Phosphatidylserine inhibits inflammatory responses in interleukin-1β–stimulated fibroblast-like synoviocytes and alleviates carrageenan-induced arthritis in rat

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ABSTRACT

Recently, phosphatidylserine (PS) has received attention for its anti-inflammatory effect; however, the molecular mechanisms of its action have not been fully understood. Thus, we hypothesized that PS might have antiarthritic and anti-inflammatory effects. To test this hypothesis, the in vitro anti-inflammatory effect of soybean-derived PS was tested on interleukin (IL)-1β–stimulated fibroblast-like synoviocytes from rheumatoid arthritis patients (RA-FLS) by measuring the levels of IL-6, IL-8, prostaglandin E2, and vascular endothelial growth factor by enzyme-linked immunosorbent assay. The analgesic and antiarthritic activities of PS were investigated in rat models of carrageenan-induced acute paw pain and arthritis. The former was evaluated with a paw pressure test; the latter, by measuring paw volume and weight distribution ratio. In addition, the participation of mitogen-activated protein kinase signaling in the anti-inflammatory and antiarthritic effects of PS was investigated in RA-FLS. Phosphatidylserine inhibited the production of inflammatory mediators IL-6; IL-8; vascular endothelial growth factor; and, in particular, prostaglandin E2 in IL-1β–stimulated RA-FLS. These effects were associated with abrogation of inhibitor of nuclear factor-κB phosphorylation and suppression of p38 and c-jun amino terminal kinase but not extracellular signal–regulated kinase 1/2 phosphorylation. In rats, PS also showed a significant inhibitory effect on arthritic and nociceptive symptoms induced by carrageenan. These findings suggest that PS has anti-inflammatory and antiarthritic effects in vitro and in vivo animal models; thus, PS should be further studied to determine its potential use as either a pharmaceutical or dietary supplement for alleviating arthritic symptoms.

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Abbreviations: ANOVA, analysis of variance; ERK, extracellular signal–regulated kinase; IκB, inhibitor of nuclear factor-κB; IL, interleukin; JNK, c-jun amino terminal kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappaB; PGE2, prostaglandin E2; PS, phosphatidylserine; RA, rheumatoid arthritis; RA-FLS, fibroblast-like synoviocytes from rheumatoid arthritis patients; VEGF, vascular endothelial growth factor; WDR, weight distribution ratio.

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1. Introduction

Rheumatoid arthritis (RA) is a chronic destructive inflammatory disease of the joints that is characterized by chronic proliferative synovitis, synovial hyperplasia, neovascularization, inflammatory cell infiltration, and destruction of cartilage and bone [1,2]. Because the exact cause of RA is not yet known, treatment of this disease has focused on relieving symptoms and reducing progression of inflammation rather than on curing the disease. In addition, nonsteroidal anti-inflammatory drugs that are widely used for treating RA are known to cause severe adverse effects, particularly in the gastrointestinal tract. Thus, an advantageous alternative with high efficacy and less severe adverse effects needs to be developed.

In joints affected by RA, fibroblast-like synoviocytes (FLS) can release various inflammatory or angiogenic factors such as interleukin (IL)-6, IL-8, vascular endothelial growth factor (VEGF), and prostaglandin E2 (PGE2) [3]. These inflammatory mediators play important roles in the pathogenesis of RA. Interleukin-6 and IL-8 most likely act as major instigators of RA joint inflammation because disruption of their functions, either by gene knockout [4] or systemic IL-4 treatment [5], leads to protection against arthritis in animal models. In addition, neovascularization, a prerequisite in the formation of nuclear factor (NF)-κB-mediated activation of inflammatory effects. Therefore, in the present study, the anti-inflammatory and antiarthritic activities of PS were evaluated in vitro via IL-1β-stimulated RA-FLS and in vivo via a carrageenan-induced arthritic rat model. To further understand the molecular mechanisms underlying the anti-inflammatory effects of PS, we examined the effects of PS on MAPK activation and inhibitor of NF-κB (IκB) phosphorylation in IL-1β-stimulated RA-FLS.

2. Methods and materials

2.1. Isolation and culture of FLS

Fibroblast-like synoviocytes were isolated from the synovial tissue of RA patients undergoing joint replacement surgery, as described previously [14], and grown in Dulbecco modified Eagle medium (low glucose; Gibco-Invitrogen, Carlsbad, CA, USA) that was supplemented with 10% (vol/vol) fetal bovine serum (Gibco-Invitrogen), 100 U/mL penicillin, and 100 μg/mL streptomycin sulphate (Gibco-Invitrogen). All experiments were performed using FLS between the 4th and 10th passages (>95% RA-FLS purity).

