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COMMENTARY

Antirheumatic Agents and Leukocyte Recruitment

NEW LIGHT ON THE MECHANISM OF ACTION OF OXACEPROL

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ABSTRACT. Most anti-inflammatory agents used in the treatment of joint diseases exert inhibitory effects on leukocyte infiltration. Methotrexate, a disease-modifying drug, and corticosteroids also inhibit leukocyte accumulation during inflammation. However, the mechanisms of action of these different compounds on leukocytes vary and in the case of non-steroidal anti-inflammatory drugs (NSAIDs) the mechanism(s) may be indirect. No current drug for inflammatory or degenerative joint disease has been proposed to act specifically by an inhibitory action on neutrophilic leukocytes. Oxaceprol is an amino acid derivative that has been used for several years for the treatment of osteoarthritis and rheumatoid arthritis, ameliorating pain and stiffness and showing good gastrointestinal safety, particularly in comparison with NSAIDs. Recent experimental studies have shown that oxaceprol does not inhibit the synthesis of prostaglandins *in vitro*, but markedly inhibits neutrophil infiltration into the joints of rats with adjuvant arthritis. These results support earlier screening data showing inhibition by oxaceprol of leukocyte infiltration into sites of acute inflammation. In studies on surgical ischemia reperfusion in hamsters *in vivo*, oxaceprol was an effective inhibitor of leukocyte adhesion and extravasation. It is proposed that oxaceprol represents a therapeutic agent for degenerative and inflammatory joint diseases, which acts predominantly by inhibiting leukocyte adhesion and migration. BIOCHEM PHARMACOL **58**;2:209–215, 1999.

KEY WORDS. oxaceprol; antirheumatic drugs; adhesion molecules; leukocyte infiltration; arthritis

Among drugs used for the treatment of rheumatic diseases, distinction is usually made among the NSAIDs,† the DMARDs, and corticosteroids. The NSAIDs are a structurally diverse group of drugs that provide symptomatic relief in inflammatory joint and active osteoarthritic disorders—reducing swelling, joint pain and stiffness and improving joint mobility—but have little effect on the underlying tissue degenerative processes that lead to cartilage loss and bone damage [1, 2]. DMARDs, on the other hand, have little or no acute anti-inflammatory or analgesic properties, but act, usually over a period of weeks or months, to slow down or stop the progression of RA [1, 3]. The mechanisms of action of DMARDs are complex and vary considerably, in many cases remaining unclear, but they all cause slowing of the progression of joint destruction and, in some patients, remission. Corticosteroids also have multiple sites of action in the treatment of rheumatic diseases. Although they inhibit disease progression [4], they are generally restricted to use in severe cases because of long-term side-effects [1].

Side-effects are a disadvantage of the other groups of drugs as well. NSAID use is frequently associated with gastrointestinal toxicity and DMARDs can cause skin, liver, kidney and gastrointestinal side-effects [1, 2]. For this reason, in mild cases of RA and particularly in OA (which is associated with less intense inflammatory lesions), simple analgesics, such as paracetamol, are frequently prescribed, predominantly in Anglo-Saxon countries [5].

Although the aetiology of OA is not entirely understood, it is associated predominantly with loss of joint cartilage. Repeated attempts have been made to develop drugs that either protect cartilage or stimulate cartilage repair and have few adverse effects. So far this approach has not met with success.

Oxaceprol, a synthetic *N*-acetylated derivative of hydroxyproline, was introduced into the therapy of degenerative joint diseases over 20 years ago and was used widely in France and Germany as a well-tolerated therapy for the symptomatic relief of joint diseases. Recent studies on oxaceprol have highlighted an anti-inflammatory mechanism of action of the drug, which indicates that it may represent a member of a novel class of drugs that act predominantly to inhibit leukocyte accumulation [6, 7]. This commentary discusses the role of inhibition of leukocyte recruitment in the effects of drugs used to treat joint disease and the position that oxaceprol may now gain among this class of compounds.

