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# Effects of tiaprofenic acid on urinary pyridinium crosslinks in adjuvant arthritic rats: Comparison with doxycycline

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**Abstract.** *Objective and Design:* To study the effects of tiaprofenic acid and doxycycline on urinary pyridinium crosslinks and paw swelling in adjuvant arthritic rats, and to gain additional information on the drugs' inhibitory potential vs. in vitro targets, such as enzyme activity of matrix metalloproteinases and cytokine generation.
*Material*: 124 male Wistar Lewis rats; for the in vitro studies human matrix metalloproteinases and human mononuclear cells were used.
*Treatment*: Arthritis was induced by injection of complete Freund adjuvant. Drugs (2, 15, 50 mg tiaprofenic acid/kg; 5, 15, 30 mg doxycycline/kg) were administered daily p.o. until day 21. In the in vitro studies 10-1000 μmoles/l of these drugs were used.
*Methods*: Urinary levels of pyridinoline and deoxypyridinoline, determined by HPLC/fluorescence, and paw volumes were the measurements in the rat study. In the in vitro studies enzyme activities were assessed using fluorogenic peptide substrates; cytokines were determined by ELISA.
*Results*: On day 21 of disease crosslink excretion was about twofold higher compared to the healthy controls. After administering daily 15 or 30 mg/kg tiaprofenic acid p.o. this increase was almost completely prevented whereas the paw volumes were suppressed by about 50%. Up to 50 mg/kg doxycycline did not display significant suppressive effects on crosslinks and paw volumes. In vitro 50-100 μmol/l of both drugs inhibited the activities of selected metalloproteinases, but only doxycycline suppressed the generation of IL-1$\beta$/TNF$\alpha$ in human mononuclear cells, whereas tiaprofenic acid was virtually inactive in that model.
*Conclusions*: In arthritic rats tiaprofenic acid has not only the capability to suppress paw inflammation, but also to prevent with high potency the excretion of pyridinium crosslinks. Doxycycline without inherent antiinflammatory activity does not exhibit such preserving effects on collagen degradation in this model. Thus the mode of action of cartilage protecting drugs within the complex pathogenesis of arthritis will need further elucidation.

**Key words:** Tiaprofenic acid – Doxycycline – Pyridinium crosslinks – Adjuvant arthritis – Human matrix metalloproteinase

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## Introduction

Chronic joint diseases such as osteoarthritis and rheumatoid arthritis are characterized by a progressive destruction of articular cartilage and bone [1, 2].

The breakdown of matrix can be visualized histopathologically and radiographically, or by measurement of biochemical markers of collagen and proteoglycan degradation in synovial fluid, plasma and urine. In particular, the quantification of certain urinary pyridinium compounds was recently reviewed as a promising non-invasive method [3]. These trifunctional cross-linking moieties are responsible for maintaining the structure of mature type I collagen in bone, and type II collagen in cartilage. Degradation of the bone matrix leads to the occurence of deoxypyridinoline (dPYR) in urine, whereas urinary pyridinoline (PYR) seems to reflect the breakdown of both cartilage and bone collagen [4, 5]. The underlying pathological processes are very complex, and may involve the action of inflammatory cytokines, such as IL-1 and TNF$\alpha$, as well as activated matrix metalloproteinases (MMPs), in particular collagenases, gelatinases and stromelysins, which degrade extracellular matrix constituents [1, 6, 7].

Adjuvant-induced arthritis in rats is one of the established experimental models for the evaluation of the antiinflammatory efficacy of agents [8]. In our study we investigated the effects of tiaprofenic acid [9], a clinically well-known nonsteroidal antiinflammatory drug (NSAID), on the urinary level of pyridinium-crosslinks. In particular, we compared the effects of tiaprofenic acid with doxycycline, since there was

reported evidence that tetracyclines, including those without antibiotic activity, may exert therapeutic potential as inhibitors of extracellular matrix breakdown [1, 10]. Effects of the drugs towards activated MMPs and cellular cytokine generation were included in our study in order to gain some insight into the potential mode of action.

## Materials and methods

### *Solvents, drugs, enzymes and reagents*

All solvents were purchased from Riedel-de Haen, Seelze, Germany, unless otherwise indicated. Tiaprofenic acid was obtained as 200 mg tablets (Surgam$^{TM}$) from a pharmacy. Doxycycline hydrochloride and mycobacterium smegmatis were ordered from Sigma, Deisenhofen, Germany which also was the source of 4-aminophenylmercuric acetate (APMA) and thermolysin (bacillus thermoproteolyticus rocco).

