Gamipaidok-san Possesses Antiallergic and Anti-inflammatory Activities

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Gamipaidok-san(GPDS) is the Hyungbangpaidok-san prescription fortified with the additional ingredients known to be effective for dermatitis. So it has been used for atopic dermatitis in the clinic work actually. In this study, we investigated the effects of GPDS on in vitro and in vivo anti-allergic effect on RBL-2H3 rat basophilic leukemia cells and on IgE-induced passive cutaneous anaphylaxis (PCA) in mice. The in vitro anti-inflammatory activity of GPDS in RAW 264.7 cells was investigated. GPDS potently inhibited β-hexosaminidase release from RBL-2H3 and the IgE-mediated PCA reaction in mice. GPDS inhibited LPS-induced NO and PGE2 production in a dose-dependent manner. Furthermore, It also inhibited inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expression in RAW 264.7 cells, and the activation of the transcription factor, NF-κB, in nuclear fraction. The antiallergic action of GPDS may originate from anti-inflammatory activities, and can improve the inflammation caused by allergies.

Key words : Gamipaidok-san(GPDS), inflammation, allergy, RAW 264.7 cells, β-hexosaminidase, Nitric oxide, PGE2, COX-2, iNOS, NF-κB

Introduction

Atopic dermatitis (AD) is a chronic or chronically relapsing inflammatory skin condition that primarily affects children. AD commonly presents in early infancy and childhood, although it can present later in life and persist into adulthood. AD is a common, chronic, relapsing, inflammatory skin disease characterized by typically distributed eczematous skin lesions with lichenification, pruritic excoriations, severely dry skin and a susceptibility to cutaneous infections[12]. AD is a highly pruritic, recurring inflammatory skin disease. It usually develops in early childhood and is frequently seen in children with a personal history of respiratory allergy and/or a family history of atopic disease[13]. The prevalence of AD has increased in recent years, and it is now estimated to affect up to 20% of the general population[20]. The term atopic dermatitis suggests a close association between AD and respiratory allergy, although there has been considerable debate over whether AD is primarily an allergen-induced disease or simply an inflammatory skin disorder found in association with respiratory allergy.

Hyungbangpaidok-san(荊防敗毒散), basic prescription of Gamipaidok-san (加味敗毒散-GPDS), is used for treatment of upper respiratory infection and dermatitis. Gamipaidok-san(GPDS) is the Hyungbangpaidok-san(荊防敗毒散) prescription fortified with the additional ingredients known to be effective for dermatitis. So it is widely used for atopic dermatitis in the clinic work actually.

Nitric oxide (NO) and prostanoids are mediators of vascular and bronchial tone that are postulated to be involved in atopy. NO is synthesized from L-arginine in the human respiratory tract by enzymes of the NO synthase (NOS) family. NOS play a major role in regulating vascular tone, neurotransmission, the killing of microorganisms and tumor cells and other homeostatic mechanisms[16]. iNOS activation catalyzes the formation of a large amount of NO, which plays a key role in a variety of pathophysiological processes including various forms of inflammation and carcinogenesis[15].

Therefore, the amount of NO produced by iNOS may be a reflection of the degree of inflammation, and therefore provide a means of assessing the effect of drugs on the inflammatory process. Because cells cannot sequester and regulate the local concentration of NO, the regulation of NO

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· Received : 2005/11/25 · Revised : 2005/12/05 · Accepted : 2005/12/14

synthesis is the key to eliciting its biological activity. NO production by iNOS is mainly regulated at the transcriptional level[14]. In macrophages, LPS activates the transcription factor nuclear factor-κB (NF-κB), which leads to the induction of expression of many immediate early genes[1].

Prostanoids, arachidonic acid metabolites produced from a variety of inflammatory cells upon stimulation, are thought to be involved in the pathogenesis of diseases. Prostanoid synthesis is regulated by two successive metabolic steps, the release of arachidonic acid from membrane phospholipids by phospholipase A2 and its conversion to prostanoids by Cyclooxygenase[7,23]. Prostaglandin E_2 (PGE_2) is widely distributed in various organs and exerts an effect on various biological activities. In inflammation processes, PGE_2 is believed to play crucial roles, since chemical mediators invoke PGE_2 synthesis in fibroblasts, endothelial cells, monocytes, and neutrophils at inflammation sites.

