# Lack of Effect of Doxycycline on Disease Activity and Joint Damage in Patients with Rheumatoid Arthritis. A Double Blind, Placebo Controlled Trial

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***ABSTRACT.*** ***Objective.*** To investigate the effects of doxycycline on disease activity and joint destruction in patients with rheumatoid arthritis (RA).

***Methods.*** A 36 week double blind, placebo controlled crossover trial was conducted. Patients (n = 66) received 50 mg doxycycline or placebo twice a day during 12, 24, or 36 weeks. Patient assessments were performed before the treatment was administered, at 6, 12, 24 and 36 weeks of treatment, and finally at 4 weeks after cessation of treatment. Patient assessments, swollen and tender joint counts, duration of morning stiffness, erythrocyte sedimentation rate, and Modified Disease Activity Score were used as measures of disease activity. Effects on joint destruction were assessed by urinary excretion of the pyridinolines hydroxylysylpyridinoline and lysylpyridinoline and by scoring radiographic damage of hands and feet before and after treatment.

***Results.*** The changes of clinical or laboratory disease activity measures, pyridinoline excretion, or progression of radiographic joint damage during doxycycline or placebo treatment did not differ significantly.

***Conclusion.*** The results indicate that 50 mg doxycycline twice a day provided no therapeutic benefit for patients with RA. (J Rheumatol 2001;28:1967–74)

*Key Indexing Terms:*
DOXYCYCLINE RHEUMATOID ARTHRITIS PYRIDINOLINES

Rheumatoid arthritis (RA) is a chronic inflammatory disease involving multiple joints, leading in most cases to irreversible destruction of the articular cartilage and bone. For patients with RA, joint destruction is an important determinant of functional capacity[1]. Arresting the progression of joint destruction is therefore an important target in treatment of RA.

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Proteolytic enzymes secreted at the site of destruction cause degradation of the articular cartilage and bone. Although all classes of proteolytic enzymes seem to be involved, cartilage destruction has been attributed in particular to matrix metalloproteinases (MMP) and serine proteases[2-4]. The MMP are a family of proteolytic enzymes that have the ability to degrade almost all proteins of the extracellular matrix. For example, collagenases (MMP-1, 8, 13) can degrade intact collagen fibrils, gelatinases (MMP-2, 9) degrade unwound collagen, and stromelysins (MMP-3, 10) degrade proteoglycans. In rheumatoid synovial tissue, increased amounts of MMP are expressed[5-7]. MMP levels in the synovium and serum are correlated with disease activity[8] and radiographic damage[9], suggesting a role for MMP in the destructive process in RA. Therefore, inhibition of MMP may be a therapeutic strategy to prevent joint destruction in RA.

As an unrelated function to their antibiotic activity, tetracyclines, in particular minocycline and doxycycline, are potent inhibitors of MMP[10-12]. In patients with joint disease, a significant reduction of MMP activity within the joints was observed after oral administration of minocycline[13] or doxycycline[14]. Double blind placebo controlled studies show that minocycline relieves clinical symptoms and reduces laboratory measures of disease activity in patients with RA[15-17]. However, in these studies the progression of radiographic damage was not significantly reduced[15,18]. Moreover, treatment with minocycline may cause specific adverse effects. Dizziness is frequently reported[15,17] and led

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in a few cases even to fractures[15]. Rare but potentially serious adverse reactions to minocycline are drug induced autoimmune syndromes such as a lupus-like syndrome[19,20] or autoimmune hepatitis[20]. In almost all cases the symptoms subside after discontinuation of minocycline, but cases of lethal minocycline induced autoimmune hepatitis have been described[20]. The lack of effect of minocycline on the progression of joint damage combined with its risk of adverse effects led to an investigation of the therapeutic effects of doxycycline in patients with RA.

To date, only a few clinical studies have evaluated doxycycline as an antirheumatic medicine[21,22]. A small uncontrolled pilot study involving 13 patients treated with low dose doxycycline (20 mg twice a day) revealed that urinary pyridinoline excretion was reduced in patients with moderate, but not severe RA[21]. These results are in agreement with observations in rats with adjuvant arthritis showing that elevated pyridinoline excretion was reduced by doxycycline[23]. Pyridinolines are collagen crosslinks that are released from the joints during cartilage and bone resorption and are finally excreted in the urine. Urinary pyridinoline excretion rates are increased in patients with RA[24-26] and correlate with the severity of joint destruction[27]. The observed reduction in pyridinoline excretion rates suggests that doxycycline may have a protective effect in joints in RA. In a recent study, a significant reduction in the number of tender joints and a significant improvement of domestic disability and vocational behavior were reported in patients with RA during doxycycline treatment[22].

