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Shengmai Suppressed Vascular Tension in Umbilical Arteries and Veins of Human and Sheep

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Abstract

Objective—The umbilical cord is a critical pathway between mothers and fetuses, and regulations of umbilical vessel tension are important for fetal growth. Shengmai is an herbal medicine being used in treatments of cardiovascular diseases. However, effects of Shengmai on human blood vessels and related pharmacological mechanisms are unclear. Methods—This study investigated the effects of related mechanisms of Shengmai and its key compounds on human and sheep umbilical arteries and veins using organ bath systems. Key Findings—Shengmai significantly suppressed phenylephrine-stimulated vasoconstriction in umbilical arteries and veins. NG-Nitro-L-arginine Methyl Estercould not change the Shengmai-suppressed vasoconstriction in human and sheep umbilical vessels. Among four key compounds of Shengmai, Ginsenoside Re, Ginsenoside Rb1, Ginsenoside Rg1, and Schisandrin, only Ginsenoside Re showed the significant effect similar to Shengmai's in the umbilical vessels. In Ca^{2+}-free solution, Ginsenoside Re did not affect vasoconstriction. In addition, caffeine- or phenylephrine-stimulated vasoconstriction were not changed by Ginsenoside Re. Either charybdotoxin or glibenclamide could inhibit Ginsenoside Re-caused inhibition of the stimulated vasoconstriction in both human and sheep umbilical vessels, where 4-aminopyridine did not show the similar inhibitory effect. Conclusion—The results provide new information on Shengmai's effects and underlying mechanisms in umbilical vessels. Importantly, the information gained offers interesting potential for developing new drugs acting on umbilical cords for fetal medicine.

Keywords

Shengmai, Umbilical Arteries and Veins, Ginsenoside Re, α-Adrenergic Receptor

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1. Introduction

The umbilical cord with its blood vessels is a major or only pathway between mothers and fetuses, and critical functions of umbilical vessels are to supply and maintain blood flow as well as oxygen and nutrition for fetuses, which is necessary for life *in utero* [1] [2]. Any condition that can influence blood vessels of umbilical cord, may affect the supply blood and nutrition for fetuses, and may cause *in utero* hypoxia or fetal growth restriction [3]. Although *in utero* hypoxia is a common condition in clinic due to multiple factors [4]-[7], there have been limited approaches in dealing with that medical problem. Therefore, finding new drugs or methods against *in utero* hypoxia related to poor supply of blood flow is a consistent effort in both basic science and clinical work.

Shengmai is one of traditional herbal medicines being used frequently in clinical practice in treatments of various of cardiovascular diseases, including ischemic heart diseases, and stroke [8] [9]. In spite of that, there exist many experimental and clinical studies in demonstration of effects and mechanisms of Shengmai in cardiovascular systems or central blood vessel systems [8], it is unknown if Shengmai has any special vascular influence or effect on blood vessels of umbilical cords. The present study is focused on this topic.

Previous work showed that Shengmai consists of at least three major components, *Panax Ginseng*, *Ophiopogon Japonicus*, *and Schisandra Chinensis* [9]-[11]. Several antioxidant ingredients have been discovered in *Panax Ginseng* and *Schisandra*, including Ginseoside Rb1, Ginseoside Rg1, and Ginseoside Re(GRe) from *Panax Ginseng* and schizandrin from *Schisandra* [12]. They have been shown in preventing the oxidative damage in heart, brain, and other tissues, and being routinely used in treatment of coronary heart disease [9]. Shengmai also was demonstrated its effects in preventing circulatory shock and brain oxidative and ischemic damage during heatstroke [8]. Moreover, administration of Shengmai right after the onset of heat stroke is still considerably effective way for improving circulatory shock and inhibiting oxidative damage in the brain [8]. As mentioned above, since there has been no data on Shengmai's effects on the umbilical vessels, no information is available on possible influence as well as mechanisms of Shengmai on umbilical vessels. Obviously, addressing such questions is important.

In the present study, we investigated Shengmai's effects on both arteries and veins from human umbilical cords first. Considering inevitable variations among different human subjects, we also used umbilical cords from experimental healthy sheep in testing Shengmai's effects on sheep umbilical vessels. Subsequent experiments determined several key compounds of Shengmai and their vascular effects on umbilical vessels. In addition, we performed preliminary study on possible mechanisms that may be involved in vascular actions of Shengmai or its ingredients on umbilical vessels. Information gained is not only contribution to vascular pharmacology, but also to perinatal medicine and clinical work related to pregnancy.

