Dual Inhibition of 5-Lipoxygenase and Cyclooxygenases 1 and 2 by ML3000 Reduces Joint Destruction in Adjuvant Arthritis

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ABSTRACT. ***Objective.*** To search for potential new therapies to inhibit the progression of joint destruction in patients with rheumatoid arthritis.

Methods. We evaluated the dual acting antiinflammatory drug ML3000 (2,2-dimethyl-6-(4-chlorophenyl)-7-phenyl-2,3-dihydro-1H-pyrrolizine-5-yl) acetic acid, a dual inhibitor of 5-lipoxygenase (5-LOX) as well as both cyclooxygenases (COX-1 and COX-2) in the rat model of adjuvant arthritis. On Day 0, female Lewis rats (5 per group) were injected intradermally with complete Freund's adjuvant at base of the tail. Treatment began on Day 2; the rats received ML3000 (20 or 80 mg/kg/day) twice daily 7 h apart for 28 days and were then sacrificed. To reduce pain, the positive control group and 2 treatment groups received paracetamol (3 mg/ml water). Joint histology was scored for synovial cell proliferation, fibroproliferative pannus, and cartilage and bone erosions, as well as diffuse leukocyte infiltrates.

Results. Daily doses of 20 or 80 mg/kg ML3000 significantly reduced the arthritis associated deficiency of body growth, the edema/erythema score, and splenomegaly. In the ankle joint, ML3000 significantly reduced the overall histological score, synovial cell proliferation, and bone/cartilage erosions, and inhibited the appearance of fibroproliferative pannus. The addition of paracetamol in the drinking water had no influence. No side effects were noted.

Conclusion. ML3000 is an antiarthritic drug with a high gastrointestinal tolerability, which can reduce synovial cell proliferation and joint erosion and is capable of markedly suppressing prostaglandin synthesis. (J Rheumatol 2001;28:2060–5)

Key Indexing Terms:
ADJUVANT ARTHRITIS 5-LIPOXYGENASE CYCLOOXYGENASES ML3000

We evaluated the dual acting antiinflammatory drug ML3000 (2,2-dimethyl-6-(4-chlorophenyl)-7-phenyl-2,3-dihydro-1H-pyrrolizine-5-yl) acetic acid, an inhibitor of 5-lipoxygenase (5-LOX) as well as both cyclooxygenases (COX) in the rat model of adjuvant arthritis (AA). ML3000 is a balanced dual inhibitor in the submicromolar range. Inhibition of COX by ML3000 is described *in vitro* in bovine and human thrombocytes, while inhibition of 5-LOX was observed *in vitro* in bovine and human granulocytes[1,2]. ML3000 inhibits 5-LOX and COX-1 and 2 of all species tested (human, rat, cow, sheep) in the submicromolar to micromolar range.

Both the 5-LOX and the 5-LOX activating protein (FLAP) genes are transcribed in osteoarthritis and rheumatoid arthritis (RA) synovial fibroblasts. The 5-LOX products (i.e., leukotrienes) are proinflammatory mediators that may participate in the process leading to joint destruction[3]. The lipoxygenase inhibitors oxphaman and oxphalin only weakly influence AA[4]. On the other hand, leukotrienes are potent bronchoconstrictors and some, like LTB4, may play a role in the development of gastrointestinal (GI) ulceration[5] during longterm treatment with nonsteroidal antiinflammatory drugs (NSAID). The 5-LOX inhibition of ML3000 was confirmed by experiments with *ex vivo* LTB4 determination in the rat after oral administration[6].

COX-1 and 2 catalyze the conversion of arachidonic acid to prostaglandin (PG) H_2, the precursor of PG and thromboxane. PG are important mediators of inflammation in arthritis, but also play important roles in normal physiological functions. An inducible form, COX-2, has been shown to be upregulated *in vitro* by various proinflammatory agents, such as lipopolysaccharide, interleukin 1, and tumor necrosis factor-α[7-11]. In the rat model of AA, PG production is associated in the affected paw with upregulation of COX-2 mRNA and protein[12,13]. In this model, 1,2-diarylcyclopentenes and sulfonamide derivatives were first shown to be potent and selective COX-2 inhibitors[14]. Numerous other selective inhibitors followed, including orally active compounds, e.g., SC-58125[12], SC-58635/celecoxib[8,15], 1,2-diarylimidazoles[16], meloxicam[17], MK-0966/rofecoxib[18], and cyclopentones[19]. Selective COX-2 inhibition reduced the production of inter-

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leukin 6 and synovial inflammatory cell infiltrates[12]. Some of these drugs have a protective effect on AA associated destruction of cartilage and bone[18] and all claimed improved GI tolerability. Some COX-1 inhibitors, e.g., 4´-meloxicam[17] and the arylacetic acid CDB[20], inhibit PG synthesis and paw swelling to a similar extent, but they appear to be associated with more GI intolerance.