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† *Abbreviations:* NSAIDs, non-steroidal anti-inflammatory drugs; DMARDs, disease-modifying antirheumatic drugs; RA, rheumatoid arthritis; OA, osteoarthritis; COX, cyclooxygenase; and PGs, prostaglandins.

ANTIINFLAMMATORY DRUG MECHANISMS AND EFFECTS ON LEUKOCYTE RECRUITMENT

The NSAIDs, as a group, are generally considered to act as anti-inflammatory, analgesic and anti-pyretic drugs through inhibition of COX, the enzyme catalysing the synthesis of PGs [8, 9]. It has been proposed that their anti-inflammatory actions are due mainly to local inhibition of inducible COX-2 [10]. Gastrointestinal toxicity of NSAIDs, on the other hand, is due to inhibition of constitutive COX-1 in the gastric mucosa, removing the gastroprotective effect of PGE_2 [11], bleeding being exacerbated by inhibition of COX-1 in platelets with prevention of pro-aggregatory thromboxane A_2 formation. Different NSAIDs vary in their potencies as inhibitors of COX-1 and COX-2, probably accounting for differences in their gastrointestinal tolerability [12].

PGE_2, the main pro-inflammatory PG, is hyperalgesic and causes vasodilation, thereby also enhancing plasma exudation during inflammation [9, 13]. Its inhibition by NSAIDs, therefore, helps to explain their relief of inflammatory pain, erythema and swelling.

Accumulation of polymorphonuclear leukocytes also is a characteristic of acute inflammatory responses, and infiltration of activated neutrophils into synovial fluid is a consistent histopathological accompaniment of active joint inflammation [14]. Vasodilation caused by PGs and other agents leads to enhancement of leukocyte infiltration in response to inflammatory stimuli [15, 16], but this enhancement is moderate and can only partially explain the ability of NSAIDs to inhibit leukocyte migration into inflamed sites. Inhibition of leukocyte infiltration by NSAIDs *in vivo* occurs at higher doses than those required to inhibit local PG formation [17], a finding that fits in with the well known fact that higher doses of NSAIDs are required to treat RA and other joint diseases than those needed to provide simple analgesia.

Several NSAIDs, at higher doses, inhibit cell–cell adhesiveness (aggregation) of neutrophils [18]. Adhesion of leukocytes to endothelium is the initial step in the process of emigration of leukocytes into the inflamed site and inhibition of this process could reduce leukocyte infiltration. Inhibition of neutrophil aggregation by high concentrations of NSAIDs is accompanied by inhibition of cell activation, which may account for the inhibition of adhesion [18]. Indeed the oxicams, such as piroxicam, have been reported to inhibit neutrophil adhesion as a result of inhibition of cell activation, probably at the level of GTP binding to G proteins [19]. Recently, it has been shown *in vitro* and *in vivo* that several NSAIDs are able to inhibit the expression of the adhesion molecule L-selectin at the neutrophil cell surface and thereby inhibit adhesion of the neutrophils to the endothelium [19].

A variety of other actions of NSAIDs, particularly the salicylates, have also been described on different enzymes with potential involvement in inflammation [19, 20]. Salicylate has been reported to be an inhibitor of kinases and of the transcription factor NF-κB [21], and indomethacin, ibuprofen and fenoprofen have been reported to inhibit monocyte cytokine release through their stimulation of the transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ) [22]. All these effects occur at concentrations of NSAIDs higher than those required to inhibit PG synthesis, but may contribute to the ability of anti-inflammatory doses of NSAIDs to inhibit leukocyte infiltration into the inflamed joint.

