Inhibitory Effects of Silsosangami on the Platelet Aggregation

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The thrombosis importantly came to the front as the risk factor of these circulation system's disease. SilsoSanGami(SSG) was used for investigating the inhibitory effect on platelet-activating factor-induced platelet aggregation about drugs that used to improvement various symptoms created by the thrombosis in oriental medicine. In this study, the water-extracted SSG was investigated for its possible antithrombotic action on platelets. The antithrombotic activity of water-extracted SSG was deduced from its ability to suppress platelet aggregation, ATP-exocytosis, and the generation of prostaglandin E_2 and thromboxane A_2 by human platelets, stimulated with arachidonic acid. Water-extracted SSG dose-dependently suppressed the aggregation of human platelets, the release of endogenous ATP, and the formation of PGE_2 and TXB_2, both the latter usually detected to estimate the activity of COX and TXS, respectively. Since the IC_{50} values necessary to inhibit COX (115 $\mu g/m\ell$ SSG) and TXS(74 $\mu g/m\ell$ SSG) were in the same range, inhibition of COX is suggested to be the primary target of water-extracted SSG, thus suppressing the formation of PGE_2 which is metabolized by TXS to TXA_2. We considerated that SSG has practical applicational value of clinical trial in the thrombosis caused by platelet aggregation.

Key words : SilsoSanGami(失笑散加味), thrombosis, platelet aggregation, COX, TXS, PGE_2.

Introduction

The cause that the increase of animality fat intakes, under exercise, fatness, adding the stress, advanced age etc., the occurrence rate of the circulation system disease has been increased[1,2]. And the thrombosis importantly came to the front as the risk factor of these circulation system's disease. Nowadays, the ischemic disease has especially discussed, for example the angina or myocardial infarction, originated in thrombosis that came from the platelet aggregation[3]. Platelet do not attache to the normal hemangioendothelial cell. But when it stimulated by endothelium peronia and so on, it attache to the injury endothelium or rise aggregation between the platelet[4]. And the activation of abnormal platelet occur the platelet grume and thrombogenesis[4]. So it bring up the occlusive angiosis, so to speak, cardiovascular disease, cerebrovascular disease, arterial sclerosis[5,6]. In oriental medical, the thrombosis in the category of blood stasis and this blood stasis present the generalize or local blood circulation disturbance that generated by all kinds of pathological fact or blood stream retention accompanying with a series of syndrome[7]. As the syndrome, stabbing pain fixed at certain region, squamous and dry skin, fullness and pain of the chest and hypochondrium, firmness and fullness of the lower abdomen, dark purple tongue or with ecchymoses and petechiae etc. have been created[7]. And it becomes the pathopoiesis cause that the convulsion and palpitation, severe palpitatiion, tympanites, the symtom complex with a mass or swelling in the abdomen, insanity, stricken by wind etc[7]. Moreover, the drugs for invigorating blood circulation and eliminating blood stasis or drugs for removing blood stasis are used for all kinds of syndrome through the blood stasis[8,9]. And the drugs for activating the blood circulation, such as *Salviae Radix, Angelicae Sinensis Radix, Persicae Semen, Cnidii Rhizoma, Carthami Flos* are used for that[10]. On this study, SilsoSanGami(SSG)[11]was used for investigating the inhibitory effect on platelet-activating factor-induced platelet aggregation about drugs that used to improvement various symptoms created by the thrombosis in oriental medicine. SSG is a formula that consisted of SilSoSan and *Paeoniae Radicis rubra, Cnidii Rhizoma, Persicae Semen, Carthami Flos, Curcumae Tuber*[11]. SSG has the action of 'the activating blood circulation, removing blood stasis' and 'disperse the accumulation of evils and alleviating pain', so used for' blood stasis and cardio-

