Rheumatology 2003;42:89–96
doi:10.1093/rheumatology/keg038, available online at www.rheumatology.oupjournals.org

The active metabolite of leflunomide, A77 1726, inhibits the production of prostaglandin E_2, matrix metalloproteinase 1 and interleukin 6 in human fibroblast-like synoviocytes

D. Burger, N. Begué-Pastor, S. Benavent, L. Gruaz, M.-T. Kaufmann, R. Chicheportiche and J.-M. Dayer

***Objectives*. To investigate the effects of the active metabolite of leflunomide, A77 1726, on fibroblast-like synoviocytes. In rheumatoid arthritis (RA) synoviocytes participate in tissue destruction by producing metalloproteinases (MMP), prostaglandin E_2 (PGE_2) and interleukin (IL) 6, which are involved in extracellular matrix degradation, resorption of the mineral phase and osteoclast-mediated bone resorption.**

***Methods*. Human synoviocytes were stimulated with IL-1α or tumour necrosis factor α (TNF-α) in the presence of A77 1726. Culture supernatants were analysed for production of interstitial collagenase (MMP-1), tissue-inhibitor of metalloproteinases 1 (TIMP-1), PGE_2 and IL-6. Total RNA was isolated and analysed for steady-state levels of MMP-1, cyclooxygenase-2 (COX-2) and IL-6 mRNA.**

***Results*. A77 1726 inhibited the production of PGE_2 in synoviocytes activated by TNF-α and IL-1α with median inhibitory concentrations (IC_{50}) of 7 and 3 μM respectively. In contrast, MMP-1 and IL-6 production was inhibited at high A77 1726 concentrations (> 10 μM), whereas TIMP-1 was not affected. The inhibition of MMP-1 and IL-6 production was due to the known inhibitory effect of A77 1726 on pyrimidine synthesis, as it was reversed by the addition of uridine. This did not apply to PGE_2 production, which was inhibited via direct action of A77 1726 on COX-2, as shown by the increasing amount of substrate (arachidonic acid) in the culture medium.**

***Conclusion*. This study shows that some of the beneficial effect of leflunomide in RA patients may be due to the inhibition of PGE_2, IL-6 and MMP-1 production in synoviocytes. This effect, coupled with its multiple inhibitory effects on T lymphocyte functions, might account for the significant reduction in the rate of disease progression in RA patients treated with leflunomide.**

KEY WORDS: Rheumatoid arthritis, PGE_2, MMP-1, IL-6, Synoviocytes.

Rheumatoid arthritis (RA) is characterized by the thickening of synovial tissue and the destruction of joint structure. The inflamed synovium thickens due to the local proliferation of synovial cells and infiltration of inflammatory cells such as lymphocytes and monocyte-macrophages [1]. We have established that contact-mediated signalling of monocytes by stimulated T lymphocytes is a potent mechanism which might play a part in the persistence of chronic inflammation, mainly in that it induces the production of large amounts of the proinflammatory cytokines tumour necrosis factor α (TNF-α) and interleukin (IL) 1β [2]. IL-1 and TNF-α are potent inducers of metalloproteinases (MMP), prostaglandin E_2 (PGE_2) and IL-6 in

Division of Immunology and Allergy, Clinical Immunology Unit (Hans Wilsdorf Laboratory), Department of Internal Medicine, University Hospital, Geneva, Switzerland.

Submitted 21 January 2002; revised version accepted 31 May 2002.

Correspondence to: D. Burger, Clinical Immunology Unit, University Hospital, 24 rue Micheli-du-Crest, CH-1211 Geneva 14, Switzerland. E-mail: danielle.burger@hcuge.ch

synoviocytes [3]. The activity of MMP is specifically controlled by tissue inhibitors of metalloproteinases (TIMP) [4], whose production is increased by IL-6 [5]. Numerous observations support the premises that in RA the resorption of the mineral phase is dependent on PGE_2, the extracellular matrix degradation is mediated by proteolytic enzymes such as MMP, and the resorption of periarticular bones is triggered by IL-6-stimulated osteoclasts [6]. Together, these mechanisms lead to tissue destruction and eventually invalidity in patients [1, 7].

Leflunomide (Arawa™) has been approved by the Food and Drug Administration for treatment of RA [8]. *In vivo* (in the cell), leflunomide is rapidly converted into its active metabolite A77 1726 [9]. Although the precise mode of action of leflunomide *in vivo* is still elusive, *in vitro* A77 1726 reversibly inhibits dihydroorotate dehydrogenase (DHODH), the rate-limiting step in the *de novo* synthesis of pyrimidines [10–12]. Indeed, most of the effects of A77 1726 can be reversed by supplying the product of DHODH activity, i.e. uridine, to target cells. Leflunomide is a potent non-cytotoxic inhibitor of the proliferation of stimulated B and T lymphocytes *in vitro*, it is effective in several rodent models of autoimmune diseases, and it prolongs graft survival in animals [9, 13]. The latter effects have drawn attention to the mechanisms of action of leflunomide in immune cells, mainly T cells, in which it also blocks TNF-mediated cellular responses by inhibiting nuclear factor-κB (NFκB), a mechanism that depends on pyrimidine biosynthesis [14, 15]. We showed previously that A77 1726 inhibited the expression of monocyte-activating factor at the surface of T lymphocytes. This in turn decreased the activation of monocyte-macrophages, thus inhibiting the production of IL-1β and MMP by monocyte-macrophages [16]. In this study, we address the question of the effect of A77 1726 on the production of PGE_2, IL-6 and MMP-1 in synoviocytes isolated from RA patients.