2. Materials and Methods

2.1. Preparation of the Umbilical Cord Vessels

Human umbilical cord samples were obtained from 105 women after delivery at term at local hospitals. All deliveries were either vaginal deliveries or elective cesarean deliveries without complications. The median gestation at delivery was 39 weeks ± 8 days. The reasons for cesarean delivery included previous cesarean section and presumed cephalopelvic disproportion. The median parity value of the women at the time of delivery was 1 (range 0 - 3). There was no evidence of hypertensive disease, gestational diabetes mellitus (GDM) and other diseases for any of the subjects. The mean body mass index (BMI) of those cases was 22.32 kg/m^2 [13]. All procedures were approved by the Institute Committee.

All procedures were approved by the Institute Animal Care Committee and were incompliance with the Guidelines for NIHC are and Use of Laboratory Animals. The animal experiments were performed in chronically instrumented conscious sheep at 128 - 134 days of gestation (term 145 days). Animals were housed in individual study cage and in a light controlled room (12 h light/dark cycles) with food and water provided libitum. All sheep deliveries were elective cesarean deliveries, and umbilical cord samples were obtained immediately. All procedures were approved by the Institute Committee and in compliance with the Guidelines for national in-

stitute of health (NIH) Care and Use of Laboratory Animals.

Approximately 10 ± 5 cm segments were excised from middle part of umbilical cords between the placenta and fetus [13]. Then samples were immediately placed in the modified Krebs-Henseleit solution (K-H solution) at 4˚C with composition (mmol/L): NaCl, 119 mmol/L, KCl, 4.7 mmol/L, $NaHCO_3$, 25 mmol/L, KH_2PO_4, 1.2 mmol/L, $CaCl_2$, 2.5 mmol/L, $MgSO_4$, 1.0 mmol/L, EDTA, 0.004 mmol/L, and D-glucose, 11 mmol/L, at 37˚C at pH 7.4 with constant bubbling with 95% O_2/5% CO_2 [14]. Samples were used immediately following the preparation.

2.2. Vascular Experiments

Umbilical veins and arteries were carefully dissected from umbilical cords by removal of surrounding tissue using micro-dissecting instruments. Vessels were cut in rings with 3 - 5 mm length [13] [15]. Rings were then suspended individually on stainless steel hooks inserted into their lumens and stretched with an initial isometric tension of 2 g, in glass-jacketed organ baths as previously reported [16] [17]. Each bath contained 5 ml of K-H solution, pH 7.35 - 7.45, at 37˚C, and constant bubbling with a mixture of 95% oxygen/5% carbon dioxide [18]. The upper hook was connected to a force transducer and changes in isometric force were recorded using PowerLab system with Chart 7.0 software (AD Instruments, Australia). Rings were allowed to equilibrate for 120 minutes. During that period bath solution was replaced every 15 minutes with fresh K-H solution.

After 120 min of equilibration, each ring was contracted using KCl (60 mM) for reaching a maximum response, and then washed out. The KCl challenge was performed three times to test functional state of the vascular tissue. Optimal tension was adjusted throughout the equilibration period. After the last KCl challenge, a 30 - 40 minutes recovery period was allowed [14]. Following drugs were tested in the experiments.

The effect of Shengmai on human umbilical vein/artery (HUV/HUA) or sheep umbilical vein/artery (SUV/SUA) rings: When the rings were equilibrated, phenylephrine (PE, 10^{-4} mol/L) was added to produce steady contraction, and then Shengmai (10^{-4} mol/L) was applied. Vascular reactions were monitored for 120 min.

When the rings were equilibrated, NG-Nitro-L-arginine Methyl Ester (L-NAME, the nitric oxide synthase (eNOS) inhibitor, 10^{-5} mol/L) for 30 minutes and PE (10^{-4} mol/L) was added to produce steady contraction,and then 10^{-4} mol/L Shengmai was applied. Vascular reactions were monitored for at least 120 minutes.

When the sheep umbilical rings were equilibrated, following PE (10^{-4} mol/L) to produce steady contraction, Following key elements of Shengmaiwere used in testing: GRe (10^{-4} mol/L), Ginsenoside Rb1 (10^{-4} mol/L), Ginsenoside Rg1 (10^{-4} mol/L), or Schisandrin (10^{-4} mol/L) was added separately. Vessel tone was monitored and recorded for at least 120 minutes after adding the drug.

$CaCl_2$ dose-effect curve: SUV/SUA rings were washed 2 - 3 times with Ca^{2+}-free K-H solution (containing 1 μM EGTA). The vessel rings were preincubated with GRe (10^{-4} mol/L) for 30 minutes before application of KCl (60 mM). $CaCl_2$ was then added cumulatively (0.25 - 5 × 10^{-5} mol/L). The vehicle, instead of GRe, was used for the control group before add KCl (60 mM).