Cyclooxygenase-2 (COX-2), which is rapidly induced in inflammatory states, may produce the prostanoids involved in immune and/or inflammatory responses. Considerable evidence has accumulated to suggest that COX-2 is important for tumorigenesis. For example, COX-2 is up-regulated in transformed cells[21,24] and various forms of cancer[18], whereas levels of COX-1 remain essentially unchanged. COX-2 deficiency also protected against the formation of extraintestinal tumors. Thus, COX-2 knockout mice developed approximately 75% fewer chemically induced skin papillomas than control mice[27]. A selective inhibitor of COX-2 caused nearly complete suppression of azoxymethane-induced colon cancer[9]. Many cell types associated with inflammation, such as macrophages, endothelial cells and fibroblasts, express the COX-2 gene upon induction[17,22].

It is well established that COX-2 is important in carcinogenesis, and is over-expressed in transformed cells as well as in various forms of cancer[22]. Because the targetedinhibition of COX-2 is a promising approach to inhibiting inflammation and carcinogenesis as well as to prevent cancer, various chemopreventive strategies have focused on inhibitors of the COX-2 enzyme activity. In macrophages, LPS activates NF-κB, eventually induces the expression of many immediate early genes[5]. Therefore, the pathways leading to NF-κB activation are frequent targets for a variety of anti-inflammatory drugs[25].

In this study, we investigated the effects of GPDS on in vitro and in vivo anti-allergic effects on rat peritoneal mast cells and on IgE-induced passive cutaneous anaphylaxis (PCA) in mice and iNOS and COX-2 in LPS-induced RAW 264.7 macrophages.

Materials and Methods

1. Materials

1) Plant Materials

The sample(GPDS) is consist of Schizonepetae herba, Ledebouriellae divaricata, Notopterygii rhizoma, Bupleuri falcatum, Peucedani radix, Auranyii fructus, Platycodon grandiflorum, Cnidium officinale, Poria, Menthae herba, Cimicifuga heracleifolia, Cicadae periostracum, Sophora flavescens, Glycyrrhiza uralensis, Lonicera japonica, Saururus chinensis, Houttuynia cordata, Ulmus devidiana, Pueraria thunbergiana, Aurantii immaturus fructus. The sample was purchased from Daejeon Medical Center.

Table 1. The Compositions of GPDS Extracts

Contents	Weight (g)
Schizonepetae herba	4
Ledebouriellae divaricata	4
Notopterygii rhizoma	4
Bupleuri falcatum	4
Peucedani radix	4
Auranyii fructus	4
Platycodon grandiflorum	4
Cnidium officinale	4
Poria	4
Aurantii immaturus fructus	4
	40
Contents	Weight (g)
Menthae herba	4
Cimicifuga heracleifolia	4
Cicadae periostracum	4
Sophora flavescens	2
Glycyrrhiza uralensis	3
Lonicera japonica	4
Saururus chinensis	4
Houttuynia cordata	4
Ulmus devidiana	4
Pueraria thunbergiana	4
	0

2) Chemicals and cell culture materials

The chemicals and cell culture materials were obtained from the following sources: Escherichia coli 0111:B4 lipopolysaccharide (LPS) from Sigma Co.; MTT-based colorimetric assay kit from Roche Co.; LipofectAMINE Plus, RPMI 1640, fetal bovine serum, and penicillin-streptomycin solution from Gibco BRL-Life Technologies, Inc. pGL3-4κB-Luc, pCMV-β-gal, and the luciferase assay system from Promega; Methyl thiazol tetrazolium assay (MTT assay) for cell viability

from Sigma-Aldrich (St. Louis, MO, USA); The enzyme-linked immunosorbent assay (ELISA) kit for PGE_2 from R&D systems. Antibodies to iNOS, COX-2, and α-tubulin from SantaCruz Biotechnology, Inc.; Western blotting detection reagents (ECL) from Amersham Pharmacia Biotech.; anti-dinitrophenyl(DNP)-IgE from Sigma Aldrich Co, Evans blue from Sigma Co p-nitrophenyl-N-acetyl-β-D-glucosaminide from Sigma Aldrich Co DNP-BSA from Calbiochem the other chemicals were of the highest commercial grade available.