Because both studies were not placebo controlled, it cannot be ruled out that the observed effects were due to other factors than the doxycycline treatment. Therefore, the present double blind, placebo controlled study was designed. We investigated whether 12, 24, or 36 weeks of adjuvant therapy with doxycycline can ameliorate clinical symptoms in RA, and can reduce the excessive excretion of pyridinolines, and whether it can slow down the progression of radiographic joint damage.

## MATERIALS AND METHODS

*Patients*. Sixty-six patients from the outpatient clinics of the Leiden University Medical Center and Jan Van Breemen Institute with active RA according to the 1987 criteria of the American Rheumatism Association[28] were studied. Inclusion criteria were: age between 18 and 85 years; stable second-line therapy for at least 10 months prior to the study; daily dose of corticosteroids < 7.5 mg; no intraarticular steroid injections during the period of 1 month prior to the trial; and a baseline score of at least 3 on the prognostic index (PI) and at least 5 on the Disease Activity Index (DAI). The PI and DAI were used to include patients with high disease activity who were likely to have high baseline rates of urinary pyridinoline excretion, since the levels of pyridinolines are correlated to levels of disease activity[25,27]. For the PI the sum of the presence of the following variables was calculated: radiographic joint damage, extraarticular manifestation (nodules, serosal disease, conjunctivitis or iridocyclitis), erythrocyte sedimentation rate (ESR) > 30 mm/h for 6 mo, C-reactive protein (CRP) > 6 mg/l, and deteriorating function[29]. The DAI was calculated by taking the sum of the scores (0 = mild, 1 = moderate, 2 = severe) for duration of morning stiffness, tender joint counts, swollen joint counts, and ESR. Patients with Steinbrocker functional stage IV disease, impaired renal or hepatic function, or active esophago-gastro-duodenal ulcer, or women who were pregnant, lactating, or planning to become pregnant were excluded from the trial.

The sample size was calculated for the expected differences in the changes of urinary hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) excretion rates. Based on previous studies[21,23], we aimed at a decrease of 40% in the doxycycline treated group compared to 0% change in the placebo treated group. With a power of 80% and a significance of 5%, 16 patients per group were required. We included 20 patients per group, but in each group several patients dropped out before the study medication was started. Unfortunately, these dropouts were not symmetrically distributed over the 4 groups and they were left out of the analysis. This left us with 15 patients in Group A, 17 in Group B, 16 in Group C, and 18 in Group D, as described below.

*Study design*. The local institutional ethics committees approved the study protocol and all subjects gave informed consent. The design was a 36 week, double blind, placebo controlled crossover trial. Patients were treated in 3 consecutive treatment periods of 12 weeks with either doxycycline (50 mg twice a day) or placebo. Patients were randomly divided into 4 treatment groups: Group A: doxycycline (Weeks 1–12), placebo (Weeks 13–24), doxycycline (Weeks 25–36); B: doxycycline (Weeks 1–24), placebo (Weeks 25–36); C: doxycycline (Weeks 1–36); D: placebo (Weeks 1–36). The patients visited the outpatient clinics on 8 occasions: 2 weeks and 1 week before the start of the study, on the first day of the study period, at 6, 12, 24 and 36 weeks during the study period, and at 4 weeks after the study period. The crossover design was used to account for the great variability in pyridinoline excretion. In this design, each patient could be his or her own control. Further, the design allowed us to compare the effects of doxycycline in various treatment periods (12, 24, and 36 weeks) and to document possible rebound effects after each treatment period.

No alterations in disease modifying antirheumatic drug (DMARD) regimen and no intraarticular injections were allowed during the trial. Concomitant therapies are listed in Table 1. Doxycycline and placebo were prepared in identical capsules and were added to the patients' therapy. The trial medication was one 50 mg capsule twice a day. Compliance was checked by pill counting.

Patients were interviewed about the appearance of new symptoms, gastrointestinal (GI) adverse effects, rash, and photosensitivity. In addition, patients were asked to report other adverse reactions they attributed to the study drug treatment.