Tenidap is a recently introduced antirheumatic agent that exhibits activities similar to those of the NSAIDs, including COX inhibition, but also lowers serum interleukin-6 (IL-6) concentrations and release of cytokines from synovial cells [23]. It thus shares the properties of NSAIDs as well as some aspects of the actions of DMARDs, although it does not exhibit immunosuppressive activity. Tenidap inhibits some of the secondary activities of neutrophils and also inhibits adhesion of neutrophils to endothelium [24]. The anti-inflammatory activity of this compound, therefore, undoubtedly includes inhibition of leukocyte infiltration.

Methotrexate is one of the most widely used DMARDs for the treatment of RA. Originally introduced as a folate antagonist for cancer chemotherapy, it subsequently has been utilized for the treatment of psoriasis and now is used in RA at doses much lower than those at which cytostatic effects are observed. Even at low doses, methotrexate still inhibits cell growth, as evidenced by side-effects that include stomatitis and leukopenia in addition to liver injury (though the latter is less frequent in RA than in psoriasis) [25]. Recently, methotrexate has also been reported to slow the clonal growth of T and B lymphocytes, but not of synovial cells from RA patients [26]. However, the most widely accepted current hypothesis on the mechanism of action of methotrexate in RA is that it inhibits methylation reactions and promotes adenosine release [27]. While inhibition of methylation may account for inhibitory effects on cytokine production, release of adenosine from connective tissue cells, as a result of inhibition of 5-aminoimidazole-4-carboxamide ribonucleotide transformylase, is sufficient to inhibit the adhesion of neutrophils [28]. This methotrexate-induced adenosine release has been shown to be sufficient to inhibit leukocyte accumulation in a mouse model of inflammation *in vivo* [29]. The actual contribution of this adenosine-mediated inhibition of leukocyte adhesion and infiltration to the therapeutic effects of methotrexate in RA has yet to be established clinically, but the association seems likely.

Other DMARDs have little anti-inflammatory activity, and their slow onset of action in RA is predominantly due to immunomodulatory actions on lymphocytes and monocytes [30]. However, corticosteroids possess both anti-inflammatory and immunosuppressive activity. The mechanism of action of corticosteroids involves the increased or decreased transcription of a variety of genes, including the reduced expression of cytokine genes [31]. It has been proposed that a major mechanism for the immunosuppres-

sive actions of corticosteroids is the inhibition of the transcription factor NF-κB, through induction of the synthesis of IκBα, the endogenous inhibitor of NF-κB [32].

In contrast, the anti-inflammatory actions of corticosteroids on leukocytes appear to be mediated by the induction of lipocortin 1 (annexin 1). The prototypic glucocorticosteroid, dexamethasone, inhibits both neutrophil and monocyte recruitment in mouse models of inflammation, actions that are reversed by immunization of the mice against lipocortin 1 [33, 34]. The inhibition of neutrophil recruitment is not due to an action on adhesion, but rather to inhibition of transmigration of the cells through the endothelium [35, 36].

This brief overview clearly indicates that whereas the mechanisms differ, all currently used drugs that exert an early anti-inflammatory action in joint diseases, together with the slower-acting agent, methotrexate, share the property of inhibiting the recruitment of neutrophilic leukocytes to the site of inflammation.

Considerable effort is being expended at present to develop novel anti-inflammatory compounds that act specifically to block leukocyte infiltration by inhibition of their adhesion to the endothelium [19, 37]. These include antibodies to adhesion molecules, carbohydrate antagonists of selectins, and low molecular weight inhibitors of integrins. Findings obtained thus far with oxaceprol suggest that this antirheumatic drug, which now has been used therapeutically for several years, acts selectively to inhibit leukocyte infiltration.

