Circulating cytokine levels in patients with rheumatoid arthritis: results of a double blind trial with sulphasalazine

V A Danis, Gradislava M Fracie, Deborah A Rathjen, R M Laurent, P M Brooks

Abstract

Interleukin 1 (IL-1), IL-6, and tumour necrosis factor (TNFα) are pleiotropic cytokines produced predominantly by macrophages, which have been implicated in the pathogenesis of rheumatoid arthritis (RA). Sulphasalazine has been shown to induce disease modifying properties and to inhibit the production of cytokines in vitro. To evaluate the effect of sulphasalazine on cytokine production in vivo, serum cytokine levels were measured in a group of patients with RA entered into a randomised controlled trial. Serum levels of IL-1α, IL-1β, IL-6, and TNFα were measured at baseline and at two monthly intervals for six months in 17 patients receiving sulphasalazine and in 22 patients treated with placebo. The two groups of patients had a similar age and sex distribution, had had RA for less than a year, had no joint erosions, and had not been treated previously with any other disease modifying drugs.

In the 19 patients studied IL-1α was detected (0.01 ng/ml) at baseline in 14 patients (median 0.04 ng/ml), IL-1β in 25 patients (median 1.0 ng/ml), TNFα in 27 patients (median 1.2 ng/ml), and IL-6 in 33 patients (median 0.44 ng/ml). In the group treated with sulphasalazine there was a progressive and significant decline in serum IL-1α, IL-1β, and TNFα levels over the six month period (median at six months were <0.01, 0.01, and 0.04 ng/ml respectively). Interleukin 6 levels were significantly reduced only at the four month time point (median level of 0.23 ng/ml) and this was associated with improvements in clinical and laboratory measures of disease activity. In contrast patients receiving placebo showed no changes in serum cytokine levels and no improvement in clinical and laboratory indices of disease activity. These results suggest that sulphasalazine may exert its disease modifying effect partly by suppressing cytokine production in vivo.

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Cytokines have been implicated as important mediators of inflammation and joint destruction in rheumatoid arthritis (RA). The development of sensitive immunoassays for cytokines has made it possible to show increased levels of some cytokines in the blood of patients with RA. More importantly increased levels of interleukin 1 (IL-1) and tumour necrosis factor α (TNFα) have been associated with disease severity in patients with RA. Although circulating IL-6 has been detected in many patients with arthritis the levels in patients with RA and those with other inflammatory arthritides were not significantly higher than in patients with non-inflammatory arthritis. Circulating levels of IL-6 in patients with RA were found to be low and not significantly different from normal control subjects. The determination of circulating cytokines may prove to have prognostic value particularly IL-1β and TNFα. There have been several anecdotal reports that the blood levels of certain cytokines (IL-1β and TNFα) can be decreased during successful treatment with disease modifying drugs. Sulphasalazine is a drug that has been found to possess disease modifying properties. It has also been shown to inhibit cytokine production in vitro (Danis V A et al, unpublished results). We performed a multicentre randomised controlled trial of sulphasalazine (salazopyrine-En, ta; Köhler Pharma) in the treatment of RA in which we showed a significant effect of sulphasalazine over placebo (Australian Multicentre Trial Group, unpublished results). We now report that patients receiving the active drug also had progressive and significant reductions in circulating cytokine levels associated with improvements in clinical and laboratory measures of disease activity.

Patients and methods

PATIENTS

Patients with clinical or definite RA of less than 12 months' duration and with no evidence of joint destruction were recruited into a controlled trial of Salazopyrine-En tabs. Patients were clinically reviewed at monthly intervals and the disease activity assessed according to a standard protocol. Cytokine blood levels were measured in serum samples (baseline, two, four, and six months) and were used as a marker of disease activity. Seventeen of these patients were receiving sulphasalazine (2 g daily). The mean (SD) age was 51 (11), the female to male ratio was 14:3, and 12 of the 17 patients were rheumatoid factor positive. The remaining 22 patients who were receiving placebo had a mean (SD) age of 55 (12) years, a female to male ratio of 16:3, and 11 of the 22 patients were rheumatoid factor positive. Standard clinical and biochemical measures of disease activity were used to weight the daily living score which was based on the health assessment questionnaire of Fries et al.

