Inhibition of Cytokine Release From Alveolar Macrophages in Pulmonary Sarcoidosis by Pentoxifylline*

Comparison With Dexamethasone

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Study objectives: Pentoxifylline (POF) has been shown to suppress the cytokine production from lipopolysaccharide (LPS)-stimulated monocytes/alveolar macrophages (AMs). Sarcoidosis is a granulomatous disease that is driven by the action of tumor necrosis factor (TNF)-α and other proinflammatory cytokines. In this study, we aimed to investigate the effects of POF on the production of TNF-α, interleukin (IL)-1β, IL-6, IL-8, IL-10, and the soluble TNF receptors (sTNFRs) 1 and 2 from AMs in sarcoidosis, and we also compared them with those of dexamethasone (DEX).

Methods: AMs from 14 patients with sarcoidosis were cultured for 24 h with RPMI medium alone or with LPS (100 ng/mL), and with POF at concentrations of 0.01, 0.1, and 1 mmol/L, or with 0.1 mmol/L DEX. Cytokines in the culture supernatants were analyzed by enzyme-linked immunosorbent assay.

Results: The results showed that POF induced a dose-dependent suppression of the spontaneous TNF-α release from AMs in sarcoidosis (p < 0.001), and that the spontaneous release of the other cytokines was unaffected by POF at all tested concentrations, but a trend for the inhibition of IL-10 production was found (p = 0.092). DEX inhibited the spontaneous release of TNF-α (p < 0.001), sTNFR2 (p < 0.05), IL-1β (p < 0.05), and IL-10 (p < 0.01). POF also suppressed the LPS-stimulated production of these cytokines except for that of sTNFR1. Similar to POF, DEX inhibited the LPS-stimulated production of these cytokines, but not that of sTNFR1 and IL-1β.

Conclusions: Compared with DEX, POF may improve therapeutic regimens in patients with sarcoidosis either by sparing or by replacing corticosteroids. However, the precise clinical value of POF in the treatment of sarcoidosis and other lung diseases will have to be determined in further clinical trials.

Key words: alveolar macrophages; cytokine production; pentoxifylline; sarcoidosis

Abbreviations: AM = alveolar macrophage; DEX = dexamethasone; IL = interleukin; LPS = lipopolysaccharide; PBMC = peripheral blood mononuclear cell; PDE = phosphodiesterase; POF = pentoxifylline; sTNFR = soluble tumor necrosis factor receptor; Th = T helper; TNF = tumor necrosis factor

Pentoxifylline (POF) is a methylxanthine that was used initially in therapy for peripheral vascular disease. It has become evident that this drug has important effects on immune cells and immune-mediated phenomena. It is able to inhibit cytokine production by macrophages/monocytes and whole blood cells. Among its immunologic effects, the inhibition of POF on tumor necrosis factor (TNF) production is of great importance. POF also suppresses the production of other cytokines from peripheral blood mononuclear cells (PBMCs) or whole-blood cells. In contrast to the well-documented and commonly accepted anti-TNF-α activity of POF, its role in the inhibition of the production of other cytokines is still unclear and is still unclear...
sometimes controversial, with divergent effects observed depending on the experimental conditions.4–8

Sarcoidosis is a multiorgan disorder of unknown origin that is characterized in the affected organs by a T-lymphocyte-mononuclear phagocyte infiltration, granuloma formation, and distortion of the normal microarchitecture.10 In the course of the sarcoid alveolitis, an abundance of cytokines, soluble cytokine receptors, and soluble adhesion molecules that are capable of attracting and activating immune cells, and of inducing and maintaining granulomas are released by alveolar macrophages (AMs), T cells, and epithelial cells. AMs behave as versatile secretory cells, which release a great variety of cytokines in sarcoidosis patients, including TNF-α, interleukin (IL)-1, IL-6, IL-8, IL-10, IL-12, IL-15, granulocyte-macrophage colony-stimulating factor, and transforming growth factor-β.11–17

So far, there are no data regarding the effects of POF on the production of these cytokines from AMs in sarcoidosis, except for our previous report, which showed that POF inhibits the spontaneous production of TNF-α from AMs in sarcoidosis.3 In this study, we were interested in investigating further the effects of POF on the soluble TNF receptors (sTNFRs) 1 and 2, on the production of other cytokines, such as IL-1β, IL-6, IL-8, and IL-10, from AMs in sarcoidosis, and in comparison with the effects of dexamethasone (DEX), which is a member of the glucocorticoid group of potent immunosuppressive and anti-inflammatory agents.