CLINICAL EFFECTS OF OXACEPROL IN JOINT DISEASES

Oxaceprol has been studied in a variety of different arthritic conditions. In early placebo-controlled, double-blind crossover studies in patients with active OA of various joints, oxaceprol (200 mg three times daily p.o.) given for 4 weeks reduced joint pain and stiffness and significantly reduced the requirement for concomitant treatment with acetylsalicylic acid [38, 39]. Further double-blind, controlled studies in OA were carried out in comparison with the NSAIDs ibuprofen and diclofenac, which are both acknowledged to provide significant improvement in joint pain and function in comparison with the placebo. In the treatment of OA of the knee or hip, oxaceprol (3 × 200–400 mg/day) was found to be as effective as ibuprofen (3 × 400 mg/day) and diclofenac (3 × 25–50 mg/day) in relieving joint pain (weight-bearing, resting, and on movement) and improving the internationally validated Lequesne index of joint function [40–43]. Relief of resting pain, pain on movement and improvement of mobility by oxaceprol (3 × 400 mg/day) in OA of the spine was also similar to that achieved with ibuprofen (3 × 400 mg/day) [44, 45].

The presence of inflammation in the OA joint is variable, and synovitis with leukocyte infiltration, although common, is rarely pronounced but frequently associated with painful activation of the disease [46, 47]. Beneficial effects of NSAIDs in OA are generally thought to be due at least partially to inhibition of this inflammation. Presumably the pain and stiffness-relieving effects of oxaceprol in OA are also related to its anti-inflammatory actions, as discussed below.

The inflammatory lesions of the joints in patients with RA are much more severe than those in OA. While neutrophilic leukocytes accumulate in synovial fluid [12], the tissue lesions are dominated by a lymphocytic and monocytic infiltrate [48]. As in OA, oxaceprol treatment (3 × 400 mg/day for 6 weeks) of patients with active RA has been shown, in a double-blind, randomized study in 401 patients, to be at least as effective treatment as with diclofenac (3 × 50 mg/day for 6 weeks) in reducing both pain (at rest and under load) and the Ritchie articular inflammation index [49]. Oxaceprol, in fact, tended to be more effective than diclofenac in reducing both pain and inflammation. Clearly, under conditions in which the NSAID diclofenac was active, oxaceprol also was able to inhibit the inflammatory process.

Moreover, in this study in RA, oxaceprol was better tolerated than diclofenac, particularly with regard to gastrointestinal complaints. Better tolerability of oxaceprol than diclofenac has also been reported in the treatment of OA patients [43], suggesting that a different mechanism of action may underlie this safety [50].*

Experimental studies have shown that the clinical efficacy and safety profile of oxaceprol is based on biological activity that differs notably in several respects from that of the classical NSAIDs.

ACTIONS OF OXACEPROL CONTRIBUTING TO ANTI-INFLAMMATORY EFFECTS

In an early study, oxaceprol (3 × 200 mg/day for 4 days) was found to inhibit by about 20% the erythema induced by the injection of a bacterial extract into the skin of human volunteers [51]. The mechanism was not clarified, although it can be assumed that, in response to a bacterial extract, leukocyte infiltration would have occurred.

Initial screening studies revealed that oxaceprol also inhibited carrageenan-induced paw oedema in rats.† Subsequent studies demonstrated that in this test model oxaceprol caused 50% inhibition at a dose of 50 mg/kg, but greater inhibition could not be achieved at higher doses [6]. Carrageenan paw oedema is the classical model for testing NSAIDs, and the efficacy of these drugs in the oedema model shows good predictability for the subsequent clinical anti-inflammatory dose [52]. It is generally considered that NSAIDs are active in carrageenan oedema through inhibition of COX, and, indeed, the rank orders of potency for inhibition of PG synthesis and inhibition of carrageenan oedema are comparable [53, 54]. Oxaceprol (up to 10^{-5}

*Hermann G, Steeger D, Klasser M, Wirbitzky J, Fürst M, Venbrocks R, Rohde H, Jungmichel D, Hildebrandt HD, Parnham MJ, Gimbel W and Dirschedl H, manuscript in preparation.