The catalytic domains of recombinant human stromelysin (hMMP-3cd; phe$^{100}$-pro$^{275}$; m.w. 19.7 kD) and neutrophil collagenase (hMMP-8cd; gly$^{99}$-gln$^{271}$; m.w. 19.9 kD) were purified according to [11] to electrophoretical homogeneity from pet-3D vector transformed E. coli received from Dr. Bartnik, Hoechst AG, Frankfurt, Germany.

The human pro-enzymes of 72 kD gelatinase A (from fibrosarcoma cells) and gelatinase B (92 kD form, from blood) were available from Boehringer Mannheim, Germany. The human interstitial collagenase MMP-1 from human fibroblast culture was received from Prof. N. Ulbrich, Berlin as the pro-enzyme.

MMP-1, gelatinase A (hMMP-2) and B (hMMP-9) were prepared by the activation procedure using APMA or trypsin as described in [12, 13].

Enzyme activity of hMMP-1, -2, -3cd, -8cd, -9 and thermolysine was determined in 96 well titer plate format in a luminescence spectrometer (LS 50B, Perkin Elmer, Langen, Germany), using the quenched fluorigenic substrate [14] (7-methoxycoumarin-4-yl) acetyl-pro-leu-gly-leu-3-(2′,4′-dinitrophenyl)-L-2,3-diaminopropionyl-ala-arg-$NH_2$ ($\lambda$ex = 328 nm, $\lambda$em = 393 nm), available from Bachem, Heidelberg, Germany. Each well contained: 70 µl buffer (0.1 mol/l tris/HCl, pH = 7.5; 0.1 mol/l NaCl; 0.01 mol/l $CaCl_2$, 0.05% Brij 35$^{TM}$), 10 µl enzyme, and 10 µl drug in 10% DMSO. After preincubation for 15 min at room temperature the reaction was started by addition of 10 µl substrate (1 mmol/l in 10% DMSO).

Initial velocity of enzymatic reaction was determined without drug (=100%) and with different drug concentrations as shown in Figures 3 and 4.

### *Animals*

A total of 124 male Wistar Lewis rats, body weight 100–150 g (Møllegaard, Skensfed, Denmark), were investigated in the animal experiments. For that limited number of animals consent was given by the Ethics Committee of the Government (permit no. H 2-1/anz.31)) to induce adjuvant arthritis by injection into the paw as described by [15].

The animals were divided in 8 groups: 1) untreated healthy animals, 2) arthritic control animals, 3), 4), 5) arthritic animals treated with 5 or 15 or 30 mg/kg tiaprofenic acid and 6), 7), 8) arthritic animals treated with 2 or 15 or 50 mg/kg doxycycline.

Briefly, 0.1 ml complete Freund adjuvant (6 mg Mycobacterium smegmatis/ml paraffine oil) was injected into the rear left paw of the rats. First drug application (1 ml/kg of a solution of doxycycline hydrochloride (50 mg/ml) in water or 1 ml/kg of a solution of tiaprofenic acid (15 mg/ml) in 1% CMC) was administered p.o. one hour before adjuvant, and subsequently daily p.o. until day 21. The animals were fed standardised food, Altromin-R$^{TM}$ (Altrogge, Lage, Germany), had free access to water, and received oral analgesic codeine (2 × 30 mg/kg) daily from the 14th to the 21th day. Body weight and paw volume were monitored during the entire period, and on day 21 the animals were scored for lesions. Animals were sacrificed after the last drug application, and 24 h urine were collected at this time point, and stored at −80 °C. Urinary creatinine was measured using a commercially available test kit (Sigma Deisenhofen, Germany), based on the established picrinic acid method [17]. Drug levels in plasma were determined similarly to [16]. 30 and 120 min after the last application of 5 mg/kg tiaprofenic acid the drug levels were 18 and 10 µg/ml. The respective values for 30 mg/kg tiaprofenic acid were 65 and 60 µg/ml plasma, and for 15 mg/kg doxycycline the drug level was 0.45 and 0.52 µg/ml. In the 50 mg/kg doxycycline group the drug level was 2.2 µg/ml plasma 45 min after drug application.

