Effect of Astragli Radix Extract on Lipopolysaccharide-Induced Inflammation in Human Amnion

Yun-Hee Shon, Jong-Ho Kim, and Kyung-Soo Nam

Department of Pharmacology, College of Medicine and Intractable Disease Research Center, Dongguk University, Sukjong-Dong 707, Kyongju 780–714, Korea and Department of Obstetrics and Gynaecology, College of Medicine, Dongguk University, Kyongju 780–714, Korea. Received August 30, 2001; accepted October 10, 2001

The effects of Astragali radix extract on interleukin (IL)-6 and tumor necrosis factor (TNF-α) productions, prostaglandin E₂ (PGE₂) biosynthesis, and leukotriene C₄ (LTC₄) production from lipopolysaccharide (LPS)-stimulated human amnion cells were investigated. Amnion cells produced detectable amounts of both IL-6 and TNF-α under LPS-stimulated conditions. Astragalus extract inhibited the production of IL-6. However, TNF-α production was not inhibited by the extract on L929 cytotoxicity assay. Treatment of amnion cells with LPS for up to 24 h resulted in an increase in PGE₂ release in a concentration- and time-dependent manner. The extract (150 mg/ml) significantly inhibited the output of PGE₂ by amnion cells (p<0.01). The arachidonate lipoxigenase metabolite (LTC₄) was increased by LPS treatment of amnion cells. Astragalus extract (30 mg/ml) inhibited LTC₄ production by approximately 65% throughout the culture period. These results suggest that Astragali radix extract may have a role in inhibiting bacterial infection-associated preterm labor by suppressing the productions of IL-6, PGE₂, and LTC₄ by human amnion cells.

Key words Astragali radix; interleukin-6; prostaglandin E₂; leukotriene C₄; preterm labor

Preterm labor and delivery remain the major cause of perinatal morbidity and mortality. A significant proportion of preterm labor is associated with intrauterine infection. Bacterial products such as endotoxins present in the amniotic fluid in cases of intraamniotic infection could stimulate host cells to produce inflammatory cytokines, such as interleukin (IL)-6, tumor necrosis factor (TNF-α), IL-8, and IL-1β, which in turn stimulate prostaglandin (PG) biosynthesis by human amnion. The prostaglandins, specifically prostaglandin E₂ (PGE₂) and prostaglandin F₂α (PGF₂α), promote uterine activity by acting locally on the myometrium. Arachidonate lipoxigenase products are generated during the course of inflammatory reactions and can be synthesized by human intrauterine tissues. These products have been found to be involved in the development of uterine contractions, and they may participate in the mechanism of labor that is associated with or the result of an inflammatory reaction. Astragali radix is the dried root of Astragalus membranaceus Bunge and is a well-known tonic in traditional Chinese medicine. A tonic is thought to nourish and strengthen the host defense systems. It was reported that Astragalus extract enhanced various types of immune response such as cytokine production by lymphocytes, restoration of macrophase function depressed by cancer cells, and cytotoxicity of natural killer or lymphokine-activated killer cells, and exert an antiinflammatory effect. Human labor at all gestational ages has strong similarities to a general inflammatory response; from this it has been suggested that antiinflammatory factors will counteract these changes and thus sustain pregnancy.

In this paper, the effects of Astragali radix extract on the production of IL-6 and TNF-α, PGE₂ biosynthesis, and leukotriene C₄ (LTC₄) production in response to lipopolysaccharide (LPS) were investigated in human amnion cells.

MATERIALS AND METHODS

Plant Material and Extraction The roots of A. membranaceus Bunge were collected in Jeong-seon, Korea. A voucher specimen (no. 00A-20) has been deposited in the herbarium of the Intractable Disease Research Center, Dongguk University, Kyongju, Korea. Dried Astragali radix (60 g) was extracted with water. The extract was filtered, and the filtrate (100 ml) was concentrated in vacuo and lyophilized. The lyophilized extract (2.92 g) of Astragali radix was dissolved in tissue culture medium. The effects of Astragalus radix extract on interleukin (IL)-6 and tumor necrosis factor (TNF-α) productions, prostaglandin E₂ (PGE₂) biosynthesis, and leukotriene C₄ (LTC₄) production from lipopolysaccharide (LPS)-stimulated human amnion cells were investigated. Amnion cells produced detectable amounts of both IL-6 and TNF-α under LPS-stimulated conditions. Astragalus extract inhibited the production of IL-6. However, TNF-α production was not inhibited by the extract on L929 cytotoxicity assay. Treatment of amnion cells with LPS for up to 24 h resulted in an increase in PGE₂ release in a concentration- and time-dependent manner. The extract (150 mg/ml) significantly inhibited the output of PGE₂ by amnion cells (p<0.01). The arachidonate lipoxigenase metabolite (LTC₄) was increased by LPS treatment of amnion cells. Astragalus extract (30 mg/ml) inhibited LTC₄ production by approximately 65% throughout the culture period. These results suggest that Astragali radix extract may have a role in inhibiting bacterial infection-associated preterm labor by suppressing the productions of IL-6, PGE₂, and LTC₄ by human amnion cells.