Materials and methods

Materials and reagents

The active metabolite of leflunomide, A771726, the kind gift of Dr M. Herrmann (Aventis, Wiesbaden, Germany), was made up as a 2 mM solution in water. Dulbecco's modified Eagle's minimal essential medium (DMEM), phosphate-buffered saline without Ca^{2+} and Mg^{2+} (PBS), fetal calf serum (FCS), penicillin, streptomycin, L-glutamine and TRIzol™ reagent were purchased from Gibco (Paisley, UK), and arachidonic acid (AA) and uridine were purchased from Sigma Fine Chemicals (St Louis, MO, USA). All other reagents were of analytical grade or better. Human recombinant IL-1α and TNF-α were obtained from Biogen (Geneva, Switzerland) and Synergen (Boulder, CO, USA), respectively.

Synoviocyte cultures

Human synoviocytes were isolated by protease treatment of surgical synovectomy specimens obtained from RA and osteoarthritis patients and cultured in DMEM supplemented with 10% FCS, 50 IU/ml penicillin, 50 μg/ml streptomycin and 2 mM L-glutamine (medium) at 37°C under an atmosphere of 5% CO_2 [17, 18]. Synoviocytes (passages 2–5) were seeded into 96-well flat-bottom plates (2×10^4 cells/well) and maintained for 48 h in medium before activation. Synoviocytes were cultured for 2 h with the indicated concentration of A77 1726 before the addition of optimal concentrations of TNF-α (10 ng/ml) or IL-1α (250 pg/ml) in a final volume of 200 μl/well [17, 18]. When required, uridine (50 or 200 μM) or AA (30 μM) was added 30 min before A77 1726. After 48 h, supernatants were analysed for PGE_2, MMP-1, TIMP-1 and IL-6 as described elsewhere [17–19]. Results are expressed as mean $\pm$ S.D. of at least three experiments carried out with three different cultures of synoviocytes.

mRNA analysis

Synoviocytes were seeded into 10 ml Petri dishes (2×10^6 cells/dish) in complete DMEM. After 48 h the cells were incubated for 30 min with or without 30 μM AA prior to the addition of 10^{-4} M A77 1726. After 2 h, cells were stimulated with 10 ng/ml TNF-α or 250 pg/ml IL-1α for 3 and 18 h before total RNA extraction by TRIzol according to the supplier's instructions (Life Technologies, Paisley, UK). Total RNA was subjected to Northern blot (10 μg) and RNase protection (2 μg) analysis as described previously [18, 20, 21].

Results

A77 1726 inhibits PGE_2, IL-6 and MMP-1 but not TIMP-1 production in synoviocytes

To determine whether A77 1726 affects the production of PGE_2, IL-6, MMP-1 and TIMP-1, isolated synoviocytes were activated by an optimal concentration of TNF-α (10 ng/ml) or IL-1α (250 pg/ml) in the presence or absence of A77 1726. In human synoviocytes, which did not produce significant amounts of PGE_2 in the absence of stimulus, TNF-α and IL-1α induced the production of 190 ± 76 and 291 ± 130 ng/ml PGE_2 respectively (Fig. 1A). PGE_2 production was completely abolished in the presence of 10^{-4} M A77 1726. Synoviocytes constitutively produced MMP-1 (1.9 ± 1.6 μg/ml, $n=5$) and its inhibitor TIMP-1 (3.0 ± 1.9 μg/ml). Depending on the synoviocyte donor/preparation, TNF-α and IL-1α increased MMP-1 production to 3.8 ± 1.7 and 3.1 ± 1.5 μg/ml respectively, and the induced but not the basal production of MMP-1 was inhibited by 10^{-4} M A77 1726 (Fig. 1B). In contrast, TIMP-1 production was neither increased by TNF-α or IL-1α nor affected by A77 1726 (Fig. 1C). IL-6 production by synoviocytes was marked in the absence of stimulus, reaching 7 ± 5 ng/ml (Fig. 1D). This basal production was increased by IL-1α and TNF-α to 346 ± 100 and 129 ± 78 ng/ml respectively. In the presence of A77 1726, the IL-6 production induced by IL-1α and TNF-α was inhibited by 47 and 63% respectively (Fig. 1D).

To ascertain whether the inhibitory effect of A77 1726 on PGE_2, IL-6 and MMP-1 production would occur at concentrations possibly reached in treated patients, the