### *Measurement of urinary pyridinoline crosslinks*

Hydrolysis of urinary collagen fragments, HPLC separation, and fluorimetric determination of pyridinolines were performed according to [5] in the following modification: 250 µl aliquots of the 24 h urine were mixed with an equal volume of 37% HCl (w/v) and hydrolyzed at 110 °C for 18 h. Samples were diluted with 2.5 ml solvent (acetic acid/acetonitrile 2/3 (v/v)) and applied to poly-prep chromatography columns (2 ml bed volume, Biorad, München, Germany) equilibrated with that solvent. After washing twice the pyridinoline crosslinks were eluted by 4 ml of aqueous 20 mmol/l heptafluorobutyric acid (Aldrich, Steinheim, Germany). The first 1 ml eluate was discarded, whereas a 100 µl aliquot of the sample fraction was directly applied to HPLC.

HPLC equipment (Waters, Eschborn, Germany) consisted of an isocratic pump no. 510, an injector no. 717, and a temperature control module. Pyridinoline crosslinks were separated on NovaPak$^{TM}$ C-18 (140*3.9 mm, 5 µm particles) isocratic by 20 mmoles/l heptafluorobutyric acid in 725 ml water/175 ml acetonitrile (1 ml/min) at 30 °C.

Crosslinks urin standard, containing 953 pmol/ml PYR and 212 pmol/ml dPYR (Biorad, München, Germany) was used for calibrating the fluorescence detector (FP 920 Jasco, Gross-Umstadt, Germany, $\lambda$ex = 290 nm, $\lambda$em = 400 nm).

The experimental results were expressed as nmol urinary pyridinium per mmol creatinine. Related to urine volume the average PYR and dPYR concentrations were found to be about 45 or, resp., 37 µmol/l. A typical separation profile is shown in Figure 1.

### *Generation of cytokines in human mononuclear cells in vitro*

The mononuclear cell fraction was purified from freshly isolated human citrated blood in accordance with a standard procedure [18] using ccntrifugation in Lymphoprep$^{TM}$ (Molter, Heidelberg, Germany). The final cell suspension, consisting of about 90% lymphocytes and 10% monocytes was adjusted to $5 \times 10^6$ cells/ml in medium (RPMI 1640, 5% FCS, 25 mmoles/L HEPES 100 µg/ml penicillin and streptomycin). The generation of cytokines was induced by adding a solution of 1 µg LPS in 0.01 ml of DMSO/water (1:10, v/v) to 0.48 ml of the cell fraction. At the same time tiaprofenic acid or doxycycline HCl, dissolved in 0.01 ml, as indicated in Table 1, was added. After a 22 h period in an incubator (37 °C, 5% $CO_2$) aliquots of the supernatants were used for the determination of IL-1$\beta$ and TNF$\alpha$, by use of a commercially available ELISA-kit (Medgenix, Fleurs, Belgium) with high specificity for either cytokine. The cytokine level measured after incubation with LPS minus that without LPS was set 100%; drug effects were calculated as % inhibition. Throughout the experiments cell viability >95% was ascertained by the LDH-method (Boehringer Ingelheim, Germany).

### *Statistics*

Results were expressed as mean ± standard deviation (SD).

Data were analysed by the two-tailed Student's t-test for unpaired samples. The experimental p-values are shown. Cut off for significance was a level of $p<0.05$; $p<0.001$ was considered as highly significant.

