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Effects of Danggi-Jakyak-San on Antiplatelet and Antihemolysis Activity of in Human blood

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We wondered whether the mechanisms of antiplatelet aggregation of DJS-WE were through multiple pathways. Danggijakyak-san(DJS) consisting of 6 herbes of *Paeoniae Radix, Poria Cocos, Angelicae Sinensis Radix, Cnidii Rhizoma, Atractylodis Macrocephalae Rhizoma* and *Alismatis Rhizoma*, is a crude mixture of a commonly used Korean herbal medicine. The water extract (DJS-WE) of DJS has been known to have an anti-platelet aggregation activity. We have reported that DJS-WE inhibited ADP-induced aggregation as well as arachidonic acid-induced aggregation of human platelet. Clinical studies on the cardiovascular effects of DJS-WE have been done in Korea. The DJS has been used as a remedy for gastrointestinal disorders (abdominal pain, dysentery), headache, amenorrhea, and postpartum hemorrhage. It has also been claimed to have a remarkable central stimulant effect, a transient hypertensive effect, and positive inotropic and chronotropic effects. In this paper, we evaluated the possible mechanisms of the antiplatelet activity of DJS-WE using human platelets. On the other hand, the role of DJS-ethanol extract on the inhibition of platelet aggregation and hemolytic effect have not yet been investigated in detail. We also used the method of activated partial thromboplastin times (APTT) for the first time to study the inhibition on platelet aggregation activity of DJS-ethanol extract. The effect of DJS-WE on hemolysis was also investigated. DJS-WE showed a high hemolysis ability on human blood.

Key words : Prescription, Danggijakyaksan(DJS, DJS-WE), aggregation, human platelet, thrombosis, mechanism, APTT, hemolysis

Introduction

Korean herbs have been widely used as remedies in oriental medicine. In recent decades, many biologically active constituents of Korean herbs have been isolated and their pharmacological actions investigated. Traditional Korean Prescription, Danggijakyak-san (DJS) consisting of 6 herbes of *Paeoniae Radix, Poria Cocos, Angelicae Sinensis Radix, Cnidii Rhizoma, Atractylodis Macrocephalae Rhizoma* and *Alismatis Rhizoma*, is a crude mixture of a commonly used Korean herbal medicine, which has been used for at least 2000 years by traditional Korean physicians to stimulate blood circulation and relieve pain[1,2]. The water extract (DJS-WE) of DJS has been known to have an anti-platelet aggregation activity[3]. We have reported that DJS-WE inhibited ADP-induced aggregation as well as arachidonic acid-induced aggregation of human platelet[4].

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Clinical studies on the cardiovascular effects of DJS-WE have been done in Korea. One study included 234 patients with acute ischemic cerebrovascular diseases who were admitted to hospitals following a cerebrovascular stroke. DJS-WE (50-500 ㎎/day) was given by oral administration for 1-3 weeks, and about 90% of the patients treated with DJS-WE showed clinical improvement of myodynamic changes and swallowing difficulties[3].

Furthermore, 50 patients with angina pectoris were treated with DJS-WE and about 90% of them showed symptomatic relief[3]. Park et al.[4] reported that extracts from "DJS-WE" were found to be effective in the treatment of angina pectoris. It was also demonstrated that it could improve changes in nail fold microcirculation of patients with acute cerebral thrombosis treated with DJS-WE[4]. Although the control groups in these clinical trials were poorly defined, the study's conclusions imply that DJS-WE may have beneficial effects in ischemic diseases. DJS-WE also had no significant adverse effects.

The DJS has been used as a remedy for gastrointestinal

disorders (abdominal pain, dysentery), headache, amenorrhea, and postpartum hemorrhage[2]. It has also been claimed to have a remarkable central stimulant effect, a transient hypertensive effect, and positive inotropic and chronotropic effects. Recently, we further reported that DJS exerts a vasodilator effect on isolated rat mesenteric arteries in a dose-dependent manner. It was also reported that DJS-WE marginally increased the release of tumor necrosis factor (TNF)-alpha, but not interferon (IFN)-gamma from leucocytes[5]. Also, DJS-WE reduced airway smooth muscle tone via a postjunctional mechanism through stimulation of the sodium pump and the subsequent hyperpolarization /repolarization of the cell membrane[6].