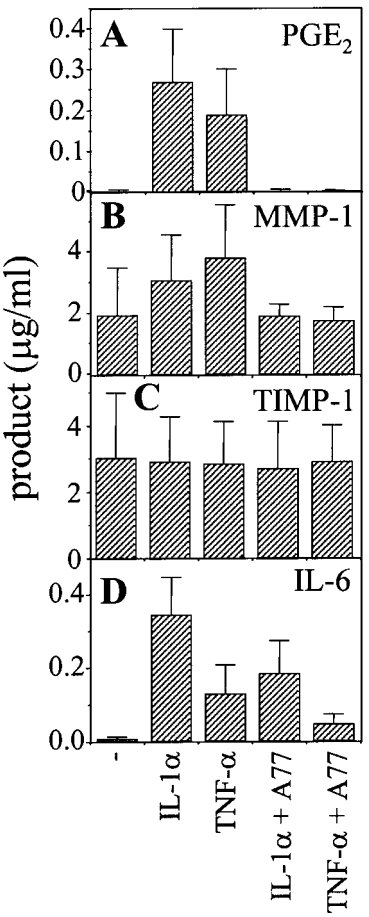

FIG. 1. A77 1726 inhibits the production of PGE_2, MMP-1 and IL-6 but not TIMP-1 in human synoviocytes. Synoviocytes were preincubated for 2 h with 10^{-4} M A77 1726 (A77) before the addition of 10 ng/ml TNF-α or 250 pg/ml IL-1α. After 48 h, supernatants were analysed for PGE_2 (A), MMP-1 (B), TIMP-1 (C) and IL-6 (D). Results are expressed as percentage of total production in the absence of inhibitor as mean ± S.D., for three experiments with three different synoviocyte cultures.

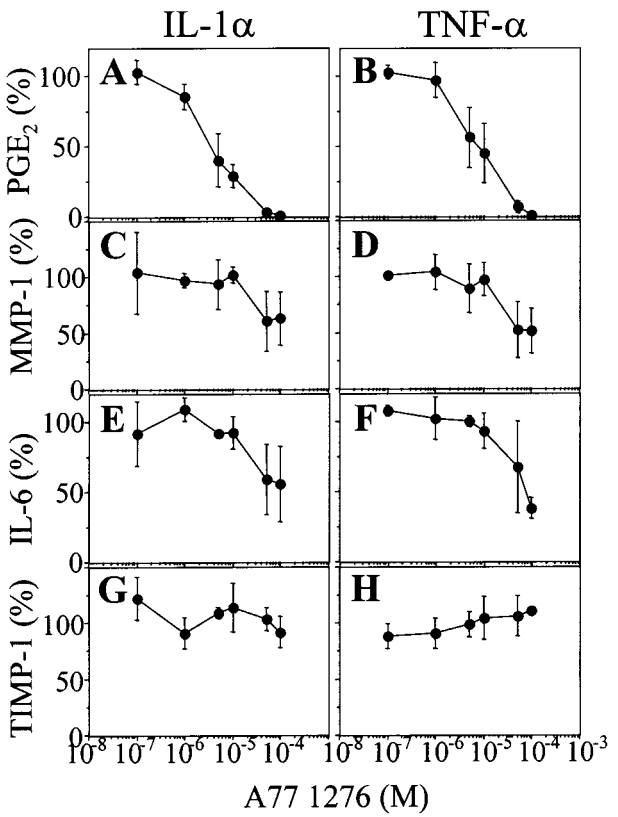

FIG. 2. The inhibition of PGE_2 and MMP-1 production by A77 1726 is dose-dependent. Synoviocytes were preincubated for 2 h with the indicated concentration of A77 1726 before the addition of 250 pg/ml IL-1α (A, C, E, G) or 10 ng/ml TNF-α (B, D, F, H). After 48 h, supernatants were analysed for PGE_2 (A and B), MMP-1 (C and D), IL-6 (E and F) and TIMP-1 (G and H), as described in the legend of Fig. 1.

inhibitory effect of serial concentrations of A77 1726 was assessed. A77 1726 diminished PGE_2 production by synoviocytes in a dose-dependent way; PGE_2 production was abolished at 10^{-4} M A77 1726 and inhibited by 100 and 99% upon stimulation by TNF-α and IL-1α respectively (Fig. 2A and B). The apparent median inhibitory concentration (IC_{50}) was 7 and 3 μM A77 1726 when synoviocytes were stimulated by TNF-α and IL-1α respectively. In contrast, the induction of MMP-1 production was inhibited at high A77 1726 concentrations only, and no inhibition was observed at concentrations $< 5\times10^{-5}$ M A77 1726 (Fig. 2C and D). Similarly, the induced production of IL-6 was only inhibited by concentrations $> 5\times10^{-5}$ M A77 1726 (Fig. 2E and F). Confirming results shown in Fig. 1, TIMP-1 production was not affected by A77 1726 (Fig. 2G and 2H), demonstrating that the marked inhibition of cytokine-induced production of PGE_2, IL-6 and MMP-1 at high A77 1726 concentration was not due to a putative cytotoxic effect of the drug.

A77 1726 inhibited PGE_2, MMP-1 and IL-6 through different mechanisms

In order to assess whether the inhibition of PGE_2, MMP-1 and IL-6 production was due to the well-described *in vitro* inhibition of pyrimidine synthesis by A77 1726, uridine was used to reverse the inhibitory effect of A77 1726. In contrast with the inhibition of MMP-1 and IL-6 production that was completely reversed by uridine (Fig. 3), the inhibition of PGE_2 production by A77 1726 was not reversed by either 50 μM (data not shown) or 200 μM uridine (Fig. 3). This suggests that the inhibition of MMP-1 and IL-6 production was due to the inhibition of DHODH, i.e. the inhibition of pyrimidine synthesis, another mechanism involved in the inhibition of PGE_2 production.

