Effect of Silsosangami on Neutrophil Functions and Cyclo-oxygenase-2

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Silsosangami(SSG) is a formula of oriental medicines as an effective biological response modifier for augmenting host homeostasis of body circulation. Also SSG has been known to have an anti-diabetic activity and anti-platelet aggregation activity. The present study was undertaken to examine the effects of a new SSG on murine macrophage and human neutrophil functions as well as on several enzymes relevant to the inflammatory process. The results of the present study indicate that SSG exerts anti-inflammatory effects related to the inhibition of neutrophil functions and of PGE2 production, which could be due to a decreased expression of and COX-2.

Key words : Silsosangami(失笑散加味), prostaglandin, cyclooxygenase, neutrophils

Introduction

Silsosangami(SSG) is a formula of oriental medicines as an effective biological response modifier for augmenting host homeostasis of body circulation[1]. SSG is consisted of crude ingredients from 7 medicinal herbs, *Typhae Pollen, Pteropi Faeces, Paeoniae Radicis rubra, Cnidii Rhizoma, Persicae Semen, Carthami Flos,* and *Curcumae Tuber*[1)2)3)]. The pharmacological action of SSG has been limitedly studied in regard to ischemic infarction[1]. This oriental medicine has been shown to express diverse activities such as immunomodulating, anti-infarction, anti-allergic and anti-inflammatory effects. The SSG widely used in traditional Korean medicine, has been known to have an anti-diabetic activity and anti-platelet aggregation activity. The study by Shin[4] has reported that SSG and each constituent herbs of SSG inhibited ADP-induced aggregation as well as arachidonic acid-induced aggregation of human platelet, but the role of each herb of SSG on the inhibition of platelet aggregation and hemolytic effect have not yet been investigated in detail. Prostaglandins exert numerous vascular and inflammatory effects. Production of prostaglandins by the constitutive isoenzymes, cyclooxygenase-1 (COX-1) is implicated in regulation of vascular tone and homeostatic functions. In contrast, COX-2 and is not generally expressed in resting cells, but are induced following appropriate stimulation with pro-inflammatory agents such as cytokines and lipopolysaccharide[5]. The activity of these inducible enzymes results in overproduction of prostaglandins, which play a key role in the pathophysiology of arthritis and other inflammatory conditions[6]. Neutrophils are essential for host defense and their contribution to the propagation and maintenance of acute and chronic inflammation includes several mechanisms. Activated neutrophils release granule constituents[7] and produce leukotrienes, which participate in the inflammation through stimulation of leukocyte functions and vascular permeability[8]. Thus, the suppression of neutrophil functions could control the inflammation. Little is known of the biological activity of SSG and previous studies have focused mainly on their anti-thrombosis[9]. There is a growing interest in the pharmacological potential of the SSG due to the recent finding by our group that SSG was able to inhibit prostaglandin E2 (PGE2) synthesis in murine peritoneal macrophages stimulated with bacterial endotoxin.

The present study was undertaken to examine the effects of a new SSG on murine macrophage and human neutrophil functions as well as on several enzymes relevant to the inflammatory process. The results demonstrated the *in vitro* inhibitory effects on cell functions exerted by this SSG, which also exhibited anti-inflammatory activity *in vivo*.

Materials and Methods

1. Materials and extraction of SSG Extracts

SSG is consisted of herbs such as *Typhae Pollen, Pteropi Faeces, Paeoniae Radicis rubra, Cnidii Rhizoma, Persicae Semen, Carthami Flos,* and *Curcumae Tuber*. A total of 28g

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of SSG was added to 500 ml of water and boiled for 2 hrs, filtered and then concentrated to 200 ml. This decoction was spray-dried to give a powdered extract. The yield was 5.2 g., which represents one human dose/day.

The aqueous extracts of SSG and its seven composed Korean herbs, which were massproduced for clinical use, were kindly supplied by the Oriental Medical Hospital of Dongguk University, Kyongju, Korea (Scheme 1).

Scheme 1. Composition of Silsosangami[1)2)]

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
Curcumae Tuber(鬱金)	4g

L[9,10-3H]oleic acid and L-3-phosphatidylcholine 1-palmitoyl -2-arachidonyl [arachidonyl-1-14C] were purchased from Du Pont (CA, USA). COX-2 specific poly-clonal antisera, N-(2-cyclohexyloxy-4-nitrophenyl) methane-sulfonamide (NS398) and N-(3-(aminomethyl)benzyl)acetamidine di-hydrochloride (1400W) were purchased from Cayman Chem. (MI, USA). The rest of reagents were from Sigma Chem. (MO, USA).