To elucidate the mechanisms of its actions, some studies have shown DJS-WE to have hypotensive effects in animal studies and an inhibitory effect in platelet aggregation. DJS-WE has also been shown to inhibit platelet aggregation induced by ADP. Uusing both arterial and venous thrombosis models in rats, we showed that DJS-WE reduced thrombus formation[7,8].

The anti-thrombotic activity was at least partly due to its antiplatelet activities, since DJS-WE inhibited platelet aggregation induced by agonists (i.e., collagen, thrombin, and ADP)[3]. Platelets that were induced to produce maximal aggregation could be dispersed by the addition of DJS-WE. Moreover, we recently found that DJS-WE has an 2-adrenergic receptor partial agonist activity. Therefore, we wondered whether the mechanisms of antiplatelet aggregation of DJS-WE were through multiple pathways. In this paper, we evaluated the possible mechanisms of the antiplatelet activity of DJS-WE using human platelets.

On the other hand, the role of DJS-ethanol extract on the inhibition of platelet aggregation and hemolytic effect have not yet been investigated in detail. In this paper, we also used the method of activated partial thromboplastin times (APTT)[9] for the first time to study the inhibition on platelet aggregation activity of DJS-ethanol extract. The effect of DJS-EE on hemolysis was also investigated. DJS-EE showed a high hemolysis ability on human blood.

Materials and Methods

1. Materials

Human thrombin, ADP, epinephrine, collagen (bovine tendon type I), DPH, prostaglandin E1 (PGE1), apyrase, bovine serum albumin, ammonium formate, myoinositol, formic acid, sodium formate, sodium tetraborate, heparin, Dowex-1 (100-800 mesh; X8, chloride form), and EGTA were purchased from Sigma (USA). Trimeresurus flavoviridis venom was purchased from Latoxan (France), and U46619 compound was obtained from Biomol. Res. Lab. (USA). Myo-2-[^{3}H]inositol was purchased from Amersham, U.K. It was dissolved in dimethyl sulfoxide (DMSO) and stored at -4℃. Formulation DJS (Specimen No. D-23-7) was obtained from Dongguk University Oriental Medical Hospital, Kyungju, Korea (Scheme 1). The APTT assay kit with a composition of actin and calcium was purchased from Dade International Inc. (manufactured by Dade Diagnostics of P.R. Inc., Aguade, PR00602-0865, USA). All other reagents used were of analytical grade.

Scheme 1. Composition of DJS (當歸芍藥散)[8-11]

Angelicae Sinensis Radix(當歸)	1.00 g
Cnidii Rhizoma(川芎)	1.00 g
Paeoniae Radix(芍藥)	2.00 g
Hoelen(茯苓)	1.33 g
Atractylodis Macrocephalae Rhizoma(白朮)	1.33 g
Alismatis Rhizoma(澤瀉)	1.66 g
Total amount	8.32 g

2. Extraction, fractionation and purification of DJS-water extract

The dried samples (100 g) of the formulation DJS (Specimen No. D-23-7) were homogenized using a mechanical disintegrator with Tekmar Tissue homogenizer (Tekmar Co., Cincinati, OH, USA) in distilled water, and the crude fraction was collected by centrifugation (7,000 rpm, for 20 min) at 4℃. The supernatant solution was concentrated to about 120 ㎖, and used for the experiments (yield: 8 g).

3. Extraction, fractionation and purification of DJS-ethanol extract

One kilogram of herbs were extracted two times with 8 ℓ of hot water (80-85℃). The extract was concentrated by vacuum evaporation and the residue (56 g) was applied to a 2 ㎏ porous resin column (porous resin, Toso Co., Tokyo, Japan), washed with 9 ℓ of water and eluted with 9 ℓ of 70% ethanol. The 70% ethanol eluate was concentrated and gave a saponin-like mixture of about 40 g.