By inhibiting both COX-1 and 2 and 5-LOX, it should be possible to decrease side effects that appear from the compensatory increase of leukotriene production induced by selectively blocking the COX/PG pathway[1]. We describe the effect of the dual inhibitor ML3000, which inhibits AA without notable side effects. In animal experiments, the compound has antiphlogistic, analgesic, antipyretic, antiasthmatic, and antiaggregative activity at a dosage that causes no GI damage[2,6,21,22].

MATERIALS AND METHODS

Animals. Five female Lewis rats per group (5 wks old, Charles River) were housed in standard cages and given food and water *ad libitum*. On Day 0, the rats were injected intradermally with Freund's complete adjuvant at base of the tail (Difco Laboratories). Treatment with ML3000 (Merckle GmbH, Blaubeuren, Germany) by oral gavage began on Day 2; the rats received 10 or 40 mg/kg ML3000 twice daily (i.e., total daily dose of 20 or 80 mg/kg) 7 h apart, for 26 days, and were then sacrificed. The suspensions of ML3000 in 1% methylcellulose (Sigma) were prepared fresh daily, stirred, and kept at 4°C in the dark until use[1,2,20,21]. The concentrations were selected on the basis of previous experiments, using complete Freund's adjuvant. Twenty mg/kg/day showed activity, which improved with higher doses of 50 and 100 mg/kg/day[22]. We selected 20 mg/kg/day as low dose for the present experiment. Based on the outcome of a 4 week toxicity experiment in the rat, with determination of clinical signs, hematology, biochemistry, macroscopic and microscopic evaluation of all major organs, we selected 80 mg/kg/day as high dose. This dose was tolerated over 4 weeks' treatment without major signs of toxicity. As required by the ethical commission of our institution to reduce pain, the positive control group and 2 treatment groups received paracetamol in drinking water 3 mg/ml (Tylenol, Janssen-Cilag AG) (Table 1). The animals absorbed a mean of 19–20 ml drinking water/day.

Macroscopic evaluation. Weight and evaluation of the hind paws for signs of edema and erythema were noted biweekly. The following scoring system was applied: 0 = no edema; 1 = slight edema of the small digital joints; 2 = edema of the digital joints and foot pad; 3 = gross edema of the entire foot pad below the ankle or elbow; 4 = gross edema of the entire foot pad including the ankle or elbow joints. In addition, erythema was scored as 0 = normal; 1 = pink; 2 = red. Two independent evaluators were blinded regarding treatment groups, and the mean score was noted.

Histologic evaluation. The left hind paws and knees were removed and fixed in 10% buffered formalin. The bones were decalcified in 10% EDTA and 10% formaldehyde and then embedded in paraffin (58°C, 3 × 45 min). Scores of joint histopathology were calculated for the hind paws and knees at the end of the experiment. We developed a scoring system for evaluating the progression or inhibition of joint destruction. The score was based on synovial hyperplasia and cartilage and bone erosions, as well as diffuse inflammatory synovitis. Two sections of each joint were evaluated for synovial cell proliferation, cartilage and bone erosions, fibroproliferative pannus, and diffuse leukocyte infiltration: 0 = normal; 1 = mild; 2 = moderate; and 3 = severe. The overall score for each animal was calculated by adding the single scores. Two blinded independent investigators evaluated treatment groups, and the mean score was noted.

Table 1. Experimental procedure.

Group	Compounds	ML3000, mg/kg
1	ML3000 and paracetamol	80
2	ML3000	80
3	ML3000 and paracetamol	20
4	Paracetamol alone	—

Statistical analysis. Box plots with interquartile ranges are shown and the Mann-Whitney U test was used for comparison. A p value < 0.05 was considered significant.

RESULTS

Macroscopic evaluation of body weight and hind limbs. In placebo (methylcellulose and paracetamol) treated positive control rats (Group 4), a growth deficiency appeared at onset of arthritis (Figure 1). Rats treated with 80 mg/kg/day ML3000 (with or without paracetamol, Groups 1 and 2) showed a normal growth ($p < 0.001$, compared with positive controls, Group 4). In contrast, rats treated with a lower dose of ML3000 (20 mg/kg/day with paracetamol, Group 3) showed reduced body weight.

