Effect of Scolopendrid Calculus Bovis-Fel Uris-Moschus Bee Venom and Sweet Bee Venom on Regional Cerebral Blood Flow after Pharmacopuncture to GV16 Pungbu and GB20 Pungji in Rat

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Key Words
bee venom; Calculus Bovis-Fel Uris-Moschus; cerebrovascular circulation; pharmacopuncture; scolopendrid

Abstract
Objectives: This study was designed to investigate the effect of four pharmacopuncture drugs (scolopendrid, Calculus Bovis-Fel Uris-Moschus (BUM), bee venom 25%, and sweet bee venom 10%) on the cerebral hemodynamics, including changes in the regional cerebral blood flow (rCBF) and in the mean arterial blood pressure (MABP).

Methods: The changes in the rCBF and the MABP were determined by using a laser-Doppler flowmeter and a pressure transducer, respectively.

Results: Scolopendrid (0.3 ml, 1 ml/kg) caused no significant changes in the rCBF and the MABP, whereas BUM (0.3 ml, 1 ml/kg) decreased the rCBF and the MABP, bee venom 25% (0.3 ml, 1 ml/kg) increased the rCBF and lowered the MABP, and sweet bee venom 10% (0.3 ml, 1 ml/kg) increased the rCBF and had no significant effect on the MABP.

Conclusions: The rCBF and the MABP were influenced differently by the administration of various pharmacopunctures. Further studies are needed to elucidate the underlying mechanism.

1. Introduction

Scolopendrid pharmacopuncture is prepared by using an extraction method of alcohol immersion, and its safety has been proven with hematologic tests, toxicity tests etc. Calculus Bovis-Fel Uris-Moschus (BUM) pharmacopuncture is prepared by using an extraction method of alcohol immersion and its safety has been proven with acute toxicity test [1,2]. Bee venom is collected from the venom sacs of worker bees; the venom is purified, frozen, and dried to yield pure bee venom powder and is then diluted in distilled water to different concentrations. Crude bee venom shows a significant cytotoxic effect at high concentrations, but no significant effect at low concentrations. Sweet bee venom is prepared by removing enzymes such as phospholipase A2 from the purified venom and undergoes the same dilution process as the conventional bee venom. It contains 12% more mellitin than crude bee venom at the same concentration [3,4]. Few studies have addressed the effects of pharmacopuncture on cerebral blood flow. One of them is a

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study about pharmacopuncture using Cervi Cornu at BL23 and BL52, and another is a study using Carthami Flos pharmacopuncture at LI4, LR3, BL23, BL62 and GV16 [5,6]. Perciae Semen pharmacopuncture at LR3, LR4 for the intravascular coagulation model and Folium Arseniziae Argyi pharmacopuncture at LR3 for the transient forebrain ischemic injury model showed significant effects in treating brain damage [7,8].

In this study, pharmacopunctures using scolopendrid, BUM, bee venom and sweet bee venom were administrated at the points corresponding to GV16 Pungbu and GB20 Pungji in rats. The regional cerebral blood flow (rCBF) and the mean arterial blood pressure (MABP) were measured. The purpose of this study was to observe the response of the brain to the pharmacopuncture and to find possible clinical applications.

2. Materials and methods

2.1. Animals
All experiments were conducted in accordance with the regulations of the Committee of Animal Care and Welfare of Dongshin University. Normally-fed male Sprague-Dawley rats weighing about 300 g (Damul Science Inc., Korea) were used. They were maintained in an air-conditioned animal room at 24 ± 2°C with 55 ± 5% relative humidity and a 12-h light-dark cycle (lights on 8:00 to 20:00). Animals received a standard laboratory diet; additionally, water was provided ad libitum.