*Clinical assessments*. At commencement the following demographic and disease characteristics were noted: sex, age, disease duration (years), the presence of erosions, the presence of a positive IgM rheumatoid factor (RF; defined as > 30 U/ml), and the use of nonsteroidal antiiflammatory drugs (NSAID), DMARD or corticosteroids. At each visit, the following clinical assessments of disease activity were performed: a patient overall assessment of current disease activity on a visual analog scale (VAS) of 0–100 mm, the duration of morning stiffness (minutes), swollen and tender joint count (28 joints), and the modified Disease Activity Score (m-DAS) using 28 joint counts[30].

*Laboratory assessments*. At each visit, serum and urine were collected and stored at –80°C until use. The ESR (mm/h) was measured and urinary pyridinolines HP and LP were measured by high performance liquid chromatography[31]. Normal values for healthy people were assessed in a group of 36 adults. To assess hepato-renal toxicity, serum alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, γ-glutamyltranspeptidase, and creatinine were measured at each visit.

*Radiographic assessments*. Radiographs of the hands and feet were made before study entry and repeated at the end of the study. All radiographs were scored separately in chronological order by the Sharp method modified by van der Heijde[32] by the same rheumatologist, who was blinded to treatment received by the patients.

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*Table 1*. Charateristics of patients in the different treatment groups at the start of the study.

| | Treatment Group† | | | |
|---|---|---|---|---|
| | A, n = 15 | B, n = 17 | C, n = 16 | D, n = 18 |
| Males/females | 8/7 | 5/12 | 7/9 | 3/15 |
| Age, yrs, mean (SD) | 62 (16) | 56 (11) | 53 (11) | 57 (9) |
| Disease duration, yrs, median (range) | 7 (1–25) | 9 (3–22) | 12 (4–47) | 9 (1–47) |
| Erosive disease, +/– | 14/0 | 17/0 | 16/0 | 17/1 |
| Sharp score, median (range) | 91 (4–324) | 50 (4–413) | 180 (23–339) | 52 (0–370) |
| RF, +/– | 15/0 | 15/2 | 13/3 | 14/4 |
| PI, median (range) | 3 (3–5) | 3 (3–5) | 3.5 (3–5) | 4 (3–5) |
| DAI, median (range) | 7 (5–8) | 6 (5–8) | 6 (5–8) | 6 (3–8) |
| Drugs during study, n (%) | | | | |
| NSAID | 10 (67)* | 13 (77)* | 16 (100) | 18 (100) |
| DMARD | 12 (80) | 17 (100) | 14 (88) | 17 (94) |
| Antimalarials | 3 (20) | 2 (12) | 2 (13) | 3 (17) |
| Sulfasalazine | 5 (33) | 8 (47) | 6 (39) | 8 (44) |
| Methotrexate | 2 (13) | 2 (12) | 1 (6) | 2 (11) |
| Gold | 2 (13) | 3 (19) | 4 (25) | 0 (0) |
| Azathioprine | 0 (0) | 4 (24) | 1 (6) | 4 (25) |
| Corticosteroids | 5 (30)* | 0 (0) | 1 (6) | 1 (6) |

† Treatment schedules: A. doxycycline-placebo-doxycycline. B. doxycycline-doxycycline-placebo. C. doxycycline-doxycycline-doxycycline. D. placebo-placebo-placebo.

*Statistic analysis*. Pearson chi-square tests were used to test for differences between the treatment groups in sex, presence of erosions, RF, the usage of NSAID, DMARD or corticosteroids, the number of patients who reported adverse effects, and the number of patients who discontinued the study. One-way ANOVA was performed to test for differences in age and in baseline values of tender and swollen joint counts, VAS, m-DAS, ESR, HP, and LP between the treatment groups. For HP and LP a natural logarithmic transformation was performed (ln) to obtain normally distributed values (lnHP, lnLP). Kruskal-Wallis tests were performed to test for differences in disease duration and Sharp score at baseline between the treatment groups. The efficacy variables were analyzed on the basis of an intention-to-treat analysis. For patients who prematurely discontinued the study, the data from the visit at which the trial medication was stopped were carried forward. Differences between courses of all outcome variables except for the Sharp score during the treatment period were evaluated by repeated measure ANOVA. The differences between the treatment groups in the changes of Sharp score were tested by one-way ANOVA. The changes ($\Delta$) of all variables during 12, 24, and 36 weeks of doxycycline treatment were compared with the changes in the placebo group. Differences were tested for significance by Student t tests. All statistical calculations were performed using SPSS 10.0 for Windows. P values $< 0.05$ were considered statistically significant.

