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Original article

Calculus Bovis-Fel Uris-Moschus Pharmacopuncture's Effect on Regional Cerebral Blood Flow and Mean Arterial Blood Pressure in Rats

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Key Words

Calculus Bovis-Fel Uris-Moschus, fengfu, pharmacopuncture, cerebrovascular circulation, hemodynamics

Abstract

Objectives: This study was designed to investigate the effects of *Calculus Bovis-Fel Uris-Moschus* pharmacopuncture (BUM) on the regional cerebral blood flow (rCBF) and the mean arterial blood pressure (MABP) in normal and cerebral ischemic rats and to investigate a possible pathway involved in the effects of BUM.

Methods: The changes in the rCBF and the MABP following BUM into Fengfu (GV16) were determined by using a laser-Doppler flow meter and a pressure transducer, respectively.

Results: BUM significantly increased the rCBF and decreased the MABP in normal rats in a dose-dependent manner. The effect on the rCBF was significantly inhibited by pretreatment with methylene blue (0.01 mg/kg, intraperitoneal), an inhibitor of guanylate cyclase, but was not affected by pretreatment with indomethacin (1 mg/kg, intraperitoneal), an inhibitor of cyclooxygenase. The BUM-induced decrease of the MABP was changed neither by methylene blue nor by indomethacin pretreatment. In the cerebral ischemic rats, the rCBF was stably increased upon cerebral reperfusion in the BUM group in contrast to the rapid and marked increase in the control group.

Conclusion: This study demonstrated that BUM into Fengbu (GV16) increased the rCBF in a dose-dependent manner in the normal state; furthermore, it improved the stability of the rCBF in the ischemic state upon reperfusion. Also, the effects of BUM on the rCBF were attenuated by inhibition of guanylate cyclase, suggesting that the effects involved the guanylate cyclase pathway.

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1. Introduction

The brain normally receives cerebral blood flow in the amount of 50 ml/100 g/min [1]. When the amount of cerebral blood flow drops under 10 ml/100 g/min, it results in energy metabolism disorders such as intracellular acidosis, often leading to fatal ischemic cranial nerve tissue impairment [2, 3]. In addition, when the decreased flow lasts longer than 5 min, brain function disorder or brain tissue impairment irreversibly occurs and results in various symptoms, including disturbance of consciousness, speech disorder, and motor disorder [4, 5]. A few studies on the effects of pharma-

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This paper meets the requirements of KS X ISO 9706, ISO 9706-1994 and ANSI/NISO Z39.48-1992 (Permanence of Paper).

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copuncture on cerebral hemodynamics exist. Cervi pantotrichum cornu pharmacopuncture at BL23 and BL52 increased the regional cerebral blood flow (rCBF) while it decreased the mean arterial blood pressure (MABP) [6]. Carthami flos pharmacopuncture at GV15 showed an anti-ischemic effect through an improvement of cerebral hemodynamics in transient cerebral ischemia [7].

Calculus Bovis-Fel Uris-Moschus pharmacopuncture (BUM) is a solution prepared by mixing dried powders of bear bile, cow bezoar, and musk. It has been used for treating musculoskeletal inflammatory and dolorific diseases such as arthritis [8]. Moschus was shown to have protective effects against brain damage induced by cerebral anoxia [9]. Cow bezoar has effects on the decrease in the blood pressure [10]. Few studies, however, have investigated the effects of BUM, as a whole, on cerebral hemodynamics and cerebral ischemia disease. Therefore, this study was designed to investigate the effects of BUM on cerebral hemodynamics in normal rats and the possible pathway, as well as its effects on the rCBF in cerebral ischemia rats upon reperfusion.