3) Animals

Male ICR mice (25~30 g) were obtained from KFDA (Seoul, Korea). The animals were allowed access to Purina Rodent Chow and tap water ad libitum. They were maintained in a controlled environment at 21 ± 2 ℃ and a 50 ± 5% relatively humidity with a 12 h dark/light cycle, and acclimatized for at least 1 week prior to use.

4) Cell culture

The mouse macrophage cell line, RAW 264.7 cells, and RBL-2H3 rat basophilic leukemia cells were obtained from the American Type Culture Collection (Bethesda, MD), and grown in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37℃ in a 5% CO_2 humidified incubator. GPDS was dissolved in dimethylsulfoxide and added directly to the culture media. The control cells were treated with the solvents only, the final concentration of which never exceeded 0.1%, which is a concentration that did not have any noticeable effect on the assay systems.

5) Nitrite assay

RAW 264.7 cells (5 X 10^5 cells/ml) were cultured in 48-well plates. After incubating for the cells for 24 h, the level of NO production was determined by measuring the nitrite level in the culture supernatants, which is a stable reaction product of a reaction between NO and molecular oxygen, using a Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in distilled water), and the absorbance of the mixture at 550 nm was determined with a microplate fluorometer. LPS was used as a positive control.

6) PGE_2 production

RAW 264.7 cells were subcultured in 24-well plates and were incubated with the chemicals and/or LPS (0.5 μg/ml) for 24 h. After incubating the cells, the PGE_2 concentration in the culture medium was determined using an enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's instructions.

7) RNA preparation and mRNA analysis by reverse transcription-polymerase chain reaction (RT-PCR)

The cells were cultured with GPDS and/or LPS (0.5 μg/ml) for 2 or 6 h. Total cellular RNA was isolated by the acidic phenol extraction procedure of Chomczynski and Sacchi[4]. cDNA synthesis, semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) for iNOS, COX-2, and β-actin mRNA, and the analysis of the results were all performed as described previously[8]. PCR reactions were electrophoresed through a 2.5% agarose gel and visualized by ethidium bromide staining and UV irradiation. Prior to analysis the PCR product band intensities were checked to ensure that they had not reached the saturation intensity.

8) Transfection and luciferase and β-galactosidase assays

The RAW 264.7 cells (5 X 10^5 cells/ml) were plated in each well of a 12-well plate, and transiently co-transfected with the plasmids, pGL3-4κB-Luc and pCMV-β-gal 12 h later using the LipofectAMINE Plus according to the manufacturer's protocol. Briefly, a transfection mixture containing 0.5 μg of pGL3-4κB-Luc and 0.2 μg of pCMV-β-gal was mixed with the LipofectAMINE Plus reagent and added to the cells. After 18 h, the cells were treated with LPS and/or GPDS for 18 h, and then lysed. The luciferase and β-galactosidase activities were determined using a method described elsewhere[10]. The luciferase activity was normalized with respect to the β-galactosidase activity and is expressed relative to the activity of the control.

9) β-hexosaminidase release assay

Inhibitory effects on the release of β-hexosaminidase in RBL-2H3 cells were evaluated by a method reported previously. Briefly, RBL-2H3 cells in 24-well plates [2×10^5 cells/well in MEM containing 10% FBS, penicillin (100 units/ml), streptomycin (100 μg/ml)] were sensitized with anti-DNP IgE (0.45 μg/ml). The cells were washed with Siraganian buffer [119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES), and 40 mM NaOH, pH 7.2] supplemented with 5.6 mM glucose, 1 mM $CaCl_2$, and 0.1% bovine serum albumin (BSA) (incubation buffer) and then incubated in 160 μl of the incubation buffer for 10 min at 37℃. After that, 20 μl of test sample solution was added to each well and incubated for 10 min, followed by an addition of 20 μl of antigen (DNP-BSA, final concn 10 μg/ml) at 37℃ for 10 min to stimulate the cells to degranulate. The reaction was stopped by cooling in an ice bath for 10 min. The supernatant (50 μl) was transferred into 96-well plate and incubated with 50 μl of substrate (1 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37℃ for 1 h. The reaction was stopped by adding 200 μl of stop solution (0.1 M $Na_2CO_3/NaHCO_3$, pH 10.0). The absorbance was measured with a microplate reader at 405 nm.