The effect of GRe on PE- or caffeine-induced contractions in SUV/SUA rings was tested in Ca^{2+}-free K-H solution. The SUV/SUA rings were washed 2 - 3 times with Ca^{2+}-free K-H solution. The rings were exposed to GRe (10^{-4} mol/L) for 30 minutes, and then add 10^{-5} mol/L PE or 20 mmol/L caffeine.

The effect of potassium channel antagonists on SUV/SUA rings was tested. Different potassium channel antagonists were applied 30 minutes before the addition of PE (10^{-4} mol/L). 4-aminopyridine (4-AP, 10^{-3} mol/L [19], Voltage-dependent K^+ (Kv) channels antagonist [19] [20], charybdotoxin (CTX, 10^{-7} mol/L [20], Ca^{2+}-activated K^+ (BKCa) channels antagonist [20]), Glibenclamide (10^{-6} mol/L [19], ATP-sensitive K^+ (KATP) channels antagonist [19] [20], or vehicle were used. Following steady vasoconstriction by PE (10^{-4} mol/L), GRe (10^{-4} mol/L) was added into the bath, and vascular responses were monitored and recorded for at least 120 minutes.

2.3. Drugs and Solutions

The modified K-H solution: NaCl, 119 mmol/L, KCl, 4.7 mmol/L, $NaHCO_3$, 25 mmol/L, KH_2PO_4, 1.2 mmol/L, $CaCl_2$, 2.5 mmol/L, $MgSO_4$, 1.0 mmol/L, EDTA, 0.004 mmol/L, and glucose, 11 mmol/L.

Shengmai was purchased from Suzhong Pharnaceutical Group (Jiangsu, China). GRe, Ginsenoside Rb1, Ginsenoside Rg1, and Schisandrol were purchased from Shanghai Sunny Biotech Co., Ltd. (Shanghai, China). Phenylephrine, NG-Nitro-L-arginine Methyl Ester (L-NAME), 4-AP, CTX, and Gli were purchased from Sigma-

Aldrich (USA).

2.4. Statistical Analysis

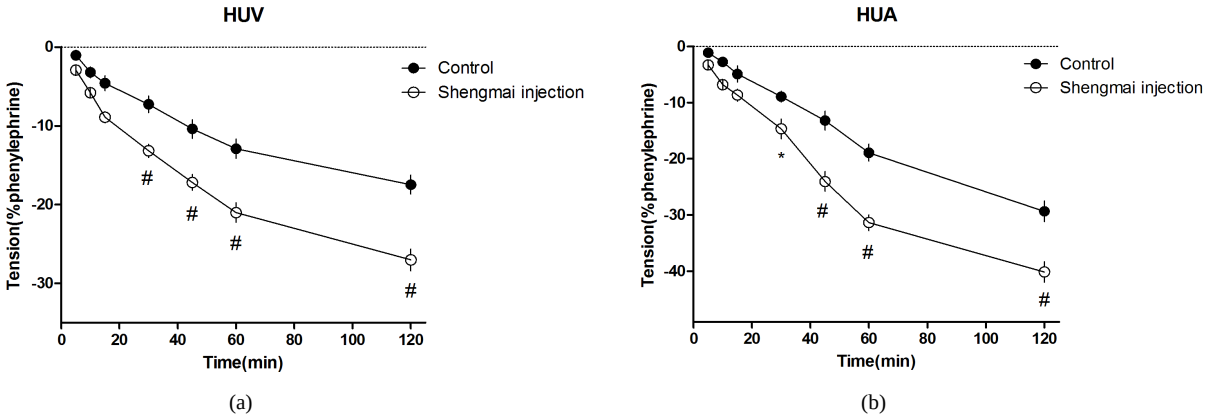
All data were expressed as means ± SEMs, the date was calculated from the concentration-response curve resulting performed by Graph Pad Prism (Version 5.01, Graph Pad Software Inc., La Jolla, CA, USA). All analog signals were recorded continuously throughout the study, and then digitized on a computer with Med-Lab acquisition software. And differences were evaluated for statistically significance ($P < 0.05$) by two-way ANOVA or *t*-test.