## RESULTS

Sixty-six patients were included and randomly divided over 4 treatment groups. The groups were not statistically different at baseline with respect to demographic and disease characteristics (Table 1). In Group C the baseline Sharp score was higher than in the other groups, but the difference was not significant (Kruskal-Wallis, $p = 0.2$). The patient groups did not differ in the usage of DMARD. The usage of NSAID at baseline differed significantly between groups (Pearson chi-square, $p = 0.008$). In Groups C and D all patients used NSAID. In Group A 10 (67%) patients and in Group B 13 (77%) used NSAID. More patients in Group A used corticosteroids (5 patients) at baseline as compared to the other treatment groups (0–1 patients; Pearson chi-square, $p = 0.05$; Table 1). There were 22 premature discontinuations: 5 in Group A, 7 in Group B, 2 in Group C, and 8 in Group D (placebo). In Group A, one patient discontinued in Week 5 during doxycycline treatment because of increase in disease activity; one patient in Week 12 at the end of doxycycline treatment for unknown reason; 3 patients in Week 24 at the end of placebo treatment because of lack of effect. In Group B, all dropouts were in the period they received doxycycline: one patient because of nausea at Week 6, 4 because of lack of effect at Weeks 12 and 24, one patient because of an intraarticular injection of corticosteroids at Week 24, and one patient for unknown reasons at Week 24. In Group C, one patient discontinued at Week 24 because of lack of effect and one patient discontinued because of GI complaints at Week 12. In Group D, 4 patients discontinued due to lack of effect: one patient discontinued at 2 weeks, one at 12 weeks, and 2 patients at 24 weeks; one patient discontinued at 24 weeks because of intraarticular corticosteroid injection, one discontinued at Week 12 because of GI complaints, and one discontinued because of surgery. There were no changes in serum levels of liver enzymes or creatinine that led to discontinuation of study medication. Forty-four patients completed the entire study period.

Adverse effects were gastrointestinal and were reported in all treatment groups including the placebo group (Table 2). There was no significant difference in the number of patients reporting adverse effects between the treatment groups. In the doxycycline treated groups the adverse effects occurred during treatment with doxycycline and not during treatment with the placebo.

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*Table 2*. Adverse effects during the 36 week study period. All adverse effects in treatment groups A, B, and C were reported during doxycycline treatment. One patient in Group C reported both stomach ache and diarrhea and discontinued the study after 12 weeks. In one patient in Group B nausea led to discontinuation of the drug treatment within 6 weeks of the study. One patient in Group D (placebo) discontinued the study due to stomach ache after 12 weeks.

| | Treatment Groups[†] | | | |
|---|---|---|---|---|
| | A, n = 15 | B, n = 17 | C, n = 16 | D, n = 18 |
| Patients reporting adverse effects (n) | 2 | 2 | 4 | 3 |
| Reported symptoms (n) | | | | |
| Nausea | 1 | 2 | 1 | 0 |
| Stomach ache | 1 | 0 | 1 | 3 |
| Diarrhea | 0 | 0 | 3 | 0 |
| Total | 2 | 2 | 5 | 3 |

[†] Treatment schedules: A. doxycycline-placebo-doxycycline. B. doxycycline-doxycycline-placebo. C. doxycycline-doxycycline-doxycycline. D. placebo-placebo-placebo.

*No effects of doxycycline on clinical disease activity variables or ESR*. To evaluate the effect of doxycycline in RA, disease activity variables (patient global assessment, m-DAS, ESR) were determined at the start, every 12 weeks, and at 4 weeks after discontinuation of the study drug treatment. For all outcome variables at baseline, there were no significant differences between the treatment groups.

Repeated measures ANOVA showed no significant differences between treatment groups in patient global assessment, swollen joint count, tender joint count, m-DAS, duration of morning stiffness, or ESR. Analysis of the changes (Δ) of these variables at the start and the end of the 12, 24, or 36 week treatment period showed no or little difference between patients in the different treatment groups (Table 3; Figure 1).