4. Preparation of Human Platelet-Rich Plasma and Platelet Suspensions

Blood was collected from healthy human volunteers who had not taken any medicine during the preceding two weeks and mixed with 3.8% (w/v) sodium citrate (9:1, v/v). Citrated blood was immediately centrifuged at 120 g for 10 minutes at 25℃ and the supernatant (platelet-rich plasma) was retained. In some experiments, washed human platelet suspensions were prepared by the method of Mustard et al.[14]. blood was mixed with acid/citrate/glucose (9:1, v/v). After centrifugation of 120 × g for 10 minutes at room temperature, the supernatant

(platelet-rich plasma) was supplemented with PGE1 (0.5 μM) and heparin (6.4 IU/㎖), incubated for 10 minutes at 37℃ and centrifuged at 500 × g for 10 minutes. The platelet pellet was suspended in 5 ㎖ of Tyrode's solution, pH 7.3 [containing (mM): NaCl (11.9), KCl (2.7), $MgCl_2$ (2.1), NaH_2PO_4 (0.4), $NaHCO_3$ (11.9), and glucose (11.1)]. Apyrase (1 U/㎖) PGE1 (0.5 μM), and heparin (6.4 IU/㎖) were then added, and the mixture incubated for 10 minutes. The washing procedure was repeated and the washed platelets were finally suspended in Tyrode's solution containing bovine serum albumin (3.5 ㎎/㎖), and adjusted to about 4.5 × 10^8 platelets/㎖. The final concentration of Ca^{2+} in the Tyrode's solution was 1 mM.

5. Platelet Aggregation by DJS-water extract (DJS-WE)

The turbidimetric method[15], with a Lumi-Aggregometer (Payton, Canada), was used to measure platelet aggregation. Platelet suspensions (0.4 ㎖) were prewarmed at 37℃ for 2 minutes (stirring at 1200 rpm) in a silicone-treated glass cuvette. herbs was added 2 minutes before the addition of platelet-aggregation inducers. The reaction was allowed to proceed for at least 6 minutes and the extent of aggregation was expressed as the percentage of the control (in the absence of herbs). The degree of aggregation was expressed in light-transmission units. While measuring ATP release, 20 ㎕ of luciferin/luciferase mixture was added 1 minute before the addition of agonists and ATP release was compared with that of the control.

6. Measurement of Platelet $[Ca^{2+}]i$ Mobilization by Fura 2-AM Fluorescence

Citrated whole blood was centrifuged at 120 g for 10 minutes. The supernatant was incubated with Fura 2-AM (5 μM), and protected from light at 37℃ for 1 hour. Then, the human platelet suspensions were prepared as mentioned. The external Ca^{2+} concentration of the platelet suspensions was adjusted to 1 mM. The $[Ca^{2+}]i$ rise was measured using a fluorescence spectrophotometer (CAF110, Jasco, Japan) at excitation and emission wavelengths of 340 and 500 nm, respectively. The $[Ca^{2+}]i$ was calculated from the fluorescence measured using 224 mM as the Ca^{2+}-Fura 2 dissociation constant[16].

7. Labeling of Membrane Phospholipids and Measurement of the Production of $[^3H]$Inositol Phosphate

Citrated human platelet-rich plasma was centrifuged at 500 g for 10 minutes at room temperature. The platelet pellets were then suspended in 1 ㎖ of a Ca^{2+}-free and bovine serum albumin-free Tyrode's solution containing $[^3H]$inositol (75 μCi/㎖). Platelets were incubated at 37℃ for 2 hours followed by centrifugation. Platelet pellets were finally resuspended in Ca^{2+}-free Tyrode's solution, and the platelet count was adjusted to 5 × 10^8 platelet/㎖. One-milliliter aliquots of platelet suspensions were prewarmed at 37℃ with 5 mM LiCl in a 3.5 ㎖ cuvette. herbs was preincubated with loaded platelets at room temperature for 2 minutes followed by the addition of collagen (5 ㎍/㎖) to trigger aggregation. Six minutes later, the reaction was stopped by adding ice-cold trichloroacetic acid (10%, w/v), followed by centrifugation at 1000 g for 4 minutes. Aliquots of 1.0 ㎖ of supernatant were transferred to test tubes. Trichloroacetic acid was removed by extraction with 10 ㎖ of ethyl ether three times. The mixture was then incubated in 80℃ waterbath to remove the residual ethyl ether. The inositol phosphates were separated on a Dowex-1 anion exchange column (50%, w/v, 1 ㎖) as described by Neylon and Summers[17]. In this experiment, only $[^3H]$inositol monophosphate was measured as an index of total inositol phosphate formation, since the levels of inositol bisphosphate and inositol trisphosphate were very low.