2.2. Animals

Five-week-old male Sprague-Dawley rats were obtained from Samtaco Co (Osan, Korea) and allowed to acclimate for 1 week before use. Animals were housed in standard cages with access to rodent chow pellets (Purina Korea, Seoul, Korea) and water ad libitum, and they were maintained at 23°C±2°C and 40%–60% humidity in a 12-hour light/dark cycle (8:00 AM-8:00 PM light, 8:00 PM-8:00 AM dark). Studies were conducted in accordance with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee of Kyung Hee University.

2.3. Treatment of PS

Soybean-derived PS was kindly provided by Doosan Co Glonet BU (Suwon, Korea) as a powder product. It was obtained by enzymatic transphosphatidylation from phosphatidylcholine purified from crude soy lecithin. The PS formula contained 90% PS, 2% phosphatidylcholine, and 6% phosphatidic acid. The fatty acid composition of PS is shown in Table 1. For cell culture experiments, PS powder was homogenously suspended in phosphate-buffered saline (pH 7.2) and sonicated for 3 minutes on ice. For rats, PS was homogenously suspended in saline using a sonicator (Vibra Cell system; Sonics and Materials, Inc, Newtown, CT, USA) and orally administered using an oral sonde needle. Phosphatidylinerine was freshly prepared before use. Celecoxib and prednisolone were used as positive controls for in vitro RA-FLS and in vivo rat experiments, respectively. In vitro RA-FLS studies, celecoxib (Celebrex) was obtained from Pfizer Inc (New York, USA) and dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL. Prednisolone (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in DMSO at a concentration of 10 mg/mL. Hockeyphore (BU (Suwon, Korea) as a powder product. It was obtained by enzymatic transphosphatidylation from phosphatidylcholine purified from crude soy lecithin. The PS formula contained 90% PS, 2% phosphatidylcholine, and 6% phosphatidic acid. The fatty acid composition of PS is shown in Table 1. For cell culture experiments, PS powder was homogenously suspended in phosphate-buffered saline (pH 7.2) and sonicated for 3 minutes on ice. For rats, PS was homogenously suspended in saline using a sonicator (Vibra Cell system; Sonics and Materials, Inc, Newtown, CT, USA) and orally administered using an oral sonde needle. Phosphatidylinerine was freshly prepared before use. Celecoxib and prednisolone were used as positive controls for in vitro RA-FLS and in vivo rat experiments, respectively. In vitro RA-FLS studies, celecoxib (Celebrex) was obtained from Pfizer Inc (New York, USA) and dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL. Prednisolone (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in DMSO at a concentration of 10 mg/mL. Hockeyphore (BU (Suwon, Korea) as a powder product. It was obtained by enzymatic transphosphatidylation from phosphatidylcholine purified from crude soy lecithin. The PS formula contained 90% PS, 2% phosphatidylcholine, and 6% phosphatidic acid. The fatty acid composition of PS is shown in Table 1. For cell culture experiments, PS powder was homogenously suspended in phosphate-buffered saline (pH 7.2) and sonicated for 3 minutes on ice. For rats, PS was homogenously suspended in saline using a sonicator (Vibra Cell system; Sonics and Materials, Inc, Newton, CT, USA) and orally administered using an oral sonde needle. Phosphatidylinerine was freshly prepared before use. Celecoxib and prednisolone were used as positive controls for in vitro RA-FLS and in vivo rat experiments, respectively. In vitro RA-FLS studies, celecoxib (Celebrex) was obtained from Pfizer Inc (New York, USA) and dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL. Prednisolone (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in DMSO at a concentration of 10 mg/mL. Hockeyphore (BU (Suwon, Korea) as a powder product. It was obtained by enzymatic transphosphatidylation from phosphatidylcholine purified from crude soy lecithin. The PS formula contained 90% PS, 2% phosphatidylcholine, and 6% phosphatidic acid. The fatty acid composition of PS is shown in Table 1. For cell culture experiments, PS powder was homogenously suspended in phosphate-buffered saline (pH 7.2) and sonicated for 3 minutes on ice. For rats, PS was homogenously suspended in saline using a sonicator (Vibra Cell system; Sonics and Materials, Inc, Newton, CT, USA) and orally administered using an oral sonde needle. Phosphatidylinerine was freshly prepared before use. Celecoxib and prednisolone were used as positive controls for in vitro RA-FLS and in vivo rat experiments, respectively. In vitro RA-FLS studies, celecoxib (Celebrex) was obtained from Pfizer Inc (New York, USA) and dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL. Prednisolone (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in DMSO at a concentration of 10 mg/mL.
NY, USA), dissolved in dimethyl sulfoxide at a concentration of 100 mg/mL, and added to the culture medium at a final concentration of 80 μg/mL. For the in vivo rat experiment, prednisolone (Sigma-Aldrich, St Louis, MO, USA) was dissolved in saline at a concentration of 200 mg/mL, and 20 mg/kg was orally administered to rats in the ART+PRED group using an oral sonde needle.

