Effects of Pulse Methylprednisolone on Macrophage Chemotactic Protein-1 and Macrophage Inflammatory Protein-1α in Rheumatoid Synovium

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ABSTRACT. ***Objective.*** To determine the effect of pulse methyprednisolone (PMP; 1000 mg) on the expression of monocyte chemoattractant protein (MCP)-1 and macrophage inflammatory protein (MIP)-1α in rheumatoid synovial membrane.

Methods. Seven patients with rheumatoid arthritis (RA) were studied. Arthroscopically-directed synovial biopsies were taken before and 24 hours after treatment with intravenous PMP. Synovial membranes were stained by immunohistochemical techniques with monoclonal antibodies against MCP-1, MIP-1α and CD68 (a macrophage marker). Quantitation of staining was performed by computer-assisted color video image analysis.

Results. PMP therapy was associated with a rapid (within 24 hours) and substantial decrease in the expression of MCP-1 and MIP-1α expression by a mean of 55% ($p = 0.05$) and 45% ($p = 0.03$), respectively, with no effect on CD68 expression in the synovial lining layer. There was no significant change in MCP-1, MIP-1α or CD68 expression in the synovial sublining.

Conclusion. PMP therapy rapidly reduces MCP-1 and MIP-1α levels in the synovial lining layer without a fall in macrophage numbers. It thus appears that the initial effect of PMP is that of reducing macrophage activation. (J Rheumatol 2001;28:2634–6)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
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MIP-1α

We have previously demonstrated that high dose pulse methylprednisolone (PMP) causes a rapid improvement in rheumatoid arthritis (RA) disease activity associated with a reduction in neutrophil ingress into the synovial space[1] probably due to a reduction in the expression of proinflammatory cytokines and cell adhesion molecules in the synovial membrane[2,3]. PMP also reduces metalloproteinase (MMP) and tissue inhibitors of metalloproteinase (TIMP) expression in the synovial membrane[4].

Monocytes/macrophages are important effector cells in RA. Macrophage infiltration in the synovial membrane predicts radiological disease progression[5]. Monocyte chemoattractant factor (MCP)-1 and macrophage inflammatory protein (MIP)-1α are members of the ß-chemokine family and are predominantly chemotactic for monocytes/macrophages[6,7]. Their role in RA is supported by *in vitro* and *in vivo* studies. Immunolocalization studies have shown that both chemokines are expressed by cells of the macrophage-rich synovial lining layer as well as by cells in the sublining region[8,9]. MCP-1 and MIP-1α are both expressed at higher levels in the synovial membrane and synovial fluid in RA compared with osteoarthritis[8,9]. Neutralizing antibodies directed against MIP-1α neutralize 36% of the chemotactic activity of RA synovial fluid for macrophages[9]. The effects of PMP on MCP-1 and MIP-1α expression have not been previously examined *in vivo*. The aim of our study was to investigate the effects of a 1000 mg intravenous PMP on the expression of MCP-1 and MIP-1α in the synovial membrane of patients with RA.

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MATERIALS AND METHODS

Patients. Seven patients all fulfilling the American College of Rheumatology criteria (1987) for RA were studied. All patients received 1000 mg of methylprednisolone intravenously as the sodium hemisuccinate salt as previously described[2,3]. Clinical assessment was performed using visual analog scores (VAS) measured on a 10 cm horizontal scale anchored at both ends for pain, generalized stiffness and well-being; as well as a tender joint count. Serum C-reactive protein (CRP) was used as a laboratory assessment of inflammation. Synovial membrane samples were obtained before and 24 hours after PMP in 7 patients using needle arthroscopic techniques and standard approaches as previously described[2,3]. This project was approved by the Repatriation General Hospital Ethics Committee and informed consent was obtained from each patient.

Immunostaining. Formalin-fixed adjacent tissue sections (4 μm thickness) were stained with murine monoclonal antibodies to MCP-1, MIP-1α, the macrophage marker CD68 (IgG1) (all purchased from ICN Biomedicals, Sydney, Australia) as well as the isotype-specific negative control X63 (IgG1, which recognizes an irrelevant mouse myeloma protein) as previously described[4]. The immunostained sections were examined by computer-assisted color video image analysis as previously described[2,3]. Measurements of the integrated optical density (IOD), which is proportional to the total amount of protein staining, were made by one blinded observer (PKKW) who was unaware of the order of biopsies from any one patient. All sections from any single patient were analyzed in the same sitting.

Statistical analysis. Results are given as mean ± SD (standard deviation) or mean ± SEM (standard error of the mean). Comparisons were made using the Wilcoxon signed rank test. Differences were considered to be significant at $p < 0.05$.

RESULTS

Six males and one female with a mean ± SD age of 70 ± 6.5 years and disease duration 5.8 ± 2.11 years were studied. Four patients were on intramuscular gold with one of each also on methotrexate and sulfasalazine. PMP administration resulted in a significant improvement in all clinical variables of disease activity 24 hours post-treatment: the CRP fell from 58 ± 15 to 20 ± 4.7 mg/dl ($p = 0.03$); the tender joint score from 22 ± 2.5 to 5.9 ± 2.5 ($p < 0.0001$); VAS for pain from 8.3 ± 0.58 to 2.6 ± 0.48 cm ($p < 0.0005$); VAS for stiffness from 8.4 ± 0.55 to 1.3 ± 0.50 cm ($p < 0.0005$). There was an increase in VAS for well-being from 0.8 ± 0.42 to 8.3 ± 0.63 cm ($p < 0.005$).