8. Measurement of Thromboxane B2 Formation

Washed human platelet suspensions (0.4 ㎖, 4.5 × 10^8/㎖) were preincubated for two minutes in the presence or absence of herbs before the addition of collagen (5 ㎍/㎖). Six minutes after the addition of the agonist, an aliquot of 2 mM EDTA and 50 μM indomethacin was added to the reaction suspension. The vials were centrifuged in an Eppendorf centrifuge (Model 5414) for 3 minutes at 14000 rpm. The thromboxane B2 levels of the supernatant were measured using an EIA kit (Cayman Co., USA) according to the instructions of the manufacturer.

9. Determination of platelet aggregation by DJS-ethanol extract (DJS-EE)

Nine volumes of healthy venous blood was added into one volume of 3.8% sodium citrate in a plastic tube. After centrifugation at 3000 rpm. for 10 min, the plasma was pipetted and then adjusted to 1.0 ㎖ aliquots containing various concentration of DJS-EE. In the control experiment, the equivalent volume of 0.9% NaCl was added to reaction mixture in place of the extracted compounds. Each 0.1 ㎖ aliquot of the reaction mixture was incubated at 37℃ for 1 min, then 0.1 ㎖ of actin was added and the mixture was incubated an addition of 2 min. After the addition of 0.1 ㎖ of 0.02 M $CaCl_2$, thromboplastin times (APTT) was recorded[18]The activated partial thromboplastin times (APTT) was performed using an activated commercial reagent (Sigma).

10. *Hemolysis measurement by DJS-ethanol extract (DJS-EE)*

Fresh blood from healthy human was collected (9 parts of blood: 1 part of 3.8% sodium citrate) in the plastic tubes, and red blood cells were separated by centrifugation at 2500 rpm for 10 min. The precipitates (red blood cells) were washed with two volume of physiological saline solution, then centrifuged at 3000 rpm. for 10 min. The red blood cells were then suspended in 0.9% saline solution (10% erythrocyte suspension). 0.25 ㎖ of 10% cells suspension was mixed with various herbs. The mixture was incubated at 37℃ for 5 min with shaking and then centrifuged at 1100 rpm for 5 min. 0.2 ㎖ of supernatant was added into 3.3 ㎖ distilled water and the absorbance of the supernatant was measured at 550 ㎚ (Asample). The percent hemolysis (H%) of each sample was calculated using the following equation: H%=Asample/A100, 100% *where A100 is the absorbance of 100% hemolysis cells,* i.e. 0.25 ㎖ of cells suspension was incubated in 8.5 ㎖ of distilled water[19].

11. Statistical analysis

The experimental results of DJS-WE are expressed as the means ± S.E.M. and accompanied by the number of observations. Data were assessed by analysis of variance (ANOVA). If this analysis indicated significant differences among the group means, then each group was compared by the Newman-Keuls methods. A P value less than 0.05 was considered significant. The significance of the difference between the DJS-EE and controls with respect to their effects on hemolysis (Fig. 1) and APTT (Table 1) were determined with the Wilconxon's test. P values less than 0.05 were considered to represent a significant difference.

Results

1. Effect of DJS-water extract (DJS-WE) on Platelet Aggregation in Human Platelet Suspensions

DJS-water extract (DJS-WE) inhibited platelet aggregation in a dose-dependent manner stimulated by a variety of aggregation inducers in washed human platelet suspensions (Fig. 1). It dose-dependently inhibited platelet aggregation induced by collagen (5 μg/㎖) and the prostaglandin endoperoxide analogue compound U46619 (1 μM). It also similarly inhibited fibrinogen (200 μg/㎖)-induced aggregation of ADP (20 μM)-stimulated platelets. Furthermore, DJS-WE also dose-dependently inhibited the ATP-release induced by agonists (i.e., collagen). The IC_{50} (μg/㎖) values of DJS-WE in *inhibiting the platelet aggregation induced by ADP (20 μM),* collagen (5 μg/㎖) and U46619 (1 μM) were estimated to be 10.6, 33, and 23, respectively (Fig. 1).

