Molecular Changes in Human Osteoarthritic Cartilage After 3 Weeks of Oral Administration of BAY 12-9566, a Matrix Metalloproteinase Inhibitor

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ABSTRACT. ***Objective.*** To determine the effect of BAY 12-9566, a matrix metalloproteinase inhibitor, on articular cartilage metabolism in patients with osteoarthritis (OA).

Methods. Thirty-five patients with OA were randomized to receive oral daily dosing of BAY 12-9566 (25, 100, or 400 mg) or placebo for 3 weeks prior to knee surgery. Cartilage samples were obtained at surgery and examined for markers of proteoglycan aggrecan turnover (846 epitope, a putative synthesis marker, and keratan sulfate epitope content) and type II collagen synthesis (C-propeptide content), cleavage by collagenase (COL 2-3/4C short), denaturation, and content (COL2-3/4m epitope). BAY 12-9566 concentrations were measured by HPLC in serum, synovial fluid, and cartilage.

Results. Comparisons between study drug and placebo treatments revealed that at the 100 mg dose there was a significant increase in the 846 epitope ($p = 0.012$). Total type II collagen content was also higher at this dosage ($p = 0.012$). Alterations in collagen degradation and synthesis were not detected.

Conclusion. BAY 12-9566 at daily doses of 100 mg significantly altered proteoglycan turnover, resulting in a cartilage composition reflected by the content of the 846 epitope that is more characteristic of a young growing individual. The increase in this epitope may signify increased matrix synthesis. The increase in type II collagen content was unexpected, since there was no other evidence for altered collagen turnover. However, increased matrix assembly would also be indicated by this increased content. (J Rheumatol 2003;30:544–9)

Key Indexing Terms:
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CARTILAGE COLLAGEN AGGRECAN CLINICAL TRIAL

Pathologically, osteoarthritis (OA) is a disorder of synovial joints characterized by the focal deterioration of articular cartilage, with sclerosis and cyst formation in the underlying bone, as well as formation of osteophytes[1,2]. Although cartilage loss is a primary feature of OA, the pathology is believed to represent an imbalance in the reparative and degenerative processes in articular cartilage[3]. Overall, OA is characterized by morphologic, biochemical, molecular, and biomechanical alterations of both the cells and extracellular matrix of cartilage[3].

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Articular cartilage is composed of an abundant extracellular matrix rich in collagen and sulfated proteoglycans (e.g., aggrecan)[3,4]. The containment of proteoglycans within the collagen network provides cartilage with the compressibility and elasticity necessary to protect and cushion the subchondral bone[3]. During the development of OA, physical characteristics of the cartilage matrix become disrupted, and loss of collagen and proteoglycan aggrecan occurs[3,4]. Specifically, the loss of collagen and proteoglycan in OA has been linked to the excessive enzymatic activity of matrix metalloproteinases (MMP)[3,4]. MMP are a family of zinc-containing endoproteinases capable of degrading collagen and proteoglycan[3-5]. A wide variety of cell types, including chondrocytes and synoviocytes, secrete these matrix proteinases *in vivo* and *in vitro*, and diseased articular cartilage is associated with increased activity of these MMP[6-8]. BAY 12-9566 inhibits a subset of MMP, including MMP-3 (stromelysin-1). It can inhibit cartilage degradation in culture[9]. Compounds of this kind may slow or halt cartilage deterioration, and perhaps disease progression, in OA.

As an initial step in identifying the effects of BAY 12-9566 *in situ*, a phase II trial was designed to not only determine concentrations of BAY 12-9566 in cartilage and synovial fluid, but also to determine if it could influence the

composition of articular cartilage following treatment over a 3 week period. Patients who were already scheduled for arthroplasty were treated with BAY 12-9566 or placebo. Their articular cartilages were removed and analyzed for molecular changes involving both the proteoglycan aggrecan and type II collagen that may signal degradation or the synthesis of these matrix molecules.

MATERIALS AND METHODS

Patient population. A total of 35 male and female patients with symptomatic and radiographic evidence of idiopathic OA of the knee joint, as defined by the American College of Rheumatology[10], who were scheduled for elective total knee replacement surgery were enrolled. All female participants were postmenopausal or surgically sterilized; male subjects were required to practice appropriate birth control methods both during and one month after study drug administration.