2.2. Acupoints and doses of pharmacopuncture
All the pharmacopunctures used in local clinics in Korea were included in the boundary of this study. Among those, Cervi Cornu Parvum (CC) pharmacopuncture was excluded due to a previous study related with cerebral blood flow and blood pressure. There was no scientific proof that safflower seed (CF) and mixture of safflower seed and deer antler (CFC) were good for the brain or the blood pressure. The eight principal pharmacopunctures and mountain ginseng, pharmacopuncture distilled from water were ruled out for the lack of medically effective ingredients. The virtues of BUM and BU are similar, so BUM was selected. Therefore, BUM, scolopendrid, sweet bee venom 10%, and bee venom 25% were chosen for this study (Fig. 1).

Two preconditions were used to choose the acupoints in this study. First, all of the acupoints are in the area of the occipital bone. Second, through a search of Korean and Chinese article databases, the acupoints must be effective for treating the brain and blood pressure. BL10 Cheonju, GB12 Wangol, GB20 Pungji, GV15 Amun and GV16
Pungbu were chosen for those reasons. Then, the number of total articles and articles related with brain or blood pressure were considered for the final selection. The number of total articles related with GB20 Pungji was four, and the number of those related with brain or blood pressure was three. The number of the total articles related with GB20 Pungji was seventeen, and the number of those related with brain or blood pressure was eight. In the conclusion, GV16 Pungbu and GB20 Pungji were chosen (Fig. 2).

GV16 Pungbu was taken directly below the occipital protuberance on the posterior midline of the head, GB20 Pungji was taken in a depression between the upper portion of the sternocleidomastoid muscle and the trapezius on the same level with GV16 Pungbu. All pharmacopunctures used in this study were prepared in the aseptic room at the Korean Pharmacopuncture Institute. The administered amounts of all pharmacopunctures were 0.3 ml (1 ml/kg), that is, 0.1 ml to GV16 and 0.2 ml to GB20. Insulin syringes were used for the administration at Pungbu and on both sides of Pungji.

2.3. Measurement of the mean arterial blood pressure
After induction of anesthesia with urethan (750 mg/kg), the rats were fixed on a heat pad in a prone position to maintain the body temperature at 37 ± 0.5°C. For 150 minutes after administration of the pharmacopuncture, the MABP was measured using a pressure transducer (Grass, USA) connected to a polyethylene tube inserted into the femoral artery. The data were acquired through MacLab.

2.4. Measurement of the rCBF
The rat was fixed in a stereotactic frame (DKI, USA), and the parietal bone was thinned to translucency over the left cerebral artery by using a dental drill (an area of 5 × 5 mm2). Changes in evoked rCBF were measured by using a laser Doppler flowmeter (Transonic Instrument, USA). The tip diameter of the laser doppler flowmeter (LDF) probe was 0.8 mm. The LDF probe was positioned over the thinned skull (over the middle cerebral artery of the brain) perpendicular to the brain surface. To ensure a stable physiological condition of the animal, we measured the rCBF at least one hour after the preparation of the parietal bone for 150 minutes after administration of the pharmacopuncture.

2.5. Statistical analyses
The data are shown as means ± standard deviations. The statistical significance was determined using the non-parametric Fridman test. P-values less than 0.05 were considered as being statistically significant.

3. Results
With administration of scolopendrid pharmacopuncture, the changes in both the rCBF (p = 0.262) and the MABP (p = 0.114) were not significant (Fig. 3). Administration of BUM pharmacopuncture lowered the rCBF (p = 0.019) and the MABP (p = 0.002) (Fig. 4). Administration of bee venom 25% pharmacopuncture increased the rCBF (p = 0.026) and, lowered the MABP (p = 0.012) (Fig. 5), while administration of sweet bee venom 10% pharmacopuncture lowered the MABP (p = 0.014), but had no influence on the rCBF (p = 0.020) (Fig. 6).