2.4. Enzyme-linked immunosorbent assay

Triplicate cultures of FLS were treated with various concentrations of PS and/or IL-1β (10 ng/mL; ProSpec, Rehovot, Israel) and then cultured for 24 hours. The supernatants were collected, centrifuged, and analyzed for IL-6, IL-8, PGE2, and VEGF expression using commercial enzyme-linked immunosorbent assay kits (BD Biosciences Pharmingen, San Diego, CA, USA). The concentrations of p-c-Jun, p-IκBα, and VEGF were measured using the Bio-Plex assay (Bio-Rad, Hercules, CA, USA). The carrageenan-induced arthritis model was used to assess the antiarthritic activity in rats as described previously[15].

2.5. Bio-Plex phosphoprotein assay

After PS and/or IL-1β stimulation, FLS cells were lysed using a Bio-Rad cell lysis kit; and the phosphorylation levels of c-Jun and IκBα, as well as 3 MAPKs (namely, c-Jun amino terminal kinase [JNK], p38, and extracellular signal–regulated kinase [ERK]1/2), were measured using the Bio-Plex assay (Bio-Rad, Hercules, CA, USA). The concentrations of p-c-Jun, p-IκBα, p-ERK1/2, p-JNK, and p-p38 were analyzed using the Bio-Plex phosphoprotein assay kit (Bio-Rad) and the Phosphoprotein Testing Reagent kit (Bio-Rad) according to the manufacturer’s instructions as described previously[16].

2.6. Mechanical hyperalgesia

Mechanical hyperalgesia was evaluated by the Randall-Selitto test using a paw pressure algosy meter (Ugo Basile, Comerio-Varese, Italy), as described previously[15]. Pressure was applied to the affected hind paw of each rat with a gradual increase (15 g/s), and nociceptive thresholds were determined as the pressure (in grams) when the rat exhibited a stereotyped flinch response and attempted to remove the foot from the apparatus. Paw hyperalgesia was induced by injection of 100 μL of 1% (wt/vol) λ-carrageenan into the left tibiotarsal ankle joint. Phosphatidylserine, homogenously suspended in saline, was orally administered once a day for 9 days from 1 day after carrageenan injection. Administration was performed 2 hours before measurement of arthritic symptoms by the paw volume, ankle flexion test, and incapacitance test. The effects of PS were compared with those of prednisolone (20 mg/kg), the positive control. After the termination of the study, rats were euthanized with CO2.

2.8. Paw volume

Paw swelling was measured by volume displacement of an electrolyte solution using a water-displacement plethysmometer (Ugo Basile), as previously described[15]. The hind paw was immersed to the line of the hairy skin, and the volumes were read on a digital display. Paw volume was expressed as relative value compared with that on day 0, which was defined as 100%.

2.9. Incapacitance test

The spontaneous pain related to inflammation of the hind limb was assessed by measuring the weight distribution ratio (WDR) using an incapacitance meter (Ugo Basile) that independently measured the weight bearing at each hind paw, as described previously[15]. The WDR was defined as the percentage weight distribution of the left hind paw and then was calculated using the following formula: WDR = left weight/(right weight + left weight) × 100. The WDR in the normal group was close to 50, indicating that 50% of the weight was carried in each hind paw. Conversely, the WDR in the arthritis group was reduced because the balance of weight was disrupted by the pain of the knee due to induction of arthritis. The WDR was measured 1 hour after PS administration. Each measurement was performed 3 times, and the value of the WDR per measurement was defined as the mean of 3 calculations of WDR. All tests were performed blind.