Patients with the following conditions were excluded from the study: secondary OA except for trauma more than 6 months prior to enrollment; use of drugs with MMP-inhibitory properties (e.g., tetracyclines or structurally related compounds) within 2 weeks of enrollment; major debilitating diseases (e.g., malignancies); failure of a major organ; active, acute, or chronic infections; neutropenia; psychosis; impaired liver function (i.e., AST or ALT ≥ 1.2 times the upper limit of the normal range); impaired kidney function (i.e., creatinine ≥ 130 µmol/l); history of gastrointestinal disorder; major cardiovascular event within 6 months of enrollment; any emotional disorder; history of substance abuse (severe depression, alcohol or drug abuse); use of oral (systemic) glucocorticoids within 28 days of enrollment; history of hypersensitivity to BAY 12-9566 or similar compounds; a significant history of drug allergies, or any clinically significant allergic disease; prior exposure to BAY 12-9566; or use of an investigational drug within 30 days prior to enrollment.

Patients undergoing nonsteroidal antiinflammatory drug (NSAID) therapy stopped NSAID prior to randomization for 2 days or for a period equal to 4 times the half-life of the particular NSAID, whichever was longer. For pain control, patients were allowed to use acetaminophen or another non-NSAID analgesic for the duration of the study. To define the patient population, all subjects completed the Western Ontario McMaster University Osteoarthritis Index (WOMAC)[11], including Patient Global Assessment, prior to receiving the first dose of study medication. Each participating center obtained approval from their institutional review board prior to enrollment of the first patient. Written informed consent was obtained from each patient prior to enrollment.

Study design and treatment assignment. This multicenter trial followed a randomized, double blind, placebo controlled parallel group design. BAY 12-9566 was administered orally, in dosages of 25, 100, or 400 mg, once daily for 3 weeks prior to surgery. Within each dose level, patients were randomized 2:1 to active drug or placebo. All study medication (BAY 12-9566 and its matching placebo) was supplied by Bayer, Inc. (Toronto, Ontario, Canada). Compliance was assessed by pill counts.

On Day 22 of treatment, articular cartilage was removed from the femoral and tibial surfaces of patients undergoing knee replacement surgery. Cartilage specimens were frozen at –20°C until assayed. The articular cartilages were chopped and combined for analysis. In the 25 mg treatment group, cartilage was obtained from both right and left knees from some subjects. Because the results were very similar for each knee, only right knee cartilage analyses are reported.

Cartilage analyses. Concentrations of type II collagen[12], putative markers for biosynthesis of type II collagen (C-propeptide of type II collagen[13]), type II collagen cleavage by collagenase neoepitope COL2-3/4C short[8], type II collagen denaturation epitope (COL2-3/4m[12]), aggrecan turnover (846 epitope[14]), and aggrecan content (keratan sulfate epitope[14]) were determined by immunoassay as described previously. DNA analyses were also performed[13].

BAY 12-9566 concentrations. Plasma BAY 12-9566 concentrations were measured at baseline and on Day 22. Study drug concentrations were derived from synovial fluid and cartilage obtained during surgery. For the plasma measurements, 10 ml of heparinized venous blood was centrifuged for 10 min at 3000 rpm. The plasma was transferred into a polypropylene storage tube and frozen at –20°C until assayed. Synovial fluids were also centrifuged at room temperature or 4°C at 3000 rpm for 10 min; the supernatant was removed and frozen at –20°C.

A validated high performance liquid chromatography (HPLC) assay for measurement of plasma, synovial fluid, and cartilage concentrations was used. Briefly, 250 µl aliquots of 0.4 M phosphate buffer, pH 7.0, containing 25 ml of a BAY 13-6465 internal standard, at 33 mg/l, were added to a glass screw-thread centrifuge tube containing 250 µl dog plasma or synovial fluid. Ethyl acetate (8 ml) was added to standard or samples and the tubes were capped and shaken on the reciprocating shaker for 10 min. After shaking, the tubes were centrifuged for 5 min, and the upper ethyl acetate layer was transferred to a clean glass tube. The ethyl acetate was evaporated under nitrogen at 37°C. A 1.0 ml aliquot of acetonitrile was used to rinse the interior walls of the tube, followed by final evaporation to dryness. The residues were dissolved in 250 ml sample solvent. The samples were then centrifuged for 10 min, and the supernatants analyzed by reverse phase HPLC.

Statistics. For each dose level, comparisons of biochemical analyses to the placebo group were made using the Mann-Whitney test.

Safety. Safety and tolerability were evaluated at each dose level. All patients who received at least one dose of study drug were evaluated for drug safety (safety population). Study drug safety was monitored by clinical observations and by monitoring hematology, biochemistry, and liver function profiles. Adverse events were ranked by the investigator according to severity and relationship to study drug.

RESULTS

Patient disposition and demographics. Among the 4 centers, a total of 35 patients were enrolled and received at least one dose of study drug (safety population). Of these, 33 patients had biochemical markers measured in cartilage. Three patients were prematurely discontinued from study drug, one in each of the BAY 12-9655 cohorts, due to edema and headache (25 mg group), angina (100 mg group), and severe anxiety (400 mg group). Data for the patient in the 400 mg group was retained in the efficacy analysis.