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abdominal pain and irregular menstruation and the stagnation *of blood' from the ancient*[11]. And also SSG is a formula applied as an effective biological response modifier for augmenting homeostasis of body circulation[12]. The pharmacological action of SSG has been limitedly studied in regard to ischemic infarction[12]. Although the effectiveness of SSG for ischemic infarction has been widely used by clinical administration, the scientific and acting mechanisms for those are not understood and elucidated. It is generally known that inflammation, hyperlipemia and arteriosclerosis induce disseminated intravascular coagulation (DIC)[13]. It was shown that the water extracts obtained from SSG was effective on endotoxin-induced experimental DIC in heperlipemia and normal rats. Also, these were inhibitory effects on collagen- and ADP-induced blood platelet aggregation, thrombin-induced conversion of fibrinogen and fibrinolysis in *in vitro* experiments (Kim et al., unpublished data). Platelet activating factor (PAF) is a fundamental mediator of mammalian cell function which is now thought to play a significant role in a variety of pathophysiological states inducing acute allergy, inflammation, asthema, gastrointestinal ulceration and toxic shock[14,15]. Several PAF antagonists such as ginkgolides, benzofuranoid neolignan and gliotoxin have been reported[14,16,17,18,19] and the studies on their biological activities and mechanisms have also been descrived. In the course of study on PAF antagonists from medicinal plants, we have found that the water extract of the SSG inhibits the platelet aggregation induced by PAF. The systematic fractionation of this extract led to a strong inhibitory activity against PAF-induced aggregation. This paper described the inhibitory effect of the water extracts and alcholic fractions of SSG on PAF-induced aggregation. The anti-thrombotic actions of the water-extracted SSG was assumed to result from an inhibition of throboxane synthase (TXS), an action which may also account for the protective properties against experimental acute and chronic ulcers in rats[12]. For examination of this hypothesis, the water-extracted SSG was investigated for its possible antithrombotic action on platelets. The antithrombotic activity of water-extracted SSG was deduced from its ability to suppress platelet aggregation, ATP-exocytosis, and the generation of prostaglandin E2 and thromboxane A2 by human platelets, stimulated with arachidonic acid.

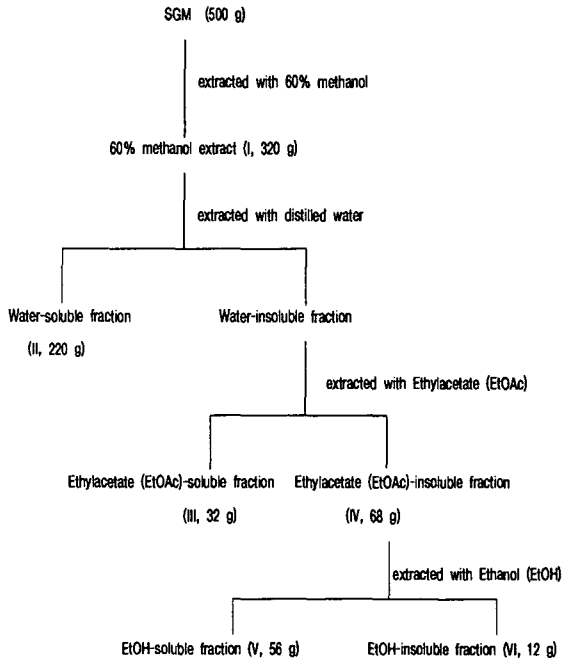
Materials and Methods

1. Materials

PAF was purchased from Calbiochem (CA, USA), and stored at -70℃ in ethanol. Verapamil, a Ca-antagonist, was obtained from Sigma (MO, USA). ortho-Phthaldialdehyde hydrochloride solution (OPT-HCl) was prepared by addition of 10 volumes of 8 N HCl to an ethanolic solution (0,5% w/v) of recrystallized ortho-Phthaldialdehyde (purchased from Wako Pure Chemicals CO., Ltd., Tokyo, Japan). ATP determination was performed with the HSII kit of Boehringer Mannheim Gmbh (Mannheim, Germany). Bovine serum albumin (BSA), ethylenediaminetetraacetic acid (EDTA), and indomethacin were purchased from Sigma Co. (MO, USA). Microtiter plate based enzyme-linked immunosorbent assay kits (ELISA) for the determination of PGE_2 and TXA_2 were provided by Promega (CA, USA). The other chemicals were supplied by Sigma and Merck (Darmstadt, Germany).