*No effect of doxycycline on pyridinoline excretion rates or progression of radiographic damage*. To assess the effects of doxycycline on progression of joint destruction urinary HP and LP excretion rates were measured at the start, at every 12 weeks, and at 4 weeks after discontinuation of study drug, and radiographs of hands and feet were made before and after the study period. There were no significant differences in HP and LP excretion rates at baseline between the treatment groups. Normal values in healthy volunteers

*Table 3*. Effects of doxycycline on disease activity or joint destruction variables. The effects of 12, 24, and 36 week treatment periods of doxycycline on the change (Δ) of disease activity variables — ESR, swollen joint count (SJ), tender joint count (TJ), VAS, DAS, and duration of morning stiffness (MS) — and on joint destruction variables (lnHP, and lnLP) were evaluated and compared to placebo. Baseline and Δ levels are presented as mean ± SD.

| | VAS, mm | MS, min | SJ, n | TJ, n | DAS Score | ESR, mm/h | lnHP, mmol/mol cr | lnLP, mmol/mol cr |
|---|---|---|---|---|---|---|---|---|
| 0–12 weeks | | | | | | | | |
| Doxy, n = 43 | | | | | | | | |
| Baseline | 49.9 ± 21.5 | 64.9 ± 61.9 | 12.8 ± 5.8 | 9.9 ± 7.0 | 5.7 ± 1.0 | 38.3 ± 26.6 | 4.4 ± 0.6 | 2.6 ± 0.6 |
| Δ | –3.5 ± 18.4* | –0.6 ± 48.7 | –0.6 ± 5.4 | –1.4 ± 6.8 | –0.3 ± 1.1 | –3.6 ± 12.6 | 0.0 ± 0.3 | 0.0 ± 0.4 |
| Placebo, n = 18 | | | | | | | | |
| Baseline | 42.1 ± 20.7 | 55.6 ± 64.4 | 12.2 ± 5.9 | 9.3 ± 6.9 | 5.6 ± 0.9 | 34.7 ± 18.7 | 4.2 ± 0.3 | 2.6 ± 0.5 |
| Δ | 8.3 ± 17.4 | –21.9 ± 45.3 | –0.1 ± 4.4 | –0.8 ± 5.4 | 0.0 ± 0.8 | –1.6 ± 16.6 | 0.0 ± 0.3 | 0.1 ± 0.2 |
| 0–24 weeks | | | | | | | | |
| Doxy, n = 33 | | | | | | | | |
| Baseline | 46.0 ± 20.8 | 62.0 ± 60.3 | 12.8 ± 5.8 | 10.7 ± 7.2 | 5.6 ± 1.0 | 36.0 ± 23.3 | 4.3 ± 0.5 | 2.5 ± 0.5 |
| Δ | –5.8 ± 19.1 | –4.8 ± 55.9 | –1.45 ± 5.9 | –2.5 ± 8.6 | –0.5 ± 1.4 | –4.2 ± 17.6 | 0.0 ± 0.3 | 0.0 ± 0.3 |
| Placebo, n = 18 | | | | | | | | |
| Baseline | 42.1 ± 20.7 | 55.6 ± 64.4 | 12.2 ± 5.9 | 9.3 ± 6.9 | 5.6 ± 0.9 | 34.7 ± 18.7 | 4.2 ± 0.23 | 2.6 ± 0.5 |
| Δ | 3.8 ± 17.8 | –16.7 ± 54.5 | –0.1 ± 3.8 | –1.7 ± 5.2 | –0.1 ± 0.8 | 1.8 ± 19.2 | 0.0 ± 0.5 | 0.1 ± 0.3 |
| 0–36 weeks | | | | | | | | |
| Doxy, n = 16 | | | | | | | | |
| Baseline | 48.1 ± 9.7 | 65.0 ± 60.8 | 13.7 ± 7.1 | 11.8 ± 7.9 | 5.6 ± 0.9 | 34.5 ± 24.5 | 4.4 ± 0.5 | 2.6 ± 0.5 |
| Δ | 0.8 ± 20.8 | –1.3 ± 50.5 | –3.3 ± 7.6 | –3.6 ± 9.0 | –0.7 ± 1.4 | –2.8 ± 15.1 | 0.0 ± 0.3 | 0.1 ± 0.4 |
| Placebo, n = 18 | | | | | | | | |
| Baseline | 42.1 ± 20.7 | 55.6 ± 64.4 | 12.2 ± 5.9 | 9.3 ± 6.9 | 5.6 ± 0.9 | 34.7 ± 18.7 | 4.2 ± 0.3 | 2.6 ± 0.5 |
| Δ | 3.4 ± 22.5 | –13.6 ± 47.6 | –0.1 ± 3.1 | –1.9 ± 7.2 | –0.2 ± 1.0 | –2.9 ± 15.6 | 0.0 ± 0.6 | 0.1 ± 0.3 |

* Student t test, $p < 0.02$. cr: creatinine.

