Inhibitory Effect of *Panax notoginseng* on Nitric Oxide Synthase, Cyclo-oxygenase-2 and Neutrophil Functions

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Our preliminary aim is to elucidate the pharmacokinetic features of the PNS(*Panax notoginseng* Buck F.H. Chen. (Arialiaceae) root). First, we assessed the prevention of neurtrophil functions. A *Panax notoginseng* inhibited neutrophil functions, including degranulation, superoxide generation, and leukotriene B4 production, without any effect on 5-lipoxygenase activity. This *Panax notoginseng* reduced nitric oxide (NO) and prostaglandin E2 production in mouse peritoneal macrophages stimulated with lipopolysaccharide, whereas no influence on the activity of inducible NO synthase, cyclo-oxygenase-2 or cyclo-oxygenase-1 was observed. *Panax notoginseng* significantly reduced mouse paw oedema induced by carrageenan. The results indicate that *Panax notoginseng* exerts anti-inflammatory effects related to the inhibition of neutrophil functions and of NO and prostaglandin E2 production, which could be due to a decreased expression of inducible NO synthase and cyclo-oxygenase-2.

Key words : PNS(*Panax notoginseng*), neurtrophil functions, nitric oxide (NO), prostaglandin E2, cyclo-oxygenase-2

Introduction

Herbal medicines that have been used in Korea for thousands of years are now being manufactured as drugs containing ingredients of standardized quality and quantity. The clinical efficacy of these medicines has been used by Korean Western-medicine practitioners for more than 20 years and is well recognized[1]. One of the herbal medicines, *Panax notoginseng*, is the most common drug to treat chronic liver disease in Korea. A Korean herbal medicine, *Panax notoginseng* Buck F.H. Chen. (Arialiaceae) root (PNS) is highly prized in Korea for its therapeutic abilities to stop haemorrhages, to influence blood circulation and to act as a tonic agent. The *Panax notoginseng* Araliaceae plant *Panax notoginseng* (Burk.) (PNS) is cultivated on a large scale in Korea. It is an oral medicine. The main root of this plant, named notoginseng, is used for treatment of trauma and bleeding due to internal and external injury. Korean herbs has many reported actions such as limitation of liver injury, anti-tumor effect, and alteration of the functional balance of the immune system[2]. Recently,

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korean herbs is widely used by patients with chronic hepatitis in Korean. The preparation prevented liver fibrosis as well as the development of HCC in patients with cirrhosis[3]. In addition, korean herbs was found to inhibit the activation of stellate cells, the rodent equivalent of human stellate cells. This is believed to be the mechanism of prevention of liver fibrosis by PNS[3].

As the principal constituents of this medicinal herb, various dammarane-type triterpene saponins were isolated from the roots, leaves, and seeds[4]. Furthermore, its immunological adjuvant activities of the principal dammarane-type triterpene saponins from notoginseng and American ginseng were characterized[5]. Because of its major pharmaceutical effects, korean herbs is presumed to generally and gradually improve biological defense mechanisms, and it has been reported to have an anti-inflammatory action via an increase in blood corticosterone levels. However, its mode of action has not been fully elucidated. It was also found that the saponin fraction from the flower buds of P. *notoginseng* showed hepatoprotective effect on liver injury induced by D-galactosamine (D-GalN) and lipopolysaccharide (LPS) in mice The flower buds of P. *notoginseng* have been used for treatment of hypertension, vertigo, tinnitus, and laryngopharyngitis, and several known dammarane-type triterpene saponins were hitherto isolated from the flower buds[6].

New new saponins from the flower buds of *P. notoginseng* as well as the hepatoprotective effects of the principal dammarane-type triterpene saponins have been characterized from the flower buds and roots[7].