2. Isolation of SSG Extracts

SilsoSanGami(加味失笑散, SSG)[11] is consisted of each herb such as *Typhae Pollen*(蒲黃) 4g, *Pteropi Faeces*(五靈脂) 4g, *Paeoniae Radicis rubra*(赤芍藥) 4g, *Cnidii Rhizoma*(川芎) 4g, *Persicae Semen*(桃仁) 4g, *Carthami Flos*(紅花) 4g and *Curcumae Tuber*(鬱金) 4g. The aqueous extracts of SSG, which was massproduced as for clinical use, were kindly supplied by the Oriental Medical Hospital of Dongguk University (Kyungju, korea). For ethanol extracts of SSG, a 60% methanol extract (I) and various fractions (II, III, IV, V and VI) derived from fraction I were prepared from crude mixture of SSG as shown in Scheme 1.



Scheme 1. Fractionation of SSG

3. Animals

Male rabbits (2-2.5kg) were purchased from DaeHan Experimental Animals and used for the preparation of platelet-rich plasma. Male ICR mice (3 weeks-old) were supplied from Genetic Resources Center, Korea Research Institute of Bioscience and Biotechnology, Korea Institute of Science and Technology (KIST) and used in PAF experiments *in vivo*.

4. Assay for platelet aggregation for rabbit platelets

A platelet-rich plasma (PRP) was prepared by centrifugation of fresh rabbit blood (190xg, for 10min) with 1/10volume of 3.8% sodium citrate solution. Platelet numbers were adjusted to $6.0x10^5$/mm3. Platelet aggregation was measured by the serotonin release according to the method[8], in which PAF (10^{-7}M) was used as an aggregating agents. A 0.2㎖ aliquot of PRP was incubated with 20㎕ of a test solution at 37℃ for 7min, then 20㎕ of PAF solution was added to the reaction mixture. Five minutes after the addition of PAF, 1.0㎖ of ice-cold EDTA (0.4% w/v solution in isotonic saline) was added to stop the reaction and the platelets were sedimented by centrifugation (1,800g for 10min). The supernatant was aspirated and the platelet pellets were lysed in 0.5㎖ of distilled water. Proteins in the lysed pellets were precipitated with 0.1㎖ of 6M trichloroacetic acid. After removing the proteins by centrifugation, 0.5㎖ of the solution was added to 3 ㎖ of OPT-HCl. The mixture was then heated at 100℃ for 10 min and cooled in an ice-bath. After washing with chloroform, fluorescence in 3㎖ samples of the aqueous phase was measured using a spectrophotometer with wavelengths of 360 and 475nm. Inhibition (%) was calculated by means of the following equation.

$$\text{Inhibiotion (\%)} = \frac{\text{(sample value-PAF value)}}{\text{(control value- PAF value)}} \times 100$$

5. Preparation of human platelets and aggregation

Platelet-rich plasma, prepared from whole blood of apparently healthy volunteers, was anti-coagulated with 1/9 volume citrate-citric acid dextrose (100mmol/l trisodium citrate, 7mmol/l citric acid, 140mmol/l dextrose, pH 6.5) by centrifugation at 100×g for 15min at room temperature. Platelets of the supernatant were sedimented by centrifugation at 1000×g for 10min. The pellet was washed 2 times with citrate-buffer [0.35% (w/v) BSA, 108mmol/l NaCl, 2.8mmol/l KCl, 1.0mmol/$CaCl_2$, 1.6mmol/l $MgCl_2$, 0.3mmol/l NaH_2PO_4, 9.5mmol/l $NaHCO_3$, 10.1mmol/l trisodium citrate, 4.6mmol/l citric acid] by centrifugation at 555×g dor 10min. The final pellet was resuspended in citrate-buffer to obtain a cell titer of about 10^9 platelets/㎖. The suspension was shaken in a petri dish until the assays were performed. For human platelet aggregation assay, an aliquot of the platelet suspension was centrifuged at 7,000×g for 1min. The pellet was resuspended in Tyrode's buffer [135mmol/l NaCl, 3.5mmol/l KCl, 1.2mmol/l $CaCl_2$, 2.0mmol/l $MgCl_2$, 0.3mmol/l NaH_2PO_4, 0.35% (w/v) BSA, 11.9mmol/l $NaHCO_3$, pH 7.4] and adjusted to 10^9 platelets/㎖. Platelet-aggregation was carried out on a dual-channel aggregometer (APACT, Ahrensburg, Germany). Aggregation of the stirred suspension was monitored in a final volume of 300㎕ at 37℃ turbidimetrically by an increase in light transmission employing cell-free plasma as a reference.