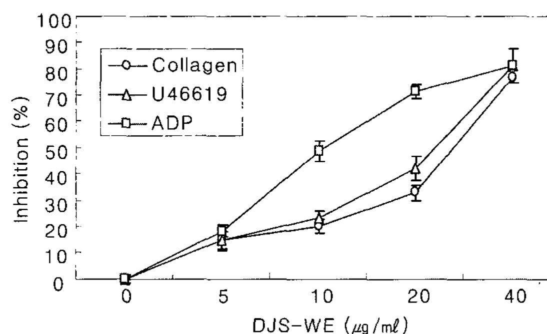

Fig. 1. Dose-inhibition curves of DJS-water extract (DJS-WE) on collagen (5 μg/㎖, ○)-, U46619 (1 μM, △) and ADP (20 μM, □)-induced aggregation of human platelet suspensions. Human platelet suspensions were preincubated with various concentrations of DJS-WE (0, 5, 10, 20 and 40 μg/㎖) at 37°C for 2 minutes followed by the addition of aggregation agonists to trigger aggregation. Data are presented as percentage of the control (means±S.E.M., n=4-5).

2. Effect of DJS-WE on Phosphoinositide Breakdown in Human Platelets

Phosphoinositide breakdown is observed in platelets activated by many agonists[15]. In this study, we found that collagen (5 μg/㎖) induced the rapid formation of radioactive inositol monophosphate, inositol bisphosphate, and inositol trisphosphate in human platelets loaded with [^{3}H]myoinositol. We measured [^{3}H]inositol monophosphate formation only as an index of the total inositol phosphate formation. As shown in Fig. 2, collagen (5 μg/㎖) caused about 3.6-fold rises in inositol *monophosphate formation, compared to that in resting platelets* (2,646±219 cpm, collagen-activated platelets vs. 727±65 cpm, resting platelets). In the presence of DJS-WE (0, 5, 10, 20 and 40 μg/㎖), inositol monophosphate formation in collagen-stimulated platelets was markedly decreased. These results indicate that DJS-WE may interfere with the phosphoinositide breakdown in platelets stimulated by agonists (i.e., collagen).

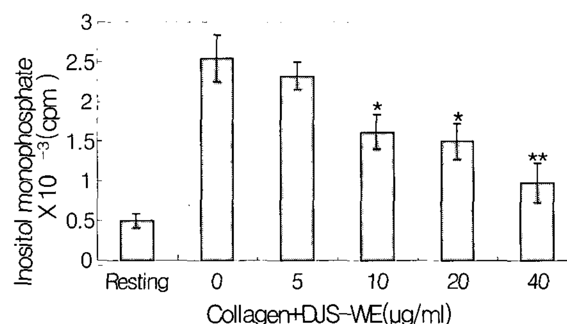

Fig. 2. Effect of DJS-WE on collagen-induced inositol monophosphate formation in washed human platelets. Platelets were labeled with [^{3}H]inositol and stimulated with collagen (5 μg/㎖) in the presence of various concentrations of DJS-WE (0,5,10,20 and 40 μg/㎖). For the detailed experimental procedure see "Materials and Methods." Data are presented as means-±S.E.M. (n=4). *P<0.05; **P<0.01 denote a significant difference compared with resting.

3. Effect of DJS-WE on Thromboxane B2 Formation

As shown in Table 1, the resting platelets showed no significant thromboxane B2 (TXB2) formation as compared with collagen (5 μg/mℓ)-activated platelets. The vehicle control DMSO (0.5%) did not significantly increase the level of TXB2. PGE1 (10 μM) inhibited TXB2 formation of collagen-activated platelets by 71% (data not shown). Furthermore, in the presence of various concentrations of DJS-WE (5-40 μg/mℓ), there were significant effects on TXB2 formation of platelets stimulated by collagen (5 μg/mℓ) (Table 1). Therefore, herbs exerts an inhibitory effect on TXA2 formation.

