Effect of Theophylline on the Production of Interleukin-1β, Tumor Necrosis Factor-α, and Interleukin-8 by Human Peripheral Blood Mononuclear Cells

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Theophylline is a mild bronchodilator and has significant extrapulmonary effects, but it may also have some anti-inflammatory properties. We investigated the immunological effects of theophylline on peripheral blood mononuclear cells (PBMC), by examining the production of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin 8 (IL-8) when PBMC were stimulated with lipopolysaccharide (LPS) or recombinant human IL-1β (rhIL-1β). At concentrations ≥50 μg/ml, theophylline suppressed the proliferative activity of PBMC stimulated with phytohemagglutinin (p < 0.05). IL-1β production showed 23% suppression by 10 μg/ml theophylline (p < 0.05), while the suppression was 26% at 25 μg/ml (p < 0.05), 30% at 50 μg/ml (p < 0.05), and 33% at 100 μg/ml (p < 0.05). TNF-α production was suppressed in a dose-dependent manner by theophylline, being decreased by 24% at 10 μg/ml (p < 0.05), by 29% at 25 μg/ml (p < 0.05), by 41% at 50 μg/ml (p < 0.01), and by 54% at 100 μg/ml (p < 0.01). IL-8 production, in contrast, was not affected by theophylline. rhIL-1β induced IL-8 production in a dose-dependent manner at concentrations of 1—100 units/ml, and theophylline (particularly at concentrations of 50 and 100 μg/ml), increased IL-8 production in the presence of rhIL-1β.

Suppression of the production of IL-1β and TNF-α by therapeutic levels of theophylline suggested that this drug might have anti-inflammatory and immunosuppressive effects.

Key words theophylline; interleukin-1β; tumor necrosis factor-α; interleukin-8; mononuclear cell

Theophylline has been widely used in the treatment of patients with bronchial asthma and chronic obstructive pulmonary disease. Although its bronchodilatory effect is considered to be its main pharmacological action, the precise mechanisms involved are uncertain. It is widely held that the bronchodilatory effect of theophylline is due to inhibition of phosphodiesterase (PDE), which breaks down cyclic nucleotides and thereby causes an increase of intracellular cyclic 3', 5'-adenosine monophosphate (cAMP) and 3',5'-guanosine monophosphate (cGMP). However, theophylline is a weak nonselective PDE inhibitor, and the total PDE activity in human lung extracts shows only 5—20% inhibition at therapeutic concentrations of this drug. Other proposed mechanisms for the bronchodilatory effect of theophylline include the translocation of intracellular calcium, prostaglandin antagonism, stimulation of endogenous catecholamine release, beta-agonist activity, and adenosine receptor antagonism.

The weak bronchodilatory action of theophylline at therapeutically relevant concentrations raises the question of whether bronchodilation is important for its antiasthma effect. There is increasing evidence that theophylline has anti-inflammatory properties other than bronchodilation, including anti-inflammatory and immunomodulatory actions. Previous studies have shown that theophylline has an inhibitory effect on the release of reactive oxygen species by human polymorphonuclear leukocytes, peripheral blood monocytes, and alveolar macrophages, as well as inhibiting the proliferative response of T lymphocytes to mitogens, the release of interleukin-2 from T lymphocytes, and the influx of inflammatory cells after allergen inhalation in animal models. Ward et al. reported that theophylline, at a mean trough serum concentration of 7.8 μg/ml, inhibited the late asthmatic reaction following allergen challenge and the allergen-induced increase of CD4+ and CD8+ lymphocytes in the peripheral blood at 48h after allergen challenge. Sullivan et al. recently reported an anti-inflammatory effect of oral theophylline in a double-blind placebo-controlled study on the inflammatory response of the bronchial mucosa to allergen inhalation in 19 atopic asthmatic subjects.

Interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and interleukin-8 (IL-8) are recognized as important monocyte/macrophage-derived cytokines, which act on various target tissues. IL-1 and TNF-α are primary regulators of inflammation and stimulate the immune system in general, while IL-8 is a neutrophil chemotactic and activating factor that has a major role in the mediation of inflammatory responses. Recently, many investigators have reported that airway inflammation is a common pathologic feature in asthma patients and these cytokines may mediate the development of asthma.

In the present study, we investigated the effect of theophylline on the production of interleukin-1β (IL-1β), TNF-α, and IL-8 by the peripheral blood mononuclear cells (PBMC) stimulated with lipopolysaccharide (LPS) to research the anti-inflammatory action of theophylline.