Because A77 1726 has been reported to inhibit the activity of cyclooxygenase (COX) 2 directly [22, 23], the inhibition of PGE_2 production by A77 1726 was assessed in the presence of an excess of the enzyme substrate AA. As shown in Fig. 4A and B, AA (30 μM)

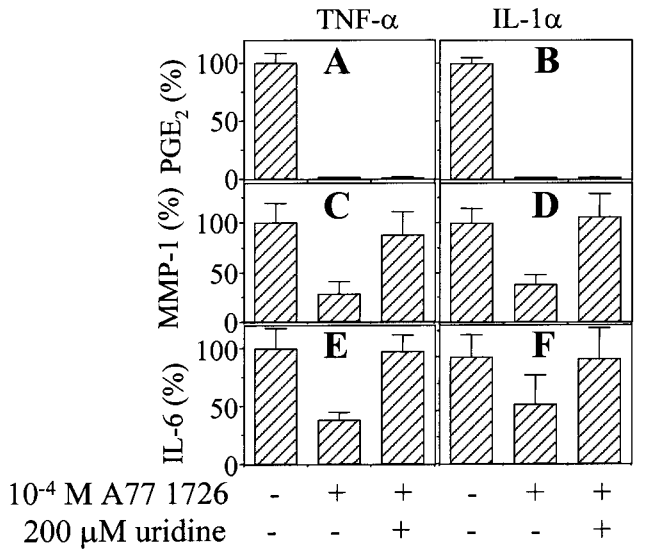

FIG. 3. Uridine reversed the inhibition by A77 1726 of MMP-1 and IL-6 but not PGE_2 production. When indicated (+), synoviocytes were preincubated for 30 min with 200 μM uridine before the addition of 10^{-4} M A77 1726. After 2 h incubation with A77 1726, cells were activated by TNF-α (A, C, E) or IL-1α (B, D, F) for 48 h. Supernatants were analysed for PGE_2 (A and B), MMP-1 (C and D) and IL-6 (E and F), as indicated in the legend of Fig. 1.

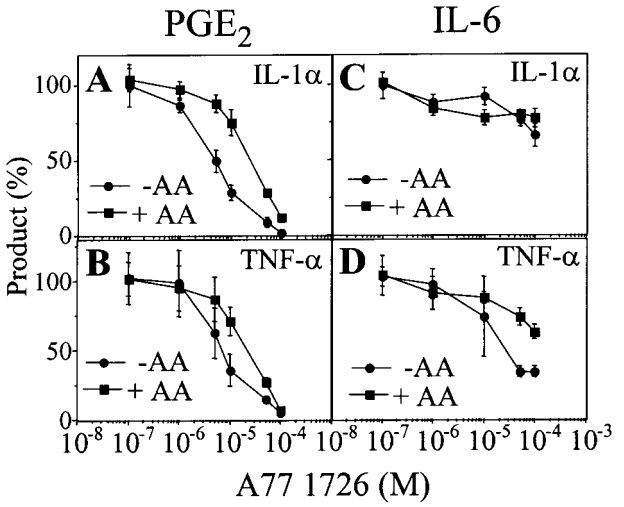

FIG. 4. Differential effect of arachidonic acid (AA) on the inhibitory activity of A77 1726. Synoviocytes were preincubated for 30 min in the presence (squares) or absence (circles) of 30 μM AA before being cultured as described in the legend of Fig. 1. Supernatants were analysed as indicated in the legend of Fig. 1. In the absence of A77 1726, PGE_2 production was 743 ± 221 and 140 ± 83 ng/ml in the presence of IL-1α plus AA and of IL-1α alone, respectively, and 406 ± 210 and 76 ± 13 ng/ml in the presence of TNF-α plus AA and of TNF-α alone, respectively. In the absence of inhibitor, IL-6 production was 340 ± 26 and 260 ± 22 ng/ml in the presence of IL-1α plus AA and of IL-1α alone, respectively, and 100 ± 13 and 60 ± 6 ng/ml in the presence of TNF-α plus AA and of TNF-α alone, respectively.

did not induce a detectable amount of PGE_2, suggesting that COX-1 expression by synoviocytes was poor. AA increased the production of PGE_2 induced by IL-1α and TNF-α, which reached 743 ± 221 and 406 ± 210 ng/ml respectively. AA shifted the IC_{50} to 20 μM A77 1726 (Fig. 4A and B), implying that the inhibitor was displaced from the enzyme and consequently that A77 1726 interacted directly with COX-2. In the presence of AA, neither the production of MMP-1 nor its inhibition by A77 1726 was affected (not shown). Upon synoviocyte stimulation by IL-1α and TNF-α, AA increased basal IL-6 production slightly (1.3-and 1.7-fold respectively, to 340 ± 26 and 100 ± 13 ng/ml). Although AA did not reverse the inhibition by A77 1726 of IL-6 production induced by IL-1α (Fig. 4C), it partially reversed the inhibition when synoviocytes were stimulated by TNF-α (Fig. 4D). This suggests that PGE_2 might enhance cytokine-induced IL-6 production, a phenomenon that was more pronounced, i.e. detectable, when cytokine stimulation was low. Indeed, the production of IL-6 induced by TNF-α was three times lower than that induced by IL-1α, suggesting that IL-1α overcame the enhancing effect of PGE_2 on IL-6 production. This was confirmed by the use of indomethacin, which inhibits the production of PGE_2. Indomethacin abolished the production of PGE_2 induced by both IL-1α and TNF-α (Fig. 5A). Because MMP-1 production was not affected, its inhibition by A77 1726 cannot result from the diminution of PGE_2