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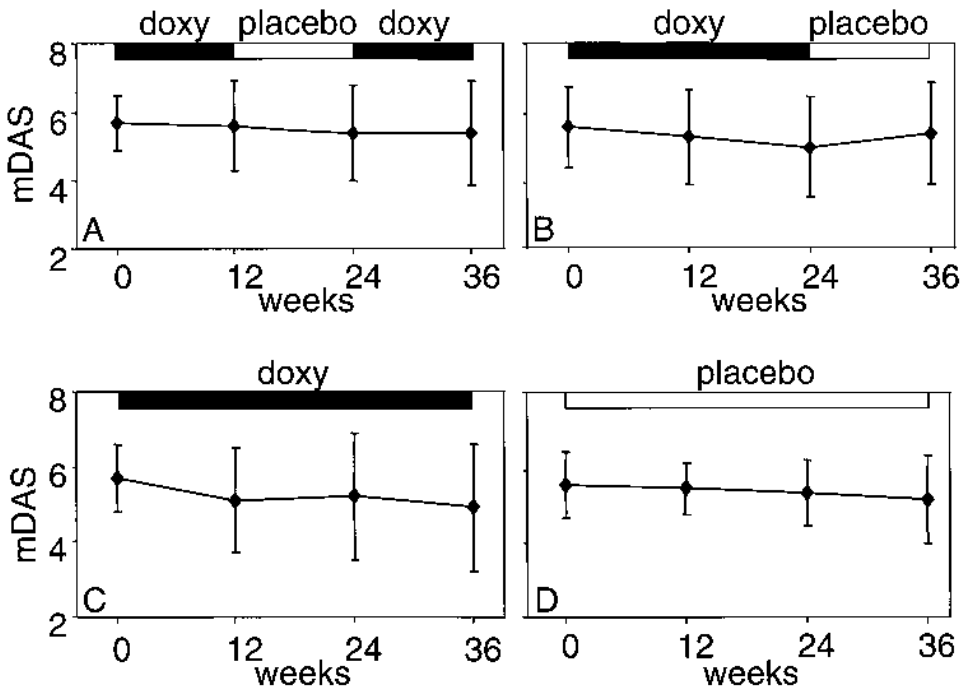


*Figure 1.* No effect of doxycycline on disease activity. The effects of 3 treatment regimens of doxycycline on modified DAS were evaluated and compared to placebo. DAS scores are depicted as means and standard deviations.

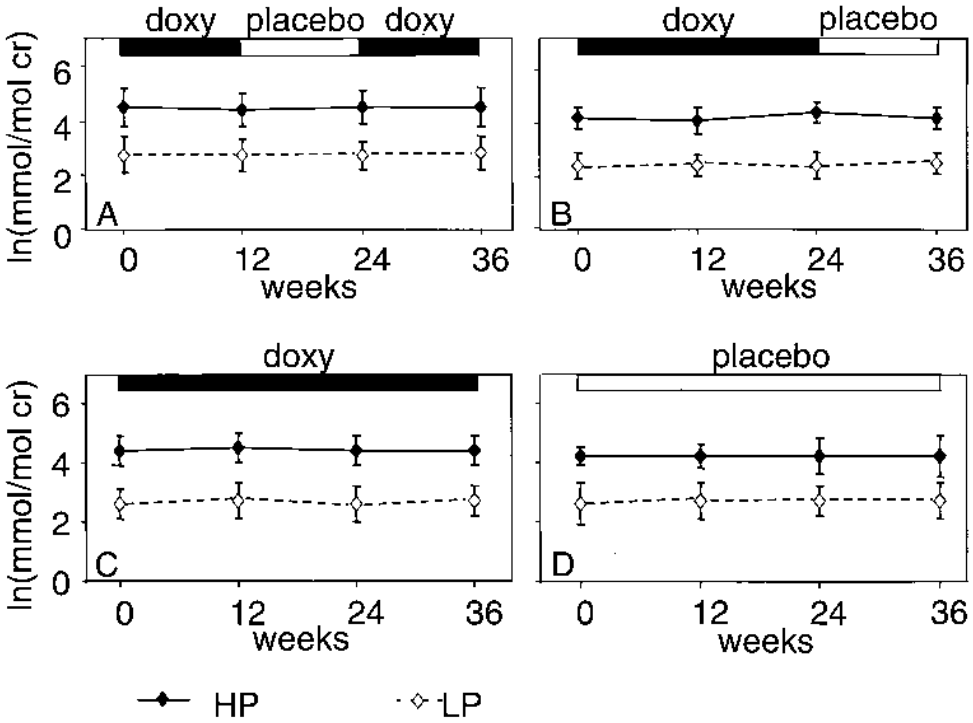


*Figure 2.* No effect of doxycycline on urinary excretion rates of pyridinolines. The effects of 3 treatment regimens of doxycycline on HP and LP were evaluated and compared to placebo. To obtain normally distributed parameters a logarithmic transformation was applied. HP and LP values are depicted as means and standard deviation of lnHP and lnLP.