3. Results

3.1. The Effect of Shanghai on HUV/HUA and SUV/SUA

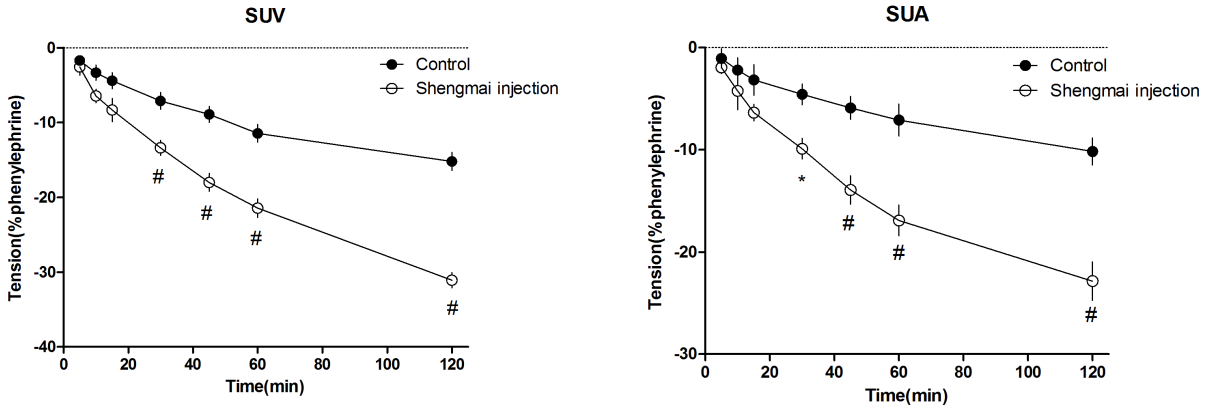
PE (10^{-4} mol/L) produced a concentration-related vasoconstriction in the HUV/HUA rings, where Shengmai (10^{-4} mol/L) could suppress PE-mediated vasoconstrictions significantly in the HUV/HUA rings (**Figure 1**).

After 120 min of equilibration, PE (10^{-4} mol/L) produced a dose-dependent vasoconstriction in the SUV/SUA rings, where Shengmai (10^{-4} mol/L) produced significant suppressed PE-mediated vasoconstriction in the SUV/SUA rings (**Figure 2**).



(a) (b)

Figure 1. Shengmai suppressed phenylephrine-stimulated vasoconstrictions in human umbilical vein/artery (HUV/HUA). ((a): HUV n = 105 rings from 94 umbilical cords; (b): HUA n = 110 rings from 94 umbilical cords). Control vs Shengmai, #P < 0.01, *P < 0.05.



(a) (b)

Figure 2. Shengmai suppressed phenylephrine-stimulated vasoconstrictions in sheep umbilical vein/artery (SUV/SUA) ((a): SUV n = 48 rings from 6 umbilical cords; (b): SUA n = 44 rings from 6 umbilical cords). Control vs Shengmai, #P < 0.01, *P < 0.05.

3.2. The effect of L-NAME on HUV/HUA and SUV/SUA

After 120 min of equilibration, PE (10^{-4} mol/L) produced dose-dependent contractions in HUV/HUA and SUV/SUA rings. There was no significant difference in the Shengmai-suppressed vascular tension between the groups with or without L-NAME (**Figure 3(A)**, **Figure 3(B)**).

3.3. The effect of Key Compounds of Shengmai on SUV/SUA

Following PE (10^{-4} mol/L)-produced steady vasoconstriction, Ginsenoside Re, Ginsenoside Rb1, Ginsenoside Rg1, or Schisandrin was added. GRe (10^{-4} mol/L) suppressed PE-stimulated vasoconstrictions in SUV/SUA rings, where Ginsenoside Rb1 (10^{-4} mol/L), Ginsenoside Rg1 (10^{-4} mol/L), and Schisandrin (10^{-4} mol/L) did not cause significant changes (**Figure 4**).

3.4. $CaCl_2$ Dose-Effect Curve

Following the rings were equilibrated in Ca^{2+}-free K-H solution, Ginsenoside Re (10^{-4} mol/L) was added 30 min before application of KCl to produce steady contraction. $CaCl_2$ was then added cumulatively. There was no statistical significance in vascular responses between the control and GRe groups (**Figure 5**).

3.5. The Effect of GRe on PE- or Caffeine-Induced Contractions in SUV/SUA

Following the rings were equilibrated in Ca^{2+}-free K-H solution and pre-treatment with GRe for 30 min, PE or