## Results

### *Urinary pyridinium levels in adjuvant arthritic rats. Effect of drugs*

Compared to the healthy control rats, adjuvant induced

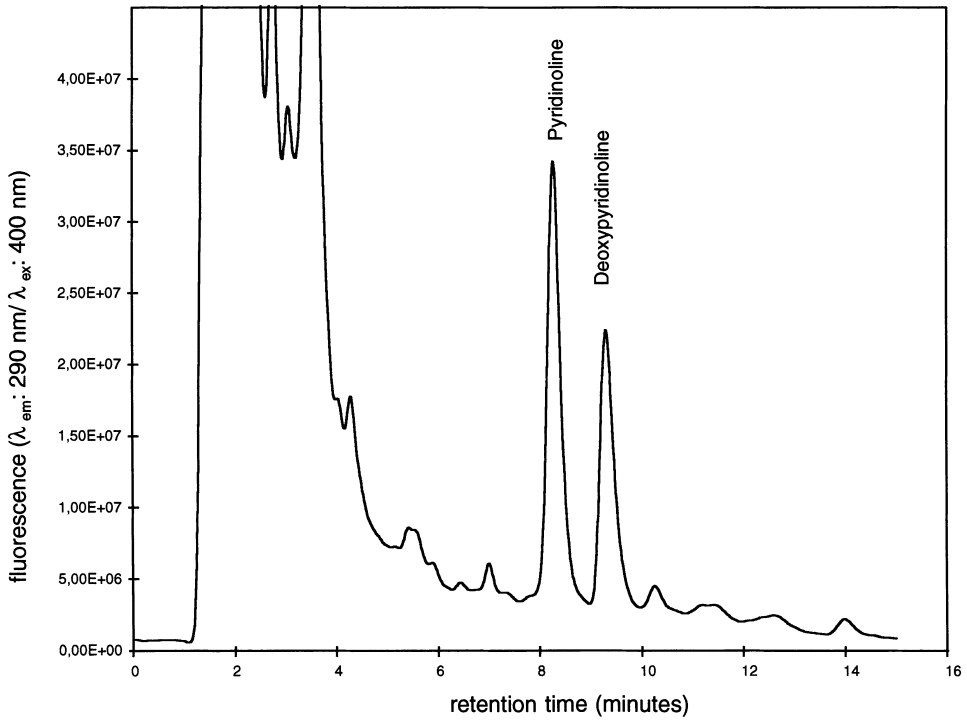


**Fig. 1.** Typical HPLC separation profile of urinary pyridinium crosslinks from untreated healthy rat, as described in detail under Methods.

| | untreated control | arthritis control | tiaprofenic acid 5 mg/kg | tiaprofenic acid 15 mg/kg | tiaprofenic acid 30 mg/kg | doxycycline 2 mg/kg | doxycycline 15 mg/kg | doxycycline 50 mg/kg |
|---|---|---|---|---|---|---|---|---|
| paw volume (ml)±SD | 1.4±0.1 p<0.001 | 4.2±0.6 | 3.3±0.2 p<0.001 | 2.6±0.4 p<0.001 | 2.4±0.1 p<0.001 | 4.5±0.4 n.s | 4.5±0.7 n.s | 4.7±0.2 p<0.05 |