In placebo treated positive control rats, arthritis appeared within 9–10 days (Figure 2). The appearance of erythema and edema scores under treatment with ML3000 (with or without paracetamol, Groups 1–3) was significantly reduced, compared with positive controls ($p < 0.001$, Group 4). In contrast to body weight, no dose-dependency was observed.

No statistically significant difference was found between the treatment with 80 mg/kg ML3000 and paracetamol (Group 1) and 80 mg/kg ML3000 alone (Group 2).

Histologic evaluation of knee joints. In rats injected with complete Freund's adjuvant, the knee joints revealed no or only very minor changes (Figure 3, upper panel). One of 5 rats in each of the Groups 2–4 showed minor synovial cell proliferation, but no erosions of cartilage or bone or mononuclear cell infiltrations. Knee joints of the other animals appeared normal.

Histologic evaluation of ankle joints. In contrast to knee joints, ankle joints of placebo treated positive control rats (Group 4) showed most significant synovial cell proliferation, fibroproliferative pannus, cartilage/bone erosions, and diffuse mononuclear cell infiltrations. For statistical reasons, the treatment groups were analyzed together. The overall score of ankle joints was significantly reduced in rats treated with ML3000 (Figure 3, lower panel). Ankle joints of rats treated with 80 mg/kg/day ML3000 and paracetamol (Group 1) or 80 mg/kg/day ML3000 alone (Group 2) appeared normal. In ankle joints of rats treated with 20 mg/kg/day ML3000 plus paracetamol (Group 3), one of 5 cases revealed minor changes, including synovial cell proliferation, cartilage/bone erosions, and diffuse inflammatory synovitis.

For statistical reasons, groups receiving ML3000 (Groups 1–3) were analyzed together. Treatment with ML3000 significantly reduced synovial cell proliferation, cartilage/bone erosion scores, and the occurrence of fibroproliferative pannus

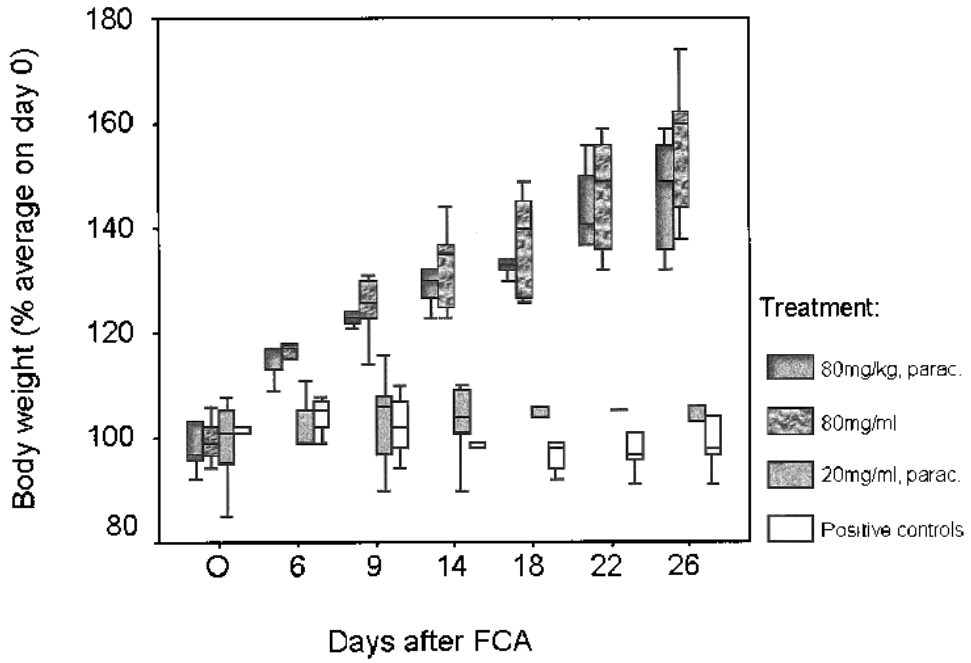

Figure 1. Box plots of body weight after intradermal injection of complete Freund's adjuvant (FCA) in female Lewis rats and effect of oral gavage with different doses of ML3000. The bar indicates the occurrence of arthritis. Significant difference between positive controls and the 2 groups treated with 80 mg/kg/day ML3000 (n = 5 each, Mann-Whitney U test). Parac: paracetamol.