6. ATP determination

ATP released from platelets was detected by a bioluminescence method[21]. An aliquot of the platelet suspension was centrifuged at 7,000×g for 1min. The platelet was resuspended in Tyrode's buffer to obtain to 109 platelets/㎖ in a final volume of 4.5㎖, which was stirred in a plastic tube located in a water bath (37℃). After an equilibration for 5min, samples of 100 ㎕ were taken as indicated, mixed with 900㎕ precooled (0℃) Tyrode's buffer and centrifuged at 7,000 ×g for 1min. For the determination of ATP, 20㎕ of supernatant were mixed with 200㎕ of 1mol/l perchloric acid and 50mmol/l EDTA for 1min to precipitate soluble proteins. The solution was adjusted to pH 7.5-8.0 by addition of 316㎕ of 1 mol/l HEPES, pH 7.75, 25℃). After an incubation for 30 imn at 0℃, the solution was centrifuged at 14,000×g for 10min to remove potassium perchlorate. Five hundred ㎕ of supernatant were added to 500㎕ HEPES buffer and the ATP content was detected by the luciferase-bioluminescene method employing the kit HSII (Boehringer Mannheim Gmbh. Mannheim, Germany) and a luminometer (LB 9502, Berthold Gmbh, Bad Wildbad, Germany). The ATP content was calculated according to calibration curve using diffeent ATP concentrations as standatds.

7. Determination of PGE_2 and TXB_2

PGE_2 and TXB_2 were determined by an enzyme-linked immunosorbent assay (ELISA) employing test kits of Progega (CA, UA). An aliquot of platelet suspension was centrifuged at 7,000×g for 1min. The pellet was resuspended in Tyrode's buffer to obtain a cell density of 1.5×10^8 platelets/㎖ on a final volume of 4㎖ and transferred into a plastic tube located in a water bath (37℃). After an equilibration for 5 min, 200㎕ of stirred suspension were taken as indicated. Considering determination of PGE_2, the TXS-inhibitor E/Z-7-phenyl-7-(pyridyl)-6-heptenoic acid[22] at a concentration of 1㎛mol/l was

added to the suspension at the onset of equilibration. For the determination of PGE_2 and TXB_2, 200 $\mu\ell$ suspension were mixed with 1800$\mu\ell$ ice-cooled stop solution (50μmol/l indomethacin, 25mmol/l HEPES, pH 7.4). Afterwards, platelets were disrupted by sonification at 0℃ for 15min to allow complete release of both eicosanoids, and the resulting homogenates were centrifuged at 5,000×g for 10min. The PGE_2 and TXB_2 contents of the supernantant were determined according to the instructions of the ELISA-kit supplier.

8. Drugs and solvents

AA was dissolved in dimethyl sulphoxide (DMSO) to obtain stock solution of 20mmol/l AA. The fincal concentration of DMSO in the platelet suspension amounted to 1% (v/v).

9. PAF-induced mortality in mice

Male ICR mice were intravenously administered 500㎎/100g or 1000mg/100g of PAF solution. All mice were observed for at least 24h after the PAF administration. Each test drug was intraperitoneally administered 15min before the PAF administration. Results were given as 24h survival rates.

10. Statistics

Results are expressed as means±SD. The dose-response curves were deduced from fitting of values according to the logic dose-response function employing the term y=a+b/(1-(x/c)d), where x represents SSG concentration, y is the pharmacologic effect expressed as % of control, and a,b,c,d are fitting constants. The IC_{50} values were calculated from individual fitted dose-response curves and expressed as means±SD.

Results and discussion

1. Effect of organic solvent(Et-OH, Me-OH, Et-OAc)-extracted SSG on platelet aggregation *in vitro*

As shown in Fig. 1, Fig. 2 and Table 1, the water and methanol extracts inhibited aggregation of platelets. Although various compounds such as swietemahonin, ginkgolides, benzofuranoid neolignans and furanoid lignans have been reported as antagonists of PAF[16,17,18,19], this results provided the first examples of water-extracted molecules having an antagonistic effect on PAF. The inhibitory effect of the MeOH extracts was much higher than that of the water extracts. Verapamil[19] as a positive control inhibited dose dependently aggregation of platelets with IC_{50} value of 30.2μg/㎖. The inhibitory activities of I-VI are weaker than that of verapamil, but the cases of III and V are significantly meaningful.