2.10. Ankle flexion test

The ankle flexion test was performed for evaluation of arthritis-induced hyperalgesia, as described previously[15]. The ankle joint of the hind limb was flexed and extended 10 times with an intertest interval of 5 seconds. Squeaking included any vocalization evoked by ankle flexion and extension. The numbers of squeaking vocalizations detected by the observer were then counted for the squeaking score. A vocalization was rated as 0 (no vocalization) or 1 (vocalization) and then calculated with a maximum value of 10. The ankle flexion was performed only once every day in each animal.

2.11. Statistical analyses

Power analysis was performed on all groups to confirm appropriateness of sample size using the G*Power 3.1.5 program.
with average values and standard deviations based on previous experience. Results are expressed as the means±SEM for each treatment group. Protein secretion and phosphorylation, and nociceptive threshold data in single time-point experiments were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. Time course data such as paw volume, WDR, and squeaking score were analyzed using one-way ANOVA with repeated measurement followed by the Tukey post hoc test. Statistical analysis was performed with the SPSS program (Version 13.0; SPSS Inc, Chicago, IL, USA). P values less than .05 were considered statistically significant.

3. Results

3.1. Effects of PS on IL-1β–induced IL-6 and PGE₂ production by RA-FLS

Phosphatidylserine (1, 5, and 10 μg/mL) alone or with IL-1β for 24 hours did not cause any significant change in RA-FLS viability compared to the normal control group (data not shown), indicating that PS was not cytotoxic to RA-FLS cells in experimental concentration. Interleukin-6 and PGE₂, highly produced in RA synovium, are well known to promote synovial inflammation, cartilage loss, and joint destruction. Thus, we first investigated whether PS can inhibit IL-1β–induced IL-6 and PGE₂ production by RA-FLS. The RA-FLS were treated with 10 ng/mL IL-1β and/or various concentrations of PS for 24 hours. Celecoxib (80 μg/mL) was used as a positive control. Stimulation of RA-FLS with IL-1β for 24 hours strongly elicited IL-6 and PGE₂ production (Fig. 1). The treatment of PS slightly, but statistically, inhibited IL-1β–induced IL-6 secretion by 18.9% in RA-FLS in a concentration-dependent manner (Fig. 1A). In addition, PGE₂ levels decreased by 29.1% in RA-FLS treated with PS and IL-1β. Phosphatidylserine inhibited production of PGE₂ more potently than that of IL-6, but showed no dose-dependent effect at the doses used in the present study (Fig. 1B).

3.2. Effects of PS on IL-1β–induced VEGF and IL-8 production by RA-FLS

Vascular endothelial growth factor and IL-8 are important angiogenic factors in the pathogenesis of RA. To test the inhibitory effects of PS on IL-1β–induced VEGF and IL-8 production by RA-FLS, RA-FLS were treated with 10 ng/mL IL-1β and/or PS for 24 hours. Celecoxib (80 μg/mL) was used as a positive control. Interleukin-1β stimulation greatly increased expression of VEGF and IL-8 by RA-FLS. The addition of PS slightly, but statistically, inhibited IL-1β–induced VEGF
and IL-8 secretion by 19.5% and 10.6%, respectively, in RA-FLS in a concentration-dependent manner (Fig. 1C, D).

### 3.3. Effects of PS on IL-1β-induced activation of MAPK signaling pathways

Mitogen-activated protein kinase pathways have important functions as mediators of cellular responses to extracellular signals in RA [7]. Thus, we investigated whether MAPK pathways were involved in the suppressive effect of PS on IL-1β-induced inflammation in RA-FLS. Activation of the JNK, p38, and ERK was detected by Bio-Plex phosphoprotein assays using phosphorylation level as an index of enzyme activity. Interleukin-1β stimulation caused phosphorylation of JNK and p38 but not ERK1/2 in RA-FLS. Treatment of RA-FLS with PS slightly inhibited IL-1β-induced phosphorylation of p38 and JNK in a concentration-dependent manner within 30 minutes, but left ERK 1/2 unaffected (Fig. 2 A–C). In addition, PS (10 μg/mL) alone had no effect on MAPK activation except for ERK1/2. These results suggest that regulation of JNK and p38 MAPK signaling cascade is a possible mechanism underlying PS inhibitory effect in IL-1β-stimulated RA-FLS. Next, because JNK is highly activated in RA-FLS and synovium, we examined the effect of PS on phosphorylation of c-Jun, which is a downstream effector of the JNK pathway. Phosphorylation of c-Jun by IL-1β stimulation was suppressed by PS treatment (Fig. 2D).