2. Preparation of human neutrophils

Venous blood was obtained, with informed consent, from healthy volunteers. Leukocytes were obtained and purified as previously described[10)] Viability was more than 95% according to the trypan blue exclusion test. The mitochondrial dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan was used to assess the possible cytotoxic effect of SSG on human neutrophils.

3. Isolation and culture of mouse peritoneal macrophages

Female Swiss mice weighing 25-30 g were used to obtain highly purified peritoneal macrophages. Cells were harvested by peritoneal lavage 4 days after i.p. injection of 1 ml of 10% thioglycolate broth. Cells were resuspended in culture medium (120 mM NaCl, 4.7 mM KCl, 1.2 mM $CaCl_2 \cdot 7H_2O$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM HEPES, 1 mM arginine, and 10 mM glucose) supplemented with 10% foetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, 100 g/ml streptomycin, and incubated at 37℃ for 2 hrs. Thrse adhrserent cells were used to perform thrse experiments described below. Cytotoxicity was assessed by thrse reduction of MTT[11)].

4. Elastase release by human neutrophils

Neutrophils (2.5×10^6 cells/ml) were preincubated with test SSG or vehicle for 5 min and then stimulated with cytochalasin B (10 μM) and N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP, 10 nM) or platelet-activating factor (PAF) (0.5 μM) for 10 min. Elastase activity was estimated in supernatants, using N-tert-butoxy-carbonyl-alanine p-nitrophenyl ester (200 μM) as substrate and p-nitrophenol release was measured. Possible direct inhibitory effects on elastase activity were also assessed[12)].

5. Synthesis and release of leukotriene B4 by human neutrophils

A suspension of human neutrophils(5×10^6 cells/ml) was preincubated with test compound or vehicle for 5 min and then stimulated with calcium ionophore A23187 (1 μM) for 10 min at 37℃. Leukotriene B4 levels in supernatants were measured by radioimmunoassay[13)]. High-speed (100,000×g) supernatants from sonicated human neutrophils were obtained and incubated under appropriate conditions with 10 μM arachidonic acid to assess 5-lipoxygenase activity[14)].

6. PGE2 production in mouse peritoneal macrophages

Peritoneal macrophages (4×10^5/well) were incubated with Escherichia coli [serotype 0111:B4] lipopolysaccharide (10 μg/ml) at 37℃ for 24 hrs in the presence of test compounds or vehicle. PGE2 levels were determined in culture supernatants by a fluorometric method[15)] and by radioimmunoassay[13)], respectively. In another set of experiments, lipopolysaccharide-stimulated cells were collected to determine COX-2 expression by Western blot analysis as described below.

7. COX-2 activity in intact cell

Macrophages stimulated with lipopolysaccharide for 24 hrs (4×10^5/well) were washed and fresh medium supplemented with L-arginine (0.5 mM) and arachidonic acid (10 μM) was added for a further 2 hrs incubation with test compounds to assess the effects of compounds on induced enzyme activity. Supernatants were collected for the measurement of nitrite and PGE2 accumulation for the last 2 hrs. PGE2 levels were assayed by radioimmunoassay.

8. COX-2 activity in broken cell preparations

Murine peritoneal macrophages stimulated with E. coli lipopolysaccharide (10 μg/ml) at 37℃ for 24 hrs were collected and sonicated at 4℃ in an ultrasonicator at maximum potency, microsomes were prepared by centrifugation at 2000 ×g for 5 min at 4℃ followed by centrifugation of the supernatant at 100,000×g for 100 min at 4℃. Microsomes (40 μg protein/tube) were incubated for 30 min at 37℃ in 50 mM

Tris HCl, pH 7.4, with arachidonic acid (5 M) and test compound or vehicle in the presence of 2 μM hematin and 1 mM L-tryptophan[16]. The reaction was stopped by boiling the samples for 5 min and PGE2 synthesis was determined by radioimmunoassay[13].

9. COX-1 activity in human platelets microsomes

Human platelets were sonicated at 4℃ in an ultrasonicator at maximum potency. Microsomes were prepared by centrifugation at 2000×g for 5 min at 4℃ follwoed by centrifugation of the supernatant at 100,000×g for 100 min at 4℃. Microsomes (20 μg protein/tube) were incubated for 30 min at 37℃ in 50 mM Tris-HCl (pH7.4) with arachidonic acid and 1 mM L-tryptopha[16]. The reaction was stopped by boiling the samples for 5 min, and thromboxane B2 levels were determined by radioimmunoassay.