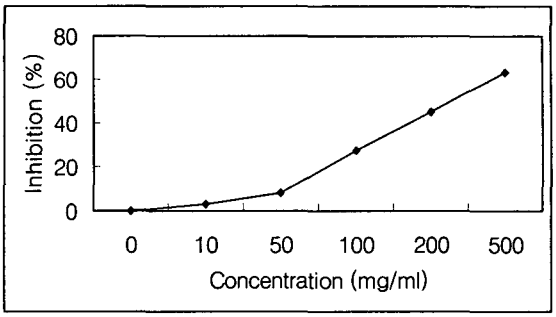


Fig. 1. Effects of the water extracts on PAF-induced aggregation of rabbit platelets *in vitro*

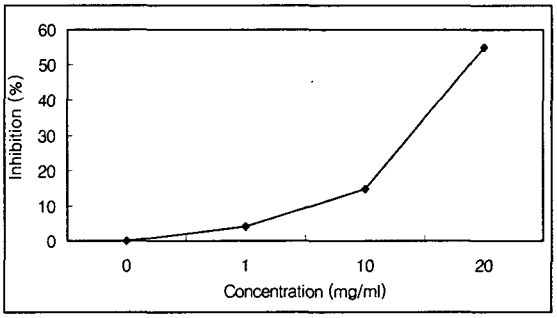


Fig. 2. Effects of the 60% MeOH extracts on PAF-induced aggregation of rabbit platelets *in vitro*

Table 1. Effects of the extracts on PAF-induced aggregation of rabbit platelets *in vitro* as shown by IC_{50} (㎎/㎖)

	IC_{50} (㎎/㎖)*
Water-Extracts	435.3
I	34.3
II	13.3
III	3.1
IV	5.3
V	0.5
VI	0.6

*IC_{50}(㎎/㎖): Concentration (㎎/㎖) required for 50% inhibition of platelets aggregation.

2. Effect of organic solvent(ETOH, MeOH, EtOAc)-extracted SSG on PAF-induced mortality in mice

For evaluation of the *in vivo* anti-PAF effect of the fractions I-VI, we have used ICR mice, which have been shown by Young et al[20]. and Ekimoto et al[19]. to be sensitive to PAF-induced mortality. Intravenous administration of PAF into ICR mice caused lethal shock. As shown in Table 2, when administered intraperitoneally, among the fractions I to VI, fraction III, V and VI specially protected against the lethality of PAF, while verapamil did not afford any protection. It is worth to note that the fractions III, V, and VI have dual activities against PAF aggregation *in vitro* and PAF mortality *in vivo*. Furthermore, it is worth to note that fractions III, V and VI have the stronger activity against *in vivo* PAF aggregation than that of fractions I, II and IV, in spite of its lower potency *in vitro* than that of verapamil. Unfortunately, however, more detailed examinations could not be undertaken because the

amount of fractions III, V and VI available was insufficient to do the anti-PAF effect *in vivo*. These findings indicate that the water extracts and alcholic-fractions inhibit the action of PAF *in vivo*, so that it may be useful in treating disorders caused by PAF.

Table 2. Effects of the extracts and fractions on PAF-induced mortality in mice

Fraction	mg/kg	route	survival	survival rate (%)
vehicle (5% DMSO-saline)	0	ip	2/14	14.2
PAF-control	0	ip	4/23	17.3
I	8	ip	7/24	29.2
II	7	ip	10/27	37.0*
III	3	ip	12/22	54.5*
IV	5	ip	2/20	10.0
V	3	ip	17/25	68.0*
VI	2	ip	15/24	62.5*
verapamil	2	ip	2/5	40.0*

PAF was administrated 200 μg/kg of solution, *Significant difference (P<0.05) from control.