**MATERIALS AND METHODS**

**Subjects**

Fourteen consecutive patients with active pulmonary sarcoidosis (six women and eight men; age range, 32 to 56 years; all nonsmokers) were investigated. The diagnosis was established on the basis of compatible clinical and radiographic features, histologic evidence of noncaseating granulomata on transbronchial biopsy specimens, or an increased CD4/CD8 ratio in BAL fluid, and the exclusion of other granulomatous lung diseases.13 The criteria of disease activity were as follows: (1) recently developed symptoms or increasing symptoms such as cough, dyspnea, weakness, fever, and arthralgia; and/or (2) chest radiographic evidence of progressive disease; and/or (3) deterioration of findings of lung function tests. According to chest roentgenographic staging, four patients had stage I disease, nine patients had stage II disease, and one patient had stage III disease. No patient was receiving treatment with steroids. Written informed consent was obtained according to institutional guidelines.

**BAL Procedure**

BAL was performed via a fiberoptic bronchoscope. Sterile isotonic saline solution was instilled into the right middle or left lingular lobe in 10 20 mL aliquots to a total volume of 200 mL, with immediate aspiration by gentle suction after the instillation of each aliquot. A volume of >50% was retrieved. The recovered BAL fluid was filtered through two layers of sterile gauze and subsequently was centrifuged at 500g for 10 min at 4°C. The cells were counted in a hemocytometer. Cell viability was assessed by Trypan blue exclusion. Cell differentials were made on smears stained with May-Grünwald-Giemsa by counting 600 cells. Immunocytochemical staining was performed to obtain a CD4/CD8 count.

**Cell Culture**

AM cell cultures were performed as previously described.14 After three washings with phosphate-buffered saline solution, the BAL cells were resuspended to a final concentration of 1 × 10^6 cells/mL in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mmol/L L-glutamine, 200 U/mL penicillin, and 200 μg/mL streptomycin (Seromed; Biochrom KG; Berlin, Germany). The cell suspension was added at 1 × 10^6 cells per well to a 24-well plastic tissue culture plate (Falcon; Becton Dickinson; Franklin Lakes, NJ) and was incubated at 37°C in a 5% CO2 humidified atmosphere for 1 h to permit the adherence of AMs. The nonadherent cells were removed by three washes with culture medium. The purity of the adherent AMs was identified to be >95% by morphology and nonspecific esterase staining. The purified AMs were incubated for an additional 24 h with 1 mL RPMI 1640 medium alone, with 1 mL RPMI 1640 medium and lipopolysaccharide (LPS) [100 ng/mL; Sigma Chemical Co; St. Louis, MO], or with 1 mL RPMI 1640 in the absence and presence of LPS (100 ng/mL) together with POF (Sigma) at concentrations of 0.01, 0.1, and 1 mmol/L, or with 0.1 mmol/L DEX (Sigma). The culture supernatants of the AMs were harvested and centrifuged, then were stored in fractions at −80°C until analysis.

**Assay for TNF-α, sTNFR1, sTNFR2, IL-1β, IL-6, IL-8, and IL-10**

The concentrations of TNF-α, sTNFR1, sTNFR2, IL-1β, IL-6, IL-8, and IL-10 in culture supernatants were quantified using commercially available human enzyme-linked immunosorbent assay kits (Endogen, Inc, Woburn, MA; sTNFR1 and sTNFR2, HyCult Biotechnology, Uden, the Netherlands) with sensitivity of 5, 25, 25, 1, 1, 2, and 3 pg/mL, respectively. The concentrations of above-measured TNF-α, sTNFR1, sTNFR2, IL-1β, IL-6, IL-8, and IL-10 were expressed as picograms per milliliters per 10^6 AMs after correction for the proportion of AMs.

**Statistical Analysis**

Data are expressed as the mean ± SEM. The figures show the median and the 25th to 75th percentiles as box-plot diagrams. Within each group, the data were analyzed using Kruskal-Wallis one-way analysis of variance on ranks. A p value of <0.05 was accepted as statistically significant.