Recent studies demonstrated various pharmacological effects of notoginsenosides: improving left ventricular diastolic function in hypertension patients, protecting the damage resulted from myocardial ischemia[2], inhibiting the increase of Ca^{2+} induced by KCl and glutamate or during hypoxia[8,9]; blocking the Ca^{2+} overload and Ca^{2+}-calmodulin complex production in nerve cell after cranial cerebral injury and thereby to protect the injured brain[10] showing an obvious anti-inflammatory effect due to reduction the level of the intracellular free calcium concentration in neutrophils[11] promoting the apoptosis of renal interstitial fibroblasts and taking effect to renal interstitial fibrosis[3]. PNS, in addition, can promote the synthesis of DNA and protein[12], modulate emotional responses in rats[13] and their metabolites show anticancer action[14]. Currently, PNS are used to treat coronary heart disease, cardiac angina, apoplexy and atherosclerosis in clinic. PNS contain several kinds of active components such as ginsenoside Rb1 and Rg1[15]. Rb1, one of the main 20 (S)-protopanaxadiol group saponins, shows effective anti-inflammatory action, obvious vasodilating effect and tranquilizing function to central nervous system. 20 (S)-protopanaxatrial group, represented by Rg1, possess the properties of exciting central nervous system, anti-fatigue and hemolysis[10]. It has not been reported about pharmacokinetics of the main saponins contained in PNS by more accurate method.

Prostaglandins and nitric oxide (NO) exert numerous vascular and inflammatory effects. Production of prostaglandins or NO by the constitutive isoenzymes, cyclo-oxygenase-1, or endothelial NO synthase, is implicated in regulation of vascular tone and homeostatic functions. In contrast, cyclo-oxygenase-2 and inducible NO synthase are not generally expressed in resting cells, but are induced following appropriate stimulation with pro-inflammatory agents such as cytokines and lipopolysaccharide[16]. The activity of these inducible enzymes results in overproduction of prostaglandins and NO, which play a key role in the pathophysiology of arthritis and other inflammatory conditions[17]. NO is also able to enhance the production of tumour necrosis factor-α and interleukin-1β, which participate in the macrophage-dependent inflammation[18].

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[19] and produce leukotrienes, which participate in the inflammation through stimulation of leucocyte functions and vascular permeability[20]. Thus, the suppression of neutrophil functions could control the inflammation.

Little is known of the biological activity of *Panax notoginseng* and previous studies have focused mainly on their anti-thrombosis[21]. There is interest in the pharmacological potential of the *Panax notoginseng* to inhibit NO and prostaglandin E2 synthesis in murine peritoneal macrophages stimulated with bacterial endotoxin. The present study was undertaken to examine the effects of a new *Panax notoginseng* 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 *Panax notoginseng*, which also exhibited anti-inflammatory activity in vivo.

Materials and Methods

1. Plant Material.

The flower buds of *Panax notoginseng* was purchased in Kyungju, Kyungbuk Province, Korea, in September 2002, and identified by one of the authors, W.H. Park. A voucher of the plant is on file in our laboratory (2002.09. PN-563). The dried flower buds of *P. notoginseng* (500 g) were finely cut and extracted three times by boiling water.

2. Materials

Loleic acid and L-3-phosphatidylcholine 1-palmitoyl-2-arachidonyl [arachidonyl-1-14C] were purchased from Du Pont (Itisa, Madrid, Spain). Inducible NO synthase and cyclo-oxygenase-2 specific polyclonal antisera, N-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide (NS398) and N-(3-(aminomethyl)benzyl)acetamidine dihydrochloride (1400W) were purchased from Cayman Chem. (MI, USA). The rest of reagents were from Sigma Chem. (MO, USA).

3. Preparation of human neutrophils

Venous blood was obtained, with informed consent, from healthy volunteers. Leukocytes were obtained and purified as previously was described[22]

Viability was greater 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[23] was used to assess the possible cytotoxic effect of *Panax notoginseng* on human neutrophils.

4. 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 $CaCl_2 \times 7H_2O$, 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 h. The adherent cells were used to perform the experiments described below. Cytotoxicity was assessed by the reduction of MTT[23].