3. Arachidonic acid induces human platelet aggregation and water-extracted SSG suppressed dose-dependently the aggregation

As examplary shown in Fig. 3, challenge of platelets with 100 μmol/l AA to human platelets induced an absolute aggregation of almost 90% within about 3min after its application as deduced turbidimetrically from the increase in light transmission. However, an application of 70 μg/mℓ SSG, 5 min before AA, suppressed aggregation. An application of SSG suppressed dose-dependently the aggregation but failed to prevent it completely. Even at a high SSG concentrations of 500 μg/mℓ, a transient aggregation of about 15% (peak maximum) could be detected, whereas the COX-inhibitor indomethacin (50 μmol/l), applied instead of SSG, prevented aggregation completely (data not shown). For calculation of the dose-response relation, the maximal aggregations including transient peak-values, observed at high SSG concentrations above 200 μg/mℓ, were expressed as % of control. As demonstrated in Fig.4, the IC_{50} necessary to diminish aggregation amounted to 74±3.4 μg/mℓ SSG.

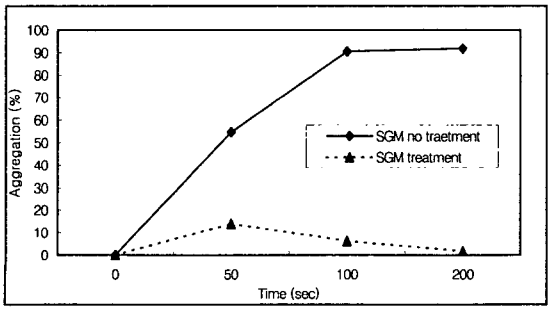


Fig. 3. Time course aggregation of human platelets by 100 μmol/l AA to human platelets

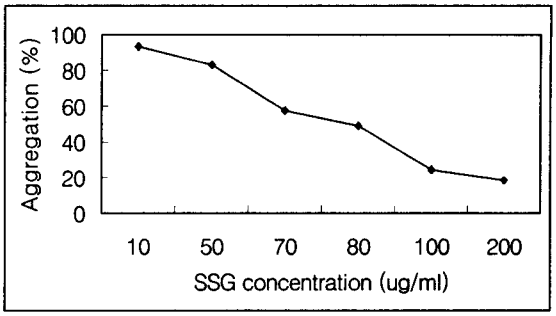


Fig. 4. Dose-dependent inhibition of human platelet aggregation by water-extracted SSG. For the calculation of the dose-response curve, SSG-dependent maximal inhibition of aggregation including peak values of transient aggregation, was expressed as % of control which represents platelets only challenged by AA. Data are depicted as means±SD (n=7).

4. Dose-dependent inhibition of ATP-exocytosis by water-extracted SSG

Similar to the time course of aggregation, AA provoked a release of endogenous ATP which was completed about 10 min after addition of AA (Fig. 5). The ATP content released from platelets, determined 5min after application of AA, amounted to 12.5±4.2 pmol ATP/10^6 platelets (n=6), which could be suppressed to 2.9±0.8 pmol ATP/10^6 platelets (n=6), if indomethacin (50 μmol/l) was added instead of SSG (Fig.5). To calculate the dose-response curve, the amount of released ATP was detected 5 min after addition of AA in the presence of different SSG concentrations. As demonstrated in Fig. 6, the IC_{50} was 108±16.2 μg/mℓ) (n= 6) SSG.

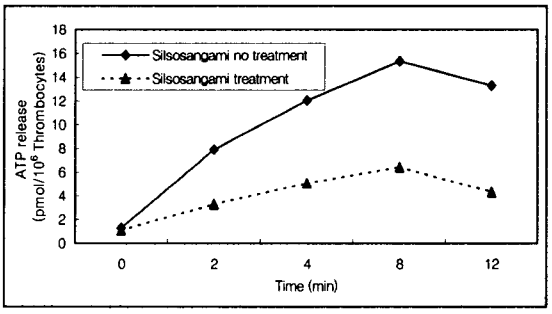


Fig. 5. Time course ATP release (ATP-exocytosis) from platelets (thrombocytes) by 100 μmol/l AA to human platelets