Key words Astragali radix; interleukin-6; prostaglandin E₂; leukotriene C₄; preterm labor

© 2002 Pharmaceutical Society of Japan
IL-6 Production Release of IL-6 into the culture medium was quantitated by using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Inc., Minneapolis, MN, U.S.A.). The manufacturer’s instructions for the kit were followed in performing the assay. Briefly, the sample media were diluted in plain media for IL-6 assay. Standard curves were developed using duplicate samples of known quantities of recombinant human IL-6. Sample concentrations were determined by correlating the absorbance obtained to the standard curve by linear regression analysis. Controls included plain media and LPS- or sample-containing media incubated in wells without tissue. Colorimetric absorption was read at 450 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, U.S.A.).

Quantitation of TNF-α The activity of TNF-α in conditioned culture medium from amnionic cells was assayed by modification of the standard L-929 cell cytotoxicity assay.4) Mouse L929 fibroblast cells in DMEM containing 10% FBS were grown overnight at 37°C in 96-well flat-bottomed trays at 5 × 10^4 cells per well in the presence of 1 μg/ml of actinomycin D in a 5% CO2 incubator. Subsequently, culture medium was removed and replaced with the conditioned culture medium of amnion cells. After 18 h incubation, viable cells were estimated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. The cells exposed to culture medium alone were set at 0% lysis.

Prostaglandin E2 Release Both the time-course and dose-response effects of LPS on PGE2 release by amnion cells were determined. To establish the effect of incubation time on LPS-stimulated PGE2 release, amnionic cells were incubated in the absence (control) or presence of LPS (100 ng/ml) for up to 24 h. Medium was collected at various times (0, 4, 8, 12, 24 h) after incubation. The incubation medium was stored at −20°C for subsequent PGE2 immunoassay.

The effect of LPS concentration on the in vitro release of PGE2 was established by incubating amnion cells in the absence (control) and presence of increasing concentrations of LPS (0.1, 1.0, 10, 100, 1000 ng/ml) for 16 h. After incubation, the medium was collected and stored at −20°C until assayed for PGE2 concentration. PGE2 release was expressed as a percentage of that released during control incubation. On the basis of the data obtained in these experiments, the effect of Astragalus extract on the release of PGE2 in LPS-treated human amnion cells was studied.

The PGE2 content of the conditioned media was assayed using the Cayman Chemical enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, U.S.A.), according to the manufacturer’s protocol. The collected conditioned media were diluted so that the levels of PGE2 were within the standard curve of the assay system. PGE2 in culture medium was assayed and compared spectrophotometrically to the standard curve. The final results are expressed as picogram per microgram of protein.

Leukotriene C4 Assay LTC4 was assayed directly in the conditioned culture media using the Cayman Chemical enzyme immunoassay kit (Cayman chemical) according to the manufacturer’s protocol. The assay is based on the competition between free LTC4 and an LTC4 tracer (LTC4 linked to an acetylcholinesterase molecule) for a limited amount of LTC4-specific rabbit antiserum binding sites. The anti-LTC4 antibody has <0.01% cross-reactivity with LTα and LTβ. The intraassay and interassay coefficients of variation are <10%. A determination range of this assay is 7.8 to 1000 pg/ml.

Statistical Analysis The data were analyzed for statistical significance using Student’s t-test. p Values less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

LPS induced IL-6 production in a concentration-dependent manner (Fig. 1). Significant, albeit modest, effect was seen at the lowest concentration tested (10 ng/ml). LPS-induced production of IL-6 was markedly inhibited by cycloheximide (Table 1), indicating that induction of production required new protein synthesis and was not a reflection of the increased release of stored material. Astragalus extract substantially inhibited the increased IL-6 output (Fig. 2).

Elevated cytokine concentrations in amniotic fluid are highly indicative of the presence of an intraamniotic infection. The measurement of IL-6 has been promulgated as useful in detecting ascending infections and predicting premortem delivery because of the very considerable increase in the level of IL-6 in amniotic fluid under conditions of infection. Our finding that IL-6 production is stimulated by LPS is consistent with the amnio’s role as a contributor to the IL-6 content of amniotic fluid in infected pregnancies.