**RESULTS**

**Effects of POF and DEX on Spontaneous Cytokine Production From AMs in Sarcoidosis**

As shown in Table 1, POF induced a dose-dependent suppression of TNF-α release. At a POF concentration of 0.1 mmol/L, the TNF-α release was 37% of the spontaneous production (p < 0.001), and
a concentration of 1 mmol/L showed almost complete inhibition (Fig 1, top, A; p < 0.001). The spontaneous production of the other cytokines was not significantly inhibited by POF at all tested concentrations, but a trend for the inhibition of IL-10 production was found (Fig 2, top, A; p = 0.092). Similar to POF, DEX suppressed the spontaneous release of TNF-α (p < 0.001). In contrast to POF, DEX also reduced the spontaneous production of sTNFR2, IL-1β, and IL-10 to 78%, 48%, and 54%, respectively, of the spontaneous production (Fig 3, top, A; p < 0.05). No inhibitory effect of DEX on sTNFR1, IL-6, and IL-8 was seen.

**Effects of POF and DEX on LPS-Stimulated Cytokine Production From AMs in Sarcoidosis**

As shown in Table 2, the LPS-stimulated production of all cytokines was significantly higher than with spontaneous cytokine production (p < 0.05 and p < 0.001, respectively). POF suppressed the release of LPS-stimulated TNF-α, sTNFR2, IL-1β, IL-6, and IL-8 production in a dose-dependent fashion (Fig 1, bottom, B, and Fig 3, bottom, B) [p < 0.05 or p < 0.001, respectively]. The LPS-stimulated release of IL-10 was inhibited by POF only at the highest dose (ie, 1 mmol/L) [Fig 2, bottom, B; p < 0.001]. Similar to POF, DEX also reduced the production of LPS-stimulated cytokines except for that of IL-1β (p < 0.001). POF and DEX showed no inhibition of LPS-stimulated sTNFR1 production.

**Discussion**

The present study showed that POF induced a dose-dependent suppression of spontaneous TNF-α release from AMs in sarcoidosis, and that there was a trend for the inhibition of IL-10. DEX suppressed the spontaneous release of TNF-α, sTNFR2, IL-1β, and IL-10 from AMs in sarcoidosis.

The rather selective inhibitory effect of POF on spontaneous TNF-α production from AMs in vitro in

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**Table 1—Effects of POF and DEX on Spontaneous Cytokine Production From AMs in Sarcoidosis**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Spontaneous Production</th>
<th>POF 0.01 mmol/L</th>
<th>POF 0.1 mmol/L</th>
<th>POF 1 mmol/L</th>
<th>DEX 0.1 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α, pg/mL/10⁶ AMs</td>
<td>496 ± 215 (100)</td>
<td>359 ± 128 (72)</td>
<td>185 ± 771 (37)</td>
<td>25 ± 111 (5)</td>
<td>197 ± 834 (40)</td>
</tr>
<tr>
<td>sTNFR1, pg/mL/10⁶ AMs</td>
<td>108 ± 24 (100)</td>
<td>90 ± 19 (83)</td>
<td>123 ± 27 (114)</td>
<td>106 ± 22 (98)</td>
<td>121 ± 31 (112)</td>
</tr>
<tr>
<td>sTNFR2, pg/mL/10⁶ AMs</td>
<td>866 ± 324 (100)</td>
<td>659 ± 201 (76)</td>
<td>769 ± 332 (89)</td>
<td>763 ± 247 (88)</td>
<td>679 ± 304 (78)</td>
</tr>
<tr>
<td>IL-1β, pg/mL/10⁶ AMs</td>
<td>472 ± 194 (100)</td>
<td>239 ± 79 (51)</td>
<td>267 ± 95 (61)</td>
<td>261 ± 87 (55)</td>
<td>226 ± 129 (48)</td>
</tr>
<tr>
<td>IL-6, pg/mL/10⁶ AMs</td>
<td>2,967 ± 1,019 (100)</td>
<td>2,876 ± 1,029 (96)</td>
<td>3,009 ± 1,017 (101)</td>
<td>3,205 ± 1,004 (107)</td>
<td>2,642 ± 1,027 (88)</td>
</tr>
<tr>
<td>IL-8, pg/mL/10⁶ AMs</td>
<td>16,918 ± 4,540 (100)</td>
<td>16,973 ± 4,681 (100)</td>
<td>17,402 ± 4,936 (103)</td>
<td>17,323 ± 4,956 (102)</td>
<td>17,160 ± 4,678 (101)</td>
</tr>
<tr>
<td>IL-10, pg/mL/10⁶ AMs</td>
<td>245 ± 159 (100)</td>
<td>169 ± 122 (69)</td>
<td>149 ± 98 (61)</td>
<td>109 ± 67 (45)</td>
<td>131 ± 102 (54)</td>
</tr>
</tbody>
</table>

*Values given as mean ± SEM (% of baseline).