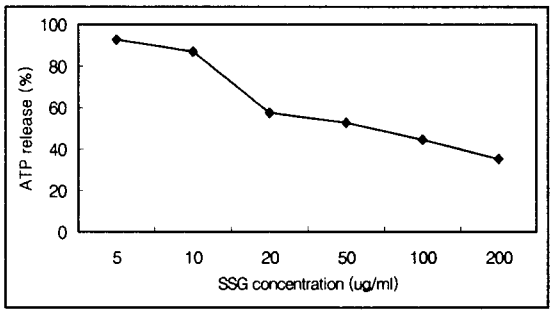


Fig. 6. Dose-dependent inhibition of ATP-exocytosis by water-extracted SSG. The dose-response relation was deduced from the amount of released ATP, detected 5min after the addition of AA in the presence of different SSG concentrations which were applied 5 min before AA. Results are shown as means±SD (n=5)

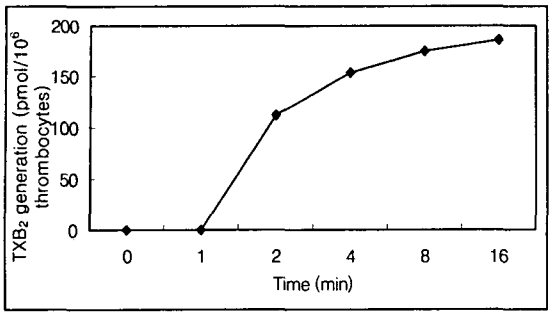


Fig. 7. Time course TXA_2 generation from platelets (thrombocytes) by 100 μmol/l AA to human platelets. Exogenously applied AA is metabolized via COX and TXS to TXA_2 which spontaneously isomerizes to TXB_2, the latter detected immunologically as a representative of TXA_2. As shown, 100 μ mol/l AA induced an increase of TXB_2 during the first minute after application which remained almost unaffected at least for 15min of incubation.

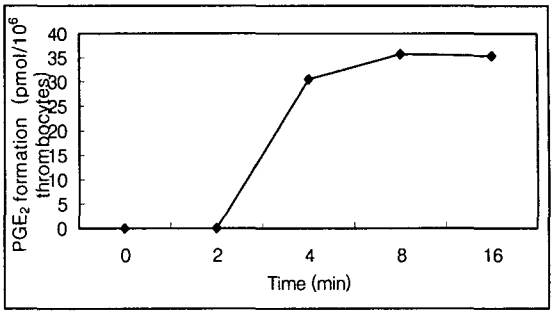


Fig. 9. Time course PGE2 generation from platelets (thrombocytes) by 100 μmol/l AA to human platelets. Addition of 100μ mol/l AA induced an immediate increase of PGE2 if platelets were incubated in the presence of TXS inhibitor E/Z-7-phenyl-7-(pyridyl)-6-heptenoic acid (1μ mol/l) which is necessary to shift enzymatic conversion of AA towards PGE2 generation.

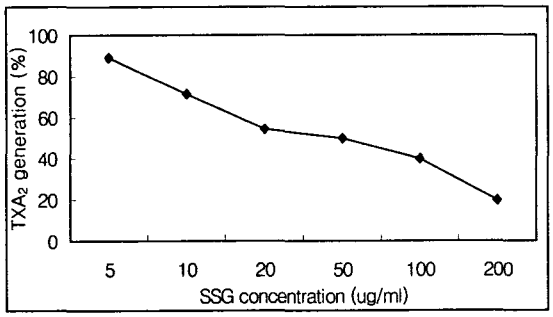


Fig. 8. Dose-dependent inhibition of TXA_2 generation by water-extracted SSG. For the calculation of the dose-response curve, the amount of TXB_2, generated 5min after AA was applied, were determined. SSG, added to platelets 5min before AA, dose-dependently diminished the generation of TXB_2, expressed as % of control which represents platelets treated with AA only. Data are means±SD (n=5).

