Anti-thrombic Actions of 70 % Methanolic Extract and Cinnamic Aldehyde from Cinnamomi Cortex

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The anti-thrombic activities of a 70% methanolic extract (CMe) from Cinnamomi Cortex on blood coagulation and fibrinolysis were investigated. CMe prevented the hepatic venous thrombosis in high butter diet-treated rats, and the decreases of blood platelets and fibrinogen in normal rats induced by endotoxin. CMe also inhibited the blood platelet aggregation induced by collagen, arachidonic acid and adenosine diphosphate (ADP) and the conversion of fibrinogen to fibrin induced by thrombin in in vitro experiments. Cinnamic aldehyde, a major essential oily component of CMe were showed stronger inhibitory activity than that of CMe on the blood coagulation. These results suggest that Cinnamomi Cortex has anti-thrombic activities.

Keywords—crude drug; Cinnamomum cassia; cinnamic aldehyde; thrombosis; blood platelet aggregation; thrombin; disseminated intravascular coagulation

Cinnamomi Cortex (the cortex of Cinnamomum cassia BLUME) is frequently used as a crude drug for treatment of inflammation, headache and pyrexia in the traditional Chinese system of medicine. Cinnamomi Cortex is also a main component in Keishi-Bukuryo-gan, which is used for Oketsu syndrome. We reported that Keishi-Bukuryo-gan possesses anti-thrombic activity.1) The possibility arises that Cinnamomi Cortex may be effective against disseminated intravascular coagulation (DIC).

The purpose of the present investigation was to study the preventive effect of Cinnamomi Cortex on experimental DIC induced by endotoxin in rats, as well as on blood platelet aggregation and the conversion of fibrinogen to fibrin (in vitro models).

Materials and Methods

Materials—A 70% methanolic extract (CMe, yield 21.6%) was prepared from Cinnamomi Cortex. The sources of other materials were as follows: cinnamic aldehyde (Kishida Chemical Co. Ltd., Japan), endotoxin (Escherichia coli 055: B5, Difco Lab., U.S.A.), dextran sulfate (Pharmacia Fine Chemicals, Sweden), adenosine diphosphate 2Na (ADP, Sigma Chemical Co., U.S.A.), collagen, arachidonic acid and aspirin (Sigma Chemical Co., U.S.A.).

Animals—Male Wistar-King strain rats weighing 150—200 g and male JW strain rabbits weighing 2—2.5 kg were used for the experiments. They were fed a standard diet (Nihon Clea, Japan) for a minimum period of 7d and then fasted for 24 h before the start of the experiments.

Endotoxin-Induced DIC in High Butter Diet-Treated Rats—Endotoxin-induced DIC model was prepared according to Renaud.2) Rats were fed on a high butter diet (salt-free butter (30%), cholesterol (15%) and bile powder (2g) in a laboratory chow) for 15 weeks. CMe (200 or 500 mg/kg) was orally administered to those rats for 7d before the intravenous injection of endotoxin (0.1 mg/kg). The animals were killed by decapitation 8 h after the injection of endotoxin, and the livers were quickly removed and subjected to microscopic examination for hepatic infarcts. The results of histological observation were rated as follows: 3, severe hepatic infarct; 2, moderate; 1, slight; 0, undetectable.

Endotoxin-Induced DIC in Normal Rats—Experimental DIC was induced by a modification of the method of
Schoendorf et al.3) CMe (50, 200 or 500 mg/kg) and cinnamic aldehyde (0.1 or 0.5 mM/kg) were administered orally to rats 1 h before the injection of endotoxin (0.1 mg/kg) into the tail vein. Blood samples were withdrawn from the heart into plastic syringes at 4 h after the injection of endotoxin, while the rats were anesthetized with pentobarbital. As anticoagulants, 0.01 M sodium ethylenediaminetetraacetic acid (EDTA) was used for platelet counts and a 1:9 volume of 3.8% sodium citrate for prothrombin time and fibrinogen determinations.

Blood platelets were counted with an automatic blood cell counter (Coulter counter, model S-Plus, Coulter Co., U.S.A.). Fibrinogen was determined according to the method of Quick.4) The prothrombin time was measured with a COAG-A-Mate dual-channel device (General Diagnostic, Warner-Lambert Co., U.S.A.). Fibrin degradation product (FDP) was determined by means of the latex aggregation test (FDPL test U, Teikoku Zoki, Japan).