10. Western blot analysis

COX-2 protein expression were studied in the cytosolic and microsomal fractions, respectively, from lipopolysaccharide -stimulated peritoneal macrophages and cell pellets obtained by centrifugation of air pouch exudates. Equal amounts of protein were loaded on 12.5% polyacrylamide gel electrophoresis-sodium dodecyl sulphate (PAGE-SDS) and transferred onto polyvinyl- idene difluoride membranes for 90 min at 125 mA. Membranes were blocked in phosphate buffer saline (0.02 M, pH7.0)-Tween-20 (0.1%) containing 3% w/v unfatted milk. For COX-2, membranes were incubated with specific anti-COX-2 polyclonal antiserum (1/1000). Membrane was incubated with peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG) (1/20,000) and peroxidase-conjugated rabbit anti-goat/sheep IgG (1/20,000), respectively. The immunoreactive bands were visualized using an enhanced chemiluminescence system (ECL, Amersham Korea, Korea).

14. Carrageenan paw oedema

The anti-inflammatory activity of SSG was assessed by the carrageenan paw oedema test in mice according to the method of Sugishita et al.[17]. SSG (10, 50, 100, 300 mg/100g by oral administration), indomethacin (5mg/kg), or vehicle (tween 80/saline 1:99, v/v) was administered intraperitoneally 1 h before injection of carrageenan (0.05 ml; 3% w/v in saline) into the subplantar area of the right hind paw. The volumes of injected and contralateral paws were measured 1, 3 and 5 hrs after induction of oedema by using a plethysmometer. The volume of oedema was expressed in each animal as the difference between the carrageenan-injected and contralateral paws. After the last determination of paw oedema (5 hrs), the animals were killed by cervical dislocation and the right hind paws were homogenized in 2 ml of saline. Aliquots of supernatants were used to determine PGE2 levels and elastase activity as above. Stomachs were homogenized in 2 ml of methanol and the content of PGE2 was measured in supernatants after centrifugation.

15. Statistical analysis

The results are presented as means±standard error. Inhibitory concentration 50% (IC_{50}) or inhibitory dose 50% (ID_{50}) values were calculated from at least four concentrations (n=6 ; n represents the number of experiments). The level of statistical significance was determined by analysis of variance (ANOVA) followed by Dunnett's t-test for multiple comparisons. P-values less than 0.05 were considered to represent a significant difference.

Results

1. MTT assay of SSG

SSG did not cause different levels of cellular toxicity with the increment of concentrations in this experiment as determined by reduction of MTT (data not shown).

2. Elastase release by human neutrophils

We assayed SSG in the degranulation process of human neutrophils activated by two different stimuli. Preincubation of isolated human neutrophils with the test compound elicited a concentration-dependent inhibition of cytochalasin B+fMLP and cytochalasin B+PAF-induced degranulation measured as elastase release. The IC_{50} was 5.2 ug/ml (Fig. 1). Direct inhibitory effects on elastase activity were not observed (data not shown).

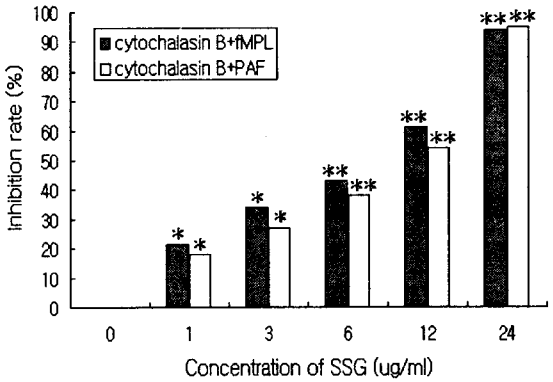

Fig. 1. Inhibition rate by SSG of neutrophil activation. Elastase release induced by cytochalasin B+fMPL and cytochalasin B+PAF respectively. Data represent means±standard error (n=4-5). * ; P-value<0.05, ** ; P-value<0.01.

3. Synthesis and release of leukotriene B4 by human neutrophils

SSG at 12 ug/ml completely abolished leukotriene B4 release by human neutrophils stimulated with ionophore A23187. The concentration-dependent study showed an IC_{50} value of 6.3 ug/ml. Nevertheless SSG failed to modify leukotriene B4 synthesis by high-speed supernatants from human neutrophils at concentrations up to 10 M (data not shown). Thus, it appears that the reduction of leukotriene B4 release by SSG in intact neutrophils is not due to direct inhibition of 5-lipoxygenase activity (Fig. 2).

