Inhibitory effect of Goshitsu-san on myometrial contraction in rats

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(Received September 29, 1993. Accepted March 7, 1994.)

Abstract

These studies were undertaken to find out whether $PGF_2\alpha$- and oxytocin-induced uterine contractions in mature, non-pregnant female Wister-Imamichi strain rats were inhibited by Goshitsu-san.

The results demonstrate a dose-dependent inhibitory effect on both the frequency and strength of contractions in the range of 4.0 to 30.0 mg/ml of Goshitsu-san.

Key words Goshitsu-san, non-pregnant rat, uterine contraction, dose-response relationship, Kampo medicines, $PGF_2\alpha$, oxytocin.

Abbreviations $PGF_2\alpha$, Prostaglandin $F_2\alpha$; Kyuki-kyogai-to (Xiong-Gui-Jiao-Ai-Tang), 芎帰膠艾湯 ; Goshitsu-san (Niu-Xi-San), 牛膝散 ; Saiboku-to (Chai-Pu-Tang), 柴朴湯 ; Toki-shakuyaku-san (Dang-Gui-Shao-Yao-San), 当帰芍薬散.

Introduction

All basic studies reported in the literature concerning the effects of Kampo medicines on uterine contractions have been limited to the pregnant uterus. However, even in the non-pregnant uterus, menstrual disorders, such as dysmenorrhea, result from strong uterine contractions and are often accompanied by severe abdominal pain.

With an organic cause of dysmenorrhea, the underlying disease can be treated, but in the case of functional dysmenorrhea, which often occurs in younger patients, treatment is primarily by symptomatic therapy. If effective Kampo medicines could be identified, then symptomatic therapy, in which analgesics and antispasmodics are administered each time dysmenorrhea occurs, could be avoided.

In this study, we have investigated the inhibitory effects of Goshitsu-san on myometrial contractions in the non-pregnant uterus of the rat. Goshitsu-san is a Kampo medicine found in ancient Kampo medical texts and is composed of eight components ; Achyranthes root, Cinnamon branch, Peony root, Peach kernel, Japanese angelica root, Moutan bark, Corydalis tuber and Saussurea root.

Materials and Methods

Myometrial specimen preparation and instruments : Laparotomies were performed on mature, female Wister-Imamichi strain rats (8-12 weeks of age) under 25 % urethane anesthesia (1 g/kg, administered intraperitoneally), and the uterus was immediately extricated. Longitudinal specimens (2 mm × 7 mm) of dissected myometrium were prepared under a dissecting binocular microscope in Krebs solution provided with an adequate supply of oxygen. Both ends of each specimen were tied with silk sutures, and the specimen was mounted horizontally in a chamber filled with 10 ml Krebs solution. One end of the suture was fixed to the chamber while the other end was attached to a force-displacement transducer (TD-111T, Nihon Kohden) connected to an amplifier (AD-

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631G, Nihon Kohden). Changes in myometrial contraction were measured isotonically with these instruments at 37 °C and were recorded with a pen recorder (SR6411, GRAPHTEC).

Solutions: Krebs solution (pH 7.40 at 37 °C) was composed of NaCl 135.5 mM, $CaCl_2$ 2.5 mM, $MgCl_2 \cdot 6H_2O$ 1.2 mM, HEPES 11.6 mM, and glucose 11.6 mM.

Prostaglandin $F_2\alpha$ ($PGF_2\alpha$, Ono Pharmaceutical Co., Ltd.) at 0.3 μg/ml and oxytocin (Teikoku Hormone Mfg. Co. Ltd.) at 1 mu/ml were used as the contraction-inducing agents.

Goshitsu-san was provided by Tsumura Co. Ltd. Nine different doses of Goshitsu-san were investigated (0.04, 0.1, 0.4, 1.0, 2.0, 4.0, 6.0, 10.0 and 30.0 mg/ml) with $PGF_2\alpha$, and 8 similar doses (except for the 2.0 mg/ml dose) of Goshitsu-san with oxytocin were investigated.