†p < 0.001 (compared with spontaneous cytokine production).

‡p < 0.05 (compared with spontaneous cytokine production).

§p < 0.01 (compared with spontaneous cytokine production).
sarcoidosis, as shown in this study, is supported by the results of similar studies in vitro and in vivo. Previous studies have demonstrated that the production of IL-1β, IL-6 and granulocyte-macrophage colony-stimulating factor from human monocytes/AMs was unaffected by POF, whereas TNF-α production was markedly suppressed by POF. A study of POF on endotoxemia in human volunteers or in OKT3-treated renal transplant recipients had shown an inhibitory effect of POF on TNF-α levels, whereas IL-6 and IL-8 were not affected. Bernard et al reported a selective inhibition of POF on TNF-α production by endothelial cells under LPS stimulation compared to that on IL-6. An in vivo study on the effects of continuous IV infusion of POF on serum levels of TNF-α, IL-6, and IL-8 in patients with septic shock showed a selective decrease of TNF-α, whereas the levels of the other two cytokines remained unaffected. The lowest effective concentration of POF in our in vitro study (0.01 mmol/L) is comparable to peak therapeutic plasma concentrations of 0.1 to 0.01 mmol/L. Other studies that investigated a comparable POF effect used higher concentrations than those required in vivo.

A trend for the inhibition of spontaneous IL-10 production was seen in AMs from our results. Other studies also have demonstrated that the modulation of cytokine release induced by POF is not solely restricted to TNF. Bienvenu et al also reported that IL-10 was significantly inhibited by POF at a concentration of 0.1 mmol/L. Benbernou et al found that POF at appropriate concentrations induced a selective suppression of IL-2 and interferon-γ, whereas at high concentrations (ie, 0.5 mmol/L) this drug could act as a suppressive agent of both Th1-derived and Th2-derived cytokines. D’Hellencourt et al demonstrated that POF...
Table 2—Effects of POF and DEX on LPS-Stimulated Cytokine Production From AMs in Sarcoidosis

<table>
<thead>
<tr>
<th>Variables</th>
<th>LPS</th>
<th>LPS + POF</th>
<th>LPS + DEX</th>
<th>DEX,</th>
<th>Spontaneous</th>
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<tr>
<td>TNF-α, pg/mL/10⁶ AMs</td>
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<tr>
<td>0.1 mmol/L</td>
<td>3.25 ± 1.22 (100)</td>
<td>2.498 ± 0.695 (45)</td>
<td>1.657 ± 0.64 (44)</td>
<td>0.815 ± 0.764 (81)</td>
<td>85 (100)</td>
</tr>
<tr>
<td>0.01 mmol/L</td>
<td>3.20 ± 0.96 (100)</td>
<td>1.699 ± 0.317 (60)</td>
<td>1.125 ± 0.371 (61)</td>
<td>0.581 ± 0.106 (38)</td>
<td>81 (100)</td>
</tr>
<tr>
<td>605 (45)</td>
<td>310 (25)</td>
<td>33 (70)</td>
<td>30 (59)</td>
<td>436 (74)</td>
<td></td>
</tr>
<tr>
<td>sTNFR1, pg/mL/10⁶ AMs</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0.1 mmol/L</td>
<td>4.007 ± 0.841 (78)</td>
<td>1.404 ± 0.289 (38)</td>
<td>1.103 ± 0.271 (60)</td>
<td>1.456 ± 0.804 (66)</td>
<td>323 ± 0.094 (69)</td>
</tr>
<tr>
<td>0.01 mmol/L</td>
<td>3.240 ± 0.84 (100)</td>
<td>1.107 ± 0.153 (84)</td>
<td>7.246 ± 0.844 (62)</td>
<td>2.863 ± 1.389 (63)</td>
<td>313 ± 0.232 (75)</td>
</tr>
<tr>
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<td>310 (25)</td>
<td>33 (70)</td>
<td>30 (59)</td>
<td>436 (74)</td>
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<tr>
<td>IL-1, pg/mL/10⁶ AMs</td>
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<tr>
<td>0.1 mmol/L</td>
<td>4.98 ± 2.15</td>
<td>1.199 ± 0.234 (50)</td>
<td>1.699 ± 0.317 (60)</td>
<td>1.456 ± 0.804 (66)</td>
<td>323 ± 0.094 (69)</td>
</tr>
<tr>
<td>0.01 mmol/L</td>
<td>4.84 ± 2.64 (100)</td>
<td>1.699 ± 0.317 (60)</td>
<td>1.125 ± 0.371 (61)</td>
<td>0.581 ± 0.106 (38)</td>
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</tr>
<tr>
<td>IL-6, pg/mL/10⁶ AMs</td>
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<tr>
<td>0.1 mmol/L</td>
<td>2,987 ± 4,840 (93)</td>
<td>1,743 ± 1,678 (100)</td>
<td>1,743 ± 1,678 (100)</td>
<td>864 ± 4,586 (62)</td>
<td>1,125 ± 1,678 (100)</td>
</tr>
<tr>
<td>0.01 mmol/L</td>
<td>1,019 ± 10,079 (100)</td>
<td>1,019 ± 10,079 (100)</td>
<td>1,019 ± 10,079 (100)</td>
<td>864 ± 4,586 (62)</td>
<td>1,125 ± 1,678 (100)</td>
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<td>605 (45)</td>
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<td>33 (70)</td>
<td>30 (59)</td>
<td>436 (74)</td>
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<tr>
<td>IL-8, pg/mL/10⁶ AMs</td>
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<tr>
<td>0.1 mmol/L</td>
<td>1,883 ± 51,795 (100)</td>
<td>11,343 ± 47,952 (100)</td>
<td>11,343 ± 47,952 (100)</td>
<td>11,094 ± 31,232 (93)</td>
<td>11,389 ± 31,232 (93)</td>
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<tr>
<td>0.01 mmol/L</td>
<td>4,840 ± 51,795 (100)</td>
<td>11,343 ± 47,952 (100)</td>
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<td>11,094 ± 31,232 (93)</td>
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</tr>
</tbody>
</table>