### 3.4. Effects of PS on IL-1β-induced activation of NF-κB pathways

Nuclear factor-κB activation is well known to be regulated by IκBα and by phosphorylation of IκBα at Ser32 and Ser36...
residues and subsequent IκBα degradation, allowing the p65 and p50 subunits of NF-κB to translocate to the nucleus [18]. In the present study, we assessed NF-κB activation by the measurement of phosphorylated IκBα. Interleukin-1β treatment markedly increased the phosphorylation of IκBα. Interestingly, this phosphorylation of IκBα, a critical step for NF-κB activation and translocation, was reversed by treatment with PS (Fig. 2E), thus indicating that PS can markedly inhibit IL-1β-stimulated nuclear translocation of NF-κB p65. In addition, PS (10 μg/mL) alone had no effect on IκBα phosphorylation. Accordingly, PS dose-dependently inhibits the NF-κB signal transduction pathway that plays a key role in the inflammation response to an IL-1β stimulus in in vitro rheumatoid synovial fibroblasts.

3.5. Analgesic effect of PS in carrageenan-induced hyperalgesia in rats

Next, we determined whether PS had antinociceptive effects in carrageenan-induced hyperalgesia in rats. Intraplantar injection of carrageenan into the left hind paw produced a significant decrease in the nociceptive threshold of all animals 3 hours after injection, indicating the induction of mechanical hyperalgesia (the contralateral hind paw appeared unaffected). Oral administration of PS at 1 hour before carrageenan injection dose-dependently increased the paw withdrawal threshold compared with the ART group (Fig. 3), which indicated that pretreatment with PS greatly prevented carrageenan-induced hyperalgesia. The efficacy at 50 mg/kg was similar to that of prednisolone, the positive control.

3.6. Antiarthritic effect of PS on carrageenan-induced arthritis in the rat

A single intraarticular injection of carrageenan into the tibiotarsal (ankle) joint led to an arthritis typified by inflammation and increased pain sensation. Thus, the in vivo antiarthritic effects of PS were tested by physical parameters such as paw volume, WDR, and squeaking score in carrageenan-induced arthritis in the rat. First, we examined the effect of PS on paw edema. Intraarticular injection of carrageenan into the tibiotarsal (ankle) joint largely increased the paw volume, with an obvious inflammatory response. This was evidenced by swelling and redness of the entire paw before reaching a plateau, which was steadily maintained throughout the experimental period. Oral administration of PS dose-dependently reduced the paw volume compared with arthritic animals (Fig. 4A). Next, we measured the effect of PS on carrageenan-induced hypersensitivity by hind limb incapacitance. Saline-injected normal rats distributed their body weight 50:50 between both hind limbs. However, carrageenan-induced arthritic rats showed a significant reduction in spontaneous weight bearing on the ipsilateral paw such that only 10% of the weight was placed on the ipsilateral leg; this reached 20% at day 9 with a slight, but progressive, increase. Oral administration of PS largely eliminated this hypersensitivity by carrageenan in a dose-dependent manner (Fig. 4B). We also recorded the vocalization response to ankle flexion to evaluate the analgesic effect of PS. The number of vocalizations in saline-treated normal animals remained almost zero throughout the experimental period. The vocalization in arthritic animals reached a maximum point on day 1 after the carrageenan injection and was sustained at a maximum level through the end of the experiment. Animals that were administered PS displayed a significant a dose-dependent decrease in the vocalization response compared with arthritic animals (Fig. 4C). In addition, the recovery effect of 50 mg/kg PS was similar to that of 20 mg/kg prednisolone, which was used as a positive control.

4. Discussion

In the present study, we investigated the anti-inflammatory effects of PS in vitro and in vivo to determine whether it had therapeutic potential for arthritis. Soybean-originated PS is commercially available as an oral supplement intended to improve cognitive function. Indeed, most research regarding PS has concentrated on memory and cognitive function. However, PS has recently been extensively demonstrated to exhibit anti-inflammatory properties. During apoptosis, exposure of PS on the outer leaflet of the cytoplasmic membrane acts as an “eat me” signal for phagocytes. Phosphatidylserine recognition of activated phagocytes inhibits proinflammatory cytokine production through induction of active anti-inflammatory or suppressive properties in macrophages [19]. In addition, PS-containing liposomes have been demonstrated to mimic the effects of apoptotic cells to induce secretion of anti-inflammatory mediators [13]. Furthermore, a few studies have addressed this question in vivo. An interesting study revealed
that PS liposomes have anti-inflammatory effects in vivo by apoptotic mimicry, which at least partly activates peroxisome proliferator–activated receptors [20]. These previous studies raise the potential of PS for treating inflammatory disorders.