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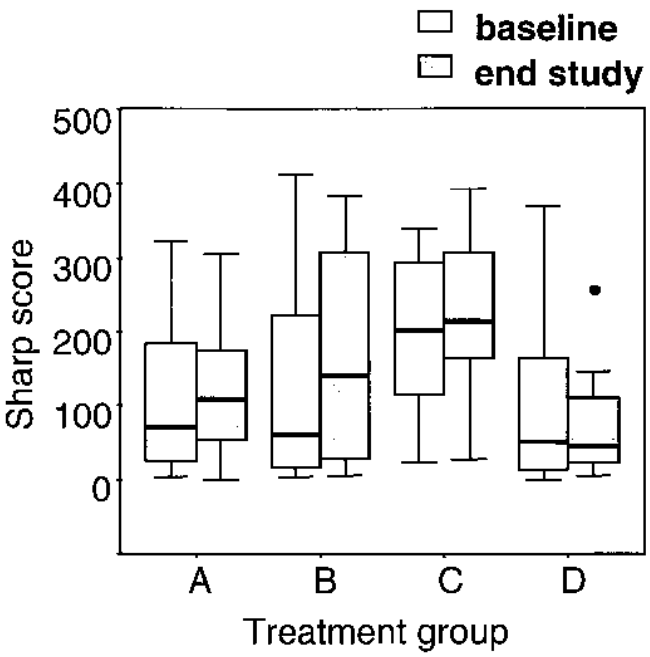


*Figure 3*. No effect of doxycycline on the progression of radiographic joint damage. The effects of doxycycline on the progression of joint destruction in the different treatment groups were evaluated and compared to placebo. Radiographic damage was scored before drug treatment (baseline) and at the end of the study period by Sharp score modified by van der Heijde. Sharp scores are depicted in box-whisker plots: horizontal line in box = median; limits of box = 25th and 75th percentiles; whiskers = highest and lowest values excluding outliers and extremes. ●: outlier (between 1.5 and 3 box lengths from the upper or lower end of the box).

were < 40 nmol/mmol creatinine for HP and < 10 nmol/mmol creatinine for LP. The urinary excretion rates of HP and LP were above normal values for 90% of the patients participating in this study. Repeated measures ANOVA showed no significant differences between the groups in the courses of the excretion rates of HP and LP during the study period. Analysis of changes of urinary excretion rates of HP and LP at the start and the end of the 12, 24, or 36 week treatment period showed no differences between patients treated with doxycycline or placebo (Table 3; Figure 2). Subgroup analysis of patients with high pyridinoline excretion rates at baseline (HP excretion rates > 80 nmol/mmol creatinine) revealed no effect of doxycycline on HP or LP excretion rates.

There was no significant difference in the progression of the Sharp score between the treatment groups (Figure 3).

## DISCUSSION

Ample evidence of the inhibitory effects of doxycycline[10,12,14,22,33] on MMP and the generally accepted concept of the involvement of MMP in joint destruction in RA[2,34] led to the hypothesis that doxycycline might be beneficial as a joint protective medicine in RA. In contrast to previous open label studies describing an effect of doxycycline on urinary pyridinoline excretion rates[21] or on joint tenderness and disability variables[22], we observed no significant effects of doxycycline on disease activity variables or pyridinoline excretion rates. Due to the size of the study, small effects of doxycycline may have been missed. However, the results do not indicate any clinically relevant effects of doxycycline that may have been observed in a larger study.

To achieve stable serum levels of doxycycline and a minimum of adverse effects, a dosage of doxycycline of 50 mg twice a day was chosen. Adverse effects, all gastrointestinal, were reported during both doxycycline and placebo treatment. Stomach ache was reported during both doxycycline and placebo treatment, suggesting that this adverse effect was not related to doxycycline treatment. Other adverse effects, such as nausea and diarrhea, were reported during doxycycline treatment only, suggesting that these adverse effects were related to doxycycline treatment. The number of patients reporting adverse effects and the number of discontinuations due to adverse effects did not differ significantly between the treatment groups. This suggests that in this patient group doxycycline in 2 daily doses of 50 mg does not produce significant adverse effects. In a previous study an effect on pyridinoline excretion rates in patients with RA taking as little as 20 mg doxycycline twice a day was reported[21]. In contrast, these results fail to confirm this effect of doxycycline in RA patients, even though a dose of more than double the amount of doxycycline was given. Moreover, one-fifth of patients treated with doxycycline discontinued the study prematurely due to lack of efficacy. This was similar to patients treated with the placebo[35]. This suggests that the effects observed in the open studies[21,22] are likely to be due to factors other than the doxycycline treatment.