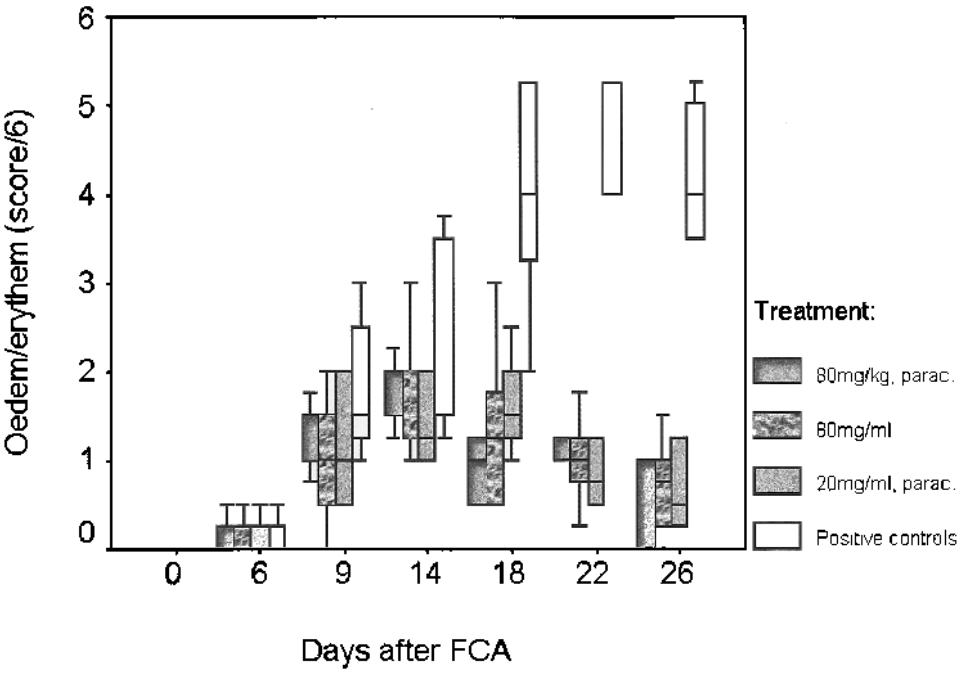

Figure 2. Box plots of the macroscopic evaluation of arthritis after intradermal injection of complete Freund's adjuvant (FCA) in female Lewis rats and effect of oral gavage with different doses of ML3000. The bar indicates the occurrence of arthritis. Significant difference between positive controls and the 3 treatment groups (n = 5 each, Mann-Whitney U test). Parac: paracetamol.

(Figure 4), whereas the difference in diffuse mononuclear cell infiltration scores was not significant (0.30 ± 0.33 for Groups 1–3 vs 0.60 ± 0.55 for Group 4).

Weights of spleen and thymus. AA was associated with splenomegaly and involution of the thymus (Group 4, Figure 5). Treatment with ML3000 (Groups 1–3) reduced