5. Elastase release by human neutrophils

Neutrophils (2.5×10^6 cells/mℓ) were preincubated with test *Panax notoginseng* or vehicle for 5 min and then stimulated with cytochalasin B (10 μM) and N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP, 10 mM) 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[24]

6. 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[25]. 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[26].

7. Nitrite and prostaglandin E2 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 h in the presence of test compounds or vehicle. Nitrite and prostaglandin E2 levels were determined in culture supernatants by a fluorimetric method[27] and by radioimmunoassay[25], respectively. In another set of experiments, lipopolysaccharide-stimulated cells were collected to determine inducible NO synthase and cyclo-oxygenase-2 expression by Western blot analysis as described below.

8. Inducible NO synthase and cyclo-oxygenase-2 activity in intact cell

Twenty-four-hour lipopolysaccharide-stimulated macrophages (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 h incubation with test compounds to assess the effects of compounds on induced enzyme activity. Supernatants were collected for the measurement of nitrite and prostaglandin E2 accumulation for the last 2 h. Nitrite concentration, as reflection of NO released, was assayed fluorometrically and prostaglandin E2 levels were assayed by radioimmunoassay.

9. Cyclo-oxygenase-2 activity in broken cell preparations

Murine peritoneal macrophages stimulated with E. coli lipopolysaccharide (10 μg/mℓ) at 37℃ for 24 h 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[28]. The reaction was stopped by boiling the samples for 5 min and prostaglandin E2 synthesis was determined by radioimmunoassay[25].

10. Inducible NO synthase activity in broken cell preparations

High-speed (100,000×g) supernatants from peritoneal macrophages stimulated with E. coli lipopolysaccharide were obtained as described above. Aliquots of supernatants were used to determine NO synthase activity by monitoring the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline[29]. Briefly, supernatants (100 g protein/tube) were incubated at room temperature for 60 min with NADPH (1 mM) and a mixture of unlabeled and L-[^{3}H]arginine (10 M, 1 μCi/mℓ). Incubations were terminated by the addition of 20 mM HEPES (1 mℓ, pH 5.5) containing 1 mM EGTA and 1 mM EDTA. L-[^{3}H]citrulline was separated from arginine by adding 1.5 mℓ of a 1:1 suspension of DOWEX (50 W) in water. Radioactivity was measured in supernatants by liquid scintillation counting.

11. Cyclo-oxygenase-1 activity in human platelets microsomes

Human platelets were sonicated at 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 (pH 7.4) with arachidonic acid and 1 mM L-tryptopha[28]. The reaction was

stopped by boiling the samples for 5 min, and thromboxane B2 levels were determined by radioimmunoassay.

12. Carrageenan paw oedema

The anti-inflammatory activity of *Panax notoginseng* was assessed by the carrageenan paw oedema test in mice according to the method of Sugishita et al.[30]. *Panax notoginseng* (10, 50, 100, 300 mg/100g by oral administration), indomethacin (5 mg/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 at 1, 3 and 5 h after induction of oedema by using a plethysmometer. The volume of oedema was expressed for each animal as the difference between the carrageenan-injected and contralateral paws. After the last determination of paw oedema (5 h), 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 prostaglandin E2 levels and elastase activity as above. Stomachs were homogenized in 2 ml of methanol and the content of prostaglandin E2 was measured in supernatants after centrifugation.

13. Statistical analysis

The results are presented as means±S.E.M.; n represents the number of experiments. Inhibitory concentration 50% (IC50) or inhibitory dose 50% (ID50) values were calculated from at least four concentrations (n=6). The level of statistical significance was determined by analysis of variance (ANOVA) followed by Dunnett's t-test for multiple comparisons.

Results

1. Elastase release by human neutrophils

We assayed *Panax notoginseng* in the degranulation process of human neutrophils activated by two different stimulation. 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 IC50 was 19.6 μg/ml(Fig. 1). Direct inhibitory effects on elastase activity were not observed (data not shown).