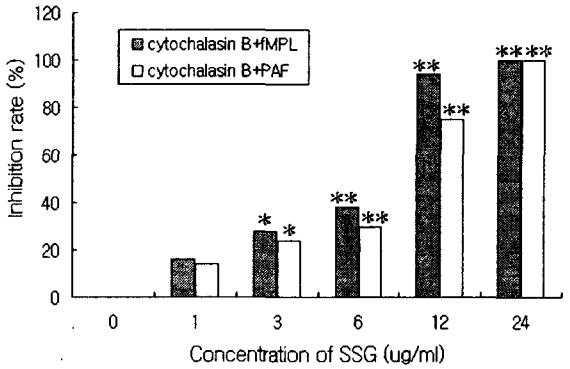

Fig. 2. Inhibition rate by SSG of release of leukotriene B4 by human neutrophils. Leukotriene B4 release induced by cytochalasin B+fMPL and cytochalasin B+PAF respectively. Data represent means±standard error (n=3-5). * ; P-value<0.05, ** ; P-value<0.01.

4. Production of PGE2 in stimulated mouse peritoneal macrophages

Incubation of 24 hrs lipopolysaccharide-stimulated mouse peritoneal macrophages with SSG caused a concentration-dependent inhibition of PGE2 production. Table 1 shows the IC50 values of test SSG for PGE2, respectively. As expected, 1400W (selective inhibitor of iNOS activity) reduced nitrite levels and NS398 (COX-2 inhibitor) showed a high inhibitory potency on PGE2 production, whereas dexamethasone inhibited metabolites at 0.9nM concentrations. None of these compounds affected cellular viability, as assessed by mitochondrial reduction of MTT after 24 hrs (data not shown) indicating that they were not cytotoxic.

Table 1. IC_{50} values for inhibition of PGE2 accumulation in stimulated macrophages

Treatments	IC_{50}[a] PGE2
SSG	1.5 ug/ml
1400W	N.D.
NS398	2.6 nM
Dexamethasone	0.9 nM

Peritoneal macrophages stimulated with lipopolysaccharide for 24 hrs produced 2.8 ng PGE2/ml, compared with 0.3 ng PGE2/ml in untreated cells. a : Fifty % Inhibitory concentration of each drug in producing PGE2

5. Effect of SSG on COX-2 activity in mouse peritoneal macrophages

The following experiments were designed to determine if the inhibition of nitrite and PGE2 production in macrophages was due either to interference with enzyme induction or to direct inhibition of enzyme activities. Cells treated with lipopolysaccharide for 24 hrs were washed and 12 ug/ml of SSG was added and 10 uM of each test products were also added, followed by 2 hrs incubation in fresh culture medium supplemented with L-arginine and arachidonic acid. No significant reduction of PGE2 levels was observed for SSG after this 2 hrs period (Table 2). Nevertheless, 1400W and NS398 caused a very significant reduction of PGE2 (68%) production respectively.

Table 2. Effects of SSG and enzyme inhibitors on COX-2 activities in intact peritoneal macrophages after 24 hrs of lipopolysaccharide stimulation

Treatments	COX-2 (ng PGE2/ml)
Basal	2.3±0.2**
Control	8.3±0.4
SSG	6.3±0.3
1400W	N.D.
NS398	4.5±0.3**

All compounds except for SSG (12 ug/ml) were incubated at 10 M for 2 hrs after the stimulation period. Data shown, means±standard error (n=4-6). Basal: cells were not stimulated with lipopolysaccharide. N.D.: not determined. ** ; P-value<0.01.

6. COX-2 activity in broken cells preparations

To confirm the results obtained with intact cells, we examined the effects of this SSG on COX-2 activity in broken cell preparations (Table 3). SSG at 10 uM was inactive in all the enzymatic activities assayed. In contrast, 1400W and NS398 reduced significantly the production of citrulline (86% inhibition), and PGE2 (53% inhibition) respectively, in these subcellular preparations.