MATERIALS AND METHODS

Materials Theophylline was obtained from Sigma. It was dissolved in RPMI 1640 medium at 1 mg/ml and further diluted in RPMI 1640 medium to reach final concentrations ranging from 5.0 to 100 μg/ml.

RPMI 1640 culture medium and phosphate-buffered

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saline (PBS) (−) were obtained from Nissui Pharmaceuticals, fetal calf serum (FCS) was from Gibco, and phytohemagglutinin (PHA) and Escherichia coli LPS were from Difco. In addition, 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and dimethylsulfoxide (DMSO) were obtained from Dojin Chemicals, lymphocyte separation medium was from Organon Teknika, and the 24-well plate came from Falcon. Recombinant human IL-1β (rhIL-1β) and the human TNF-α enzyme-linked immunosorbent assay (ELISA) kit were from Genzyme, the human IL-1β ELISA kit was from Immunotech, and the human IL-8 ELISA kit was from R & D Systems. A Fujirebio MPR A4i microplate reader was used for the assays.

Preparation of Peripheral Blood Mononuclear Cells
PBMC were obtained from the heparinized peripheral blood from healthy donors using lymphocyte separation medium. The cells were then washed with PBS (−) and resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 50 U/ml penicillin, and 50 μg/ml streptomycin. Then they were cultured at 37 °C in a humidified atmosphere of 5% CO₂/95% air.

Proliferative Activity of PBMC
PBMC (2 × 10⁵ cells/ml) were incubated with or without various concentrations of theophylline and 5 μg/ml PHA. After 72 h incubation, proliferative activity was determined by the MTT assay.²³

MTT was dissolved in PBS (−) to a concentration of 5 mg/ml, and 100 μl was added to each well of a 24-well plate, after which incubation was performed for 4 h at 37 °C. Then the plate was centrifuged for 15 min at 2000 rpm, the supernatant was removed, and 1500 μl of DMSO was added to dissolve the MTT formazan produced. When the formazan was fully dissolved, the absorption was measured at a wavelength of 540 nm and a reference wavelength of 620 nm using a microplate reader.

Production and Assay of Cytokines
Preliminary experiments on an appropriate concentration of LPS and incubation time were made to examine the effects of theophylline on the production of IL-1β, TNF-α, and IL-8 by PBMC. To avoid any waste of PBMC, we decided on the following culture conditions: PBMC were cultured at 37 °C in a 24-well plate with 10 μg/ml LPS in a humidified atmosphere of 5% CO₂/95% air. Culture supernatant was collected by centrifugation at 2000 rpm for 10 min, and was stored at −30 °C until use. The levels of IL-1β, TNF-α, and IL-8 were measured using a human IL-1β ELISA kit, a human TNF-α ELISA kit, and a human IL-8 ELISA kit, respectively. The detection limit for IL-1β, TNF-α, and IL-8 was 5, 15, and 31.3 pg/ml, respectively. In some experiments, we examined the effect of theophylline on the rhIL-1β-induced IL-8 production by the same method as described above.

Statistical Analysis
Data were analyzed using the analysis of variance (ANOVA)-based Student’s paired t-test. Results were expressed as the mean ± standard error of the mean (S.E.M.) for four experiments.

RESULTS
The effect of theophylline on the proliferative activity of PBMC stimulated with or without PHA was measured by the MTT assay after incubation for 72 h. As shown in Fig. 1, theophylline had almost no effect on unstimulated PBMC after 72 h of incubation. However, in the case of PHA-stimulated PBMC, theophylline inhibited proliferative activity at concentrations of more than 50 μg/ml, with more than 50 μg/ml causing a significant decrease (p < 0.05).

We examined the effects of various concentrations of theophylline on the production of IL-1β, TNF-α, and IL-8 by PBMC which had been preincubated with or without theophylline for 2 h and stimulated with LPS for 24 h. IL-1β production showed 23% suppression by 10 μg/ml theophylline (p < 0.05), while the suppression was 26% at 25 μg/ml (p < 0.05), 30% at 50 μg/ml (p < 0.05), and 33% at 100 μg/ml (p < 0.05) (Fig. 2). TNF-α production was suppressed in a dose-dependent manner by theophylline, being decreased by 24% at 10 μg/ml (p < 0.05), by 29% at 25 μg/ml (p < 0.05), by 41% at 50 μg/ml (p < 0.01), and by
Table 1. IL-8 Production by PBMC Stimulated with Various Concentrations of rhIL-1β

<table>
<thead>
<tr>
<th>rhIL-1β (units/ml)</th>
<th>IL-8 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>976 ± 178</td>
</tr>
<tr>
<td>1</td>
<td>983 ± 203</td>
</tr>
<tr>
<td>10</td>
<td>2465 ± 451</td>
</tr>
<tr>
<td>50</td>
<td>6988 ± 890</td>
</tr>
<tr>
<td>100</td>
<td>7140 ± 865</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of four experiments.