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**Clinical and laboratory measures of disease activity in the patients with rheumatoid arthritis at baseline and after six months**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline</th>
<th>Six months</th>
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<tbody>
<tr>
<td>Ritchie total score</td>
<td>125 (10)</td>
<td>92 (7) (p&lt;0.001)</td>
</tr>
<tr>
<td>Pain score</td>
<td>30 (19)</td>
<td>24 (14) (p&lt;0.001)</td>
</tr>
<tr>
<td>ESR (mm)</td>
<td>7.3 (11)</td>
<td>0.3 (1) (p&lt;0.001)</td>
</tr>
<tr>
<td>CRP (mg)</td>
<td>20 (15)</td>
<td>13 (10) (p&lt;0.001)</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>3 (2)</td>
<td>0.7 (0.4) (p&lt;0.001)</td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>36 (20)</td>
<td>13 (9) (p&lt;0.001)</td>
</tr>
<tr>
<td>RF (IU/ml)</td>
<td>326 (285)</td>
<td>256 (249) (p&lt;0.001)</td>
</tr>
<tr>
<td>Anti-CCP (IU/ml)</td>
<td>5.2 (0.9)</td>
<td>5.7 (2.0) (p&lt;0.001)</td>
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</table>

Statistically significant compared with baseline.
index and the activities of daily living score there were no improvements in any of the laboratory parameters. In fact changes in the erythrocyte sedimentation rate and serum hyaluronic acid levels indicate the continuing progression of disease. In contrast patients receiving sulfasalazine showed improvements in all clinical and laboratory parameters. Stable levels of serum hyaluronic acid indicate control of disease progression. Therefore changes in serum cytokine levels in patients receiving sulfasalazine are clearly associated with clinical improvement.

Discussion
We have shown that successful treatment with the disease modifying drug sulfasalazine is associated with reductions in serum levels of IL-1α, IL-1β, and TNF α if these cytokines are present in detectable amounts before treatment. At least one of these cytokines was detected in most patients (56/59). Although IL-6 was detected in the serum of most patients with RA (33/39), it was unaffected by treatment with sulfasalazine. This is consistent with our observations on the effects of sulfasalazine on cytokine production in vitro where we found that much higher concentrations of sulfasa-
lazine (50 μg/ml) were required to inhibit IL-1 production compared with IL-1 or TNF α (production (12-25 μg/ml) (Danis V A et al, unpublished results). The upper range of serum concentrations of sulfasalazine in patients is 12-25 μg/ml.25 26 The source of the cytokines in RA patients but activating macrophages may be the main cellular source. Endothelial cell stimulation of cytokines produces significant amounts of IL-627 but only small amounts of IL-1 and TNF α.28

In this study there was a significant decrease in the standard clinical and laboratory para-

imeters in patients treated with sulfasalazine. Although we have to be cautious in the interpretation of these results due to the limited number of patients treated, sulfasalazine has been shown to be beneficial in the treatment of RA.24-25 (Australian Multicentre Trial Group, unpublished results) and of ankylosing spondylitis.29 30 In a comparative study sulfasalazine significantly slowed the rate of skin erosion in patients with RA compared with hydroxychloroquine.20 Like all disease modifying drugs, sulfasalazine has a spectrum of adverse reactions including rash and gastrointestinal events. It is, however, well tolerated by patients compared with similar drugs.31 32

This study shows that sulfasalazine may inhibit cytokine production in vivo, though we do not know if this is a primary effect of the drug in vivo or a change secondary to the suppression of inflammation. Cytokine levels were measured after only four to six months of treatment with sulfasalazine. Disease activity, however, was not assessed until after four and six months where there were significant improvements in the clinical and laboratory measurements (data presented only after six months). Therefore it was not possible to determine whether the changes in serum cyto-
kine levels preceded clinical improvement. Out in vitro studies suggest that sulfasalazine may inhibit cytokine production as a primary mode of action of the drug (Danis V A et al, unpublished results). Moreover the minimal effect of sulfasalazine treatment on circulating IL-6 levels is also consistent with the in vitro data (Danis V A et al, unpublished data). There is preliminary evidence to suggest that IL-6 may have a protective and anabolic role in connective tissues—for example, by stimulating chondrocyte growth, the production of transforming growth factor β, and the tissue infiltration of metalloproteinases by chondrocytes (P.A. Greiser et al, unpublished results). Inter-

leukin 1 may have a protective role in arthritis and it may not be desirable to suppress its production with disease modifying drugs. Suppression of the production of potentially pathogenic cytokines (IL-1 and TNF α) may be one mechanism of the disease modifying action of sulfasalazine in patients with RA.

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