Experimental schedule: All experiments were performed according to the following time schedule. Each specimen was left to stand for 30-60 min in the chamber to allow it to stabilize. After stabilization, the contraction-inducing agents, $PGF_2\alpha$ at 0.3 μg/ml or oxytocin at 1 mu/ml, was applied for 10 min (a). The specimen was then washed with normal Krebs solution for 30 min, and the appropriate dose of Goshitsu-san then added to the solution. After 40 min, the particular contraction-inducing agent was applied for 10 min in the presence of Goshitsu-san (b). After removal of Goshitsu-san and the inducing agent by washing with normal solution for 30 min, the inducing agent was then allowed to act for 10 min (c).

Analysis: The frequency (F) and strength (S) of contractions were obtained from the number of contractions over a 10 min period, and from the area under the contraction wave over 10 min, respectively. The effects of Goshitsu-san on the frequency of contractions were investigated by calculating the response ratio of Fb to the mean value (control) of Fa and Fc, (Fb/ {1/2 (Fa+Fc)}). The effects on the strength of contractions were investigated by a calculation similar to that of the frequency, (Sb/ {1/2 (Sa+Sc)}).

Results

The effects of 9 different doses of Goshitsu-san on $PGF_2\alpha$-induced contraction and of 8 doses on

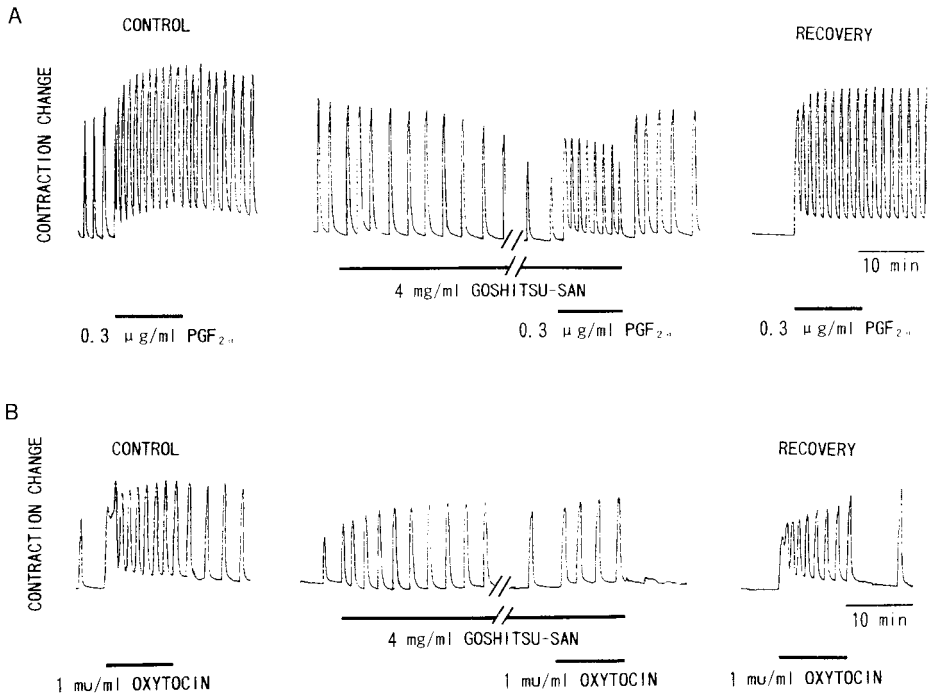

Fig. 1 An example of the inhibitory effects of 4 mg/ml Goshitsu-san on the myometrial contractions induced by 0.3 μg/ml $PGF_2\alpha$ (A) and by 1 mu/ml oxytocin (B) in a myometrial specimen from a non-pregnant rat.

oxytocin-induced contraction were examined. Thus, when $PGF_2\alpha$ at 0.3 μg/ml or oxytocin at 1 mu/ml was allowed to act in the presence of Goshitsu-san 40 min after administration, the effects of Goshitsu-san were determined by comparing the frequency and strength of the myometrial contractions to those when $PGF_2\alpha$ or oxytocin was administered alone. An example of a contraction wave pattern, in which the inhibitory effects of Goshitsu-san (4 mg/ml) on $PGF_2\alpha$ -(Fig. 1A) and oxytocin (Fig. 1B)- induced myometrial contractions were observed, is shown in Fig. 1.

