td>
</tr>
</tbody>
</table>

Notes: DSWST: administration of Danggui Sini plus Wuzhuyu Shengjiang Tang extraction 3 g with 150 mL of water. Control: administration of 150 mL of Water. EI: elongation index of erythrocyte deformability. %: percent change of erythrocyte deformability compared to baseline. DSWST: Danggui Sini plus Wuzhuyu Shengjiang Tang. Vertical bars represent standard error of the mean. *$P = 0.009$ by repeated measure analysis of variance (compared to baseline). **$P < 0.001$ by paired t-test (compared to the water intake).

Figure 1 Elongation index and change of erythrocyte deformability
A: elongation index; B: change of erythrocyte deformability. DSWST: administration of Danggui Sini plus Wuzhuyu Shengjiang Tang extraction 3 g with 150 mL of water. Control: administration of 150 mL of Water. EI: elongation index of erythrocyte deformability. %: percent change of erythrocyte deformability compared to baseline. DSWST: Danggui Sini plus Wuzhuyu Shengjiang Tang. Vertical bars represent standard error of the mean. *$P = 0.009$ by repeated measure analysis of variance (compared to baseline). **$P < 0.001$ by paired t-test (compared to the water intake).

In this study, it was shown that DSWST has a transient effect on erythrocyte deformability in young healthy subjects. After 2 h following oral administration of DSWST, the elongation index of erythrocyte deformability improved significantly compared to baseline ($P = 0.009$), and the percent change was also significant compared to the water intake ($P < 0.001$).

The results of this study suggest that DSWST could be used for treatment of diseases associated with reduced erythrocyte deformability, such as RP. In this study, DSWST improved microcirculation by affecting the erythrocyte itself, since erythrocyte deformability is a property determined by geometric and material properties of the erythrocyte cell membrane.

DISCUSSION
In Korean medicine, herbal medicine is widely used to treat symptoms such as sensitivity to coldness. There are many medicines to choose from, that differ for each patient, but DSWST, which is a compound prepared from 9 plants, is one of the most used herbal medicine in these cases. Despite the fact that DSWST has been used for treating RP for a long time, the mechanisms underlying the effects have not been clearly reported. In one in vitro study, it was found that DSWST inhibits cold-induced responses in endothelial cells and vascular smooth muscle cells.

In this study, it was shown that DSWST has a transient effect on erythrocyte deformability in young healthy subjects. After 2 h following oral administration of DSWST, the elongation index of erythrocyte deformability improved significantly compared to baseline ($P = 0.009$), and the percent change was also significant compared to the water intake ($P < 0.001$).

The results of this study suggest that DSWST could be used for treatment of diseases associated with reduced erythrocyte deformability, such as RP. In this study, DSWST improved microcirculation by affecting the erythrocyte itself, since erythrocyte deformability is a property determined by geometric and material properties of the erythrocyte cell membrane. However, a previous in vitro study explored the effects of DSWST on endothelial cells and vascular smooth muscle cells. It showed that the compound inhibits cold-induced activation of RhoA in both vascular smooth muscle cells and endothelial cells, and blocks endothelin-1-mediated paracrine path for cold response on vessels. RhoA is a GTPase protein that activates Rho kinase, which eventually leads to reorganization of the actin cytoskeleton of cells. Activation of Rho/Rho-kinase signaling pathway has...
been reported to decrease erythrocyte deformability, hence inhibiting the activation of RhoA may help increase the erythrocyte deformability.11 As mentioned before, the mechanisms behind the Raynaud’s phenomenon is complex and the pathophysiology is contributed by imbalance between vasoconstriction and vasodilation, and reduced blood flow.12 Thus, DSWST may have effects on various mechanisms including Rho/Rho-kinase signaling pathway. However, this conclusion must be drawn with caution and further studies are needed to fully understand the therapeutic mechanisms of DSWST.

It is also hard to say which bioactive components of DSWST are responsible for these effects. A previous study has shown that angelica extract prevents the decrease of erythrocyte deformability in vitro, so Angelica Radix may play a key role in effecting erythrocyte deformability.13 Further studies will be needed to find out the bioactive components responsible for these effects. This study was performed on healthy young patients, whose erythrocyte deformability was in the normal range. To prove the effects of DSWST on erythrocyte deformability, patient-based trials will be needed. Further, this study only showed the acute effects of DSWST. Hence trials will be needed to show the long-term effects of the compound.

In conclusion, this study showed that DSWST has a transient effect on erythrocyte deformability in young healthy subjects. Thus, DSWST improves microcirculation by improving erythrocyte deformability and may be effective for patients with diseases associated with decreased erythrocyte deformability.

REFERENCES