2. Synthesis and release of leukotriene B4 by human neutrophils

Panax notoginseng at 40 μg/ml completely abolished leukotriene B4 release by human neutrophils stimulated with

ionophore A23187. The concentration-dependent study showed an IC50 value of 36.3 μg/ml. Nevertheless *Panax notoginseng* failed to modify leukotriene B4 synthesis by high-speed supernatants from human neutrophils at concentrations up to 5 μg/ml (data not shown, Fig. 2). Thus, it appears that the reduction of leukotriene B4 release by *Panax notoginseng* in intact neutrophils is not due to direct inhibition of 5-lipoxygenase activity.
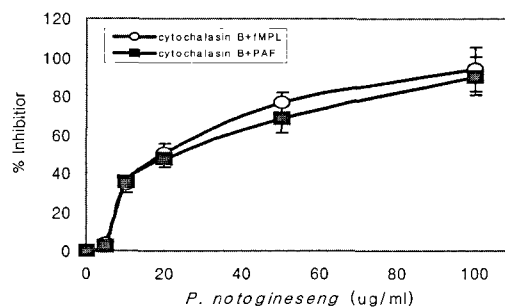

Fig. 1. Inhibition by *Panax notoginseng* of neutrophil activation: elastase release induced by cytochalasin B+fMPL (dotted line □) or cytochalasin B+PAF (solid line ■). Data represent means±S.E.M., n=4-5. *P<0.05, **P<0.01.
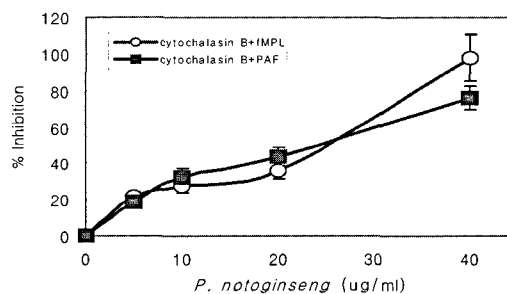

Fig. 2. Inhibition by *Panax notoginseng* of release of leukotriene B4 by human neutrophils. elastase release induced by cytochalasin B+fMPL (dotted line □) or cytochalasin B+PAF (solid line ■). Data represent means±S.E.M., n=3-5. *P<0.05, **P<0.01.

3. Production of nitrite and prostaglandin E2 in stimulated mouse peritoneal macrophages

Incubation of 24 h lipopolysaccharide-stimulated mouse peritoneal macrophages with DTD caused a concentration-dependent inhibition of nitrite (as index of NO generation) and prostaglandin E2 production. Table 1 shows the IC50 values of test *Panax notoginseng* for nitrite and prostaglandin E2, respectively. As expected, 1400W (selective inhibitor of inducible NO synthase activity) reduced nitrite levels and NS398 (cyclo-oxygenase-2 inhibitor) showed a high inhibitory potency on prostaglandin E2 production, whereas dexamethasone inhibited both metabolites at nM concentrations. None of these compounds affected cellular

viability, as assessed by mitochondrial reduction of MTT after 24 h (data not shown) indicating that they were not cytotoxic.

Table 1. IC50 values for inhibition of nitrite and prostaglandin E2 accumulation in stimulated macrophages.

시료	IC50^a mitrite	IC50^a prostaglandin E2
P. notoginseng	18.7 μg/ml	8.3 μg/ml
1400W	2.1 μM	N.D.
NS398	N.D.	2.6 nM
Dexamethasone	32.5 nM	0.9 nM

Twenty-four-hour lipopolysaccharide-stimulated peritoneal macrophages produced 978.3 ng nitrite/ml and 2.7 ng prostaglandin E2/ml, compared to 83.6 ng nitrite/ml and 0.4 ng prostaglandin E2/ml in untreated cells Panax notoginseng

4. Effect of *Panax notoginseng* on inducible NO synthase and cyclo-oxygenase-2 activity in mouse peritoneal macrophages

The following experiments were designed to determine if the inhibition of nitrite and prostaglandin E2 production in macrophages was due either to interference with enzyme induction or to direct inhibition of enzyme activities. Twenty-four-hour lipopolysaccharide-treated cells were washed and *Panax notoginseng* was added at 20 μg/mℓ and all other test products were added at 10 uM, followed by 2 h incubation in fresh culture medium supplemented with L-arginine and arachidonic acid. No significant reduction of either nitrite or prostaglandin E2 levels was observed for *Panax notoginseng* after this 2 h period (Table 2). Nevertheless, 1400W and NS398 caused a very significant reduction of nitrite (66%) and prostaglandin E2 (68%) production, respectively.