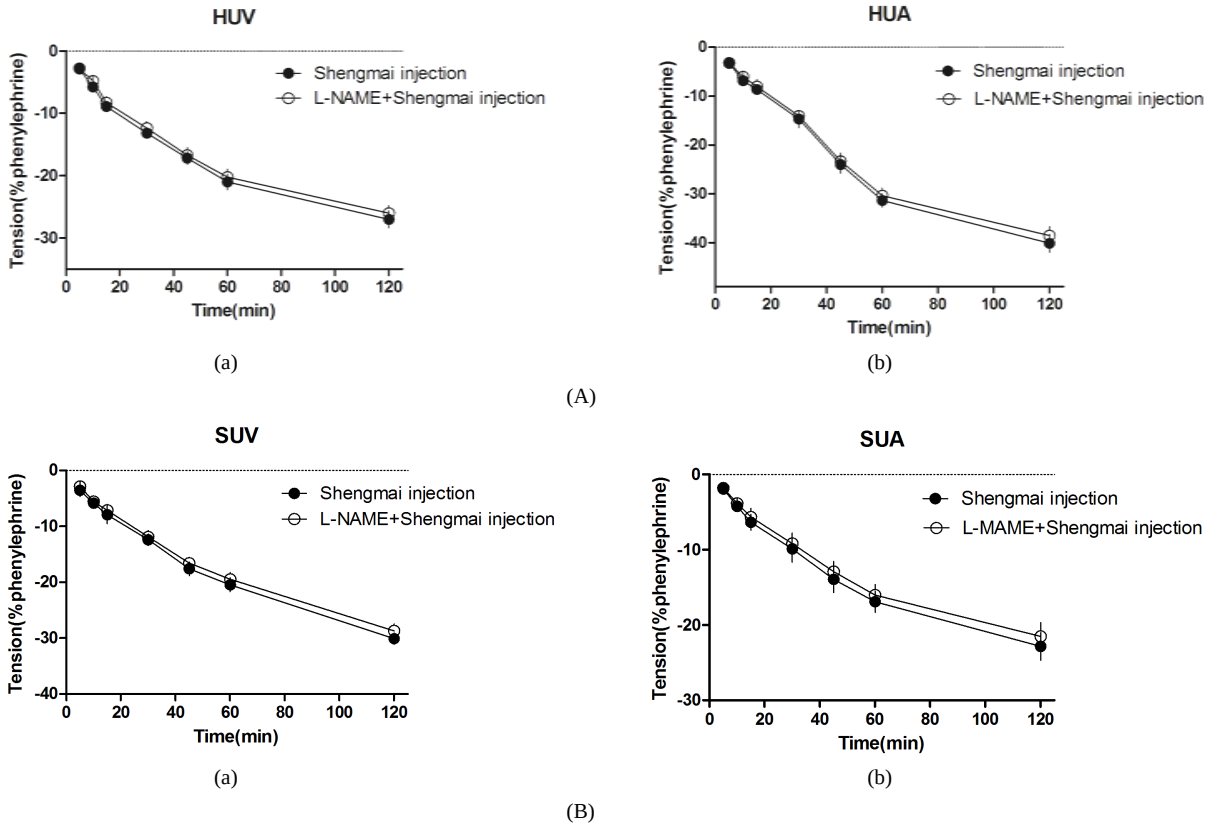
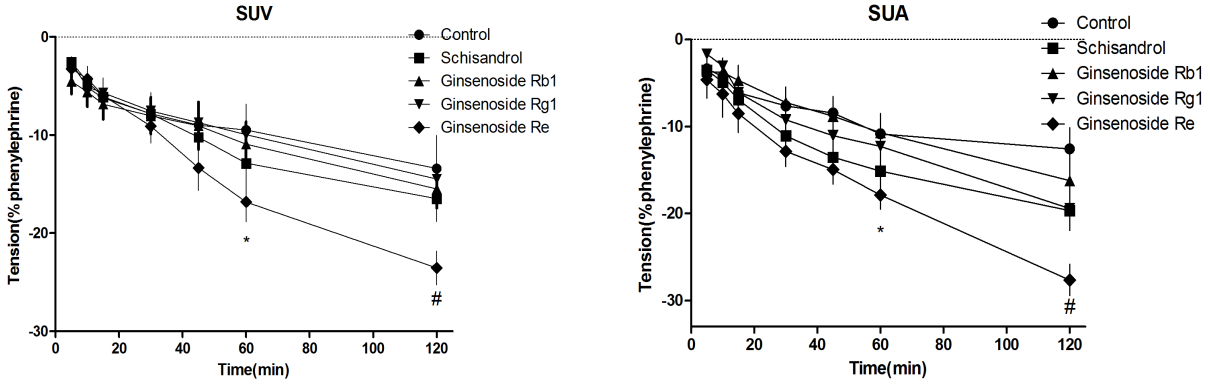
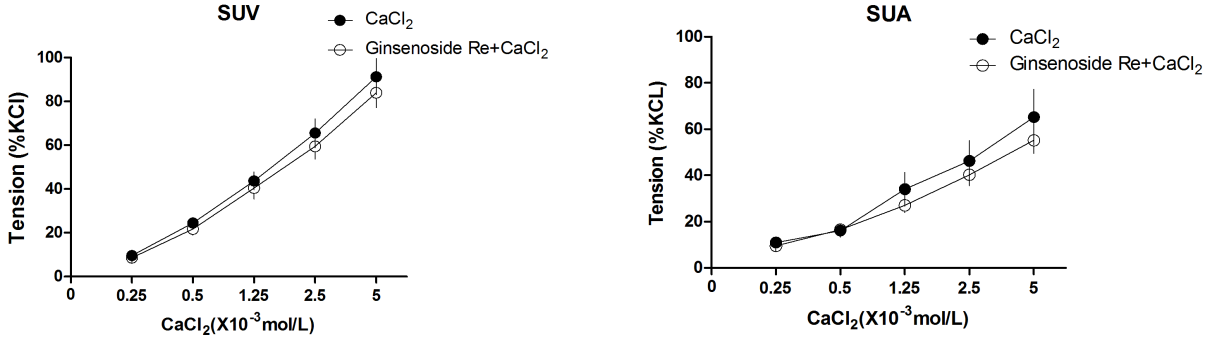