Euglobulin Lysis Time (ELT) in Normal Rats—Whole blood samples were collected in plastic syringes from the heart of rats anesthetized with pentobarbital at 1 h after the oral administration of CMe (50, 200 or 500 mg/kg) or cinnamic aldehyde (0.1 or 0.5 mM/kg). One-tenth volume of 3.8% sodium citrate was added to the blood sample and the mixture was centrifuged at 4000 rpm at 4°C for 10 min. Using the plasma thus obtained, ELT was measured in the manner reported by Kaulla and Schultz.5) After addition of 9.8 ml of precooled water, the plasma was incubated at 4°C for 5 min in a stream of CO₂ gas, then centrifuged at 4000 rpm for 10 min. The resulting precipitates were dissolved in 0.7 ml of 1/15 N phosphate buffer solution, and 40 μl of thrombin solution (125 U/ml) was added. The coagulating plasma was incubated at 37°C, and the ELT was measured.

Whole blood samples obtained from the heart at 30 min after intravenous injection of dextran sulfate (1 or 10 mg/kg) were treated in the same manner as mentioned above, and the ELT was measured.

Blood Platelet Aggregation Test—Whole blood samples were collected from pentobarbital-anesthetized rabbits. Nine ml of the blood of rabbit and 1 ml of sodium citrate (3.8%) was transferred into a plastic tube, and centrifuged at 1000 rpm for 10 min to obtain platelet-rich plasma (PRP). PRP was removed with a siliconized pipet, and stored in a plastic test tube with a screw cap. The remaining red cell precipitate of the blood samples was further centrifuged at 3000 rpm for 30 min to give platelet-poor plasma (PPP), which was used as a maximal transmittance standard.

The platelet aggregation test described by Born and Cross6) was performed with collagen (500 μg/ml), arachidonic acid (50 mM) and ADP (2 μM) as aggregating agents. A 0.2 ml aliquot of PRP was placed in a test tube and the content was stirred at 1200 rpm for 3 min at 37°C, then a 10 μl aliquot of a test solution was added. After 3 min, an aggregating agent was added to the reaction mixture. Changes in the light transmittance of the reaction mixture were continuously recorded with a Husm system platelet aggregometer (Rika Electric Co., Japan) and the transmission at the maximal aggregation after the addition of an aggregating agent was recorded. Platelet aggregation was expressed as the percent increase in the transmittance taking the transmittance of a control mixture containing no test solution as zero. An anti-platelet aggregating agent, aspirin, was used as a standard drug.

Thrombin-Induced Conversion of Fibrinogen to Fibrin—Fibrinogen (500 mg) was dissolved in 100 ml of 0.05 M NaCl containing 0.05 M Tris-acetate buffer (pH 7.4). A test solution (0.1 ml) was added to 1.8 ml of the fibrinogen solution with stirring. After 1 min, 0.1 ml of thrombin solution (0.2 U/ml) was added to the mixture and the whole was gently stirred until a fibrin clot appeared. The time required for clotting was recorded. An anti-thrombin agent, heparin, was used as a standard drug.

Results

Endotoxin-Induced DIC in High Butter Diet-Treated Rats

In high butter diet-fed rats, intravenous injection of endotoxin induced hepatic infarct...
with partial or straggling thrombus and hemorrhage (Fig. 1). Table I showed that CMe (500 mg/kg) significantly inhibited the formation of hepatic infarct in high butter diet- and endotoxin-treated rats.

### Endotoxin-Induced DIC in Normal Rats

It was shown that DIC could be induced by injection of endotoxin (0.1 mg/kg) into the tail vein, resulting in decreases of blood platelets and fibrinogen, prolongation of prothrombin time and an increase of FDP. Before the injection of endotoxin, CMe (50, 200 or 500 mg/kg) or cinnamic aldehyde (0.1 or 0.5 mm/kg) was administered, and its preventive effect against the endotoxin-induced DIC was examined (Table II).

The blood platelet count was $115 \pm 4\times 10^4$/mm$^3$ in normal rats injected with saline only. It was reduced to $62 \pm 7\times 10^4$/mm$^3$ in rats injected with endotoxin (0.1 mg/kg). When rats were given CMe (500 mg/kg) or cinnamic aldehyde (0.5 mm/kg), the reduction of the blood platelet count by endotoxin was significantly smaller.