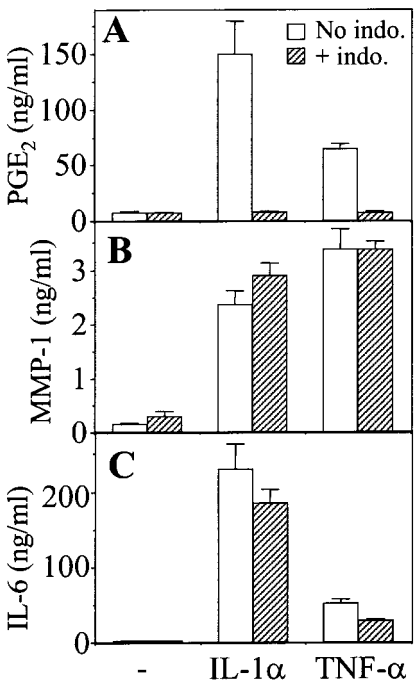

FIG. 5. Inhibition of MMP-1 production does not depend on the inhibition of PGE_2 production. Synoviocytes were preincubated for 1 h in the presence (▨) or absence (□) of 5 μg/ml indomethacin prior to activation with 250 pg/ml IL-1α or 10 ng/ml TNF-α for 48 h. Supernatants were analysed for PGE_2 (A), MMP-1 (B) and IL-6 (C).

concentration in the medium (Fig. 5B). However, the specific inhibition of PGE_2 production by indomethacin decreased IL-6 production by 20 and 42% when synoviocytes were stimulated by IL-1α and TNF-α respectively, demonstrating that the production of IL-6 due to endogenously produced PGE_2 was higher when cells were stimulated by TNF-α than by IL-1α.

To confirm that different mechanisms were involved in the inhibition of PGE_2, MMP-1 and IL-6 in synoviocytes, the effect of A77 1726 on steady-state levels of mRNAs for COX-2, MMP-1 and IL-6 was assessed by either Northern blot or RNase protection analysis. COX-2 mRNA was not expressed in synoviocytes, whether unstimulated or cultured in the presence of AA (Fig. 6A and B). Upon stimulation for 3 h by IL-1α or TNF-α, COX-2 mRNA was expressed. Levels of MMP-1 mRNA were high in unstimulated synoviocytes, and 2- to 2.5-fold increase was observed in the presence of cytokines (Fig. 6C and F). However, in the presence of A77 1726, AA, or both, there were no changes in the steady-state level of either COX-2 or MMP-1 mRNA, as shown by Northern blot analysis (Fig. 6A–C). Similarly, after 15 h of incubation the steady-state level of MMP-1 mRNA was not affected by A77 1726, uridine, or both, as shown by RNase protection assay (Fig. 6D and F). This contrasted with the fact that MMP-1 production induced by cytokines was indeed inhibited by A77 1726 (Figs 1 and 2) and that uridine reversed this inhibition (Fig. 4). The steady-state level of IL-6 mRNA was inhibited by A77 1726, an effect that was reversed by uridine (Fig. 6D and E). This confirms that A77 1726 inhibited IL-6 production through the inhibition of DHODH, i.e. the inhibition of pyrimidine synthesis.

Discussion

The salient result of this study is that leflunomide modulates the production of destructive factors by synoviocytes. Indeed, the active metabolite of leflunomide, A77 1726, inhibits PGE_2, IL-6 and MMP-1 production in synoviocytes. Although the inhibition of MMP-1 and IL-6 production only occurred at high A77 1726 concentrations, PGE_2 production was inhibited by A77 1726 with an IC_{50} <10 μM when either IL-1α or TNF-α was used as a stimulus. This effect was not reversed by uridine, contrary to other inhibitory activities of A77 1726 that have been described [9, 15], which have an IC_{50} in the same range. However, the inhibition of MMP-1 and IL-6 production was fully reversed by uridine, demonstrating that the inhibition of MMP-1 and IL-6 expression was due to the blockade of pyrimidine synthesis. The inhibition of MMP-1 and IL-6 production was observed in the presence of $\geqslant 5\times10^{-5}$ M A77 1726, roughly equal to the maximal plasma concentration in treated patients, i.e. around 50 μM [24]. This suggests that part of the beneficial effects of A77 1726 in RA patients might be due to the inhibition of PGE_2, MMP-1 and IL-6 production in synoviocytes.

High concentrations of A77 1726 are required to inhibit the induced production of MMP-1 and IL-6. However, even at these high drug concentrations, the production of TIMP-1 was not affected by A77 1726, showing that high levels of A77 1726 did not display cytotoxicity towards synoviocytes. Furthermore, from the premise that TIMP-1 production remained unchanged upon A77 1726 treatment, it follows that the drug tends to favour factors that counteract the enzymatic