2. Materials and Methods

Male rats in the Sprague, Dawley family with weights of approximately 250 g (Samtaco, Korea) were purchased as subject animals and were used for the experiments. The animals were used after having been stabilized for more than 5 days in a breeding environment (interior temperature 24℃, humidity 55 ± 5%, and 12-h dark/light cycle). The BUM pharmacopuncture solution was prepared in the aseptic room of the Korean Pharmacopuncture Institute [11]. BUM, 1 ul, 5 ul, 25 ul, and 125 ul, was administrated to Fengfu (GV16) by using an insulin syringe (ultrafine 2, BD, U.S.A.), following dilution to 1 ml. Fengfu (GV16) was taken between the external occipital protuberance and the atlas on the posterior midline of the head. The rats were anesthetized with 750 mg/kg of urethane (Sigma, U.S.A.) by using peritoneal injections. Then, the parietal bone was exposed by dissecting the scalp along the center line. At a point 4-6 mm to the side and 0.2-1 mm to the front of the bregma, the operation for a 5- to 6-mm skull window was conducted. The needle probes for the laser Doppler flow meter (Transonic Instrument, U.S.A.) were carefully inserted toward the cerebral pial artery and were carefully kept perpendicular to the parietal cortical surface. After having been stable for certain periods of time, the rCBF was monitored for BUM injected into Fengfu (GV16), the concentrations of which was increased by 5 times in 30-min intervals. To measure the MABP, we fixed the anesthetized rats on heat pads to stabilize the body temperature at 37.5℃. A polyethylene tube was inserted in the aorta femoralis of the rat, and the data were transmitted to the data acquisition system (Maclab, U.S.A.) through a pressure transducer (Grass, U.S.A.). The MABP was measured with BUM in the same manner as the rCBF was measured. Methylene blue (0.01 mg/kg, intraperitoneal, MTB, Sigma), a inhibitor of guanylate cyclase, and indomethacin (1 mg/kg, intraperitoneal, IDN, Sigma), a inhibitor of cyclooxygenase, were used to pre-treat normal rats, and the rCBF and the MABP were observed following the injection of 25 ul of BUM. For cerebral ischemia induction, the middle cerebral artery (MCA) closing method according to Longa et al. was used [12]. Thirty min after the MCA fundus had been closed, a 1-ml saline solution containing 25-ul BUM was injected. One hundred twenty min after the closing, the blood flow was reperfused, and the change in the rCBF was monitored for 240 min.

The data for the rCBF and the MABP were analyzed by using the SAS program (ver. 9.2). The statistical significance was determined using the repeated measure ANOVA. *P*-values less than 0.05 were considered as being statistically significant.

3. Results

Compared to the rCBF of normal rats without BUM (100 ± 18%), the rCBFs were 98.74 ± 0.02%, 102.58 ± 0.64%, 112.14 ± 0.28%, and 122.08 ± 0.71% with 1 ul, 5 ul, 25 ul, and 125 ul of BUM, respectively, indicating significant increases in a dose-dependent manner ($P = 0.003$; Fig. 1A). On the other hand, the MABP decreased to 93.00 ± 0.67%, 88.94 ± 0.32%, 89.14 ± 0.26%, and 87.38 ± 0.97% with 1 ul, 5 ul, 25 ul, and 125 ul of BUM, respectively, compared to that without BUM, indicating significant decreases in the blood pressure in a dose-dependent manner ($P = 0.047$; Fig. 1B). To find the effective mechanisms for the increased rCBF and MABP in normal rats to which BUM had been administered, we set the normal rats' rCBF and MABP as the control and the rCBF and the MABP of the normal rat changed by BUM after pretreatment with indomethacin as the experimental group. No significant difference was observed between the two groups (Fig. 2A, Fig. 2B). However, the rCBF, which was significantly improved with BUM, was significantly suppressed by pretreatment with methylene blue, an inhibitor of guanylate cyclase (Fig. 3A). However, according to the MABP results between the two groups, no significant difference wais found (Fig. 3B).