†Internal report 1987, Chephasaar, St. Ingbert, Germany.

mol/L), however, had no effect on PGE_2 or leukotriene C_4 production by ionophore-stimulated mouse peritoneal macrophages *in vitro*, a system in which COX inhibitors are consistently inhibitory [6]. Oxaceprol is thus devoid of COX inhibitory activity, explaining its limited efficacy in carrageenan paw oedema, and pointing to an anti-inflammatory mechanism of action distinct from that of NSAIDs. Since the gastrointestinal toxicity of NSAIDs is also closely related to inhibition of COX-1 in the gastrointestinal mucosa [11, 12], the lack of effect of oxaceprol on PG synthesis probably also explains its good tolerability in clinical studies.

A possible clue to the mechanism of action of oxaceprol was obtained from studies in carrageenan-induced pleurisy in the rat.* Here, dose-dependent inhibition of both exudation and neutrophil infiltration (ED_{50} = 16 mg/kg) was observed following oral oxaceprol (2–32 mg/kg) administration 5 hr previously. Ibuprofen, given as a reference compound, inhibited exudation and neutrophil infiltration by 33–40% at 50 mg/kg.

Thus, despite its lack of inhibitory activity against COX, oxaceprol is still able to inhibit both exudation and neutrophil infiltration in a manner similar to that of the NSAIDs. Neutrophils are an important stimulus to plasma exudation and other tissue-damaging processes in inflammation [55, 56]. Consequently, the effects of oxaceprol on exudation may be secondary to inhibition of neutrophil infiltration.

Such an action of oxaceprol is suggested by its effects on adjuvant arthritis in the rat, a traditional model of immunologically driven polyarthritis [57]. Oxaceprol (6–54 mg/kg p.o.) administered daily for 15 days to arthritic rats markedly inhibited secondary tail and ear lesions, but had little effect on the primary paw swelling due to adjuvant injection [6]. Double-blind, histological analysis of the knee joint on day 15, however, revealed marked inhibition of neutrophilic leukocyte infiltration of the synovial membrane and periarticular soft tissue, which was comparable to that achieved with indomethacin (3 mg/kg/day) (Fig. 1). Oxaceprol also inhibited synovial membrane proliferation, but had little effect on cartilage damage in this model. Stimulatory rather than inhibitory effects of oxaceprol on cartilage proteoglycan metabolism could be demonstrated at high concentrations *in vitro* [58]. Adverse effects on cartilage *in vivo* are not observed with oxaceprol [59], in contrast to some NSAIDs [60].

Thus, the available experimental data suggest that the anti-inflammatory activity of oxaceprol is closely related to its ability to inhibit neutrophil infiltration into the inflamed site, independent of PG synthesis and cartilage catabolism.

*Internal report 1991, Chephasaar, St. Ingbert, Germany.