Human labor at all gestational ages has strong similarities to a general inflammatory response; from this it has been suggested that antiinflammatory factors will counteract these changes. There are many reports that Astragalus extract enhances various types of immune response.5,6) It was demon-

Table 1. Effect of Cycloheximide on Basal and LPS-Stimulated Production of IL-6 by Amnion Cells in Culture

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IL-6 (pg/μg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td>Cycloheximide (10 μg/ml)</td>
<td>2.7±0.7</td>
</tr>
<tr>
<td>LPS (100 ng/ml)</td>
<td>11.3±1.0</td>
</tr>
<tr>
<td>LPS + cycloheximide</td>
<td>4.1±0.2</td>
</tr>
</tbody>
</table>

Fig. 1. Concentration-Dependent Effect of LPS on IL-6 Production by Amnion Cells in Primary Culture
Data shown are mean values with bars indicating the S.D. of the mean (n=3). 
*p<0.05, **p<0.01 compared with control.
strated that antiinflammatory T helper 2 (Th2) cell activity in normal and immunodepressed mice is increased by *Astragalus* extract.5,7) A series of studies have shown that IL-10 can inhibit the production of other inflammatory cytokines (IL-6, IL-8, and TNF-α) from human fetal membranes.8—10) It is possible that the inhibition of IL-6 production in LPS-stimulated human amnion cells is mediated by *Astragalus* extract induced amplification of cytokines that have antiinflammatory activities such as IL-10 or IL-4.

TNF-α release from amnion cells was also increased in response to LPS in this study (data not shown). However, *Astragalus* extract did not inhibit the increased TNF-α output by LPS (Table 2). Romero et al.11) suggested that preterm labor and premature rupture of the membrane with microbial invasion of the intraamniotic cavity were associated with increased concentrations of TNF-α in the amniotic fluid. The inducibility of TNF-α mRNA expression by LPS in amnion after 48 h in culture has been documented, whereas control amnion is negative for this mRNA after 48 h.12) To establish the time course of the effect of LPS on the release of PGE2, amnion cells were incubated in the absence (control) or presence of 100 ng LPS/ml for up to 24 h. Figure 3 summarizes the effect of LPS on the release of PGE2 into incubation medium. In the presence of LPS (100 ng/ml), the rate of PGE2 release during the incubation period was 3.5-fold greater than that observed in control incubations. Comparison of group means revealed no statistically significant difference between the release by control and LPS-treated amnion cells up to and including 4 h of incubation; however, all comparisons for time points after 4 h of incubation showed significant differences (*p*<0.05, *p*<0.01). Figure 4 illustrates the effect of LPS on production of PGE2 by amnion cells. Endotoxin consistently and significantly increased PGE2 production. The results of this study demonstrate that LPS can increase the rate of biosynthesis of PGE2 by human amnion cells. Coincubation with *Astragalus* radix extract diminished the effect of LPS on PGE2 production (Fig. 5).

Although the mechanisms responsible for the onset of labor in patients with intraamniotic infection are poorly un-
understood, a role for PG is suggested by the increased concentration in amniotic fluid in women with intraamniotic infection. The mechanisms for the increased bioavailability have not been elucidated. Thus far, research has focused on the role of bacteria. Microorganisms indigenous to the genital tract have been found to have phospholipase activity and to stimulate the production of PGE₂ by amnion cells in culture.

IL-4 has several antiinflammatory properties, including suppression of the production of TGF-α, IL-1, and PGE₂ and up-regulation of the synthesis of IL-1 receptor antagonist. Thus Astragalus extract may induce amplification of IL-4 and inhibit PGE₂ production.

We next evaluated the action of LPS on LTC₄ production in the cells. Our data indicate that LPS stimulates LTC₄ production from amnion cells (Fig. 6). These findings are consistent with the possibility that LTC₄ may play a role in preterm labor. A clinical study suggests some relationship between preterm labor and LTC₄ generation. A study of women with and without preterm labor showed that those with preterm labor responsive to tocolytic agents have lower amniotic fluid concentrations of LTC₄ than do those who failed tocolysis (13) suggesting that preterm labor with delivery is associated with activation of the 5-lipoxygenase pathway. Intraamniotic infection may thus elicit a host response that includes secretion of inflammatory cytokines from gestational tissues, which in turn may stimulate LTC₄ production and inhibit PGE₂ production.

We conclude that Astragalus radix extract has antiinflammatory effect in LPS-stimulated human amnion cells. Astragalus radix may be considered a promising agent to prevent preterm labor because of its multiple antiinflammatory activities in human fetal membrane. However, analysis of the effects of Astragalus radix at the cellular and molecular levels is likely to provide an understanding of the basic mechanisms needed for rational application of Astragalus radix extract in modern medicine.

Acknowledgment This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (01-PJ2-PG3-21602-0004).

REFERENCES