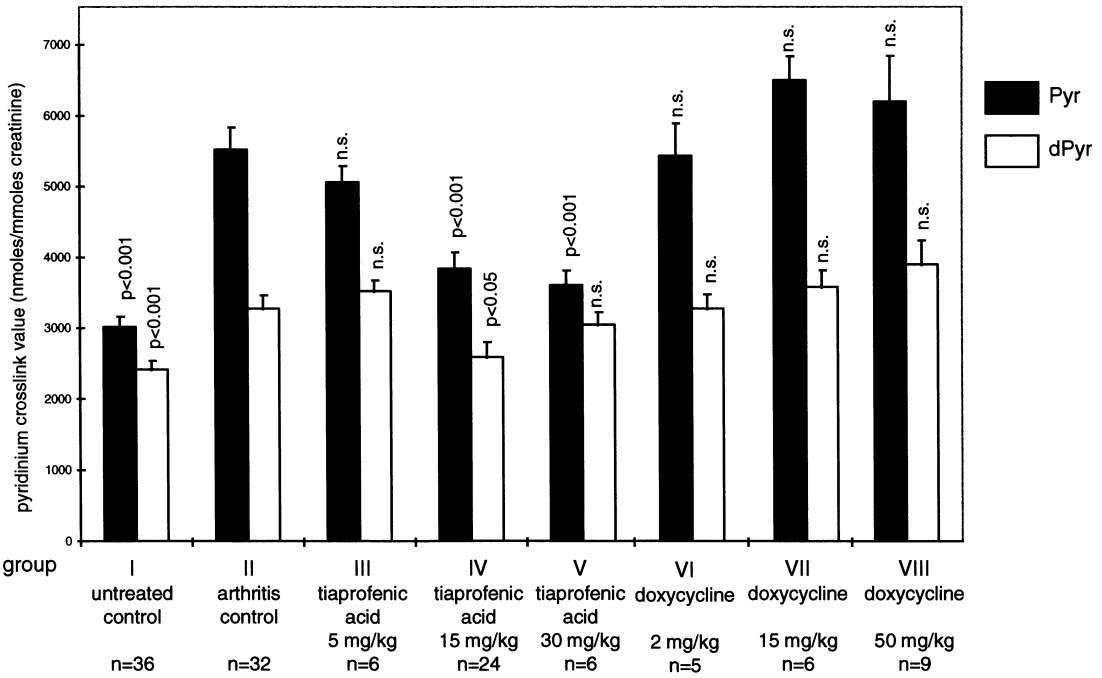


**Fig. 2.** Effect of drugs on paw volume (see insert above) and urinary pyridinium levels in adjuvant arthritic rats. Doxycycline and tiaprofenic acid were administered daily per os in the indicated amounts, as described in detail under Methods. The data shown represent the measurements on day 21. p-values were calculated against arthritis control group, n.s. = not significant.

arthritis led to a more than threefold increase in the volumes of the left paws on day 21, whereas the daily treatment p.os of the arthritic rats with 5, 15 or 30 mg tiaprofenic acid/kg body weight suppressed this increase in a dose dependant manner. In accordance with published data [10, 19], treatment in the same way with 2, 15 or 50 mg doxycycline/kg body weight did not reveal such antiinflammatory effects, see table inserted in Figure 2.

With respect to the urinary pyridinium crosslinks we could demonstrate (Fig. 2) that in the arthritic rats on day 21 the level of PYR/creatinine was enhanced by about 2.3 times, and dPYR/creatinine by about 1.4 times, compared to healthy controls. Tiaprofenic acid almost normalized the level of PYR/creatinine and dPYR/creatinine.

No such clearcut effects could be seen by the applied doxycycline dosage. On day 21 the alteration of urinary levels of PYR and dPYR were statistically insignificant compared to the arthritis controls.

*Human MMPs in vitro*

The inhibitory activity of tiaprofenic acid and doxycycline was assayed in vitro towards human MMP-1, -2, -9, -3, -8, (the latter two as the recombinant catalytic domains), and the bacterial Zn protease thermolysin [20].

As shown in Figure 3, tiaprofenic acid exerted against all these proteases, including thermolysin, a significant inhibitory potency in the range of $IC_{50}$ 200 μmoles/l.

Concentrations in the same magnitude were necessary to inhibit these proteases by doxycycline, as demonstrated in Figure 4. The $IC_{50}$ ranged from about 100 μmoles/l to 200 μmoles/l. Inhibition was significant, as assessed for both drugs (Fig. 3, 4).

*Cytokine generation in human mononuclear blood cells (hMNC)*

10 or 50 μmoles/l doxycycline inhibited significantly the liberation of IL-1$\beta$ or TNF$\alpha$ from LPS-stimulated hMNC by about 50%, whereas no significant inhibitory effects were seen with tiaprofenic acid at such concentrations. 100 μmoles/l tiaprofenic acid exerted a significant but weak inhibition (16%) on IL-1$\beta$ (Table 1).

Measurement of cellular LDH liberation revealed that practically no cell toxicity was seen even at highest drug levels.

## Discussion

The clinically established NSAIDs exert various effects on cartilage degradation. For instance, acetylsalicyclic acid accelerated the destruction of canine osteoarthritic cartilage [21]. Similarly, in patients with knee osteoarthritis indomethacin increased the rate of radiological deterioration of joint space, whereas tiaprofenic acid, a NSAID of the propionic acid family, did not [9, 22]. In animal models tiaprofenic acid showed protective effects on proteoglycans in the immobilised rabbit joint [23], and in the degenerating disc in a porcine model [24] as well as on type II collagen content in chondrosarcoma cells [25].

The tetracyclines, including those without antibacterial activity, represent another medicinal class that are comprehensively discussed as potential inhibitors of extracellular matrix breakdown (reviewed in [1]). Tetracyclines were shown to prevent collagen breakdown in diabetic rats [26]. Investigations in canine models of osteoarthritis revealed beneficial effects by oral tetracycline therapy [27].