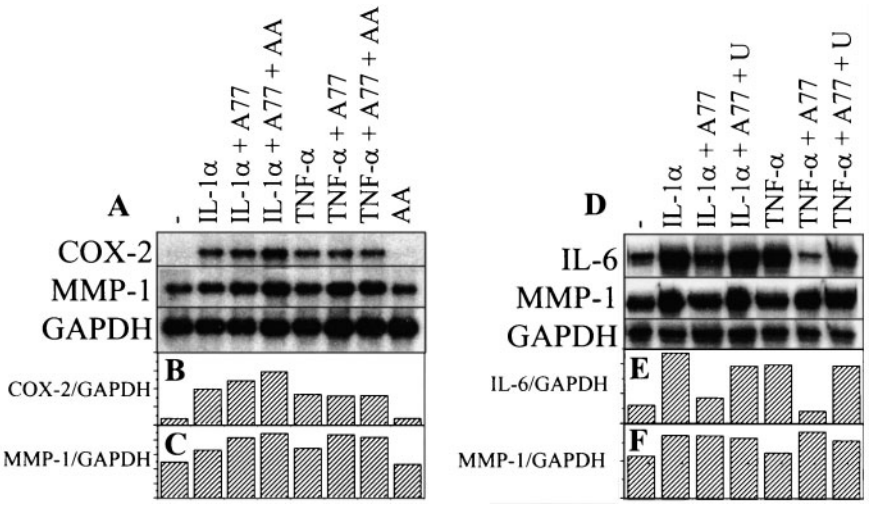

FIG. 6. Northern blot and RNase protection analysis of the steady-state level of mRNAs for COX-2, IL-6 and MMP-1. When indicated, synoviocytes were cultured for 30 min with 30 μM arachidonic acid (AA) or 200 μM uridine before the addition of 10^{-4} M A77 1726. After 2 h incubation with A77 1726 (A77), cells were stimulated or not with 250 pg/ml IL-1α or 10 ng/ml TNF-α for 3 h (A–C) or 18 h (D–F). (A) Northern blot autoradiography and (B and C) densitometric analysis of autoradiographic data shown in A normalized for densitometry of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA = 1. (D) Autoradiography of RNase protection gel and (E and F) densitometric analysis of autoradiographic data shown in D normalized for densitometry of GAPDH mRNA = 1.

degradative processes. This is reminiscent of previous results we obtained about the effect of A77 1726 on the expression of monocyte-stimulating factors on the surface of activated T lymphocytes [16]. Indeed, in that study we showed that in the presence of 10^{-5} M A77 1726 the molar ratios of IL-1 receptor agonist/IL-1β and TIMP-1/MMP-1 produced by the monocytic cells were increased around 4- and 2-fold, respectively. This demonstrated that in the T cell/monocyte-macrophage system also, A77 1726 tends to favour the inhibition of proinflammatory and matrix-destructive factors over that of anti-inflammatory factors and metalloproteinase inhibitors, thus interfering with both inflammation and tissue destruction.

A77 1726 inhibited the activity of COX-2 directly. Indeed, an excess of exogenous COX-2 substrate (AA) increased the cytokine-induced production of PGE_2 in the absence of A77 1726 and increased the IC_{50} of A77 1726 2- to 6-fold. The direct inhibition of COX-2 by A77 1726 has been described in other cellular systems. A77 1726 inhibits the activity of COX-1 and -2 isolated from sheep, although with an IC_{50} of 742 and 2766 μM respectively, suggesting that A77 1726 is a weak inhibitor of PGE_2 synthesis [22]. This low inhibitory potency was attributed in part to the premise that A77 1726 displays extremely high levels of binding to serum proteins [25]. More recently, A77 1726 was shown to inhibit COX-2 activity in the presence of 10% FCS in two cell lines, J774.2 murine macrophages and A549 human epithelial cancer cells, with IC_{50} values comparable to those observed here, i.e. in the micromolar range [23]. Although A77 1726 has been shown to inhibit PGE_2 production directly in several types of cell, this is the first observation of such inhibition occurring in synoviocytes, which are primarily involved in tissue degradation processes in RA. A77 1726 has been shown to inhibit both COX-1 and COX-2 activity [22]. It is, however, very unlikely that it inhibits a putative COX-1 activity in synoviocytes as there was no constitutive production of PGE_2 in these cells and AA did not induce any detectable PGE_2 production in the absence of cytokine. Therefore, as A77 1726-inhibition of PGE_2 production was observed in the presence of 10% FCS in the micromolar range, it might occur *in vivo* and thus account for part of the beneficial effect of leflunomide in RA. New drugs which specifically inhibit COX-2 but not COX-1 activity have been shown to display anti-inflammatory activities in RA patients [26, 27]. A77 1726 is not a specific inhibitor of COX-2, as it has also been shown to inhibit COX-1 [22]. However, it displays weak inhibitory activity compared with other COX inhibitors with IC_{50} values in the micromolar range [22]. However, we cannot rule out the possibility of synergism or an additive effect due to the inhibition of PGE_2 when leflunomide is administered together with other NSAIDs.

Although A77 1726 inhibited MMP-1 production, it did not decrease the level of MMP-1 mRNA, suggesting that the inhibition of MMP-1 production occurred at a post-transcriptional level. This contrasts with data showing that A77 1726 blocks NFκB activation in T cells, MMP-1 promoter containing an NFκB-responsive element [14, 28]. Whether A77 1726 inhibits NFκB nuclear translocation in synoviocytes remains to be determined. A77 1726 might inhibit MMP-1 production because of decreased production of pyrimidine sugars [29]. Indeed, pyrimidine sugars are important in the synthesis of the *N*-glycans that are present on at least some MMP-1s [30, 31]. Thus, considering its inhibitory effect on DHODH, A77 1726 could decrease the concentration of one or the other of the pyrimidine sugars, triggering misglycosylation of MMP-1, which in turn could hamper its production.