4. Discussion
Many trials with interventions using herbal drugs, acupuncture and foods to increase rCBF have been conducted. Samul-tang and Bambusae caulis in liquamen are known prescriptions to increase rCBF by dilating the cerebral artery through cyclooxygenase, a biosynthetic enzyme producing prostaglandin [9]. Chukdamtang-gamibang has been prescribed to increase the rCBF through biosynthesis of soluble guanylyl cyclase and nitric oxide. Single herbal drugs or foods, such as pomegranate, Nelumbo nucifera, Schizandrae fructus, or Rhizoma acori graminei, have also been reported to increase rCBF [10-14]. Acupoints related with the rCBF are ST36, LI4, and GV20 [15-17].

The rCBF is directly proportional to the blood pressure and the diameter of the blood vessel. The higher the blood
Figure 3 Effects of scolopendrid pharmacopuncture on the rCBF and the MABP. The arrow heads (▲) indicate the pharmacopuncture of scolopendrid (0.3 ml, 1 ml/kg), that is, 0.1 ml to GV16 and 0.2 ml to GB20. All values are presented as means ± SDs. The p-value was calculated by using the Friedman test.

Figure 4 Effects of BUM pharmacopuncture on the rCBF and the MABP. The arrow heads (▲) indicate the pharmacopuncture of BUM (0.3 ml, 1 ml/kg), that is, 0.1 ml to GV16 and 0.2 ml to GB20. All values are presented as means ± SDs. The p-value was calculated by using the Friedman test.
Figure 5 Effects of bee venom 25% pharmacopuncture on the rCBF and the MABP. The arrow heads (▲) indicate the pharmacopuncture of bee venom 25% (0.3 ml, 1 ml/kg), that is, 0.1 ml to GV16 and 0.2 ml to GB20. All values are presented as means ± SDs. The *p*-value was calculated by using the Friedman test.

Figure 6 Effects of sweet bee venom 10% pharmacopuncture on the rCBF and the MABP. The arrow heads (▲) indicate the pharmacopuncture of sweet bee venom 10% (0.3 ml, 1 ml/kg), that is, 0.1 ml to GV16 and 0.2 ml to GB20. All values are presented as means ± SDs. The *p*-value was calculated by using the Friedman test.
pressure is, the more the rCBF is. The wider the diameter of blood vessel is, the more the rCBF is. The dilation of the blood vessel arises from relaxation of smooth muscles by secretion of prostaglandin endothelium-derived relaxing factor (EDRF). The blood pressure is controlled by the heart rate, the contraction of the myocardium, the peripheral blood vessel, the tensity of the smooth muscles and the intrinsic active substances in the body [18,19]. In the physiological state, the rCBF maintains its stability in spite of thermodynamic changes, for example, vascular resistance and blood pressure. On the other hand, in the pathological state, the rCBF changes according to the blood pressure, the heart rate, the effect of the autonomic nerve system, and the partial pressure of carbon dioxide [20,21]. Stimulation of free oxygen to the endothelial cell of the blood vessel generates powerful substances that dilate the blood vessel. Then, the rCBF is increased. Nitric oxide, guanylyl cyclase, guanosine triphosphate, cyclic guanosine monophosphate and calcium ions are involved in this process [22,23].

In this study, bee venom 25% pharmacopuncture lowered the blood pressure and increased the rCBF while BUM pharmacopuncture lowered the blood pressure and decreased the rCBF. Sweet bee venom 10% pharmacopuncture lowered the blood pressure and had no effect on the rCBF. The results arise from the different effects of each pharmacopuncture on the blood pressure and the diameter of the blood vessel. Bee venom 25% pharmacopuncture has a strong effect on lowering the blood pressure and a weak effect on dilating the blood vessel. However, BUM pharmacopuncture has a weak effect on lowering the blood pressure and a strong effect on dilating the blood vessel.

In conclusion, bee venom 25% pharmacopuncture and BUM pharmacopuncture are different in effects on cardiovascular circulation. Further studies are needed to elucidate the underlying mechanism.

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References

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