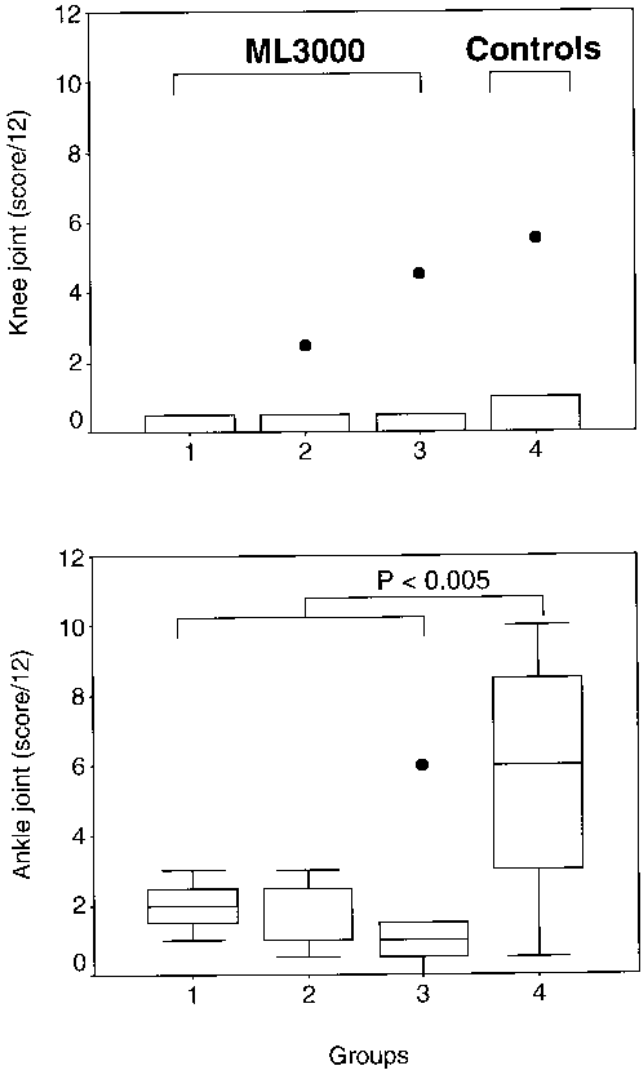

Figure 3. Box plots of the histologic evaluation of the knee and ankle joints (overall score), 28 days after intradermal injection of complete Freund's adjuvant in female Lewis rats, and effect of oral gavage with different doses of ML3000 (Group 1, 80 mg/kg/day plus paracetamol; Group 2, 80 mg/kg/day alone; Group 3, 20 mg/kg/day plus paracetamol; Group 4, placebo treated positive controls). Significant difference between positive controls (n = 5) and the treatment groups combined (n = 15, Mann-Whitney U test).

splenomegaly and involution of the thymus by 25–30%. The reduction in splenomegaly was significant ($p < 0.05$).

DISCUSSION

The course of macroscopic edema and erythema scores, as well as the histological score at end of the experiment (Day

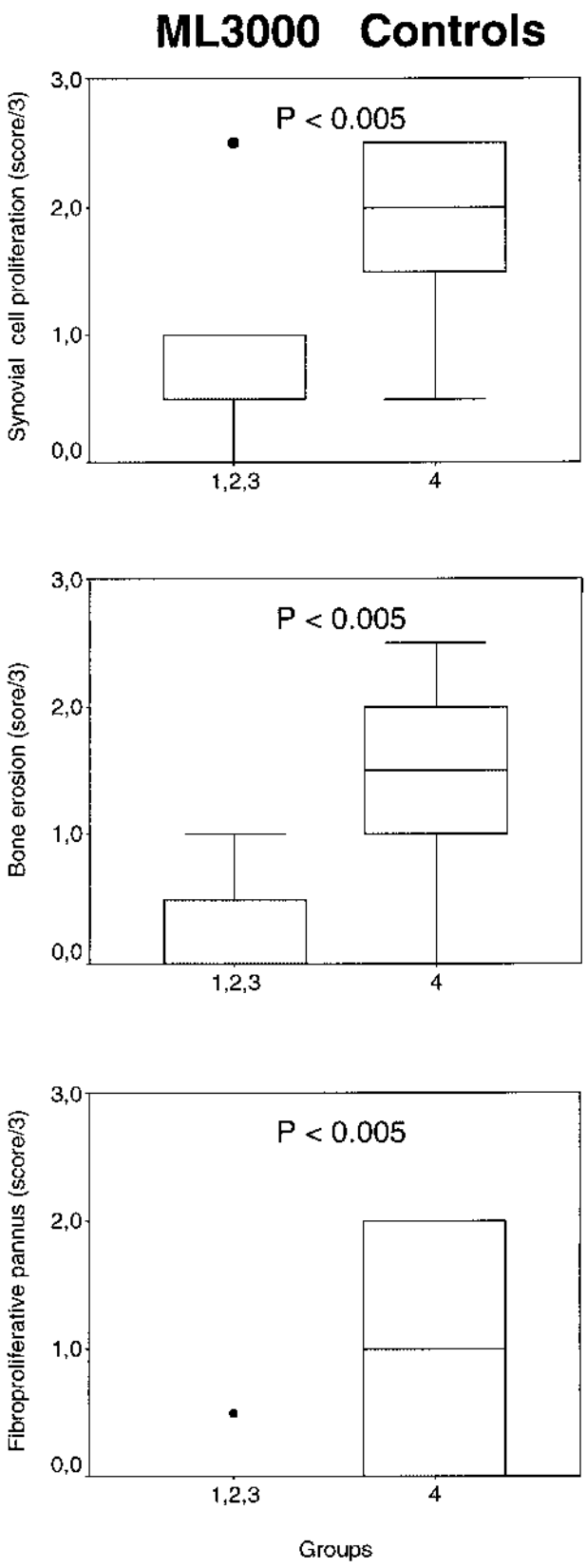

Figure 4. Box plots of the evaluation of ankle joint synovial cell proliferation, bone erosion, and fibroproliferative pannus, 28 days after intradermal injection of complete Freund's adjuvant in female Lewis rats, and effect of oral gavage with different doses of ML3000. Significant differences between positive controls (n = 5) and the treatment groups combined (n = 15, Mann-Whitney U test).