Table 1. Effect of DJS-WE on collagen (5 μg/mℓ)-induced thromboxane B2 formation in washed human platelets

Treatment	Thromboxane B2 (ng/ml)
Resting	6.0±0.5 (5)
DMSO (0.5%)	8.3±1.2 (4)
Collagen	198±21.4 (4)
+DJS-WE 10 μg/ml	104±12.5* (5)
+DJS-WE 20 μg/ml	103±13.7* (5)
+DJS-WE 40 μg/ml	83±7.3** (5)

DJS-WE (0, 5, 10, 20, and 40 μg/ml) was added to washed human platelet suspensions (4.5×10^8/ml) 2 minutes before the addition of collagen (5 μg/ml). The reaction was termined 6 minutes after the addition of collagen and the platelet suspensions were collected for the determination of the thromboxane B2 level. Data are presented as means±S.E.M. (n). *P<0.05; **P<0.01 as compared with collagen (5 μg/ml).

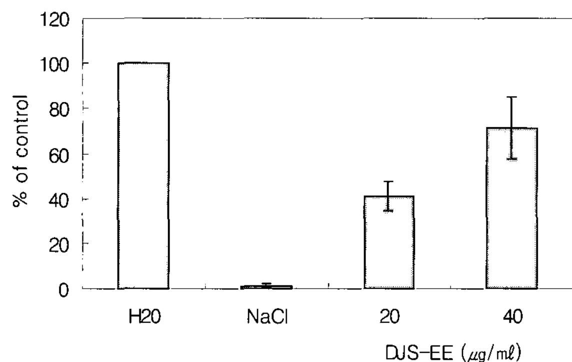

Fig. 3. Effect of DJS-ethanol extract on hemolysis of human erythrocytes. The assays were carried out as described under Materials and methods. The final concentrations of the DJS-EE were 20 μg/ml and 50 μg/ml, respectively. In the control experiment, the same volume of 0.9% NaCl was added. Hemolysis by water was shown as 100%. The results of five independent experiments were averaged and summarized in a bar figure (mean±S.E.M P<0.01).

4. The effect of DJS-ethanol extract on hemolysis of erythrocytes and on the activated partial thromboplastin times (APTT)

The effect of DJS-ethanol extract (DJS-EE) on hemolysis of erythrocytes is shown in Fig. 3. DJS-EE showed high hemolysis ability on human blood. The effect of DJS-EE on hemolysis depends on the increment of concentrations of DJS-EE. The DJS-EE is resistant to dissolve in water, suggesting that DJS-EE is hydrophobic and has strong interaction with the membrane. Therefore, the effect of DJS-EE on the activated partial thromboplastin times (APTT) was also investigated. Antithrombin activity in DJS-EE-treated plasmas was measured as described in the Materials and methods. The inhibition effect of the DJS-EE was shown in Table 2. DJS-EE showed marked inhibiting effect on platelet aggregation, and activated partial thromboplastin times (APTT) were sensitive to the presence of DJS-EE. The plasma incubated with DJS-EE showed significant longer APTT than that of control. Using an in vitro system, APTT was delayed with the increment of concentrations of DJS-EE (P<0.01).

Table 2. Effect of DJS-EE on platelet aggregation

Samples	APTT (s)	Delaya(s)
0.9% NaCl	28.03±3.21	0.00
DJS-EE (20μg/ml)	29.44±2.65	2.25
DJS-EE (40μg/ml)	31.21±1.73	2.89

a The time compared with the APTT induced by the same volume of 0.9% NaCl. Date are presented as mean±SD (n=5), all APTT values are significantly different from control.

Discussion

DJS is an active ingredient of a Korean traditional herbal medicine. It relaxes cardiovascular and uterine smooth muscle [20]. In animal models, DJS has been demonstrated to be an effective antithrombotic agent[3]. The antithrombotic activity of DJS-WE may be due, at least partly, to inhibition of platelet aggregation and facilitation of platelet disaggregation. DJS-WE exerts antiplatelet activity in a dose-dependent manner induced by agonists.