The main finding of the present study is direct evidence that PS has anti-inflammatory effects. Phosphatidylserine was effective not only in reversing joint hyperalgesia and inflammatory signs in rats with an inflamed ankle joint but also in inhibiting IL-1β–induced inflammation in RA-FLS.

Because FLS are key players in the propagation of joint inflammation and joint destruction in RA [3], the anti-inflammatory effects of PS were examined in IL-1β–stimulated FLS from RA patients. Because IL-1β is believed to play a major role in synovial inflammation, RA-FLS stimulated with IL-1β in vitro have been used to mimic the synovial proliferation that occurs in RA [21]. We found that PS slightly, but statistically, inhibited production of 2 important proinflammatory mediators, IL-6 and PGE2, in IL-1β–stimulated RA-FLS. Inhibition of PGE2 production is important because it is a key mediator of inflammatory pain sensitization [22]. This result was consistent with antinociceptive effects in animal models. Two animal models, carrageenan-induced hyperalgesia and carrageenan-induced acute arthritis, were used to test the in vivo analgesic or antiarthritic effects of PS. Phosphatidylserine showed a significant analgesic effect in carrageenan-induced hyperalgesia and an effective improvement in arthritic symptoms in carrageenan-induced acute arthritis, on par with prednisolone.

In addition, PS showed a slight, but statistically significant, inhibition of VEGF and IL-8, which are important angiogenic mediators [23]. This suggests that neovascularization may be inhibited in the inflamed joints of arthritic rats. Interestingly, PS did not affect the expression of matrix metalloproteinase 1 and matrix metalloproteinase 13 in IL-1β–stimulated RA-FLS (data not shown). They play an important role in the pathogenesis of RA because they are rate-limiting components in the collagen-degradation process [24]. Thus, PS may have an antiarthritic effect with respect to inhibition of inflammation and neovascularization but not collagen degradation.

Little is known regarding the uptake and pharmacokinetics of PS following oral administration in humans. In rats, however, although most exogenous PS after oral administration is likely extensively hydrolyzed to lysoPS and degraded in the small intestine, small fractions of PS remain available and may reach the systemic circulation [25]. In addition, lysoPS, after irreversible diffusion into intestinal cells, is sequentially converted into PS and phosphatidylethanolamine or further degraded [26]. Furthermore, in vitro studies have shown that exogenous PS is rapidly integrated into cell membranes [27]. Therefore, repeated administration of PS may have caused an increase in the PS content in the systemic circulation and incorporation of PS within cells in the arthritic joint. However, the possibility still cannot be excluded that the antiarthritic effect of PS is caused by the fatty acid composition of PS.

To further understand the possible mechanism of PS in IL-1β–mediated inflammation in FLS, the effect of PS on intracellular signal transduction was tested. The MAPKs are induced by proinflammatory cytokines such as IL-1β and regulate expression of multiple proinflammatory genes, matrix metalloproteinases, and adhesion molecules [7]. Phosphatidylserine completely inhibited phosphorylation of JNK and p38 stimulated by IL-1β. However, phosphorylation of
c-Jun, a downstream effector of the JNK pathway, by IL-1β was only partially inhibited by PS. Although c-Jun is mainly phosphorylated by JNK, it could also be phosphorylated by kinases such as ERK1/2 and IκB kinase ε [28,29], which could explain the imbalanced correlation between them. The phosphorylated form of IκB was upregulated by IL-1β stimulation, and this was inhibited by PS treatment. These in vitro data demonstrated that PS treatment in RA-FLS could lead to inhibition of NF-κB activation.

Our results support the hypothesis that PS is capable of reducing inflammatory response via inhibition of the NF-κB and JNK/p38 MAPK pathway, resulting in antiarthritic effects. However, this study is limited by the fact that the analgesic and antiarthritic effects of PS were verified in carrageenan-induced inflammatory arthritic rats using only physical parameters such as edema and pain. The effects of PS at the cellular and molecular levels and the molecular mechanisms by which PS suppress arthritic symptoms remain to be elucidated in vivo and should be investigated in the near future.

In closing, these results indicate that PS has antiarthritic effects in animal models and anti-inflammatory effects in IL-1β-stimulated FLS by inhibition of IκBα phosphorylation and JNK and p38 MAPK phosphorylation. Thus, PS has the potential to be a therapeutic drug or dietary supplement for treatment of inflammatory diseases such as RA.

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