Modulation of cell recruitment by anti-inflammatory agents in antigen-induced arthritis


CONCISE REPORT

Objective: To study the effect of meloxicam (MXC) and diclofenac (DCF) on the recruitment of leucocytes during acute experimental arthritis.

Methods: Rabbits with antigen-induced arthritis were treated with MXC, DCF, or not treated. After 48 hours, synovial fluid (SF) leucocyte influx and prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) levels were evaluated. Interleukin 8 (IL\textsubscript{8}) and monocyte chemotactic peptide-1 (MCP-1) expression and synthesis were studied in the inflamed tissues.

Results: Arthritic knees showed synovial effusion with a high leucocyte count and PGE\textsubscript{2}, and an increased expression of IL\textsubscript{8} and MCP-1. Both non-steroidal anti-inflammatory drugs (NSAIDs) reduced PGE\textsubscript{2}, levels and the polymorphonuclear cell (PMN) concentration in SF, while the mononuclear cell (MN) concentration was unchanged in the treated groups in comparison with controls. A definite reduction of IL\textsubscript{8} levels was obtained with the treatments, but the drugs did not prevent the up regulation of MCP-1.

Conclusion: The effect of these NSAIDs in acute arthritis may be related to the down regulation of IL\textsubscript{8} production. The results suggest a differential effect of anti-inflammatory drugs on PMN and MN recruitment to the joint.

RESULTS

Infiltration of leucocytes and production of PGE\textsubscript{2}

Untreated rabbits showed a large synovial effusion that was reduced by MXC treatment, but not by DCF (table 1). A high cell concentration was found in the SF in the untreated group (80% of the total cells were polymorphonuclear cells (PMN)). Only MXC administration diminished total cell count and PMN concentration (table 1). None of the drugs diminished the SF MN influx compared with the untreated group (table 1).

Tissue damage was partially prevented by both NSAIDs (web extra fig W1). MXC treated rabbits showed a diminution in the PMN infiltration. DCF did not have a significant effect. Neither MXC nor DCF modified SM MN cell infiltration (table 1).

Determination of PGE\textsubscript{2} in synovial fluid

PGE\textsubscript{2} concentration in the synovial fluid (SF) was determined by PGE\textsubscript{2} enzyme immunoassay (Assay Designs, Inc).

Identification of recruited cells and chemokines in the synovial membrane

Paraffin embedded synovial membrane (SM) was stained with haematoxylin and cosin. A 0–3 points scoring scale estimated the density of leucocyte populations.

Immunoreactivity to IL\textsubscript{8} and MCP-1 was assessed by peroxidase techniques.\textsuperscript{1} The density of IL\textsubscript{8} and MCP-1 staining was scored 0–4.

RT-PCR studies

Total RNA from the SM was isolated, reverse transcribed and amplified with Access RT-PCR System (Promega),\textsuperscript{2} employing specific primers of rabbit IL\textsubscript{8} and MCP-1.\textsuperscript{1}

Statistical analysis

Data were expressed as mean (SEM). Descriptive statistics, analysis of variance test, and comparison of means by independent \textit{t} test were carried out. Differences were considered significant for \textit{p}<0.05.

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Abbreviations: COX, cyclo-oxygenase; DCF, diclofenac; IL, interleukin; MCP-1, monocyte chemotactic peptide-1; MN, mononuclear cells; MXC, meloxicam; NSAIDs, non-steroidal anti-inflammatory drugs; PGE\textsubscript{2}, prostaglandin \textsubscript{E}; PMN, polymorphonuclear cells; SF, synovial fluid; SM, synovial membrane
Figure 1  IL8 (A–D) and MCP-1 (E–H) SM immunostaining (×200). Healthy controls (A, E); untreated group (B, F); MXC treated rabbits (C, G); DCF treated rabbits (D, H). Semiquantitative scoring for IL8 (I) and MCP-1 (J) of the different groups (*p<0.05 v non-treated).