The level of fibrinogen was $201 \pm 20$ mg/dl in normal rats, while it was prolonged to $127 \pm 20$ mg/dl in DIC rats. No shortening of prothrombin time was observed in rats given CMe (50, 200 or 500 mg/kg) or cinnamic aldehyde (0.1 or 0.5 mm/kg), as compared with the control.

The FDP level was $0.1 \pm 0.1\mu g/ml$ in normal rats injected with saline only. The level

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<th>Table I. Effect of CMe from Cinnamomi Cortex on the Endotoxin-Induced DIC in High Butter Diet-Treated Rats</th>
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<td><strong>TABLE II. Effects of CMe and Cinnamic Aldehyde from Cinnamomi Cortex and Aspirin on the Endotoxin-Induced DIC in Normal Rats</strong></td>
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Each value is the mean ± S.E. Significantly different from the control, a) p < 0.05, b) p < 0.01.
increased to 1.6 ± 0.4 μg/ml in the DIC rats. When CMe (50, 200 or 500 mg/kg) or cinnamic aldehyde (0.1 or 0.5 mM/kg) was administered to rats 1 h before the injection of endotoxin, the FDP levels were not reduced.

A clear preventive effect of aspirin (used as a standard drug) was recognized on blood platelets and fibrinogen, but not on prothrombin time or FDP.

**ELT in Normal Rats**

As shown in Table III, ELT was 108 ± 13 min in the normal rats orally given water only. When 10 mg/kg dextran sulfate (as a standard drug) was injected into rats, the ELT was significantly shortened to 36 ± 4 min. It was not shortened when 200 or 500 mg/kg of CMe or 0.1 or 0.5 mM/kg of cinnamic aldehyde was given.

**Collagen-Induced Blood Platelet Aggregation**

As shown in Table IV, the incubation of CMe (50 to 100 μg/ml) with PRP weakly inhibited the blood platelet aggregation induced by collagen. Cinnamic aldehyde (1.0 mM), as well as the active control agent, aspirin, showed an inhibitory effect on blood platelet aggregation induced by collagen.

**Arachidonic Acid-Induced Blood Platelet Aggregation**

As shown in Table V, CMe (100 μg/ml) showed weak inhibition of arachidonic acid-
induced blood platelet aggregation. Cinnamic aldehyde and the active control agent, aspirin, both showed potent inhibition of blood platelet aggregation induced by arachidonic acid.

**ADP-Induced Blood Platelet Aggregation**

As shown in Table VI, CMe (100 μg/ml) inhibited blood platelet aggregation induced by ADP. Cinnamic aldehyde also had an inhibitory effect, but aspirin did not.

**Thrombin-Induced Conversion of Fibrinogen to Fibrin**

As shown in Table VII, the clotting time of the control without addition of any test solution was 150±3 s. The clotting time was prolonged significantly by incubation with 10 U/ml of heparin, an anti-thrombic agent, before addition of thrombin. CMe at a concentration of 50 or 100 μg/ml inhibited the conversion of fibrinogen to fibrin. Cinnamic aldehyde at a concentration of 0.5 or 1.0 mM also significantly prolonged the clotting time.

**Discussion**

CMe of Cinnamic Cortex inhibited the hepatic venous thrombosis in high butter diet-treated rats, and the decreases of blood platelets and fibrinogen induced by endotoxin in normal rats. CMe also inhibited collagen-, arachidonic acid- and ADP-induced blood platelet aggregation and thrombin-induced conversion of fibrinogen to fibrin in vitro.

However, CMe had no effect on euglobulin lysis time in rats. Cinnamic aldehyde, major essential oily component which is contained in CMe prepared from Cinnamomi Cortex, showed greater inhibitory activity than CMe on the blood coagulation in vitro and endotoxin-induced DIC in normal rats.

Consequently, cinnamic aldehyde seems to be an active principle of CMe.

Among the five crude drugs (Moutan Cortex, Cinnamomi Cortex, Paeoniae Radix, Persicae Semen and Hoelen) that make up Keishi-Bukuryo-gan, Moutan Cortex extract was previously shown in exhibit anti-thrombic activity.7,8) In the present study, Cinnamomi Cortex was also found to have such activity.

**References**