The effects of Goshitsu-san on the frequency and the strength of $PGF_2\alpha$ - induced contractions are shown in Figs. 2A and 2B. Significant inhibitory effects on both the frequency and strength of contractions were found between 1.0 and 2.0 mg/ml Goshitsu-san ($p<0.01$), but not between doses below 1.0 mg/ml. An inhibition of 50 % on both the frequency and strength of contractions was obtained with Goshitsu-san between 4.0 and 10 mg/ml.

Similar effects of Goshitsu-san on the frequency

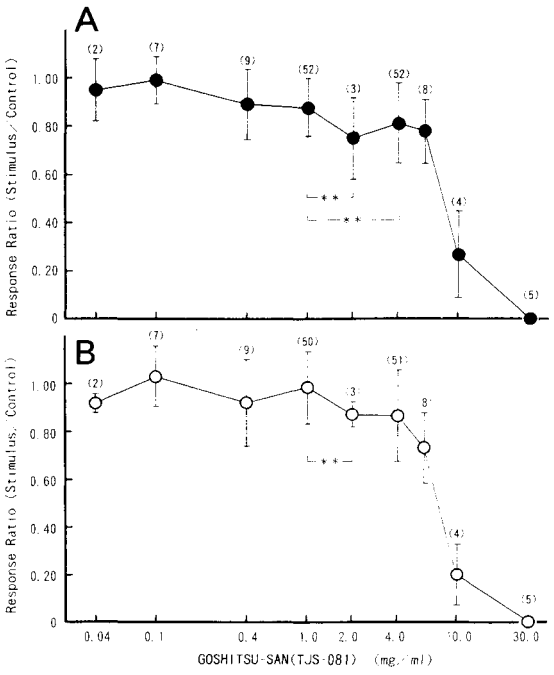

Fig. 2 Dose-response curve of Goshitsu-san to the frequency (A ; closed circles) and strength (B ; open circles) of myometrial contraction induced by 0.3 μg/ml $PGF_2\alpha$, (* * : $p<0.01$)

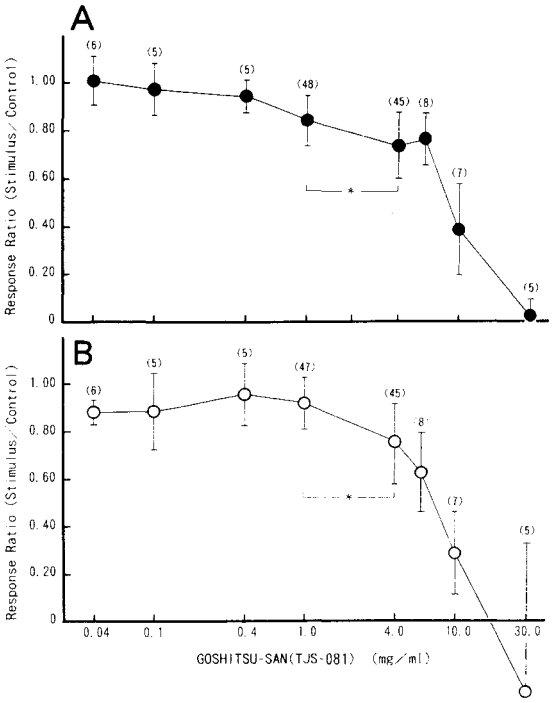

Fig. 3 Dose-response curve of Goshitsu-san to the frequency (A ; closed circles) and strength (B ; open circles) of myometrial contractions induced by 1 mu/ml oxytocin. (* : $p<0.05$)

and strength of oxytocin-induced myometrial contractions are shown in Figs. 3A and 3B, respectively. Significant inhibitory effects on both the frequency and strength of contractions were found between 1.0 and 4.0 mg/ml of Goshitsu-san ($p<0.05$), but not significant at doses below 1.0 mg/ml. As with $PGF_2\alpha$-induced contractions, a 50 % inhibitory effect on the frequency and strength of contractions was obtained with Goshitsu-san between 4.0 and 10.0 mg/ml.

Similar patterns were found in both contraction frequency and strength between the dose and the effects of Goshitsu - san, and a linear relationship between the dose and reaction was obtained between 1.0 and 30.0 mg/ml. It was therefore clear that the myometrial contractions induced by $PGF_2\alpha$ or oxytocin were inhibited in a dose-dependent manner by Goshitsu-san.