The lack of effect on disease activity observed in this study is in contrast to findings with minocycline. The effects of minocycline in RA have been extensively studied in double blind placebo controlled trials. Effects of minocycline (100 mg twice a day) were observed on clinical symptoms and laboratory disease activity variables such as improvement of swelling and tenderness of the joints[17], patient and physician global assessment of disease activity[16], CRP, hemoglobin, platelet count, and ESR[15]. None of these studies demonstrated a significant effect of minocycline on the progression of radiographic joint destruction. The discrepancy between effects of minocycline on disease activity and the lack of effect on joint destruction may be explained by other properties of tetracyclines besides MMP inhibition. Tetracyclines have various immunomodulating properties, such as inhibition of the proinflammatory enzyme phospholipase $A_2$, suppression of neutrophils and T lymphocytes, antioxidative effects, inhibition of proliferation of peripheral blood lymphocytes[36], inhibition of nitric oxide synthases[37], and induction of apoptosis in activated T lymphocytes[38]. Even though doxycycline has such immunomodulating properties, we observed no effects

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of doxycycline on disease activity measures in this study. An explanation for this may be that the dosage we used was not sufficient to provide an antiinflammatory effect as shown for minocycline. However, recent studies investigating the effects of doxycycline in intravenous doses of 200 and 300 mg over 12 weeks also failed to show effects of doxycycline on clinical and laboratory disease activity variables[35,39], suggesting that even in higher doses doxycycline is not capable of suppressing inflammation in RA. It is unclear why doxycycline, in contrast to minocycline, does not influence disease activity in RA. Perhaps differences in chemical properties, for instance, minocycline being more lipophilic than doxycycline[40], result in a more favorable distribution of minocycline within the synovium. Moreover, in contrast to doxycycline, minocycline penetrates the blood–brain barrier easily, which accounts for the frequently reported minocycline induced vertigo[41]. One can speculate that, if minocycline interacts with parts of the central nervous system that influence the immune system[42], this may contribute to its immunosuppressive effects.

The lack of effect of doxycycline and minocycline on joint destruction are disappointing, since studies describing potent inhibitory effects of tetracyclines on MMP and on joint destruction both *in vitro* and *in vivo* appeared so promising[43]. An explanation for the lack of effect of minocycline and doxycycline may be that the dose regimens used in these studies did not provide sufficient MMP inhibitory levels within the joints to slow the rate of joint destruction in RA. However, for minocycline it was observed that 100 mg twice a day was sufficient to provide significant inhibition of collagenase activity in RA synovial tissue cultures[13]. For doxycycline, it was shown that a dose of 20 mg twice a day achieved *in vivo* inhibition of excess MMP activity in patients with periodontitis[44,45]. Hence an effect of 50 mg doxycycline twice a day on MMP activity in the joints of patients with RA was expected. Unfortunately, synovial fluid or biopsies from patients in our study were not obtained, so it cannot be ruled out that MMP inhibition in the joint by doxycycline was insufficient. Recent observations indicate daily intravenous administration of as much as 200 or 300 mg doxycycline also produces no effects on cartilage and bone resorption[35,39], suggesting that even in high doses doxycycline is not capable of inhibiting joint destruction in RA. Possibly, proteinases that are not inhibited by doxycycline are important mediators of joint destruction in RA. MMP-1 and MMP-3 are not effectively inhibited by doxycycline[14,49,50], whereas MMP-2, MMP-9, MMP-8, and MMP-13 are effectively inhibited in therapeutically attainable concentrations[12,46-48]. The inability of doxycycline to inhibit MMP-1 and MMP-3, both implicated in RA[8,9], may explain why doxycycline was not effective as a joint protective drug in this study.

Our results show that 12, 24, or 36 weeks of doxycycline in a dose of 50 mg twice a day does not relieve clinical symptoms, has no effect on ESR, and does not slow the progression of joint destruction in RA. Further studies are needed to determine how the excess of proteolytic activity at the site of joint destruction in RA is best inhibited.

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