Table 2. Effect of *Panax notoginseng* and enzyme inhibitors on inducible NO synthase and cyclo-oxygenase-2 activities in intact peritoneal macrophages after 24 h of lipopolysaccharide stimulation.

Treatment	Inducible NO synthase (ng/nitrite/ml)	Cycloxygenase-2 (ng PGE2/ml)
Basal	1.6±0.2^b	2.3±0.2^b
Contral	43.6±5.4	8.7±0.9
P. notoginseng	35.2±2.7	6.7±0.6
1400W	15.3±1.3	N.D.
NS398	N.D.	4.5±0.3^b

All compounds except for *Panax notoginseng* (40 μg/ml) were incubated at 10 mM for 2 h after the stimulation period. Data shown, means±S.E.M. (n=4-6). N.D., not determined. Basal: cells not stimulated with lipopolysaccharide. ^{b} P(0.01.

5. Inducible NO synthase and cyclo-oxygenase-2 activity in broken cells preparations and synthesis of thromboxane B2 by human platelet microsomes

To confirm the results obtained with intact cells, we examined the effects of this *Panax notoginseng* on inducible NO synthase and cyclo-oxygenase-2 activity in broken cell preparations (Table 3). Panax notoginseng at 20 μg/mℓ was inactive on all the enzymatic activities assayed. In contrast, 1400W and NS398 reduced significantly the production of citrulline (86% inhibition), and prostaglandin E2 (53% inhibition), respectively, in these subcellular preparations.

Synthesis of thromboxane B2 by cyclo-oxygenase-1 present in microsomes from human platelets was significantly inhibited by the reference compound, indomethacin (86%), whereas *Panax notoginseng* was inactive (Table 3), suggesting that *Panax notoginseng* does not reduce prostaglandin E2 generation by inhibition of cyclo-oxygenase-1 activity

Table 3. Effect of *Panax notoginseng* and enzyme inhibitors on inducible NO synthase and cyclo-oxygenase-2 activities in high speed supernatants or microsomes of 24 h lipopolysaccharide-stimulated macrophages, respectively, and on cyclo-oxygenase-1 activity in human platelet microsomes.

Treatment	Inducible NO syntase (pmol citrulline/mg protein, min)	Cyclooxygenase-2 (ng PGE2/ml)	Cyclooxygenase-1 (ng TBX B2/mg protein)
control	16.2±3.2	17.7±2.2	121.5±12.4
P. notoginseng	13.4±4.4	15.6±4.3	80.6±7.7
1400W	1.9±0.3b	N.D.	N.D.
NS398	N.D.	7.4±0.6^b	N.D.
Indomethacin	N.D.	N.D.	37.3±2.8^b

Data shown, means±S.E.M. (n=4-5). N.D., not determined. Panax notoginseng was assayed at 40 μg/ml and at 10 mM. TBX, thromboxane. ^{b}P(0.01.
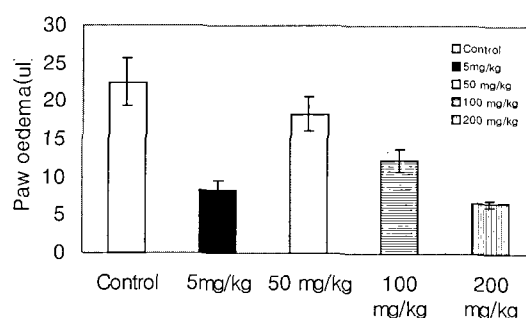
6. Carrageenan paw oedema