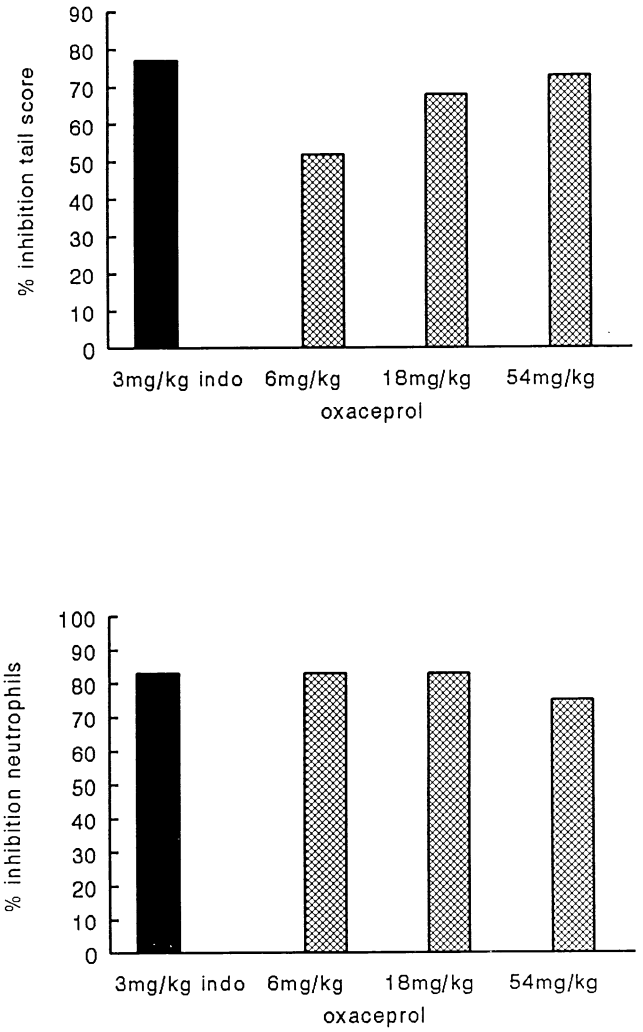

FIG. 1. Inhibition of tail lesions and synovial membrane neutrophil infiltration in day 15 adjuvant arthritic rats by daily administration of indomethacin or oxaceprol [6].

EFFECT OF OXACEPROL ON LEUKOCYTE ADHERENCE

The process of extravasation of leukocytes during inflammation is closely regulated by the expression (under stimulation by cytokines) of adhesion molecules on the leukocytes and endothelial cells [61]. Binding of L-selectin on neutrophils to its ligand on endothelial cells causes rolling of the neutrophils along the endothelium and adherence is mediated by the tighter interactions between intercellular adhesion molecule-1 (ICAM-1) on the endothelium and β_2-integrins (CD11a,b,c/CD18) on the neutrophils [19].

In a study in hamsters fitted with a dorsal skinfold chamber, 4 hr ischemia of the chamber followed by reperfusion, resulted in rolling and adherence of leukocytes, with subsequent leukocyte extravasation (Fig. 2). The rolling was not influenced by oxaceprol. All the subsequent responses, together with postcapillary venule leakage, were inhibited significantly 0.5, 2.0 and 24 hr after reperfusion by a bolus intravenous dose of 50 mg/kg of oxaceprol