To observe BUM's effect on the rCBF change in rats with induced cerebral ischemia, we observed the changes in the rCBF after reperfusion following the administration of BUM to the rats in which cerebral ischemia had been in-

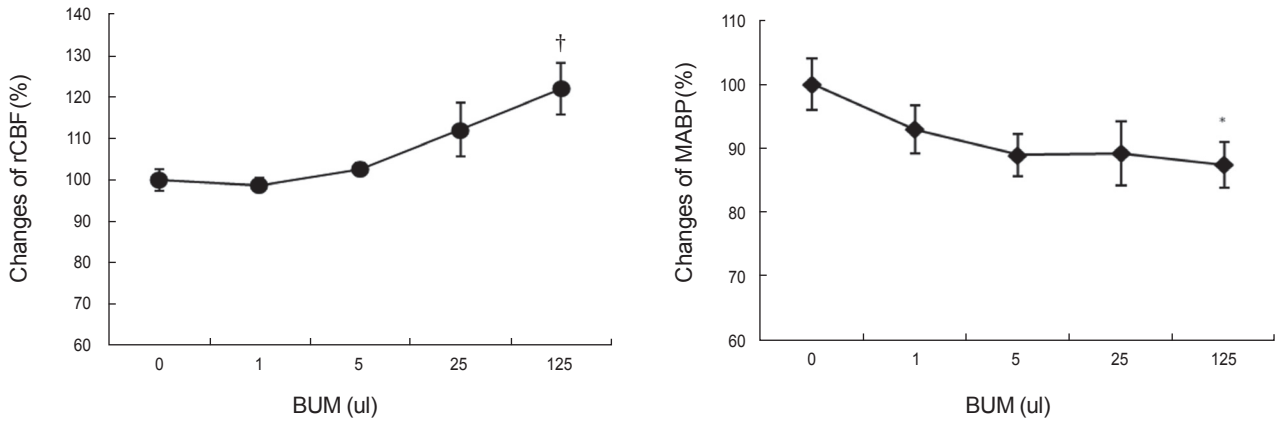

Figure 1 Effects of BUM on the (A) rCBF and the (B) MABP in normal rats. The present data were expressed as means ± SEs (n = 8). rCBF means regional cerebral blood flow, and MABP, the mean arterial blood pressure. *Statistical significance compared with the base group ($P < 0.05$); † Statistically significant compared with the base group ($P < 0.01$).

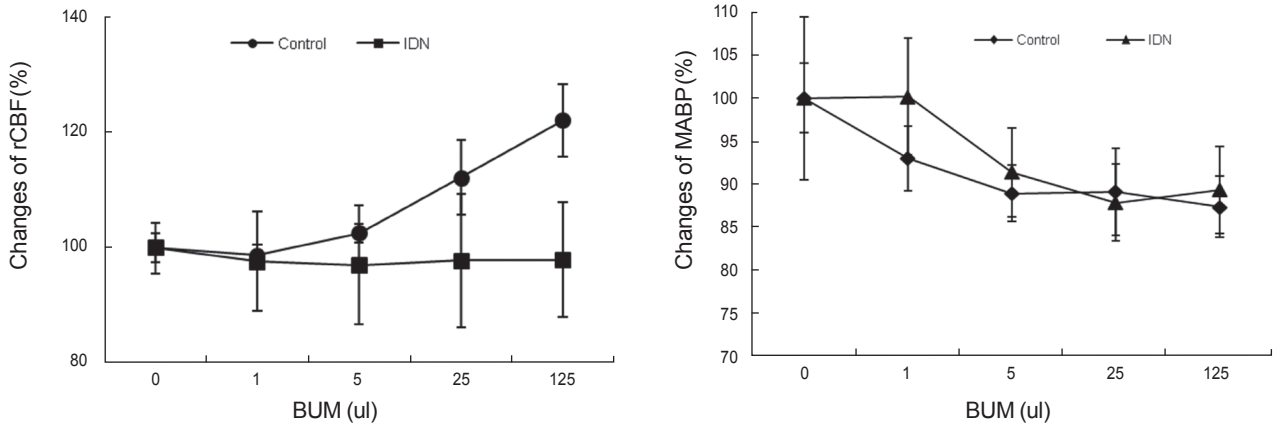

Figure 2 Effects of pretreatment with indomethacin on the (A) rCBF and the (B) MABP when BUM is administered in normal rats. The data are expressed as means ± SEs (n = 8/group). Control is non-treated group; IDN is the indomethacin (1-mg/kg, intraperitoneal)-treated group; rCBF means regional cerebral blood flow; MABP is mean arterial blood pressure.