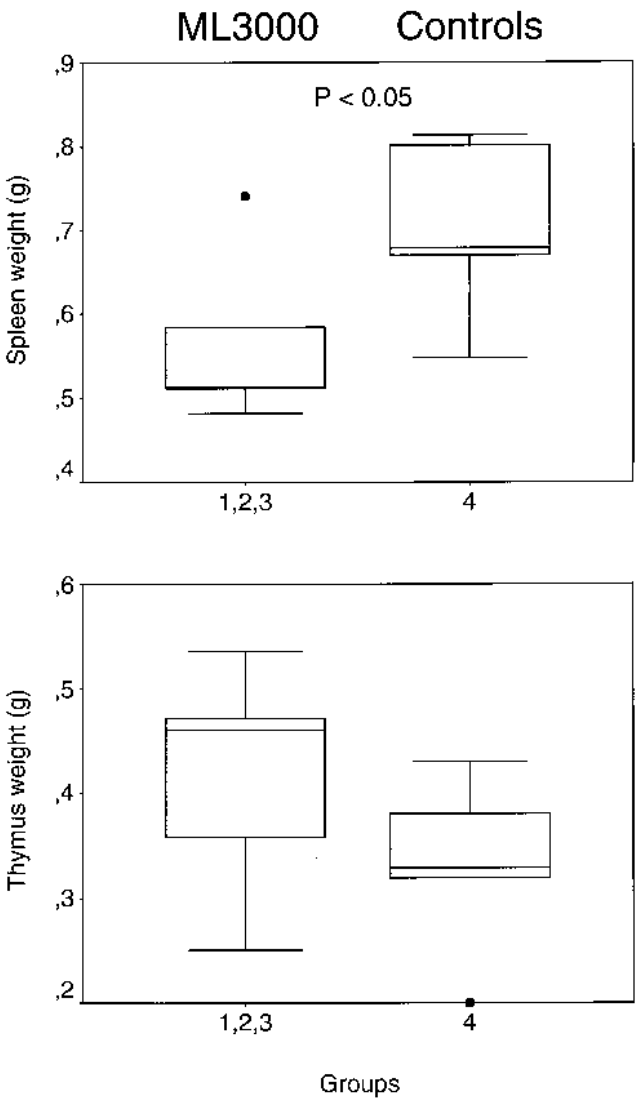

Figure 5. Box plots of weights of spleen and thymus, 28 days after intradermal injection of complete Freund's adjuvant in female Lewis rats, and effect of oral gavage with different doses of ML3000. Regarding spleen weight, significant difference between positive controls (n = 5) and the treatment groups combined (n = 15, Mann-Whitney U test).

26), illustrated the beneficial effect of ML3000 in the rat model of AA. The drug significantly reduced the arthritis associated deficiency of body growth, edema/erythema scores, and splenomegaly. In ankle joints, ML3000 significantly reduced the overall histological score, synovial cell proliferation, and bone/cartilage erosions and inhibited the appearance of fibroproliferative pannus. Our data also pointed out that scores of synovial cell proliferation and bone/cartilage erosions do not necessarily correlate with the degree of mononuclear cell infiltration. In human whole blood, ML3000 is not effective in inhibiting lipopolysaccharide induced interleukin 1ß release, and the drug has only minor effects on inducible nitrite oxide synthetase production by *S. typhosa* endotoxin stimulated murine macrophages (unpublished data). Taken together, these results suggest that ML3000 acted mainly by altering synovial fibroblast activities.

It is now known that inhibition of COX is the principal mechanism for both the efficacy and the toxicity of NSAID. The major drawbacks of these NSAID are severe mechanism based side effects including GI ulcerations and bronchospasms. Compelling evidence suggests that COX-1 synthesizes PG that are involved in the regulation of normal cell activity (including gastrointestinal cytoprotection), whereas COX-2 appears to produce PG mainly at sites of inflammation[8,23]. In RA, as observed in AA, COX-2 is expressed in synovial lining cells, lymphoid aggregates, and vascular endothelial cells[13]. These findings led to the search for compounds that would inhibit COX-2 without affecting COX-1. Arguments have been made that more selective inhibitors of COX-2 will be safer than less selective ones. In humans and rodents, rankings of the COX-2/COX-1 inhibition ratios of various NSAID as they relate to the agents' toxicity have been used as evidence that COX-2 is an important factor in the upper GI safety of some NSAID[9]. None of these claims has been supported conclusively by endoscopy studies[24]. The results of many of these studies depend on the models used, and there appears to be no clear reproducible and commonly accepted assessment of COX-2/COX-1 inhibition ratio by NSAID. With respect to ML3000, the situation is similar. Depending on the models used, the ratio for COX-2/COX-1 inhibition differs in a wide range (unpublished data).