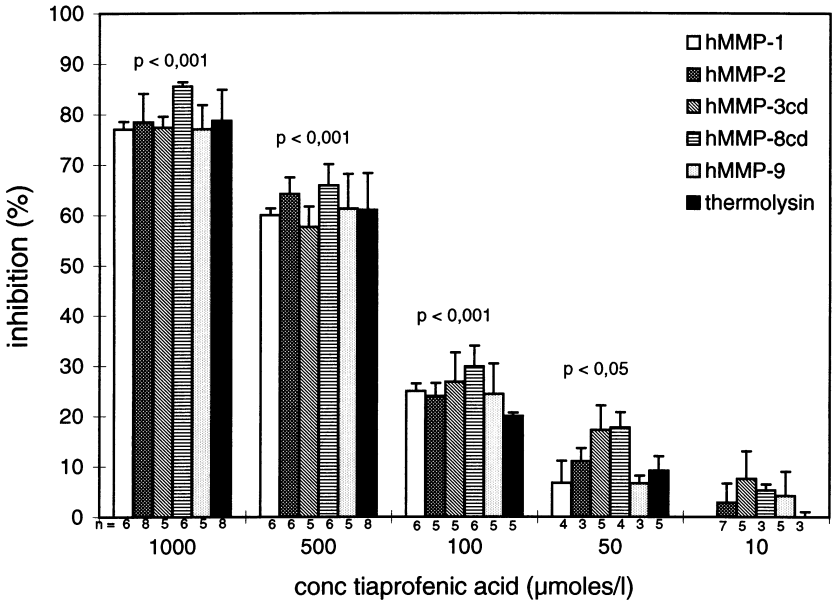


**Fig. 3.** Inhibitory effects of tiaprofenic acid in indicated concentrations on human MMPs (interstitial collagenase (hMMP-1); gelatinase A (hMMP-2); catalytical domain of stromelysin (hMMP-3cd); catalytical domain of neutrophil collagenase (hMMP-8cd); gelatinase B (hMMP-9)), and on thermolysin of bacterial origin. p-values were calculated against enzyme activity without inhibitor. See Methods for experimental details.

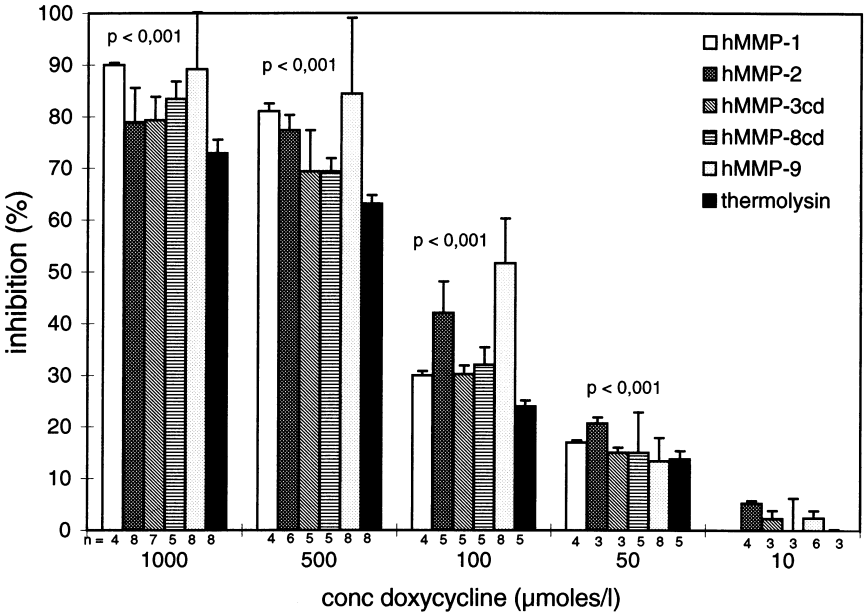


**Fig. 4.** Inhibitory effects of doxycycline in indicated concentrations on humanMMPs (interstitial collagenase (hMMP-1); gelatinase A (hMMP-2); catalytical domain of stromelysin (hMMP-3cd); catalytical domain of neutrophil collagenase (hMMP-8cd); gelatinase B (hMMP-9)), and on thermolysin of bacterial origin. p-values were calculated against enzyme activity without inhibitor. See Methods for experimental details.