10) Passive Cutaneous Anaphylaxis Reactions

This model was based on a protocol previously

described[11]. Ten ng of anti-DNP IgE diluted in 10 μl of PBS alone was injected intradermally in both ears of mice with a 0.3 ml insulin syringe. One day later, 100 μg of DNP-BSA was injected i.v. in 200 μl of PBS with 0.5% Evans blue. Thirty minutes after challenge, both ears were cut and incubated at 80℃ in 1 ml of formamide for 2 h. The mixture was homogenized with an Ultra Turax (M. Zipper) and centrifuged at 20,800 Xg for 10 min. The absorbance of the supernatant was measured at 620 nm. The relation between Evans blue concentration and absorbance was linear, indicating that absorbance represented the quantity of Evans blue extravasation.

11) Statistical Analysis

All the experiments were repeated at least three times. The data is presented as mean ± SD of at least three different sets of plates and treatment groups. A Student's t-test was used to examine the statistical significance of the differences. A $p < 0.01$ was considered significant.

Results

1. Inhibition of LPS-induced NO and PGE_2 production in RAW 264.7 macrophages by GPDS

To evaluate the effect of GPDS on NO production in LPS-induced RAW 264.7 macrophages, nitrite accumulation was examined by the Griess assay. Fig. 2. shows that LPS (0.5 μg/ml) treatment for 24 h triggered significant nitrite accumulation, which was effectively inhibited in a dose-dependent manner by treatment with GPDS. GPDS alone did not affect NO production. However, GPDS inhibited LPS-induced NO production in a dose-dependent manner in RAW 264.7 cells (Fig. 1A). We investigated the possibility that GPDS could inhibit LPS-induced PGE_2 synthesis in RAW 264.7 macrophages. When incubated with vehicle alone, the cells yielded 2.52 ± 0.21 ng/ml of PGE_2. Treatment of the cells with 0.5 g/ml LPS produced 24.8 ± 1.8 ng/ml of PGE_2, a 9 fold increase of PGE_2 production compared to the control. When treated with LPS following pre-treatment with GPDS (100~400 μg/ml), however, the cells showed markedly decreased production of PGE (Fig. 1B). Suppression of PGE_2 production by concentration of GPDS was significant as compared to cells receiving LPS treatment alone.

2. Inhibition of iNOS and COX-2 gene expression by GPDS

In order to determine whether GPDS regulates regulated NO production at the mRNA level, a RT-PCR assay was conducted with LPS as a positive control. Consistent with the results obtained from the proinflammatory cytokines production assays, LPS-inducible iNOS and COX-2 mRNA levels were found to be markedly suppressed by GPDS treatment(Fig. 2). The control β-actin was constitutively expressed and was unaffected by the GPDS treatment. Therefore, a decrease in the iNOS and COX-2 levels by GPDS is believed to be regulated by the transcriptional activation.