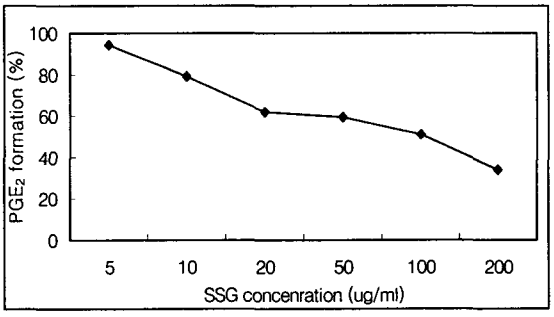


Fig. 10. Dose-dependent inhibition of PGE2 formation by water-extracted SSG. Since PGE2 remained stable at least for 15 min of incubation, the amount of PGE2 was determined 5 min after application of AA. SSG, added 5 min prior to AA, dose-dependently suppressed PGE2-formation of platelets, expressed as % of control which are platelets challenged by AA only. Data are means±SD (n=6).

6. Inhibition of PGE_2 formation by water-extracted SSG

Since a synthesis of TXA_2 from exogenously applied AA dependes on both the activity of COX and TXS, SSG- dependent suppression of TXB_2 formation may also be explained by an inhibition of COX due to SSG. Therefore, to estimate the action of SSG on COX, PGE_2 was determined in the presence of different concentrations of SSG and 1 μmol/l of the TXS-inhibitor E/Z-7-phenyl-7-(pyridyl)-6-heptenoic acid, both applied 5min before AA. The application of the TXS-inhibitor was necessary to shift the formation of AA metabolites from thromboxanes towards prostaglandins, which assures sufficient formation of PGE_2 usually detected as an indicator of COX activity[23]. Challenge of platelets with AA enhanced PGE_2 immediately from a basal value of 1.5±1.0 to 32.5±12.5pg $PGE_2/10^6$ platelets (n=5), the latter value determined 5 min after the addition of AA (Fig. 9). Since PGE_2 remained stable under the incubation procedure at least for 10 min after addition of AA (Fig.9), the amount of generated PGE_2 in the presence of SSG was determined 5 min after AA-application to calculate the dose-response curve. As depicted in Fig. 10, SSG dose-dependently diminished the formation of PGE_2 with an IC_{50} of 72±21 μg/㎖) (n= 5).

Conclusion

The anti-thrombic properties of the Korean herbal medicine, SilsoSanGami(SSG), which is consisted of 7 herbs of *Typhae Pollen, Pteropi Faeces, Paeoniae Radicis rubra, Cnidii Rhizoma, Persicae Semen, Carthami Flos and Curcumae Tuber,* were investigated. Water extracts from SSG inhibited the platelet-activating factor (PAF)-induced platelet aggregation. SSG was extracted with methanol and further fractionated by EtOAc. A methanol and EtOAc extracts showed an inhibition against PAF-induced aggregation. Exogenously applied AA to platelets was reported to be metabolized by COX and TXS to prostaglandins and thromboxane A_2[23], the latter released from platelets[24]. Binding TXA_2 to its receptors causes an increase of cytosolic Ca^{2+}[25] which triggers exocytosis of inducers like ATP and PAF, thus amplifying aggregation of platelets. In the current investigation, water-extracted SSG dose-dependently suppressed the aggregation of human platelets, the release of endogenous ATP, and the formation of PGE_2 and TXB_2, both the latter usually detected to estimate the activity of COX and TXS, respectively[23]. Since the IC_{50} values necessary to inhibit

COX (115 μg/mℓ SSG) and TXS(74 μg/mℓ SSG) were in the same range, inhibition of COX is suggested to be the primary target of water-extracted SSG, thus suppressing the formation of PGE_2 which is metabolized by TXS to TXA_2. An additional substantial inhibition of TXS by water-extracted SSG might be excluded, otherwise IC_{50} inhibition should be significantly smaller than that obtained for COX inhibition because of limited PGE_2 due to reduced COX-activity. Besides the enzyme activities, aggregation of platelets could also be suppressed at an IC_{50} of 72 μg/mℓ SSG, a value closely related to COX-inhibition, thus supporting the idea that inhibition of COX is the only cause of the toxicating SSG constituents against inflammation of the genito-urinary system, rheumatism, and inflammatory wounds (data not shown), the anti-phlogistic properties of water-extracted SSG might be explained by an inhibition of COX comparable to non-steroid analgesic like acetylsalicylic acid. Like this, as confirm SSG's restraint effect of on platelet-activating factor-induced platelet aggregation. We considerated that SSG has practical applicational value of clinical trial in the thrombosis caused by platelet aggregation.

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