**Table 1.** Effects of tiaprofenic acid and doxycycline on LPS induced liberation of cytokines. Inhibitory activity of drug in the indicated concentration is shown together with corresponding p-value as percent reduction of LPS stimulated cytokine level without drug (10.0 ng TNF $\alpha$/ml, SD $\pm$ 2.9 ng; 8.85 ng IL-1$\beta$/ml, SD $\pm$ 2.8 ng). See Methods for further experimental details.

| | TNF$\alpha$ | | | | IL-1$\beta$ | | |
|---|---|---|---|---|---|---|---|
| | Concentration ($\mu$mol/l) | Inhibition (%) | $\pm$ SD | n | Inhibition (%) | $\pm$ SD | n |
| Tiaprofenic acid | 100 | 0 | 15 | 4 | 16 ($p < 0.05$) | 7 | 4 |
| | 50 | 0 | 8 | 4 | 6 (n.s.) | 4 | 4 |
| | 10 | | | | 2 (n.s.) | 4 | 4 |
| Doxycycline | 100 | 78 ($p < 0.001$) | 15 | 4 | | | |
| | 50 | 43 ($p < 0.05$) | 29 | 6 | | | |
| | 10 | 13 (n.s.) | 13 | 4 | 44 ($p < 0.001$) | 21 | 12 |
| | 5 | | | | 22 ($p < 0.05$) | 19 | 12 |
| | 1 | | | | 11 (n.s.) | 25 | 12 |

n.s. = not significant.

Our data presented here demonstrate that in the adjuvant induced arthritis model the urinary pyridinium levels reflecting collagen breakdown products are significantly increased compared to the healthy control rats. The nearly twofold enhanced level of urinary dPYR reflects in accordance with published work [4, 15] an accelerated turnover and degradation of the collagen type I in bone, whereas the more than twofold increase in the urinary PYR seems to be derived from bone and cartilage destruction. The markedly increased PYR/dPYR ratio in the arthritis group compared to controls may support the hypothesis that in the diseased animals cartilage is more susceptible to destruction than bone.

The applied precise separation procedure for the hydrolyzed urinary pyridinium compounds by HPLC, originally published by [5], enabled us to investigate therapeutic agents in the adjuvant arthritic rat. As shown in Figure 2 the daily application of tiaprofenic acid over a 21 day-period led to a dose dependent significant reduction in the urinary pyridinium compounds. dPYR was reduced almost down to the level seen in the healthy controls, whereas the effect on PYR was insignificantly smaller. Due to the drug's inherent antiinflammatory activity [9] the tiaprofenic acid treated animals exhibited significantly reduced paw volumes compared to the arthritis controls.

Concerning doxycycline it was published [19], that the 21 days regimen of 20 mg/kg doxycycline led to a normalization of the urinary PYR levels. With reference to that study Greenwald [10] reported similar effects with chemically modified tetracyclines, whereas two antiinflammatorics, flurbiprofen and ibuprofen, did not alter the PYR levels.

In contrast, our study with 2, 15 or 50 mg/kg doxycycline did not show such effects on both PYR and dPYR.

In addition we could not detect significant effects on paw swelling after the treatment with doxycycline. Here our results confirm the doxycycline experiments described in [19].

The plasma levels attained in the adjuvant arthritic rats were up to 200 $\mu$moles/l for tiaprofenic acid, but we observed only up to about 5 $\mu$moles/l for doxycycline. In the animal group dosed with 2 mg/kg doxycycline we found no detectable drug levels. This group was included in our

study in reference to the reported low dose doxycycline regimen (20 mg orally b.i.d.), that has been used to inhibit PYR excretion in selected patients with rheumatoid arthritis [28]. Low plasma levels of doxycycline after oral ingestion are known from literature. However, this does not a priori exclude an accumulation of the drug in the desired target tissues.

Thus the discrepancies concerning doxycycline's effect on excretion of pyridinium crosslinks might be explained by technical differences in the applied animal model, such as the nature and mode of application of the adjuvant. For instance, adjuvant injection into the base of the tail causes weaker effects compared to the direct injection into the paw as performed in our lab. Moreover, as recommended recently by Greenwald [3], such animal studies now probably require HPLC rather than the formerly used ELISA based on polyclonal antibodies.

In our experiments we used the sensitive HPLC procedure, described by [5] in the modification outlined above. In conclusion, our results demonstrate that, if studied under the same conditions, tiaprofenic acid was able to significantly reduce the urinary level of pyridinium crosslinks, whereas concerning doxycycline our study could not verify the reported effects.