However, little information relating to the detailed mechanisms of DJS-WE on antiplatelet aggregation has been reported. The principal objective of this study was to ascertain the detailed mechanisms involved in the inhibition of agonist (i.e., collagen, ADP, and U46619)-induced human platelet aggregation by DJS-WE. In this study, both platelet aggregation and ATP-release reactions induced by these agonists (i.e., collagen) were apparently affected by DJS-WE (5-40 μg/mℓ). Therefore, it is inferred that DJS-WE may affect Ca^{2+} release from intracellular Ca^{2+}-storage sites (i.e., dense tubular system or dense bodies) and this is in accord with the concept that intracellular Ca^{2+} release is responsible for the ATP-release reactions[21]. TXA2 is an important mediator of release reaction and aggregation of platelets[22]. Formation of TXB2, a stable metabolite of TXA2, induced by collagen was markedly inhibited by DJS-WE. This indicates that the antiplatelet effect of DJS-WE is due, at least partly, to the inhibition of TXA2 formation.

It has been demonstrated that phosphoinositide breakdown can induce thromboxane A2 formation via free arachidonic acid release by diglyceride lipase or by endogenous phospholipase A2 from membrane phospholipids

[23]. Furthermore, stimulation of platelets by agonists (i.e., collagen) results in phospholipase C-catalyzed hydrolysis of the minor plasma membrane phospholipid phosphatidylinositol 4,5-bisphosphate, with concomitant formation of inositol 1,4,5-trisphosphate and diacylglycerol[24]. There is strong evidence that inositol 1,4,5-trisphosphate induces the release of Ca^{2+} from intracellular stores[25].

On the other hand, diacylglycerol activates protein kinase C, inducing protein phosphorylation and a release reaction. Although phosphoinositide turnover is believed to be an earlier event than thromboxane synthesis, thromboxane A2 can, however, also activate platelets, leading to phosphoinositide breakdown[26]. In this study, both thromboxane B2 formation and phosphoinositide breakdown of collagen-activated platelets were inhibited by DJS-WE, suggesting that inhibition of platelet aggregation by DJS-WE is related to inhibition of thromboxane A2 formation or phosphoinositide breakdown. Although the action mechanisms of various platelet aggregation inducers, such as collagen, ADP, and U46619, are different; DJS-WE at a higher concentration (40 µg/㎖) inhibited platelet aggregation stimulated by all of them, not only in washed platelet suspensions but also in platelet-rich plasma (data not shown). It is implied that DJS-WE may block a common step shared by these inducers. This also indicates that the site of action of DJS-WE is not at the individual agonist receptor level.

Thrombosis plays a major role in ischemic heart disease. Patients who have sustained an acute myocardial infraction are at increased risk of developing vascular events such as sudden death, recurrence of infraction and thromboembolic stroke. The results of recent clinical studies indicate that antithrombotic therapy significantly reduces the number of vascular events in these patients. The activated partial thromboplastin time (APTT) is a rapid test and the most widely used screening test to detect hereditary and acquired coagulation defects. APTT is also used to adjust heparin sodium dosage[27]. DJS-ethanol extract (DJS-EE) showed marked inhibiting effect on platelet aggregation, and activated partial thromboplastin times (APTT) were sensitive to the presence of DJS-EE. The plasma incubated with DJS-EE showed significant longer APTT than that of control. Using an in vitro system, APTT was delayed with the increment of concentrations of DJS-EE.

Conclusion

In conclusion, platelet aggregation plays a pathophysiological role in a variety of thromboembolic disorders, including myocardial infarction, cerebrovascular diseases, and atherosclerosis. Therefore, prevention of platelet aggregation by herbs should provide effective prophylactic and/or therapeutic means of treating such disorders. The present observations suggested that DJS inhibition of platelet aggregation may involve two mechanisms: At a lower concentration (10 µg/㎖), DJS inhibits platelet aggregation, possibly by the inhibition of phosphoinositide breakdown and TXA2 formation. These results indicate that a novel approach of antiplatelet aggregation of DJS could be taken in the design of a clinical application.

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