After *i.p.* administration, *Panax notoginseng* caused a dose-dependent reduction in carrageenan-induced oedema at 3 and 5 h after induction of inflammation (Fig. 2). The greatest effect was observed at 3 h, with percent inhibitions of 20.7%, 36.7% and 40.5% at the doses of 40, 100 and 200 mg/100 g, respectively. Indomethacin (5 mg/kg *i.p.*) was assayed as reference compound, showing a significant reduction in swelling at 3 (56.6%) and 5 h (44.5%) after the administration of carrageenan.

A) 1h after the induction of inflammation
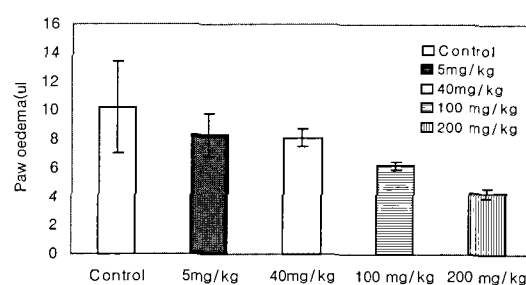
B) 3h after the induction of inflammation
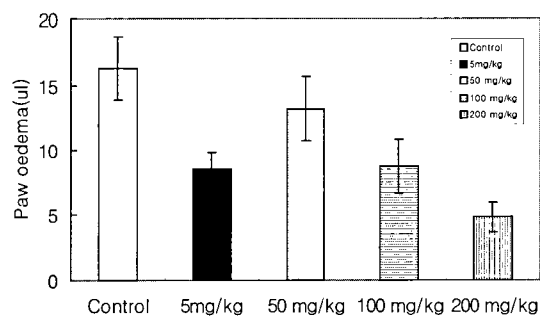
C) 5h after the induction of inflammation

Fig. 3. Effect of *Panax notoginseng* and indomethacin on carrageenan mouse paw oedema, 1, 3 and 5 h after the induction of inflammation. Control (□). *$P < 0.05$, **$P < 0.01$ (n = 6-7 animals). *Panax notoginseng* at the doses of 40, 100 and 200 mg/100 g was administrated orally. Others were i.p. administrated.

7. Inhibition by *Panax notoginseng* and indomethacin of elastase activity and PGE2 levels inflamed paws.

The last evaluation of oedema (5 h) was followed by killing of the animals and the paws injected with carrageenan were homogenized to determine the levels of elastase and prostaglandin E2 (Table 5). The results indicate that elastase activity was significantly and dose dependently decreased by the three doses of *Panax notoginseng* assayed. Indomethacin also significantly reduced elastase activity in homogenates of inflamed paws. This reference compound strongly reduced the levels of prostaglandin E2 at the dose assayed, whereas *Panax notoginseng* reduced the levels of this prostanoid at the doses of 100 mg/100 g and 200 mg/100 g. On the other hand, the content of prostaglandin E2 in stomach homogenates (Table 5) was not significantly affected by the administration of *Panax notoginseng*, in contrast to indomethacin, which clearly reduced the levels of this metabolite.

Table 4. Inhibition by *Panax notoginseng* and indomethacin of elastase activity (control value 154.7±3.6 nmol p-nitrophenol released/mℓ) and prostaglandin E2 levels in homogenates of inflamed paws or stomachs (control values 92.2±5.4 and 16.4±1.4 ng/mℓ, respectively).

Treatment	Inducible NO syntase (pmol citrulline/mg protein, min)	Cyclooxygenase-2 (ng PGE2/ml)	Cyclooxygenase-1 (ng TBX B2/mg protein)
control	16.2±3.2	17.7±2.2	121.5±12.4
P. notoginseng	13.4±4.4	15.6±4.3	80.6±7.7
1400W	1.9±0.3[b]	N.D.	N.D.
NS398	N.D.	7.4±0.6[b]	N.D.
Indomethacin	N.D.	N.D.	37.3±2.8[b]

Data represent means±S.E.M. (n = 5-6 animals). [a] $P < 0.05$, [b] $P < 0.01$.