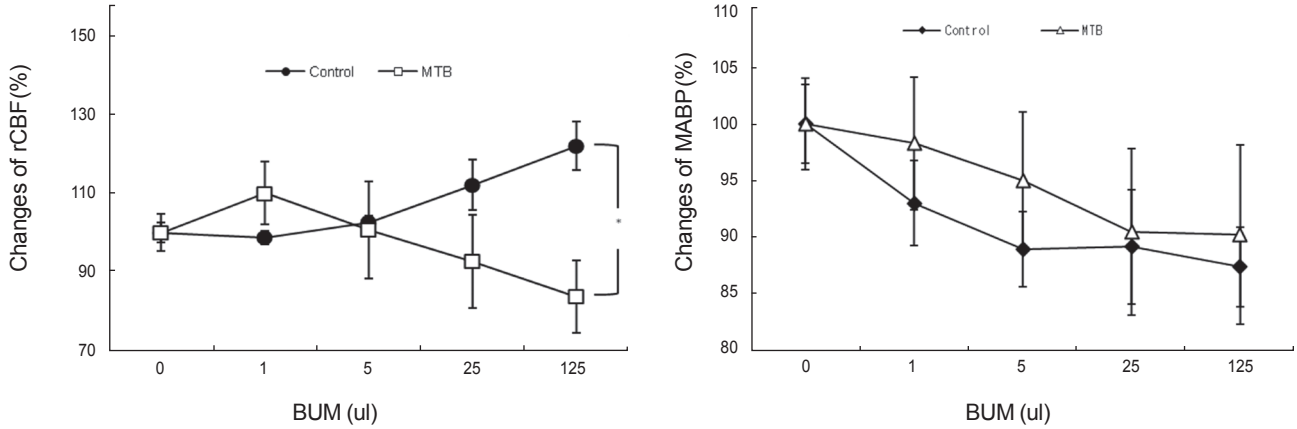

Figure 3 Effects of pretreatment with methylene blue on the (A) rCBF and the (B) MABP when BUM is administered in normal rats. The data are expressed as eans ± SEs (n = 8/group). Control is the non-treated group; MTB is the methylene-blue (0.01-mg/kg, intraperitoneal)-treated group, rCBF means regional cerebral blood flow; MABP is mean arterial blood pressure. *Statistically significant compared with the control group ($P < 0.001$).

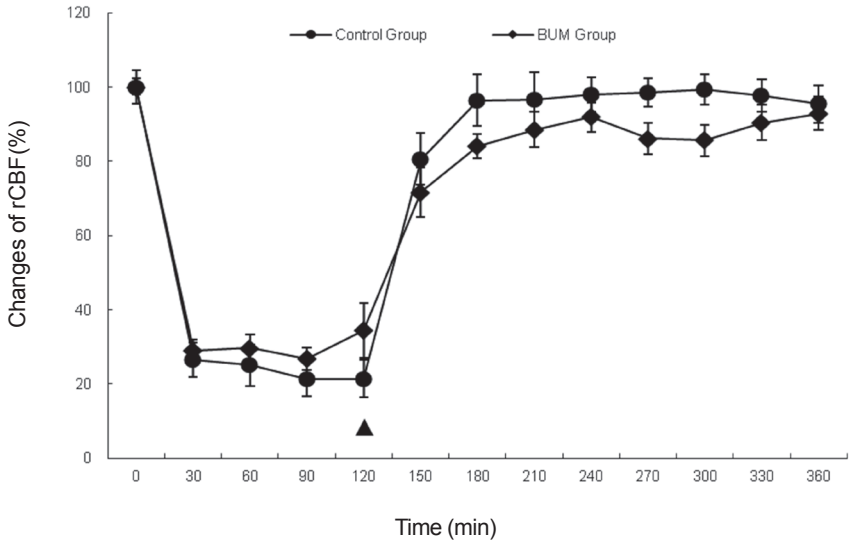

Figure 4 Effect of BUM on the rCBF response in cerebral ischemic rats. Right middle cerebral artery occlusion was exerted for 120 min; then, reperfusion was exerted (▲, refusion; control group, non-treated group; BUM Group, group treated with BUM 25 ul; rCBF, regional cerebral blood flow. *Statistically significant compared with the control group ($P < 0.05$).