Figure 3. (A) Pre-treatment with NG-Nitro-L-arginine Methyl Ester (L-NAME) had no influence on Shengmai-suppressed vasoconstriction by phenylephrine in human umbilical vein/artery (HUV/HUA) ((a): HUV n = 105 rings from 90 umbilical cords; (b): HUA n = 110 rings from 94 umbilical cords); (B) Pre-treatment with NG-Nitro-L-arginine Methyl Ester (L-NAME) had no influence on Shanghai-suppressed phenylephrine-stimulated vasoconstrictions in sheep umbilical vein/artery ((a): SUV n = 48 rings from 6 umbilical cords; (b): SUA n = 44 rings from 6 umbilical cords). L-NAME + Shangmai: Following pre-treatment with L-NAME, Shanghai was added into the organ bath.



(a) (b)

Figure 4. The effect of Ginsenoside Re, Ginsenoside Rb1, Ginsenoside Rg1, or Schisandrin on sheep umbilical vein/artery (SUV/SUA) stimulated by phenylephrine ((a): SUV n = 43 rings from 10 umbilical cords; (b): SUA n = 45 rings from 10 umbilical cords). Control vs Ginsenoside Re, #P < 0.01, *P < 0.05.



(a) (b)

Figure 5. The effect of $CaCl_2$ on sheep umbilical vein/artery (SUV/SUA) ((a): SUV n = 24 rings from 6 umbilical cords; (b): SUA n = 20 rings from 6 umbilical cords).

caffeine was then applied. There was no significant difference in vascular responses between the control and GRe group (P > 0.05) in both SUV and SUA (**Figure 6**).

3.6. The Effect of Potassium Channel Antagonists on Ginsenoside Re-Induced Relaxation in SUV/SUA

Either CTX (10^{-7} mol/L) or Glibenclamide (10^{-6} mol/L) significantly reduced the GRe-suppressed vascular tension in SUV/SUA. However, the pre-treatment with 4-AP (10^{-3} mol/L) did not change the vascular response-caused by GRe (**Figure 7**).

4. Discussion

A number of clinical and experimental investigations have demonstrated the cardiovascular effects of Shengmai, and Shengmai has been often used in clinical treatments against stroke and cardiovascular diseases, including coronary heart disease, atherosclerosis, and hypertension [8] [9]. It is recently confirmed that blood vessels in umbilical cords can act differently from other vascular systems to chemical stimulation. However, there was no information regarding effects of Shengmai on either arteries and veins of umbilical cords as background of this study. Importantly, the umbilical cord serves as only important pathway between pregnant mothers and fetuses for supply of blood, oxygen, and nutrition. Thus, understanding influence of various chemicals or drugs on umbilical vessels is very important to perinatal medicine.

In the present study, Shengmai induced a significant decrease of vascular tone-stimulated by phenylephrine in both human and sheep umbilical arteries and veins, demonstrating that Shengmai could cause vasodilation or