Due to tissue availability, synovium from only 6 patients could be analyzed for MCP-1 and CD68 whereas 7 patients were analyzed for MIP-1α. In the synovial lining layer, PMP administration resulted in a significant reduction in MCP-1 and MIP-1α expression by a mean of 55% ($p = 0.05$) and 45% ($p = 0.03$), respectively, (Figure 1a). There was no effect on CD68 expression in the synovial lining layer (Figure 1a). There was no significant change in MCP-1, MIP-1α or CD68 expression in the synovial sublining layer (Figure 1b).

DISCUSSION

The most significant finding of this study was that PMP reduced MCP-1 and MIP-1α expression in the synovial lining layer within 24 hours of administration. This may be due to either a direct effect on chemokine production or an indirect effect on other proinflammatory mediators that regulate chemokine expression. Evidence to support a direct effect comes from *in vitro* studies in which glucocorticoids reduced MCP-1 gene expression in cultured rheumatoid synoviocytes[10]. An *in vitro* study has demonstrated that tumor necrosis factor-α (TNF-α) upregulates MCP-1 and MIP-1α expression in rheumatoid synoviocytes[8,9] while an *in vivo* study found that TNF-α blockade reduced MCP-1 expression in

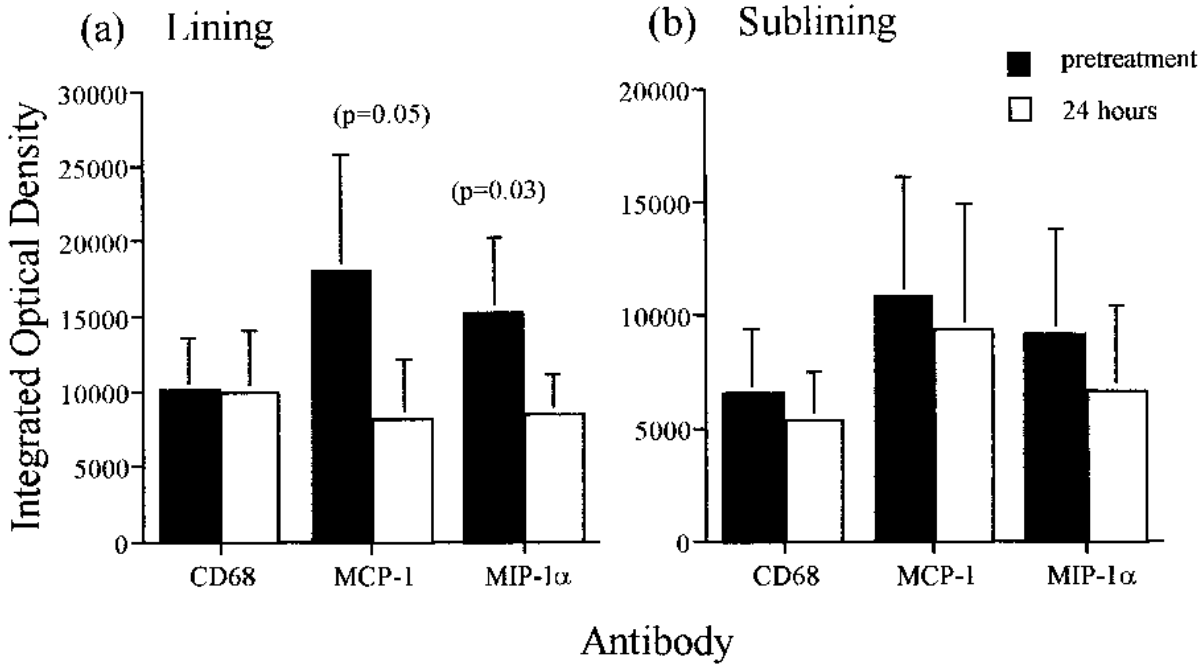

Figure 1. Changes in macrophage numbers and the expression of chemokines (mean ± SEM) in (a) the synovial lining layer; and (b) the synovial sublining layer after pulse methylprednisolone therapy.

rheumatoid synovial membrane[11]. We have previously demonstrated that PMP markedly reduces TNF-α expression in the synovial membrane within 24 hours[3]. This may then lead to a reduction in MCP-1 and MIP-1α expression.

We found no change in macrophage numbers in the synovial membrane. It appears that the initial effect of PMP is that of reducing macrophage activation as evidenced by a reduction in the production of several proinflammatory mediators by these cells after treatment. It is possible that this would then be followed by a reduction in macrophage numbers due to a fall in macrophage chemokine expression. In order to demonstrate this, we would need to study the rheumatoid synovium at later time points after PMP, which we have not as yet done.

In summary, we have demonstrated that PMP is associated with a reduction in ß-chemokine expression in the synovial lining layer.

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