The efficiency of leflunomide in the treatment of RA patients might be due to the fact that it acts at several levels of the inflammatory cascade [32]. Indeed, in addition to the inhibition of production of PGE_2, MMP-1 and IL-6 in synoviocytes, A77 1726 diminishes the ability of stimulated T cells to induce the production of proinflammatory cytokines in monocyte-macrophages [16], a mechanism highly relevant to chronic inflammation [2, 33–35]. Inhibiting the induction of cytokine production in monocyte-macrophages by A77 1726 in turn lowers the levels of inducers of metalloproteinases, IL-6 and PGE_2 in synoviocytes, and because this affects PGE_2 synthesis directly in the latter cells the production of inflammatory factors is decreased still further. Together, these effects might account for the significant reduction in the rate of disease progression in RA patients treated with leflunomide [36–38].

In conclusion, this study demonstrates that leflunomide may affect functions of stromal cells involved in tissue destruction in RA. This suggests that leflunomide not only acts on several types of cells involved in the inflammatory cascade but also interferes with different intracellular processes, and may thus display broad efficacy in RA.

Acknowledgements

The authors are grateful to Dr M. L. Herrmann (Aventis, Wiesbaden, Germany) for his generous gift of A77 1726, and to Mrs Roswitha Rehm for skilful reading of the manuscript. This work was supported in part by a grant from the Swiss National Science Foundation (320-068286.02), the Hans Wilsdorf Foundation and a grant from the Swiss Society for Multiple Sclerosis.

References

1. Krane SM, Goldring SR, Dayer JM. Interactions among lymphocytes, monocytes and other synovial cells in rheumatoid synovium. In: Pick E, Landy M, eds. Lymphokines, Vol. 7. New York: Academic Press, 1982:75–136.