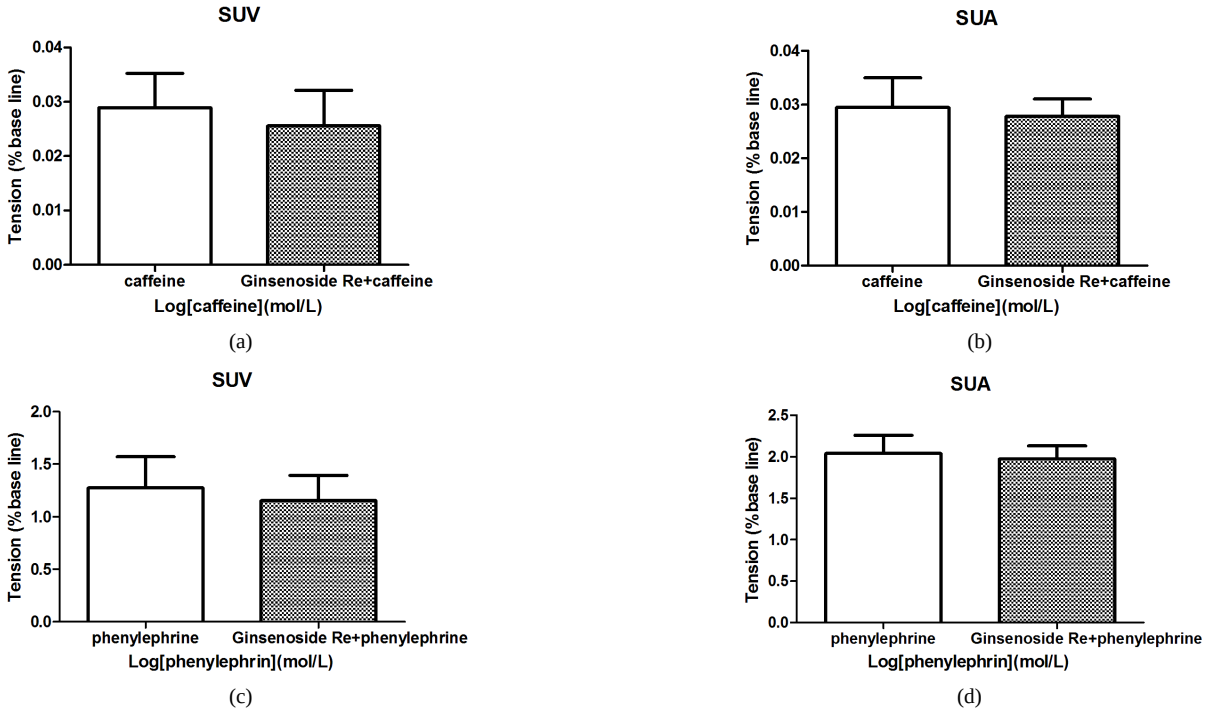


Figure 6. The effect of Ginsenoside Reon sheep umbilical vein/artery (SUV/SUA) stimulated by phenylephrine or caffeine ((a): SUV n = 24 rings from 6 umbilical cords; (b): SUA n = 20 rings from 6 umbilical cords).

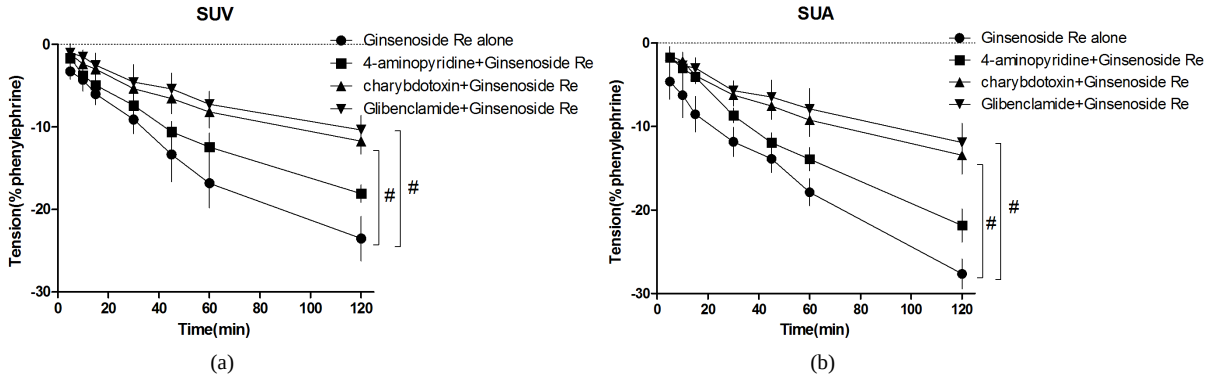


Figure 7. The effect of potassiumchannels antagonists on Ginsenoside Re-suppressed vascular tension in sheep umbilical vein/artery (SUV/SUA) ((a): SUV n = 36 rings from 8 umbilical cords; (b): SUA n = 34 rings from 8 umbilical cords). Ginsenoside Re alone vs charybdotoxin; Ginsenoside Re vs Glibenclamide, #P < 0.01.

suppress phenylephrine-produced vascular tension. Notably, this was the first study to show the effects of Shengmai on umbilical cords. Significance of the findings includes that may offer new opportunities for developing novel drugs or approaches targeting at regulations of umbilical cord blood vessels. For example, many clinical conditions may lead to *in utero* hypoxia as well as fetal growth restriction [4]. Increasing of umbilical blood flow could be helpful for those conditions. Thus, the Shengmai's effects on umbilical vessels may have great potentials in the field.

In the present study, possible mechanisms of Shengmai-suppress vasoconstriction by PE in the umbilical cord vessels were determined. The first possibility we considered was contribution from nitric oxide (NO) signaling pathway. It is well known that NO mainly from the endothelium in vascular systems, and plays critical roles in vascular relaxation [21] [22]. Damage of vascular endothelium or NO pathways may cause disability of vascular functions, particularly vasodilation [23]-[26]. In our experiments, following the pre-treatment with L-NAME in both human and sheep vessels, no significant changes was observed in Shengmai-mediated vascular responses,

suggesting that Shengmai-produced vascular relaxation may not rely on NO.