Table 1  DCF and MXC effect on leucocyte infiltration and PGE₂ production. Values are shown as mean (SEM)

<table>
<thead>
<tr>
<th></th>
<th>SF volume (µl)</th>
<th>SF PGE₂ (ng/ml)</th>
<th>SF PMN (cells/ml × 10⁶)</th>
<th>SF MN (cells/ml × 10⁶)</th>
<th>SM PMN (0–3)</th>
<th>SM MN (0–3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treated</td>
<td>324 (24)</td>
<td>16 (4)</td>
<td>88 (13)</td>
<td>22 (3)</td>
<td>1.8 (0.4)</td>
<td>0.8 (0.3)</td>
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<tr>
<td>MXC</td>
<td>177 (29)*</td>
<td>3 (0.4)*</td>
<td>46 (8)*</td>
<td>21 (3)</td>
<td>0.5 (0.2)*</td>
<td>1.2 (0.3)</td>
</tr>
<tr>
<td>DCF</td>
<td>229 (53)</td>
<td>1.7 (0.4)*†</td>
<td>54 (10)</td>
<td>32 (9)</td>
<td>1.3 (0.3)</td>
<td>1.4 (0.2)</td>
</tr>
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</table>

*p<0.05 v untreated rabbits; †p<0.05 v MXC.
Increased levels of PGE\(_2\) were found in the SF of untreated rabbits (16 (4) ng/ml). Both NSAIDs reduced PGE\(_2\) concentration. DCF induced a greater fall in PGE\(_2\) levels than MXC.

**IL8 and MCP-1 immunolocalisation**

IL8 and MCP-1 followed a similar pattern of distribution. Mononuclear cells and the lining layer were strongly positive (fig 1). Extracellular IL8 was largely detected in neutrophilic collections and in the endothelium. Both NSAIDs down regulated IL8 immunoreactivity (figs 1A–D, I) mainly by a reduction in the areas corresponding to neutrophilic collections. For the MCP-1 signal, there were no differences between the groups (figs 1E–H, J).

**IL8 and MCP-1 gene expression in the SM**

The high immunoreactivity to IL8 and MCP-1 in the injured tissues was accompanied by an enhancement in the expression of both genes. IL8 expression was reduced with both treatments, although it was more evident in MXC treated animals. None of the drugs diminished the MCP-1 message (fig 2).

**DISCUSSION**

We have studied the effect of two NSAIDs on cell infiltration, local PGE\(_2\), and IL8 and MCP-1 synovial expression in a model of experimental arthritis. The clinical benefit of both drugs was related to a diminution of joint swelling, reduction of the effusion and infiltration by PMN. Only MXC treatment showed significant effects on SF volume and PMN infiltration. The MXC group showed a smaller diminution of PGE\(_2\) levels in SF than did DCF. Our results showed a lack of correlation between PGE\(_2\) inhibition and clinical improvement. Previous evidence has shown that PGE\(_2\) depletion may lead to an up regulation of several proinflammatory mediators, such as tumour necrosis factor \(\alpha\) and IL1\(\beta\) in the inflamed SM.\(^7\) COX derived eicosanoids exert some regulating functions important for the resolution of inflammation.\(^7\)

We found a differential regulation of PMN and MN migration by the NSAIDs. Cell count and density scoring in each group paralleled the respective induction of the chemokines studied. NSAIDs action appeared selective for PMN influx, and related to a down regulation of IL8 local expression. MCP-1 expression and synthesis remained largely activated in the SM of treated groups. This may explain why both treatments increase MN density in the SM and SF. This is in agreement with previous data describing a lack of effect of NSAIDs at the chronic stage of this experimental disease, when inflammatory events are dominated by macrophages and lymphocytes. Recent unpublished data from our laboratory show that MXC and DCF further increase MCP-1 expression in IL1\(\beta\) stimulated synoviocytes. These and other data suggest that PGE, may act as an MCP-1 repressor agent.\(^7\) This effect seems to be related to the PGE, synthesis inhibition afforded by NSAIDs.

Our results indicate that a total PGE, depletion may not be desirable. We have shown that NSAIDs down regulate IL8 production in antigen arthritis, but may favour the recruitment of MN. A full understanding of prostaglandin activities as stimulating or repressor factors of inducible genes is desirable to design a better therapeutic approach with NSAIDs in joint diseases.

The synovium histopathology of rabbits with acute antigen arthritis is shown on the web at [www.annrheumdis.com](http://www.annrheumdis.com)
REFERENCES

UNUSUAL AND MEMORABLE

Case Number 25: A butterfly rash

Series editor: Gary D Wright

A 33 year old woman attended the rheumatology clinic for assessment of her cutaneous lupus. The diagnosis of cutaneous lupus had been made by her dermatologist five years beforehand; this was confirmed by biopsy. She subsequently developed a positive anti-DNA antibody at low titres, with negative extractable nuclear antigen, but had no other clinical symptoms of systemic lupus.

The figure shows one of several active cutaneous lupus lesions overlying a butterfly tattoo, which had predated any of her symptoms.

Acknowledgement
Photograph by IM Chalmers, University of Manitoba, RR149, 800 Sherbrook Street, Winnipeg, MB Canada R3A 1M4. Tel. 204 787 2208.

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