Discussion and Conclusion

The extract from the flower buds of *P. notoginseng* cultivated in Kyungbuk province of Korea was partitioned into a water to furnish a soluble fraction. In the present work, the respiratory burst elicited in human neutrophils by TPA was potently inhibited by *Panax notoginseng*, showing a minor scavenging action in the cell-free system. Panax notoginseng 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. *Panax notoginseng* may either prevent or slow the progression of neutrophil-mediated tissue injury. *Panax notoginseng* seems to affect cell activation at a site common to different signaling pathways as it inhibited responses induced by fMLP, PAF, TPA or ionophore A23187.

The induction of NO synthase and cyclo-oxygenase-2 greatly increases the synthesis of NO and prostaglandins. Inducible NO synthase inhibition results in modulation of the inflammatory response and delayed paw swelling induced by carrageenan in mice[31]. Furthermore, NO has been shown, in *in vitro* and *in vivo* studies, to increase the production of pro-inflammatory prostaglandins[23]. On the other hand, overproduction of prostaglandins by cyclo-oxygenase-2 expression in vivo has been reported for chronic inflammatory conditions such as rheumatoid arthritis[17] and experimental models of inflammation[32]. *Panax notoginseng* inhibited the production of NO and prostaglandin E2 in murine peritoneal macrophages stimulated by lipopolysaccharide. The inhibition was dose-dependent without any evidence of a cytotoxic effect. Western blot analysis of mouse peritoneal macrophages lysates showed that inducible NO synthase and cyclo-oxygenase-2 protein expression was reduced by the presence of *Panax notoginseng* during lipopolysaccharide treatment, indicating that *Panax notoginseng* inhibits the induction rather than the activity of both enzymes. This hypothesis was confirmed by the fact that *Panax notoginseng* was inactive on inducible NO synthase and cyclo-oxygenase-2 activity in a cell-free system (broken cell preparations). In addition, *Panax notoginseng* did not modify the arachidonic acid pathway by a direct action on the activity of enzymes such as phospholipase A2, 5-lipoxygenase, or cyclo-oxygenase-1.

In a model of inflammation, the mouse paw oedema induced by carrageenan, *Panax notoginseng* exerted potent inhibitory effects. Interestingly, *Panax notoginseng* reduced the elastase content in the inflamed paw, an index of migration. In addition, the inhibition of cyclo-oxygenase-2 expression by *Panax notoginseng* may account for the anti-inflammatory effects of this on mouse paw oedema, as evidenced by the observed reduction of prostaglandin E2 levels in the inflamed paw. NO or prostaglandin E2 overproduction can be controlled

by NO synthase or cyclo-oxygenase-2 inhibitors, respectively. Nevertheless, at the doses normally used they can also inhibit constitutive isoforms, which leads to detrimental effects. Our results indicate that *Panax notoginseng* can control NO and prostaglandin E2 overproduction by selective inhibition of the enhanced expression of both enzymes, thus providing a possible strategy in the treatment of inflammatory diseases.

In summary, the present study demonstrated that *Panax notoginseng* can control NO and prostaglandin E2 overproduction by selective inhibition of the enhanced expression of both enzymes. *Panax notoginseng* inhibited the oxidative burst in human neutrophils and murine peritoneal macrophages. *Panax notoginseng* exerts acute anti-inflammatory effects by reduction of leucocyte activation and inhibition of inducible NO synthase and cyclo-oxygenase-2 expression. Reactive oxygen species and reactive nitrogen intermediates have been implicated in the synthesis of different pro-inflammatory mediators and thus it is known that these species do not operate solely as end-stage effector molecules, but also as mediators regulating cytokine gene expression[33]. Further studies are required to find if the inhibition of enzyme expression by DTD is related to an effect on the generation of reactive oxygen species.

Acknowledgments

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