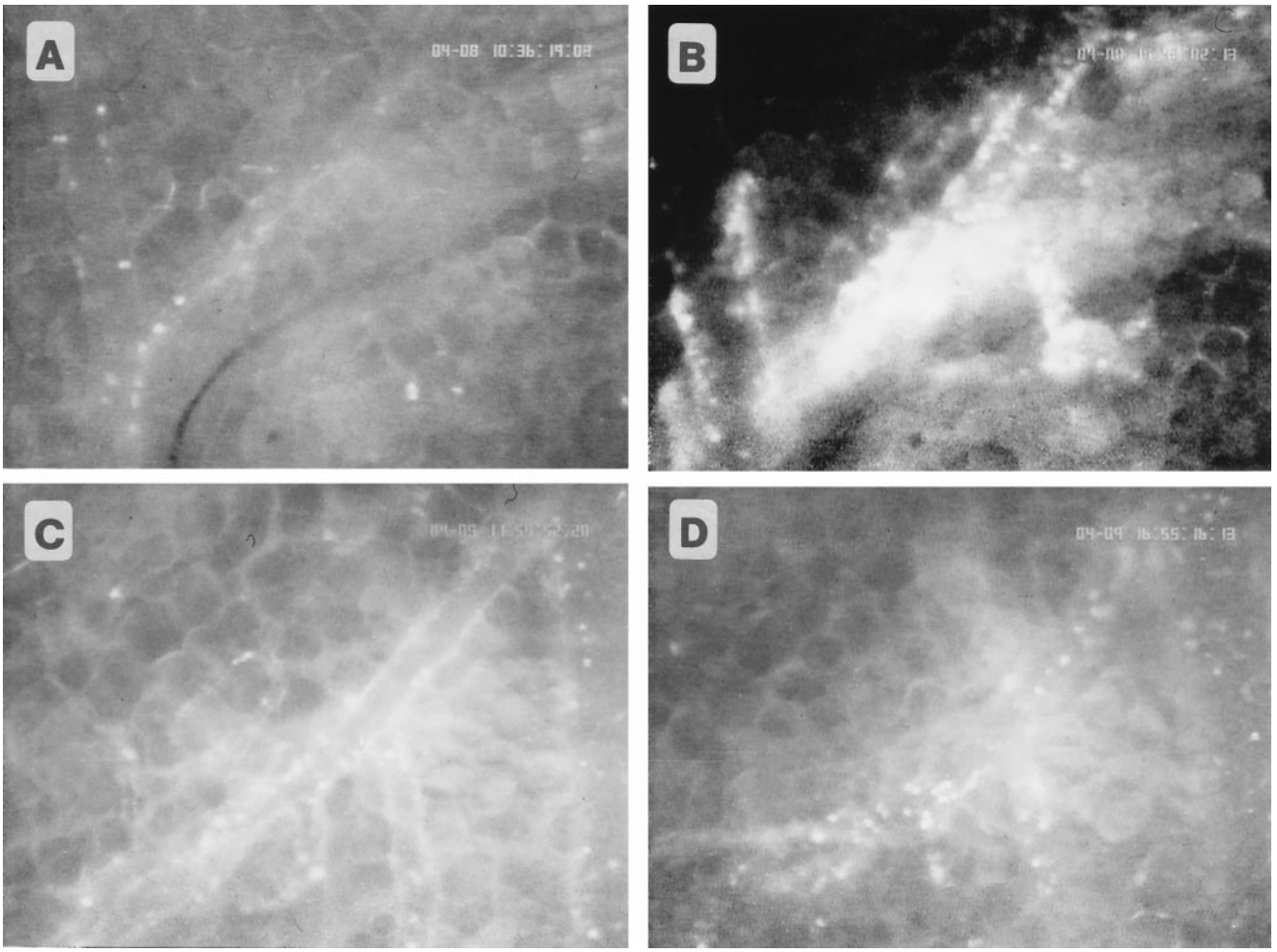

FIG. 2. Inhibition by oxaceprol of ischemia/reperfusion-induced leukocyte adhesion in a hamster dorsal fold skin chamber. In untreated hamsters, leukocytes bound massively to the blood vessel endothelium when reperfusion was induced after a 4-hr ischemia (A: before ischemia; B: after reperfusion). In oxaceprol-treated hamsters, leukocyte adhesion after reperfusion was similar to that before the ischemia (C: before ischemia; D: after reperfusion). Photographs were taken after rhodamine staining [7] and are courtesy of Dr. A. Harris and Prof. Dr. K. Messmer, Institute of Surgical Research, Klinikum Großhadern, Ludwig-Maximilians-University, Munich, Germany (published with permission).

followed by a 45-min infusion at the same dose [7]. The mean number of extravasated leukocytes was reduced from 2.0/vessel to 0.25/vessel.

Consequently, oxaceprol is able to inhibit, from the earliest stage of leukocyte adherence, both the process of leukocyte infiltration of damaged tissues and the associated plasma exudation. Although the precise mechanism of action remains to be established, it does appear that the function of adhesion molecules during inflammation is reduced by oxaceprol.

CONCLUSIONS

Most drugs used for the treatment of joint diseases are able to inhibit the infiltration of leukocytes into the inflamed tissue. The mechanisms whereby these inhibitory effects occur are still unclear, although the opinion is widespread that the major mode of action of NSAIDs involves inhibition of PG synthesis. Methotrexate is thought to act by releasing adenosine. The balance of experimental data on oxaceprol, which is used for the treatment of joint disease, indicates that this drug acts selectively to inhibit the extravasation of neutrophilic leukocytes, probably by reducing the adhesion of leukocytes to the endothelium. Oxaceprol, a synthetic amino acid derivative, thus, represents a potentially novel class of anti-inflammatory agents.

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