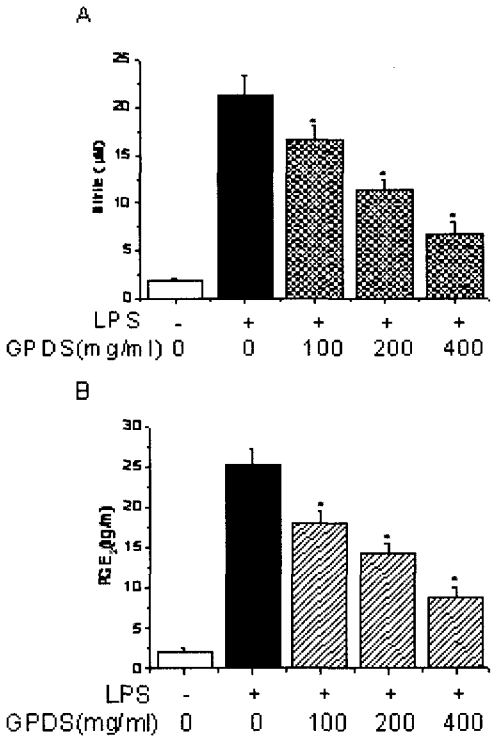

Fig. 1. Effects of GPDS on NO and PGE_2 production. (A). RAW 264.7 cells (5×10^5 cells/ml) were treated with GPDS in the presence of LPS (0.5 μg/ml). Nitrite production was measured by the Griess reaction assay as described in the methods section. Data were obtained from three independent experiments and were expressed as means ± SD. *P < 0.01 significantly different from the LPS. (B). The supernatants were harvested 24 h later and assayed for PGE_2 production. PGE_2 concentrations in the culture medium were measured by ELISA as described in Materials and methods. The values are expressed as means ± SD of triplicate experiments. *P<0.01, significantly different from the LPS.

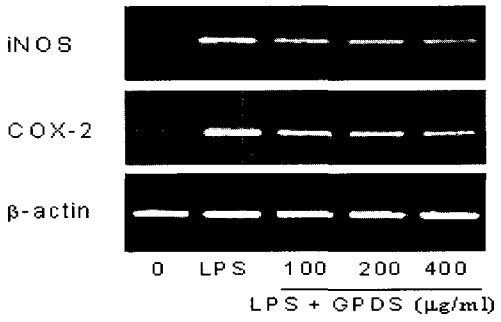

Fig. 2. Effect of GPDS on LPS-induced expression of iNOS and COX-2 mRNA. RAW 264.7 cells were treated with GPDS in the presence of LPS (0.5 μg/ml). Total RNA was prepared and RT-PCR was performed as described in Materials and methods. The PCR products were separated on a 2.5% agarose gel and stained with ethidium bromide. β-actin was used as an internal control.

3. Inhibition of LPS-induced NF-κB activation by GPDS

The transcription factor NF-κB is activated in response to

stimulation by LPS, and this activation is an essential step in the induction of iNOS and COX-2 gene expression. Transient transfection with a NF-κB-dependent luciferase reporter plasmid was done to confirm whether GPDS inhibited the NF-κB binding activity in LPS-activated macrophages. As shown in Fig. 3, GPDS inhibited the LPS-activated NF-κB transcriptional activity in a dose-dependent manner. These results suggest that the suppression of iNOS and COX-2 gene expression by GPDS occurred via the prevention of NF-κB activation.

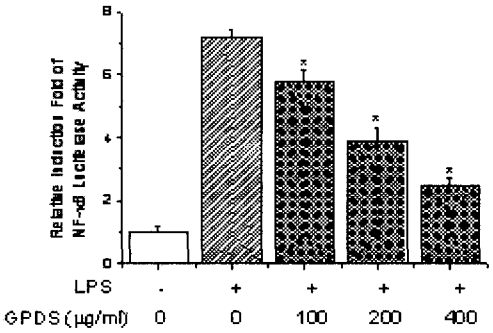

Fig. 3. Effects of GPDS on NF-κB-dependent luciferase gene expression in macrophages. The RAW 264.7 cells (5 X 10^5 cells/ml) were transiently co-transfected with pGL3-4κB-Luc and pCMV-β-gal. After 18 h, the cells were treated with the indicated concentrations of GPDS (100, 200, 400 μg/ml) and/or LPS (0.5 μg/ml). The cells were then harvested, and their luciferase and β-galactosidase activities were determined. The luciferase activity was normalized with respect to the β-galactosidase activity and expressed relative to the activity of the control. Each bar shows the mean ± S.D. of three independent experiments, performed in triplicate. *P<0.01, significantly different from the LPS.