Discussion

The inhibitory effects of Goshitsu - san on the

myometrium have not been previously reported. The results of the present experiments demonstrate, however, that myometrial contractions induced by $PGF_2\alpha$ or oxytocin were clearly inhibited by Goshitsu-san, and that the effects were dose-dependent.

Chimura [1] investigated the effects of Toki-shakuyaku-san and some of its components ; Japanese angelica root, Peony root and Cnidium rhizome on spontaneous uterine contractile waves at the end-stage of gestation in rats. Although marked changes in contractile patterns were not observed with the individual components, a contraction inhibitory effect was found with 1 mg/ml Toki-shakuyaku-san. In addition, when Kyuki-kyogai-to, which also contains Japanese angelica root, Peony root and Cnidium rhizome, was investigated with similar equipment, multiple doses of 2 mg/ml were required to induce an effect which was still not completely inhibitory.[2]

Futhermore, Chimura *et al.*[3] investigated the effects of Saiboku-to and showed that a 4 mg/ml dose completely inhibited pregnant uterine spontaneous contractions 13.4±3.8 min after administration. They also demonstrated an inhibitory pattern, 2-3 min after administration of three 2 mg/ml doses on the contractile waves induced by 5 mu/ml oxytocin. Saiboku-to is composed of the ten components ; Bupleurum root, Pinellia tuber, Hoelen, Scutellaria root, Magnolia bark, Jujube, Ginseng, Glycyrrhiza, Perilla herb and Ginger, but does not contain Japanese angelica root, Peony root and Cnidium rhizome as do Toki-shakuyaku-san and Kyuki-kyogai-to. However, clinically, Saiboku-to acts on bronchial asthma. According to Chimura, the inhibitory wave form of Saiboku-to on uterine contractions differs from that during administration of Toki-shakuyaku-san and Kyuki-kyogai-to, but is similar to the wave form during the administration of β_2 stimulants, that is, the wave form which involves changes in the intracellular c-AMP concentrations.

In a report by Sankawa,[4] in which the PGE_2 biosynthesis inhibitory activities of Kampo medicines were compared, high PGE_2 biosynthesis inhibition rates of 61.6 % were observed with Scutellaria root, 57.1 % with Magnolia bark and 47.8 % with Ginger, components that comprise Saiboku-to. Japanese angelica root and Peony root were not investigated. However, Ukita[5] reported that in human clinical trials of 23 cases of threatened premature labor (23 - 33 weeks of gestation), blood levels of PGE_2 were significantly reduced ($p<0.05$) to 100±18 pg/ml after 2-3 weeks of administration of Toki-shakuyaku-san compared with the mean value of 195±35 pg/ml before administration.

Goshitsu-san, which we investigated in this study, contains Japanese angelica root and Peony root, as do Toki-shakuyaku-san and Kyuki-kyogai-to, and inhibits $PGF_2\alpha$-induced contractions in a dose-dependent manner from over 1.0 mg/ml. The relationship between Achyranthes root, the principal component of Goshitsu-san, in addition to Japanese angelica root and Peony root, and $PGF_2\alpha$ is particularly interesting. As with Chimura's results, a clear contraction inhibitory effect of the individual components ; Japanese angelica root, Peony root and Cnidium rhizome, on pregnant rats were found, but a definite inhibitory effect was observed when they were combined in unique proportions in Toki-shakuyaku-san. Such apparently-contradictory findings are difficult to explain in terms of Western medicines, but it is conceivable that the effects of Kampo medicines are not the products of the effects of the individual components of the drug. Consequently, when investigating the mechanism of action of Kampo medicines, not only should the effects of the individual components be investigated, but also the effects of the medicine as a whole.

Conclusion

Goshitsu-san inhibited $PGF_2\alpha$- and oxytocin-induced uterine contractions in mature, non-pregnant female Wister-Imamichi strain rats.

The effects were dose-dependent in the range of 4.0 to 30.0 mg/ml.

The results of this study strongly suggest that Goshitsu-san can be used as an agent to treat dysmenorrhea caused by strong uterine contractions.

和文抄録

ウイスター・今道株成熟雌非妊娠ラットにおいて，$PGF_2\alpha$ または oxytocin 惹起子宮収縮を牛膝散が抑制

するか否かを検討した。

その結果，収縮強度および頻度共に牛膝散 4.0～30.0 mg/ml の範囲で容量依存性に抑制効果を認めた。

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