From research into the difference between COX-1 and COX-2, new insights into the role of each isoform in normal homeostasis and in their responses to exogenous stimuli have emerged. In AA, there is a centrally mediated neurological component that is mediated at least in part by COX-2[25,26]. COX-2 is also now known to be induced in intestinal epithelium after bacterial infection, as well as in colon adenoma, carcinoma cells, and breast cancer[10,11]. Thus, even selective COX-2 inhibitors could be associated with side effects due to blockade of physiological functions. PG have been proven to be cytoprotective and antisecretory[27], thus a decrease of these mediators by inhibition of COX disturbs this cytoprotective mechanism. Another hypothesis focuses on the so-called shunt to the leukotrienes, metabolites of arachidonic acid formed by 5-LOX. Inhibiting COX may increase the metabolism of arachidonic acid via the leukotriene pathway[28]. By inhibiting both COX-1 and 2 and 5-LOX, it should be possible to improve efficacy and reduce side effects compared to selective COX inhibitors. Indeed, previous studies emphasize that ML3000 at pharmacologically active doses of up to 100 mg/kg orally is better tolerated by the gastrointestinal tract than other NSAID. The compound proved in different studies to be clearly better tolerated than reference compounds and to be in the range of the control animals[2,6,21]. While indomethacin causes a significant increase in leukocyte adherence to mesenteric venules, and diclofenac causes penetrating ulcer

formation in the gastric antrum, ML3000 did not[6]. It is noteworthy that several other dual inhibitors of COX and 5-LOX have reduced irritant effects on the stomach compared to nonselective inhibitors[29,30]. In our study using repeated oral administration of ML3000, no side effects were noted. The most reasonable explanation of the beneficial action on both joint destruction and high GI tolerability, as shown previously[2,6,21] and in unpublished data, is the dual mechanism of ML3000.

Both 5-LOX and COX-1 may also play a role in inflammatory processes[3,17,26]. The 5-LOX mRNA is upregulated in RA synovial tissue, and COX-1 is constitutively expressed in synovial tissues, particularly in synovial lining cells[4,7]. It should be expected, therefore, that a dual inhibitor like ML300 would be a more potent antiarthritic drug than a selective COX-2 inhibitor.

In summary, ML3000 is an antiarthritic drug that can reduce synovial cell proliferation and joint erosion and is capable of markedly suppressing PG synthesis without significant gastrointestinal side effects.