It was published, too, that tetracyclines exert inhibitory effects on the generation of cytokines, such as IL-1 [29] or TNF$\alpha$ [30], that may stimulate matrix degradation in several model systems [1]. Using the LPS induced generation of cytokines in human mononuclear cells in vitro we could confirm such inhibitory effects towards IL-1$\beta$ and TNF$\alpha$ for doxycycline (Table 1). However, the concentrations necessary to achieve inhibitory effects were far above the $\mu$mol/l range.

In the same system tiaprofenic acid showed practically no effects on these two cytokines (Table 1). Thus, concerning tiaprofenic acid, our experiments do not support the formerly discussed potential mechanism, that based on tetracycline as an anti-TNF$\alpha$-drug [1]. The fact that we compared a rat model with a human cell system may also limit too extensive conclusions.

Involved in the matrix degradation are proteolytic enzymes belonging to the group of MMPs. These are secreted as inactive proenzymes and can be activated to the free collagenases, gelatinases and stromelysins under a variety of biological conditions, including the action of cytokines [1, 6, 7]. In our experiments we investigated the active forms of interstitial collagenase (MMP-1), gelatinase A and B (MMP-2,-9) of human origin as well as the catalytic domains fo recombinant human stromelysin (MMP-3) and neutrophil collagenase (MMP-8). Each of these enzymes was inhibited in a dose dependent manner by tiaprofenic acid and by doxycycline (Fig. 3, 4). However, rather high drug amounts at the 50 $\mu$moles/l level were necessary to show a marked inhibition.

It was interesting to observe that the inhibitory potency of both drugs against all the investigated enzymes were in the same range. Throughout our investigation tiaprofenic acid exerted against the MMPs a weak inhibitory potency with $IC_{50}$ about 200 $\mu$moles/l (Fig. 3). As demonstrated in Figure 4 the $IC_{50}$ for doxycycline ranged from 100 $\mu$moles/l to 200 $\mu$moles/l. Since this was also found for the bacterial metalloproteinase thermolysin, there is obviously no preferred specificity of the drugs towards one special member of this group of enzymes in our investigation. In this context it should be pointed out, that our study included the active catalytic domain of MMP-8. A more selective participation of the full MMP-8 enzyme complex is suggested by reports evidencing a somewhat more active inhibition of the activated full length MMP-8 [31] by doxycyclin ($IC_{50}$ 10–30 $\mu$moles/l). Moreover, it was described that the $IC_{50}$ could be shifted towards 5–12 $\mu$moles/l, when pro-MMP-8 was preincubated with doxycycline, since the pro-form is reduced to inactive fragments upon activation in the presence of doxycycline [32]. In line with this a several day regimen of tetracycline, given to patients requiring hip replacement, decreased collagenase activity by about 60% in extracts of joint tissues [33].

However, the $IC_{50}$ values measured in our experiments do not exclude a potential secondary inhibition mechanism versus metalloproteinases that could at least in part contribute to the in vivo effects of the drugs, and this is supported by data in the literature. Concerning tiaprofenic acid, there were anti-MMP effects seen in cultivated rabbit chondrocytes [34], and in human osteoarthritic cartilage [35], too. In canine osteoarthritis collagenase and gelatinase levels were reduced in extracts of articular cartilage by tetracyclines [27]. It could be shown in conditioned media of bovine articular cartilage that high concentrations of several tetracyclines but not tiaprofenic acid were able to inhibt stromelysin activity [36]. Recently a systematic in vitro investigation of a series of chemically modified tetracyclines on gelatinase B [37] revealed remarkable differences between the tetracycline analogs, and evidenced certain structures exceeding the effect of doxycycline on this enzyme.

The mode of action of drugs within the complex pathogenesis of adjuvant arthritis and the involved progression of degradation of cartilage matrix is not nearly elucidated. Effects on cytokines an proteolytic enzymes reflect only parts of the elements involved. In our experimental protocol the drug was started at the time of adjuvant injection. Thus tiaprofenic acid prevented the disease progression. It should be pointed out, however, that the drug almost completely suppressed the increase of pyridinium crosslinks, whereas the effect on paw volume was only about 50%. In order to get a better separation between these events it is recommendable to apply a curative treatment protocol, i.e. to applicate the drug in animals with established arthritis. The already mentioned ineffectiveness of flurbiprofen and ibuprofen on the PYR excretion [10] may indicate that antiinflammatory effects do not generally lead to a suppression of such effect. Our experiments have to be continued to gain a better insight into the potential mode of action of tiaprofenic acid on collagen degradation.

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