2. Burger D. Cell contact-mediated signaling of monocytes by stimulated T cells: a major pathway for cytokine induction. Eur Cytokine Netw 2000;11:346–53.
3. Dayer JM, Arend WP. Cytokines and growth factors. In: Kelley WN, Harris ED Jr, Ruddy S, Sledge CS, eds. Textbook of rheumatology. Philadelphia: W.B. Saunders, 1997:267–86.
4. Nagase H, Woessner JF Jr. Matrix metalloproteinases. J Biol Chem 1999;274:21491–4.
5. Lotz M, Guerne PA. Interleukin-6 induces the synthesis of tissue inhibitor of metalloproteinases-1/erythroid potentiating activity (TIMP-1/EPA). J Biol Chem 1991; 266:2017–20.
6. Matsuda T, Hirano T. IL-6. In: Oppenheim JJ, Feldmann M eds. Cytokine reference. New York: Academic Press, 2000:DOI: 10.1006/rwcy.2000.06001.
7. Harris ED. Enzymes responsible for joint destruction. In: Harris ED, ed. Rheumatoid arthritis. Philadelphia: W.B. Saunders, 1997:168–75.
8. Fox RI, Herrmann ML, Frangou CG *et al*. Mechanism of action for leflunomide in rheumatoid arthritis. Clin Immunol 1999;93:198–208.
9. Herrmann ML, Schleyerbach R, Kirschbaum BJ. Leflunomide: an immunomodulatory drug for the treatment of rheumatoid arthritis and other autoimmune diseases. Immunopharmacology 2000;47:273–89.
10. Bartlett RR, Brendel S, Zielinski T, Schorlemmer HU. Leflunomide, an immunorestoring drug for the therapy of autoimmune disorders, especially rheumatoid arthritis. Transplantation Proc 1996;28:3074–8.
11. Cherwinski HM, Byars N, Ballaron SJ, Nakano GM, Young JM, Ransom JT. Leflunomide interferes with pyrimidine nucleotide biosynthesis. Inflamm Res 1995; 44:317–22.
12. Williamson RA, Yea CM, Robson PA *et al*. Dihydroorotate dehydrogenase is a target for the biological effects of leflunomide. Transplantation Proc 1996; 28:3088–91.
13. Breedveld FC, Dayer JM. Leflunomide: mode of action in the treatment of rheumatoid arthritis. Ann Rheum Dis 2000;59:841–9.
14. Manna SK, Aggarwal BB. Immunosuppressive leflunomide metabolite (A77 1726) blocks TNF-dependent nuclear factor-κB activation and gene expression. J Immunol 1999;162:2095–2.
15. Manna SK, Mukhopadhyay A, Aggarwal BB. Leflunomide suppresses TNF-induced cellular responses: effects on NF-κB, activator protein-1, c-Jun N-terminal protein kinase, and apoptosis. J Immunol 2000;165:5962–9.
16. Déage V, Burger D, Dayer JM. Exposure of T lymphocytes to leflunomide but not to dexamethasone favors the production by monocytic cells of interleukin-1 receptor antagonist and the tissue-inhibitor of metalloproteinases-1 over that of interleukin-1β and metalloproteinases. Eur Cytokine Netw 1998;9:663–8.
17. Burger D, Chicheportiche R, Giri JG, Dayer JM. The inhibitory activity of human interleukin-1 receptor antagonist is enhanced by type II interleukin-1 soluble receptor and hindered by type I interleukin-1 soluble receptor. J Clin Invest 1995;96:38–41.
18. Burger D, Rezzonico R, Li JM, Modoux C, Welgus HG, Dayer JM. Imbalance between interstitial collagenase (MMP-1) and tissue inhibitor of metalloproteinases-1 (TIMP-1) in synoviocytes and fibroblasts upon direct contact with stimulated T lymphocytes: involvement of membrane-associated cytokines. Arthritis Rheum 1998; 41:1748–59.
19. Vey E, Zhang JH, Dayer J-M. IFN-gamma and 1,25(OH)2D3 induce on THP-1 cells distinct patterns of cell surface antigen expression, cytokine production, and responsiveness to contact with activated T cells. J Immunol 1992;149:2040–6.
20. Rezzonico R, Burger D, Dayer JM. Direct contact between T lymphocytes and human dermal fibroblasts or synoviocytes down-regulates types I and III collagen production via cell-associated cytokines. J Biol Chem 1998; 273:18720–8.
21. Jungo F, Dayer JM, Modoux C, Hyka N, Burger D. IFN-β inhibits the ability of T lymphocytes to induce TNF-α and IL-1β production in monocytes upon direct cell–cell contact. Cytokine 2001;14:272–82.
22. Curnock AP, Robson PA, Yea CM *et al*. Potencies of leflunomide and HR325 as inhibitors of prostaglandin endoperoxide H synthase-1 and -2: comparison with nonsteroidal anti-inflammatory drugs. J Pharmacol Exp Ther 1997;282:339–47.
23. Hamilton LC, Vojnovic I, Warner TD. A771726, the active metabolite of leflunomide, directly inhibits the activity of cyclo-oxygenase-2 in vitro and in vivo in a substrate-sensitive manner. Br J Pharmacol 1999; 127:1589–96.
24. Weinblatt ME, Kremer JM, Coblyn JS *et al*. Pharmacokinetics, safety, and efficacy of combination treatment with methotrexate and leflunomide in patients with active rheumatoid arthritis. Arthritis Rheum 1999;42:1322–8.
25. Lucien J, Dias VC, LeGatt DF, Yatscoff RW. Blood distribution and single-dose pharmacokinetics of leflunomide. Ther Drug Monit 1995;17:454–9.
26. Schnitzer TJ, Truitt K, Fleischmann R *et al*. The safety profile, tolerability, and effective dose range of rofecoxib in the treatment of rheumatoid arthritis. Phase II Rofecoxib Rheumatoid Arthritis Study Group. Clin Ther 1999;21:1688–702.
27. Luong BT, Chong BS, Lowder DM. Treatment options for rheumatoid arthritis: celecoxib, leflunomide, etanercept, and infliximab. Ann Pharmacother 2000;34:743–60.
28. Vincenti MP, Coon CI, Brinckerhoff CE. Nuclear factor-κB/p50 activates an element in the distal matrix metalloproteinase 1 promoter in interleukin-1β-stimulated synovial fibroblasts. Arthritis Rheum 1998;41:1987–94.
29. Abeijon C, Hirschberg CB. Topography of glycosylation reactions in the endoplasmic reticulum. Trends Biochem Sci 1992;17:32–6.
30. Saarinen J, Welgus HG, Flizar CA, Kalkkinen N, Helin J. N-glycan structures of matrix metalloproteinase-1 derived from human fibroblasts and from HT-1080 fibrosarcoma cells. Eur J Biochem 1999;259:829–40.
31. Wilhelm SM, Eisen AZ, Teter M, Clark SD, Kronberger A, Goldberg G. Human fibroblast collagenase: glycosylation and tissue-specific levels of enzyme synthesis. Proc Natl Acad Sci USA 1986;83:3756–60.
32. Dayer JM, Feige U, Edwards III CK, Burger D. Anti-interleukin-1 therapy in rheumatic diseases. Curr Opin Rheumatol 2001;13:170–6.
33. McInnes IB, Leung BP, Liew FY. Cell–cell interactions in synovitis. Interactions between T lymphocytes and synovial cells. Arthritis Res 2000;2:374–8.
34. Parry SL, Sebbag M, Feldmann M, Brennan FM. Contact with T cells modulates monocyte IL-10 production. Role of T cell membrane TNF-α. J Immunol 1997;158:3673–81.

35. Burger D. Meeting report: Cell contact interactions in rheumatology. The Kennedy Institute for Rheumatology, London, UK, 1–2 June 2000. Arthritis Res 2000; 2:472–6.
36. Strand V, Cohen S, Schiff M *et al*. Treatment of active rheumatoid arthritis with leflunomide compared with placebo and methotrexate. Leflunomide Rheumatoid Arthritis Investigators Group. Arch Intern Med 1999;159:2542–50.
37. Smolen JS, Kalden JR, Scott DL *et al*. Efficacy and safety of leflunomide compared with placebo and sulphasalazine in active rheumatoid arthritis: a double-blind, randomised, multicentre trial. European Leflunomide Study Group. Lancet 1999;353:259–66.
38. Emery P, Breedveld FC, Lemmel EM *et al*. A comparison of the efficacy and safety of leflunomide and methotrexate for the treatment of rheumatoid arthritis. Rheumatology 2000;39:655–65.