Table 3. Effect of SSG and enzyme inhibitors on COX-2 activities in high speed supernatants or microsomes of 24 hrs lipopolysaccharide-stimulated macrophages, respectively, and on COX-1 activity in human platelet microsomes

Treatments	COX-2 (ng PGE2/ml)	COX-1 (ng TBX B2/mg protein)
Control	17.6±0.6	118.5±6.4
SSG	14.5±1.5	112.2±8.4
1400W	N.D.	N.D.
NS398	7.4±0.6**	N.D.
Indomethacin	N.D.	35.6±0.8**

Data shown, means±standard error, (n=4-5). N.D. ; not determined. SSG was assayed at 24 ug/ml and at 10 uM. TBX ; thromboxane. ** ; P-value<0.01.

7. Synthesis of thromboxane B2 by human platelet microsomes

Synthesis of thromboxane B2 by COX-1 present in microsomes from human platelets was significantly inhibited by the reference compound, indomethacin (86%), whereas SSG

was inactive (data not shown), suggesting that SSG does not reduce PGE2 generation by inhibition of COX-1 activity

8. COX-2 protein expression in mouse peritoneal macrophages

Western blot assays of 24 hrs lipopolysaccharide-stimulated cells were performed to assess possible effects on COX-2 protein expression. Fig. 3 shows a representative experiment where co-incubation of SSG at 12 ug/ml with lipopolysaccharide (10 ug/ml) for 24 hrs, caused reduction in the expression of both isoenzymes. As expected, dexamethasone at 10 uM was very effective.

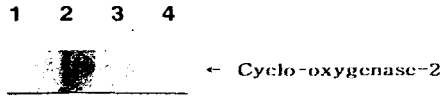

Fig. 3. Effect of SSG and dexamethasone (DX) (10 uM) on COX-2 expression on 24 hrs lipopolysaccharide (LPS)-stimulated murine peritoneal macrophages. The figure is representative of three similar experiments.

9. Carrageenan paw oedema

After i.p. administration, SSG caused a dose-dependent reduction in carrageenan-induced oedema 3 and 5 hrs after induction of inflammation (Table 4).

Table 4. Effect of SSG and indomethacin on carrageenan mouse paw oedema, 1, 3 and 5 hrs after the induction of inflammation

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Time(hr)	Control	Indomethacin (5mg/kg)	SSG(mg/100g) 60	120	360
1 hr	9.5±0.7	8.3±0.4	7.9±0.4	7.3±0.7	4.9±0.3*
3 hrs	20.8±0.4	8.8±0.3**	16.9±0.5*	11.5±1.1*	7.7±0.6**
5 hrs	16.0±0.3	8.9±0.4**	10.0±1.4**	7.3±1.0**	5.3±1.0**

Control : vehicle (tween 80/saline 1:99, v/v) was administered intraperitoneally 1 h before injection of carrageenan (0.05 ml; 3% w/v in saline) into the subplantar area of the right hind paw. * : P-value<0.05, ** : P-value<0.01 (n=6-7 animals). SSG at the doses of 60, 120 and 360 mg/100g was administrated orally. Others were i.p. administrated.

The greatest effect was observed 3 hrs later, with the inhibition percent of 18.7%, 44.7% and 63% at the doses of 60, 120 and 360 mg/100g, respectively. Indomethacin (5 mg/kg i.p.) was assayed as reference compound, showing a significant reduction in swelling 3 (57.7%) and 5 hrs (44.4%) after the administration of carrageenan. The last evaluation of oedema (5 hrs) was followed by killing of the animals and the paws injected with carrageenan were homogenized to determine the levels of elastase and PGE2 (Table 5). The results indicate that elastase activity was significantly and dose dependently decreased by the three doses of SSG assayed. Indomethacin also significantly reduced elastase activity in homogenates of inflamed paws. This reference compound strongly reduced the levels of PGE2 at the dose assayed, whereas SSG reduced the levels of this prostanoid at the doses of 120 mg/100g and 360 mg/100g. On the other hand, the content of PGE2 in stomach homogenates was not significantly affected by the administration of SSG, in contrast to indomethacin, which clearly reduced the levels of this metabolite (Table 5).

Table 5. Inhibition by SSG and indomethacin of elastase activity (control value 153.6±6.5 nmol p-nitrophenol released/ml) and PGE2 levels in homogenates of inflamed paws or stomachs (control values 92.2±5.4 and 16.4±1.4 ng/ml, respectively)

Dose	% Inhibition: Inflamed paws Elastase	Inflamed paws PGE2	Stomachs PGE2
SSG (60 mg/100g) oral	28.4±2.2*	16.5±1.7	0
SSG (120 mg/100g) oral	44.4±4.2*	24.6±2.2**	7.7±1.6
SSG (360 mg/100g) oral	53.2±4.3*	34.4±2.2*	14.5±2.1
Indomethacin (5.0mg/kg) i.p	40.3±2.4*	87.3±2.5*	72.2±0.8

Data represent means±standard error, (n=5-6 animals). * : P-value<0.05, ** : P-value<0.01.