4. Inhibition of β-hexosaminidase release by GPDS

Rat basophilic leukemia (RBL-2H3) cells display properties of mucosal-type mast cells. Therefore, we used RBL-2H3 cells as a model cell line for β-hexosaminidase release. To determine whether GPDS can modulate DNP-BSA-induced β-hexosaminidase release, the RBL-2H3 cells were pretreated with various concentrations of GPDS for 20 min prior to DNP-IgE antigenic stimulation. Culture supernatants were assayed for β-hexosaminidase. GPDS significantly decreased on DNP-DSA-induced β-hexosaminidase release and IC_{50} value was 162.5± 0.3 in RBL-2H3 cells(Table 1).

Table 1. Inhibitory effects of GPDS on DNP-BSA-induced release of β-hexosaminidase from RBL-2H3 cells.

Compound	Inhibition(%)				IC_{50} (μg/ml)
	0μg/ml	100μg/ml	200μg/ml	400μg/ml	
GPDS	0±0.3	35.5±1.3*	63.5±2.1*	85.5±2.5*	162.5±0.3

Each value represents the mean ± SEM. *p<0.01 significantly different from the control

5. Inhibition of PCA Reaction by GPDS

PCA is one of the most useful in vivo models for anaphylaxis in local allergic reactions[11]. As described in Materials and methods, 10 ng of anti-DNP IgE diluted in 10 μl of saline alone was injected intradermally in both ears of mice with a 0.3 ml insulin syringe. One day later, 100 μg of DNP-BSA was injected i.v. in 200 μl of PBS with 0.5% Evans blue. GPDS inhibited the PCA reaction in mice. GPDS showed thatstrong inhibitory activity (64.8±5.1%), significantly inhibiting the PCA at a dose of 2 mg/kg(Table 2).

Table 2. Inhibitory effects of GPDS on ear PCA reaction in mice.

Treatment(Dose)	Anti DNP-IgE +DNP-BSA	Leakage of dye (% of control)
None(saline)	+	100.0±3.8
GPDS 0.5mg/kg	+	75.3±1.9*
GPDS 1mg/kg	+	55.5±6.8*
GPDS 2mg/kg	+	35.2±4.5*

Each value represents the mean ± SEM. *p<0.01 significantly different from the saline value

Discussion

Gamipaidok-san is a Korean traditional prescription used to treat dermatosis such as inflammation, itching, eczema, and pruritic eruption. It is composed of *Schizonepetae Herba, Ledebouriellae Divaricata, Notopterygii Rhizoma, Bupleuri Falcatum, Peucedani Radix, Auranyii Fructus, Platycodon Grandiflorum, Cnidium Officinale, Poria, Menthae Herba, Cimicifuga Heracleifolia, Cicadae Periostracum, Sophora Flavescens, Glycyrrhiza Uralensis, Lonicera Japonica, Saururus Chinensis, Houttuynia Cordata, Ulmus devidiana, Pueraria Thunbergiana, and Aurantii Immaturus Fructus.*

The etiology of the allergy reactivity is based on the IgE-mediated pharmacological processes of a variety of cell populations, such as mast cells and basophils. Degradation of mast cells and basophils with antigen-cross-linked IgE releases histamine, prostaglandins, leukotrienes and cytokines. Finally, the cytokine-induced reaction causes tissue inflammation. These cytokines activate the chemotaxis and phagocytosis of neutrophils and macrophages. The RBL-2H3 cells contain several hundred thousand IgE receptors on the membrane surface, and after sensitization with mouse monoclonal IgE, the cells respond to antigen and release -hexosaminidase. Therefore, we used RBL-2H3 cells as a model cell line for -hexosaminidase release. Antiallergic agents, with anti-inflammatory actions, may be beneficial in allergic diseases. In this study, we demonstrated that GPDS inhibited β-hexosaminidase release from rat peritoneal mast cells and the IgE-mediated PCA reaction in mice. These results suggest that the inhibitory action of GPDS on the release of β-hexosaminidase may be due to the protection of the cytolytic response of the antigen-IgE. PCA reaction is one of the models

most frequently used to evaluate anti-allergic drugs11). In the immediate phase of PCA, passively sensitized mast cells in skin are activated by intravenously administered specific antigen, followed by the release of vasoactive mediators such as histamine, which increase vascular permeability at the sensitized skin site.

