Effects of Kangen-karyu on Coagulation System and Platelet Aggregation in Mice

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Kangen-karyu (KGK) is a herbal formula created under the theory of traditional Chinese herbal medicine to invigorate the blood and dispel blood stasis. It contains 6 herbs: peony root, cnidium rhizome, safflower, cyperus rhizome, saussurea root (JP XIV), and Salvia miltiorrhiza root. The present study has been conducted to evaluate the in vivo anti-thrombotic activity of KGK using normal mice. Three consecutive days of oral administration of KGK to mice significantly extended tail-bleeding time and suppressed ex vivo platelet aggregation, while it did not extend the prothrombin time of plasma. It was revealed that the anti-thrombotic effects of KGK did not depend on the downregulation of the coagulation system, but depended in part on the inhibition of platelet aggregation. These results explain one of the pharmacological activities of KGK to invigorate the blood and dispel blood stasis.

Key words Salvia miltiorrhiza; anti-thrombosis; anti-coagulant; anti-platelet; Guan-xin No. 2

In traditional Chinese herbal medicine, there are herbal formulas to invigorate the blood and dispel blood stasis (活血化瘀方剂). These formulas are used to treat patterns of blood stagnation and stasis, which represent progressive stages in the impairment and blockage of the flow of blood.1) Kangen-karyu (冠元顆粒, KGK) is a prescription created under this theory. It contains 6 herbs: peony root, cnidium rhizome, safflower, cyperus rhizome, saussurea root (JP XIV, respectively), and Salvia miltiorrhiza root. It is commonly used to treat symptoms related to blood circulation deficiencies, and is reputed to reduce blood and plasma viscosity, and thus improve microcirculation.2) A previous clinical report showed that KGK improved hypertension, arteriosclerosis, headache, and dizziness,3) and the animal experiment demonstrated that KGK recovered the learning and memory impairment in senescence accelerated mouse by preserving the activities of choline acetyltransferase and superoxide dismutase.4) However, the pharmacological mechanisms of KGK in improving microcirculation had not been studied. In the present study, we discuss the anti-thrombotic effect of KGK by evaluating its in vivo effects on the coagulation system and platelet-aggregative activity.

MATERIALS AND METHODS

Materials KGK in the form of a dried powder extract was supplied from Iskra Co., Ltd. (Tokyo, Japan). The human daily dose (4.5 g) of KGK contained an extract of the following herbs: peony root (2.25 g), cnidium rhizome (2.25 g), safflower (2.25 g), cyperus rhizome (1.125 g), saussurea root (1.125 g) and Salvia miltiorrhiza root (4.5 g). Warfarin and ticlopidine hydrochloride were purchased from Sigma (St. Louis, MO, U.S.A.) and Wako (Osaka, Japan), respectively. Ketamine hydrochloride (Ketalar®) was bought from Sankyo Co. Ltd., Tokyo. Six-week-old male ddY mice were obtained from Japan SLC (Hamamatsu, Japan).

Experiment 1: Bleeding Time Mice tail-bleeding time was measured by the slightly modified method of Hornstra et al.5) KGK (0.5 or 2 g/kg) or ticlopidine hydrochloride (20 mg/kg) were orally administered 3 times at 24 h-intervals to the mice (n = 12). The dosage of KGK (0.5 g/kg) and ticlopidine hydrochloride corresponded to about 5-times the amount of human daily dosages. One hour after the final administration, mice were anesthetized by intraperitoneral injection of ketamine hydrochloride (100 mg/kg). Then, the tail was transected at 2 mm from the tip, and the distal 3 cm of the tail was immersed in saline at 37 °C. The period between transection and the moment bleeding stopped was taken as the bleeding time.

Experiment 2: Coagulation System KGK (1 g/kg b.w.) or vehicle (distilled water) was orally administered 6 times at 12-h intervals to the mice (n = 8). Blood (0.45 ml) was collected from the heart 1 h after the final KGK-administration with a plastic syringe containing 0.05 ml of 3.13% sodium citrate, and immediately centrifuged (14000 rpm, 5 min). For the positive control, warfarin (1 mg/kg) was orally administered to the mice, and the blood was collected 24 h after warfarin-treatment. The plasma (0.1 ml) was pre-warmed for 2 min at 37 °C, the mixed with 0.2 ml of recombinant thromboplastin reagent (Orth-Recombi-Plastin 1.0, Orth Clinical diagnostics, Tokyo, Japan). Times were recorded until a clot was formed, and the average of two independent measurements was regarded as the prothrombin time.