duced by using the MCA closing method. The rCBF results for the two groups showed significant differences in the group (F = 0.60, $P = 0.452$), the time (F = 138.48, $P < 0.001$) and the interaction according to the group and the time (F = 2.31, $P = 0.036$). Thus, the rCBF change was observed to be improved stably in the BUM-treated group compared to the control group in which the changes were unstable when going through the reperfusion in the cerebral ischemia state (Fig. 4).

4. Discussion

The rCBF is directly proportional to the blood pressure and the diameter of the blood vessel. The higher the blood pressure is, the more the rCBF is. The dilation of the blood vessel arises from relaxation of smooth muscles by secretion of prostaglandin, an endothelium-derived relaxing factor. Prostaglandin is an effective drug for the rennin and angiotensin system and acts to relax vessels and to lower the blood pressure. The blood pressure is controlled by the heart rate, the contraction of the myocardium, the peripheral blood vessel and the intrinsic active substances in the body [13,14]. This study shows that the rCBF increases to a value significantly higher than the base value when BUM is administrated to normal rats. Considering that the cerebral blood flow is proportional to the mean blood pressure and the cerebrovascular diameter, this result suggests that BUM may dilate the brain's blood vessels.

In this study, to verify what mechanism causes BUM to increase the rCBF significantly, we pretreated the rats with indomethacin [15], an inhibitor of cyclooxygenase involved in vessel dilation, and with methylene blue [16], an inhibitor of guanylate cyclase, and we observed the mechanism. Considering such results, according to the report by Shin et al. stating that the reduction of the rCBF by methylene-blue pretreatment was related to guanylate cyclase, an enzyme creating cyclic guanosine monophosphate [17-19], and reports by Bakalova [20] and Okamoto et al. [21] stating that the reduction of the rCBF with indomethacin treatment was related to cyclooxygenase, the significantly increased rCBF after BUM administration is considered to be the result of cerebrovascular dilation due to vitalized guanylate cyclase.

The ischemic brain impairment occurring due to the reduction of cerebral blood flow is more serious when the blood is re-supplied than when the cerebral blood flow is reduced [22-24]. The reperfusion of the ischemia area intensifies the neuron impairment by causing biochemical and cytogenic reactions in series and by causing impairments not only in the regionally relevant tissues, but also in other organs. This action is assumed to be related to non-specific toxins, vasoreactive materials, lactic acid and oxygen-free radicals. Therefore, the maintenance of the stable rCBF after reperfusion may become a critical index for treatments to prevent brain impairment [25]. In this study, the rCBF of the BUM group was significantly improved and became stable while the rCBF of the control group under a cerebral ischemia condition showed an unstably increasing state. The results may suggest that BUM controls the impairment of brain nerve cells caused by non-specific toxins, vasoreactive materials and oxy-

gen-free radicals occurring due to cerebral ischemia, although whether the stimulation by needles *per se*, beside BUM injection, added any effects cannot be ruled out. In conclusion, when brain impairment occurs due to cerebral ischemia, BUM is shown to have an anti-ischemic effect by dilating the diameters of cerebrovascular vessels, which is related to guanylate cyclase, to provide a stable cerebral blood flow.

5. Conclusion

This study demonstrated that injection of BUM into Fengbu (GV16) increased the rCBF in a dose-dependent manner in the normal state; furthermore, it improved the stability of the rCBF in the ischemic state upon reperfusion. Also, the effects of BUM on the rCBF were attenuated by inhibition of guanylate cyclase, suggesting that the effects involved the guanylate-cyclase pathway.

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