DISCUSSION

The results presented here show that theophylline inhibits IL-1β and TNF-α (but not IL-8) production by PBMC stimulated with LPS, and increases rhIL-1β-induced IL-8 production. Theophylline suppressed TNF-α production in a dose-dependent manner at concentrations ranging from 10 to 100 μg/ml (Fig. 3), while it had a limited suppressive effect on IL-1β production (Fig. 2). Its suppressive effect on the production of these cytokines was probably based on decreased gene transcription, as has been demonstrated for pentoxifylline. A therapeutic serum level of theophylline is usually considered to be 10–20 μg/ml. Sennier et al. have reported that theophylline suppressed LPS-induced production of TNF-α but not IL-1β by human mononuclear cells. However, we found that 10 μg/ml theophylline achieved 23% and 24% inhibition of IL-1β and TNF-α, respectively (Figs. 2 and 3), which suggests that therapeutic levels of theophylline should be sufficient to inhibit the production of these cytokines by PBMC. The difference in the results may be due to the differences in experimental conditions such as the concentration of LPS and patient background. In any event, our present data suggest that theophylline may have an anti-inflammatory effect even at relatively low plasma concentrations.
Theophylline is a nonselective PDE inhibitor and it causes an increase of intracellular cAMP. Evidence that cAMP inhibits production of TNF-α by LPS-stimulated murine peritoneal exudate macrophages and human monocytes has been obtained by treating these cells with dibutyl cAMP or prostaglandins to stimulate adenylate cyclase.\textsuperscript{20-22} Either of these treatments reduces the amount of detectable mRNA for TNF-α, which indicates that cAMP regulates this cytokine at the transcriptional level.\textsuperscript{21} Conflicting reports have been published regarding the inhibition of IL-1 production by cAMP-regulating compounds. Some authors report that total IL-1 production is not inhibited in human mixed mononuclear cells or adherent monocytes after treatment with prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) or nonspecific PDE inhibitors.\textsuperscript{22,23} However, Vihervuoto et al.\textsuperscript{24} reported that cAMP and PGE\textsubscript{2} reduce the amount of secreted IL-1β from LPS-stimulated adherent monocytes without affecting steady-state mRNA levels or cell-associated IL-1β, and cAMP interferes with secretion of IL-1β rather than with other steps in the biosynthetic pathway. Although the present study did not look into how theophylline regulates the production of TNF-α and IL-1β, intracellular cAMP elevated by theophylline would contribute to the suppressive effect on production of these cytokines. 

IL-1β and TNF-α are nonchimeratic cytokines, but they induce production of IL-8 (a potent neutrophil chemotactic and activating factor), by LPS-stimulated monocytes or endothelial cells \textit{in vitro}.\textsuperscript{25,26} In addition to being a potent neutrophil chemoattractant, IL-8 also has a wide range of other pro-inflammatory effects. For example, it induces the expression of CD11/CD18 cell adhesion molecules and enhances the adherence of neutrophils to endothelial cells and subendothelial matrix proteins.\textsuperscript{27} Although theophylline suppressed IL-1β and TNF-α production by LPS-stimulated PBMC, it had no direct effect on IL-8 production. IL-8 production could be suppressed by decreasing IL-1β or TNF-α, which are IL-8 inducers, but it was not affected by theophylline. Therefore, we examined the effect of theophylline on rhIL-1β-induced IL-8 production (Fig. 5). In this case IL-8 production was potentiated by theophylline. This may be the reason why theophylline did not affect the LPS-induced IL-8 production.

We conclude that theophylline suppresses IL-1β and TNF-α production by LPS-stimulated PBMC, suggesting that it may have an anti-inflammatory effect. Although theophylline is an old drug, we still have much to learn about its mechanism of action in asthma. Further studies are necessary to evaluate its anti-asthma properties other than the well-known bronchodilatory effect.

REFERENCES