Experiment 3: Platelet Aggregation KGK (0.5 or 2 g/kg) or ticlopidine hydrochloride (20 mg/kg) was orally administered 3 times at 24 h-intervals to the mice (n = 12). One hour after the final administration, mice were anesthetized and 0.9 ml of the blood was collected from the abdominal aorta by a plastic syringe containing 0.1 ml of 3.13% sodium citrate. Platelet-rich plasma (PRP) was obtained by centrifugation of the blood at 1000 rpm for 10 min at room temperature. Platelet-poor plasma (PPP) was obtained by further centrifugation at 2500 rpm for 20 min. The number of platelets in PRP was adjusted to 3 × 10^10 platelets/ml by dilution with PPP. Forty-five microliters of PRP was mixed with 5 μl of 100 μM adenosine 5′-diphosphate (ADP) (final concentration of ADP, 10 μM), and the optical density (OD) at 620 nm was sequentially measured under agitation at 37 °C by a mi-
Anti-thrombotic therapy is applied to prevent atherosclerotic diseases, including myocardial and cerebral infarction. This therapy includes the suppression of primary hemostasis and suppression of the coagulation system using anticoagulants such as warfarin and heparin.6) In the present study, we examined the anti-thrombotic effects of KGK and its pharmacological mechanisms to improve blood microcirculation by evaluating the coagulation system and platelet-aggregative activity of normal mice, respectively.

In the first experiment, we evaluated the effect of KGK on the tail-bleeding time of the mice. Three consecutive days of administration of KGK significantly extended the tail-bleeding time (Fig. 1). In order to further evaluate the ability of KGK to cause prolongation of tail-bleeding time, we measured the prothrombin time of the plasma and ex vivo platelet aggregation. There was no statistical difference in the prothrombin time between control and KGK-treated mice, although warfarin significantly extended the time (control, 9.6 ± 0.67 s; KGK-treated group, 10.6 ± 0.40 s; warfarin-treated group, 20.4 ± 3.5 s, p < 0.05), suggesting that KGK could not affect the coagulation system. On the other hand, 3 consecutive days of KGK significantly suppressed ex vivo platelet aggregation (Fig. 2). It is expected that KGK would extend bleeding time at least via inhibition of platelet function.

Platelets play important roles in thrombosis at damaged blood vessels, since thrombus formation occurs through the activation and aggregation of platelets. Thus, platelet aggregation is the major pathogenic mechanism in thrombosis.6,7) Considering the importance of thrombosis in cardiovascular disorders, screening for better anti-platelet drugs from national products were successfully conducted, such as from Ginkgo biloba,8) garlic,9) etc. Several herbal components prescribed in KGK have an inhibitory effect on platelet aggregation in vitro. The decoction of *Salvia miltiorrhiza* root inhibited ADP- or epinephrine-induced platelet aggregation in vitro via the inhibition of cyclic adenosine 5′-monophosphate (cAMP) phosphodiesterase,10) and magnesium lithopermate B was isolated as an active constituent.11) The decoction of peony root also inhibited ADP-induced platelet aggregation in vitro.12) However, most herbal medicines are usually taken orally. Since not all of the constituents in an herbal decoction are absorbed from the gastrointestinal tract into the circulation,13) these in vitro results, which were obtained by the direct addition of the herbal decoction into platelet-rich plasma, were very limited. Moreover, in vivo experiences are very important in order to evaluate the anti-platelet effect of drugs, since ticlopidine hydrochloride, which is one of the most widely prescribed anti-platelet drugs and used as a positive control in this study, did not suppress platelet aggregation in vitro.14) The present study is the first to report the suppressive effect of orally administered KGK on platelet aggregation, and supports the clinical report that KGK would improve symptoms related to blood circulation-deficiencies to prevent atherosclerotic diseases such as myocardial and cerebrovascular infarction.2,3)

In the clinical case, it is considered that *Salvia miltiorrhiza* root, the main component of KGK, can affect hemostasis by the inhibition of platelet aggregation, interference with extrinsic blood coagulation and the promotion of fibrinolytic activity.15) Zou et al. showed that intravenous injection of rosmarinic acid, which is a minor constituent of this herb, to rats suppressed platelet aggregation and promoted fibrinolytic activity,16) with the expectation that KGK would improve microcirculation via not only inhibition of platelet aggregation...
but also by the promotion of fibrinolysis. The present study revealed that the anti-thrombotic effects of KGK depended not on downregulation of the coagulation system, but in part on the inhibition of platelet aggregation; however, the effect of orally administered KGK on the promotion of fibrinolysis has not yet been evaluated, and further studies are needed.

In conclusion, KGK has an anti-thrombotic effect via the inhibition of platelet aggregation. This herbal prescription would be a promising agent to improve blood viscosity and microcirculation and to prevent atherosclerotic diseases such as myocardial and cerebral infarction.

REFERENCES