Values given as mean ± SEM (% of baseline).

* p < 0.001 (compared with LPS-stimulated cytokine production).
‡ p < 0.001 (compared with LPS-stimulated cytokine production).
§ p < 0.01 (compared with LPS-stimulated cytokine production).

POF, which is known for its multiple immunosuppressive and anti-inflammatory activities, has been widely used in the treatment of allergic and inflammatory granulomatous diseases. It has been shown to inhibit the production and gene expression of many cytokines, which are known to induce inflammatory or immunologic responses.9,27–33 The molecular mechanism of the action of DEX is comparatively well-understood through its effect on the transcription factor nuclear factor-κB, which has been implicated in the activation of multiple cytokine genes. It has been demonstrated that DEX and POF inhibit TNF-α production at both the transcriptional and translational levels, with the effect of DEX being more pronounced at the translational level, but with POF inhibiting predominantly the transcription of the TNF-α gene.2,4,34 Although POF and DEX inhibit TNF-α production by distinctly different mechanisms, our results indicate that these drugs inhibit the production of the spontaneous TNF-α and other cytokines to a similar extent.

Shown by our in vitro studies of the LPS-stimulated cells and in line with those studies reported in the literature, POF not only inhibits TNF-α production, but also down-regulates other cytokines.4–9,22 Based on these results, POF has complex effects on the
cytokine network. The cellular environment and the mechanisms that trigger cytokine production seem to greatly influence these effects.

In sarcoidosis, AMs, AM-derived TNF-α, and other cytokines play a crucial role in orchestrating inflammatory cell accumulation, granuloma formation, and the fibrogenesis of sarcoidosis. Accordingly, the results of our in vitro study may have important implications for the potential use of POF in the treatment of sarcoidosis, and also may explain in part the beneficial effect of POF on sarcoidosis patients what was observed in a 1997 open trial. Compared with DEX, POF has no severe side effects and, thus, has a broader therapeutic window. However, the full details of the mechanism of how POF may be a promising immunosuppressive agent for the treatment of sarcoidosis have not yet been elaborated.

In conclusion, the results of this in vitro study show that POF not only inhibits the spontaneous production of TNF-α from AMs, but also suppresses the LPS-stimulated production of TNF-α and other cytokines from AMs. Compared with DEX, POF shows similar effects in regard to the inhibition of spontaneous and LPS-stimulated cytokine production. POF may improve the therapeutic regimens for sarcoidosis either by sparing or by replacing corticosteroids. However, the precise clinical value of POF in the treatment of sarcoidosis and other lung diseases will have to be determined in further clinical trials.

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