REFERENCES

1. Laufer SA, Augustin J, Dannhardt G, Kiefer W. (6,7-Diaryldihydropyrrolizin-5-yl) acetic acids, a novel class of potent dual inhibitors of both cyclooxygenase and 5-lipoxygenase. J Med Chem 1994;37:1894-7.
2. Laufer SA, Tries S, Augustin J, Dannhardt G. Pharmacological profile of a new pyrrolizine derivative inhibiting the enzymes cyclo-oxygenases and 5-lipoxygenases. Drug Res 1994;44:629-36.
3. Bonnet C, Bertin P, Cook-Moreau J, Chable-Rabinovich H, Treves R, Rigaud M. Lipoxygenase products and expression of 5-lipoxygenase and 5-lipoxygenase-activating protein in human cultured synovial cells. Prostaglandins 1995;50:127-35.
4. Bekemeier H, Hirschelmann R, Langner A, et al. The pharmacology of the lipoxygenase inhibitors oxphaman and oxphalin. Pharmazie 1989;44:550-5.
5. Asako H, Kubes P, Wallace J, Gaginella T, Wolf RE, Granger N. Indomethacin-induced leucocyte adhesion in mesenteric venules: role of lipoxygenase products. Am J Physiol 1992;262:G903-8.
6. Wallace JL, Carter L, McKnight W, Tries S, Laufer SA. ML3000 reduces gastric prostaglandin synthesis without causing mucosal injury. Eur J Pharmacol 1994;271:525-31.
7. Crowford LJ. COX-2 in synovial tissues. Osteoarthritis Cartilage 1999;7:406-8.
8. Geis GS. Update on clinical developments with celecoxib, a new specific COX-2 inhibitor: what can we expect? Scand J Rheumatol 1999;109 Suppl:31-7.
9. Kaplan-Machlis B, Klostermeyer BS. The cyclooxygenase-2 inhibitors: safety and effectiveness. Ann Pharmacother 1999;33:979-88.
10. Hla T, Bishop-Bailey D, Liu CH, Schaefers HJ, Trifan OC. Cyclooxygenase-1 and 2 isoenzymes. Int J Biochem Cell Biol 1999;31:551-7.
11. Crofford LJ, Lipsky PE, Brooks P, Abramson SB, Simon LS, van de Putte LBA. Current comment: basic biology and clinical application of specific cyclooxygenase-2 inhibitors. Arthritis Rheum 2000;43:4-13.
12. Anderson GD, Hauser SD, McGarity KL, Bremer ME, Isakson PC, Gregory SA. Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin-6 in rat adjuvant arthritis. J Clin Invest 1996;97:2672-9.
13. Kang RY, Freire-Moar J, Sigal E, Chu CQ. Expression of cyclooxygenase-2 in human and an animal model of rheumatoid arthritis. Br J Rheumatol 1996;35:711-8.
14. Li JJ, Anderson GD, Burton EG, et al. 1,2-Diarylcyclopentens as selective cyclooxygenase-2 inhibitors and orally active anti-inflammatory agents. J Med Chem 1995;38:4570-8.
15. Penning TD, Talley JJ, Bertenshaw SR, et al. Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzene sulfonamide (SC-58635, celecoxib). J Med Chem 1997;40:1347-65.
16. Khanna IK, Weier RM, Yu Y, et al. 1,2-Diarylimidazoles as potent, cyclooxygenase-2 selective, and orally active anti-inflammatory agents. J Med Chem 1997;40:1634-47.
17. Pairet M, van Ryn J, Schierol H, Mauz A, Trummlitz G, Engelhardt G. Differential inhibition of cyclooxygenases-1 and 2 by meloxicam and its 4ß-isomer. Inflamm Res 1998;47:270-6.
18. Chan CC, Boyce S, Brideau C, et al. Rofecoxib [Vioxx, MK-0966; 4-(4'-methylsulfonylphenyl)-3-phenyl-2-(5H)-furanone]: a potent and orally active cyclooxygenase-2 inhibitor. Pharmacological and biochemical profiles. J Pharmacol Exp Ther 1999;290:551-60.
19. Black WC, Brideau C, Chan CC, et al. 2,3-Diarylcyclopentenones as orally active, highly selective cyclooxygenase-2 inhibitors. J Med Chem 1999;42:1274-81.
20. Cutler SJ, DeWitt-Blanton C Jr, Akin DT, et al. Pharmacological evaluation of 1-(carboxymethyl)-3,5-diphenyl-2-methylbenzene, a novel arylacetic acid with potential anti-inflammatory properties. Inflamm Res 1998;47:316-24.
21. Laufer SA, Tries S, Augustin J, et al. Gastrointestinal tolerance of (2,2-dimethyl-6-(4-chlorophenyl-7-phenyl-2,3-dihydro-1H-pyrrolizine-5-yl) acetic acid in the rat. Drug Res 1994;44:1329-33.
22. Laufer SA, Tries S, Augustin J, et al. Acute and chronic anti-inflammatory properties of 2,2-dimethyl-6-(4-chlorophenyl-7-phenyl-2,3-dihydro-1H-pyrrolizine-5-yl)-acetic acid. Drug Res 1995;45:27-32.
23. Simon LS. Role and regulation of cyclooxygenase-2 during inflammation. Am J Med 1999;31:106 Suppl:37S-42S.
24. McKenna F. COX-2: separating myth from reality. Scand J Rheumatol 1999;109 Suppl:19-29.
25. Beiche F, Brune K, Geisslinger G, Goppelt-Struebe M. Expression of cyclooxygenase isoforms in the rat spinal cord and their regulation during adjuvant-induced arthritis. Inflamm Res 1998;47:482-7.
26. Smith CJ, Zhang Y, Koboldt CM, et al. Pharmacological analysis of cyclooxygenase-1 in inflammation. Proc Natl Acad Sci USA 1998;95:13313-8.
27. Cryer B, Feldmann M. Effect of nonsteroidal anti-inflammatory drugs on endogenous gastrointestinal prostaglandins and therapeutic strategies for prevention and treatment of nonsteroidal anti-inflammatory drug-induced damage. Arch Intern Med 1992;152:1145-55.
28. Kuehl FA, Daugherty HW, Ham EA. Interactions between prostaglandins and leucotrienes. Biochem Pharmacol 1984;33:1-5.
29. Whittle BJR, Higgs GA, Eakins KE, Moncada S, Vane JR. Selective inhibition of prostaglandin production in inflammatory exudates and gastric mucosa. Nature 1980;284:271-3.
30. Wallace JL, Whittle BJR. Role of prostanoids in the protective actions of BW755C on the gastric mucosa. Eur J Pharmacol 1985;115:45-8.