Shengmai is a traditional herb medicine with many ingredients. Major biological compounds in Shengmai include Ginsenoside Re, Ginsenoside Rb1, Ginsenoside Rg1, and Schisandrin [12]. Previous study using those key compounds of Shengmai showed vascular effects on other vessel systems [27]-[30], including anti-oxidative influence [31] and inhibition of P-glycoprotein [32] [33]. It was unknown whether any of those four elements play major roles in Shengmai-mediated vascular dilation in umbilical cords. In the present study, we found Shengmai-produced vasodilation mainly depend on GRe, not other three compounds tested. Again, this is a new finding in demonstration of that GRe in Shengmai is critical for the suppression of PE-induced vasoconstriction effect in umbilical cord vessels.

After excluding possibility of NO involved, other mechanisms, including calcium and potassium signaling pathways, were considered in smooth muscle of umbilical vessels. Vascular smooth muscle requires Ca^{2+} for constrictions either from intracellular stores or the influx of extra-cellular Ca^{2+}. The major routes of Ca^{2+} influx include receptor-operated Ca^{2+} channel (ROCC) [34] and voltage-gated Ca^{2+} channel (VGCC) [35] [36]. PE is a α-adrenergic receptor agonist [37] that produces vasoconstriction mainly via VGCC. Extra-cellular high potassium makes VGCCs to open in response to membrane depolarization and allow Ca^{2+} ions to enter cells [38] and induce vasoconstriction. $CaCl_2$ could cause dose-dependent vasoconstriction linked to Ca^{2+} influx [36] [39]. Our study demonstrated that Shengmai and Ginsenoside Re could suppress PE-induced vasoconstriction in umbilical vessel rings. When the vessel rings were equilibrated in Ca^{2+}-free K-H solution with GRe, $CaCl_2$ was then added into the bath cumulatively. This treatment could not change the Ca^{2+} influx induced vasoconstriction in both umbilical arteries and veins, indicating that GRe-suppressed vascular tension may not be related to VGCC-mediated intracellular Ca^{2+} influx.

The sarcoplasmic reticulum (SR) can release stored Ca^{2+} in cells [40]. Caffeine could induce transient vasoconstriction mainly by Ca^{2+} release from the SR, where the SR Ca^{2+} flux is mediated by ryanodine receptors that can be opened in response to small trigger Ca^{2+} stimulation [41]. In Ca^{2+}-free K-H solution, PE-induced vasoconstriction was induced by activating IP3 (1, 4, 5-trisphosphate)-sensitivity calcium channels [42]. In the present study, GRe could not affect caffeine- or PE-induced vasoconstriction in Ca^{2+}-free K-H solution, suggesting that GRe-induced vascular tension may not be related to the Ca^{2+} release via the SR or IP3-sensitivity calcium pool [43].

Potassium channels integrate a variety of vasoactive signals to dilate or constrict blood vesselsvia regulations of the membrane potential (depolarization or hyper-polarization) in smooth muscle cells [20] [44]. In order to test the relationship between Shengmai- or GRe-induced vasodilation and K^+ channels, we determined the effects of the different antagonists on K^+ channels, including 4-AP, Glibenclamide, and CTX. The pre-treatment of vessel rings with 4-AP had no effects on the GRe-suppressed vascular tension. However, either CTX or Glibenclamide significantly reduced GRe-suppressed vessel tension inumbilical vessels. The results demonstrated that the Shengmai-mediate umbilical vascular response was related to potassium channel pathways. Since 4-AP had no influence on GRe-produced vascular changes, while CTX and Glibenclamide inhibited the effect of GRe, the data indicated that Ca^{2+}-activated K^+ channels and ATP-sensitive channels, not voltage-dependent K^+ channels, might play a role in the GRe- and Shengmai-suppressed vascular tension.

5. Conclusion

This was the first study to show that Shengmai and its compound GRe could suppress vascular tension-generated by PE in the umbilical cord. Since Shengmai could be used during pregnancy, the data increased understanding the effects of this herb drug on umbilical vessels. The possible mechanisms for Shengmai-reduced vascular tonemay not be related to NO pathways in the umbilical cords, and may not be linked to release of intracellular Ca^{2+} orentry of extra-cellular Ca^{2+}. However, potassium channels, particularly BKCa channels and ATP-sensitive channels, may play an important role in the GRe-suppressed vascular tension. Although further studies are required for detailed mechanisms of Shengmai on umbilical vessels, the new information gained in this study offers insight for understanding Shengmai's effects on umbilical cords.

Acknowledgements

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Conflict of Interest

The authors declare that they have no conflict of interest.

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