Discussion

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. 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 and so forth have been created. And it becomes the pathopoiesis cause that the symtom complex with a mass or swelling in the abdomen, apoplexy etc18). 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[19)20)].

On this study, SSG was used for investigating reaction of enzymes acted on mechanism of thrombosis formation. SSG is consisted of *Typhae Pollen, Pteropi Faeces, Paeoniae Radicis rubra, Cnidii Rhizoma, Persicae Semen, Carthami Flos,* and *Curcumae Tuber*[1)2)3)]. SSG has been reported to have a hypolipidemic effect in patients with hypercholesterolemia and in cholesterol-induced experimental models[1)]. Also SSG has been reported to have an inhibitory effects on the atherosclerosis in hypercholesterolemic rabbits[4)]. The results of recent clinical studies indicate that antithrombotic therapy significantly reduces the number of vascular events in these patients. As explanation in introduction, neutrophils are essential for host defense and their contribution to the propagation and maintenance of acute and chronic inflammation includes several mechanisms. It is generally accepted that recruitment and activation of leukocytes

contribute to tissue damage in inflammation. Neutrophils migrate to the site of inflammation and upon activation by different stimuli, generate large amount of reactive oxygen species, and release granular enzymes such as elastase and myeloperoxidase, which mediate tissue injuries. In the present work, the respiratory burst elicited in human neutrophils by TPA was potently inhibited by SSG, showing a minor scavenging action in the cell-free system. SSG reduced the degranulation induced by cytochalasin B+fMLP or cytochalasin B+PAF, as well as the leukotriene B4 synthesis induced by ionophore A23187, thus exerting inhibitory effects on neutrophil functions triggered by structurally divergent agonists. SSG may either prevent or slow the progression of neutrophil-mediated tissue injury. DTD seems to affect cell activation at a site common to different signaling pathways as it inhibit responses induced by fMLP, PAF, TPA or ionophore A23187. The induction of COX-2 greatly increase the synthesis of prostaglandins. On the other hand, overproduction of prostaglandins by COX-2 expression in vivo has been reported as being observed in chronic inflammatory conditions such as rheumatoid arthritis[6] and experimental models of inflammation[30]. SSG inhibited the production of PGE2 in murine peritoneal macrophages stimulated by lipopolysaccharide. The inhibition was dose-dependent without any triggering cytotoxic effect. Western blot analysis of mouse peritoneal macrophages lysates showed that COX-2 protein expression were reduced by the presence of SSG during lipopolysaccharide treatment, indicating that SSG inhibits the induction rather than the activity of both enzymes. In a model of inflammation, the mouse paw oedema induced by carrageenan, SSG exerted potent inhibitory effects. Interestingly, SSG reduced the elastase content in the inflamed paw, an index of migration. In addition, the inhibition of COX-2 expression by SSG may account for the anti-inflammatory effects of this on mouse paw oedema, as evidenced by the observed reduction of PGE2 levels in the inflamed paw. In sum, the present study demonstrated that SSG can control PGE2 overproduction by selective inhibition of the enhanced expression of two enzymes. SSG inhibited the oxidative burst in human neutrophils and murine peritoneal macrophages. SSG exerts acute anti-inflammatory effects by reducting of leukocyte activation and by inhibiting of COX-2 expression.

Conclusion

SSG is a dried decoctum of a mixture of 7 Korean herbal medicine, SSG, which is consisted of 7 herbs (indicated as concentrations) of *Typhae Pollen, Pteropi Faeces, Paeoniae Radicis rubra, Cnidii Rhizoma, Persicae Semen, Carthami Flos,* and *Curcumae Tuber*. A SSG inhibited neutrophil functions, including degranulation, superoxide generation, and leukotriene B4 production, without any effect on 5-lipoxygenase activity. This SSG reduced PGE2 production in mouse peritoneal macrophages stimulated with lipopolysaccharide, without the influence on the activity of COX-2 and COX-1 being observed. SSG significantly reduced mouse paw oedema induced by carrageenan. Western blot analysis showed that SSG reduced the expression of COX-2. The results indicate that SSG exerts anti-inflammatory effects related to the inhibition of neutrophil functions and of PGE2 production, which could be due to a decreased expression of and COX-2.

Acknowledgments

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