Cytokine-activated macrophages synthesize various mediators that modulate the inflammatory response. NO and prostaglandins are two pleiotropic mediators produced by NOSs and COXs, respectively, at inflammatory sites. NOSs can be classified into two major groups. Neuronal and enthothelial NOSs are in general constitutively expressed, and the NO produced by such isoforms is a key regulator of homeostasis. Conversely, the iNOS plays an important role in the cytotoxic activity of activated macrophages. Stimuli, such as cytokines and/or bacterial LPS, induce iNOS protein expression, which once synthesized is responsible for the prolonged, high-output production of NO. Despite its beneficial role in host defense, the sustained production of NO can be deleterious to the host, and has been implicated in the pathogenesis of various inflammatory diseases. COX, which catalyzes the conversion of arachidonic acid to PGE_2, is a rate-limiting enzyme in the biosynthesis of prostaglandins. Two isoforms of this enzyme have been described: the constitutive enzyme COX-1, which is present in almost all cell types,is thought to be involved in homeostatic prostanoid biosynthesis, whereas COX-2 is rapidly induced after stimulation with cytokines and/or LPS. COX-2 is predominantly expressed in the cells involved in inflammatory reactions, such as macropharges. GPDS inhibits the production of NO and PGE_2, in LPS-stimulated RAW264.7 cells, and the activity of iNOS. Also, GPDS inhibited the expression of iNOS and COX-2 mRNA and proteins. GPDS inhibited the activation of the NF-κB transcription factor, which regulates iNOS and COX-2 gene expression on the RAW264.7 cells stimulated with LPS. These results suggest that GPDS may express anti-inflammatory activity via the regulation of the signal transduction related to the activation of NF-κB. From this point of view, we suggest that the presence of GPDS is responsible for their strong anti-inflammatory properties. The promoter of the iNOS gene contains two major and discrete regions that function synergistically in the binding of transcription factors[26]. Among these transcription factors NF-κB, which is a primary transcription factor activated by LPS, and regulates various genes, is important in immune response and inflammation. GPDS also inhibited LPS-induced NF-κB activation therefore inhibited expression of iNOS and COX-2 expression. The expression of iNOS and COX-2 in murine macrophages has been shown to be dependent on NF-κB activation[1]. The possibility that GPDS might inhibit the activity of NF-κB was examined. The results indicate that the inhibition by GPDS on the expression of the iNOS and COX-2 proteins and iNOS, COX-2 and inflammatory cytokines mRNA was most likely due to the suppression of NF-κB. The role of NF-κB should be seen as an amplifying and perpetuatingmechanism that will exaggerate the disease-specific inflammatory process. Some anti-inflammatory drugs have actually been tested in human clinical trials to prevent cancer and numerous pharmaceutical companies are developing new drugs targeting NF-κB, iNOS, COX-2, etc. Many signaling pathways and molecules, however, play dual roles in inflammation and carcinogenesis. For example, inhibition of NF-κB activation or iNOS promotes inflammation and carcinogenesis under certain circumstances[6]. This is consistent with previous reports that NF-κB response elements are present on the promoters for the iNOS, COX-2 and inflammatory cytokine genes[3,19,26]. These observations suggest that GPDS exert an anti-inflammatory action through regulation of the NF-κB inactivation. In conclusion, theseresults of the present study indicate that GPDS is a potent inhibitor of the LPS-induced NO, PGE_2 and inflammatory cytokines production via gene expression and this inhibition was found to be caused by the blocking of NF-κB activation in RAW 264.7 macrophages. The beneficial effect of GPDS in the treatment of atopy seems to be due to its actions as an antiallergy and anti-inflammatory agent. Further studies are clearly needed to identify the molecular mechanisms by which chronic inflammation increases cancer risk and to develop new and more effective strategies for cancer prevention on the basis of these findings.

In conclusion, the GPDS has an antiallergy/anti-inflammatory activity due to inhibitions of β-hexosaminidase release, NO, and PGE_2 synthesis. Also, we believe that the GPDS have extensive antiallergic properties, and candidate therapeutic agent for allergies and inflammation.

Acknowledgments

This work was supported by RIC(R) grants from Traditional and Bio-Medical Research Center, Daejeon University(RRC04700, 2005) by ITEP.

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