The demographics and baseline medical characteristics of the study population revealed that the majority of patients were Caucasian women close to 70 years of age and did not have generalized OA (3 or more joints involved) (Table 1). Of 33 patients who were valid for efficacy assessment, 60% were female, 89% were Caucasian, mean age was 70 years, OA duration was 15 years, and 74% had generalized OA (≥ 3 joints affected). The mean total baseline WOMAC score for the efficacy-valid population was similar for the placebo (52.09), 25 mg (51.33), and 400 mg groups (42.60); the 100 mg group had a slightly higher score (62.83), indicating more impairment.

Safety analysis. At least one adverse event was reported by 100%, 86%, 86%, and 100% of patients in the placebo, 25 mg, 100 mg, and 400 mg BAY 12-9566 groups, respectively. The most common drug related events included nausea and anemia (Table 2). Two patients had adverse events considered serious by the physician investigator; however, neither

Table 1. Demographic variables for patients valid for safety analysis.

Variable	Placebo, n = 11	Bay 12-9566 25 mg/day, n = 7	Bay 12-9566 100 mg/day, n = 7	Bay 12-9566 400 mg/day, n = 10
Age, years, mean ± SD	71 ± 7	71 ± 7	68 ± 11	71 ± 6
Male, n	4	4	1	5
Female, n	7	3	6	5
Race				
Caucasian, n	11	4	6	10
Non-Caucasian, n	0	3	1	0
Generalized OA				
No, n	9	5	5	7
Yes, n	2	2	2	3
Duration of symptoms in any joint, years, mean ± SD	18 ± 13	14 ± 16	10 ± 3	16 ± 13
Duration of symptoms in knee, years, mean ± SD	13 ± 14	11 ± 15	10 ± 3	14 ± 14
Total WOMAC* (0–96), mean ± SD	52.09 ± 10.09	51.33 ± 14.87	62.83 ± 14.33	42.60 ± 17.13

Table 2. Incidence rates of adverse events (observed in ≥ 15% of patients valid for safety analysis).

Adverse Event	Placebo (n = 11), n (%)	Bay 12-9566 25 mg/day (n = 7), n (%)	Bay 12-9566 100 mg/day (n = 7), n (%)	Bay 12-9566 400 mg/day (n = 10), n (%)
Allergic reaction	2 (18.2)	0 (0)	0 (0)	0 (0)
Asthenia	2 (18.2)	0 (0)	1 (14.3)	0 (0)
Headache	1 (9.1)	2 (28.6)	0 (0)	0 (0)
Hypertension	0 (0)	0 (0)	0 (0)	2 (20.0)
Nausea	3 (27.3)	2 (28.6)	2 (28.6)	4 (40.0)
Anemia	3 (27.3)	0 (0)	3 (42.9)	4 (40.0)
Hypochromic	2 (18.2)	0 (0)	0 (0)	1 (10.0)
Dizziness	3 (27.3)	0 (0)	0 (0)	1 (10.0)
Urinary retention	2 (18.2)	0 (0)	0 (0)	0 (0)

event was considered to be drug related. Three subjects prematurely discontinued the study due to adverse events that were not considered drug related. Clinically significant changes in routine hematologic or biochemistry laboratory studies were not observed during the study.

BAY 12-9566 concentrations. The median concentrations in the serum, synovial fluid, and cartilage indicate dose-dependent penetration into both synovial fluid and cartilage. As shown in Table 3, serum levels of BAY 12-9566 were roughly 2.5- and 4.5-fold greater in the 100 mg and 400 mg groups compared with the 25 mg dosage, respectively. Similarly, less than dose-proportional increases were also observed in synovial fluid and cartilage. The cartilage concentrations ranged from 0.31 µg/g (25 mg dose) to 3.16 µg/g (400 mg dose).

Efficacy analysis. The effects of BAY 12-9566 on cartilage metabolism are summarized in Table 4. There were no effects on DNA content and data were therefore normalized to DNA content. The changes illustrated in Figure 1 were observed for patients treated with 100 mg of BAY 12-9566 once daily. In these patients, the aggrecan 846 epitope was increased compared to placebo (p = 0.012), but this was not

Table 3. Median Bay 12-9566 concentrations in cartilage, plasma, and synovial fluids.

Source	Bay 12-9566 25 mg/day	Bay 12-9566 100 mg/day	Bay 12-9566 400 mg/day
Plasma, µg/ml	11.7	33.5	54.8
Synovial fluid, µg/ml	6.6	16.9**	43.1
Cartilage, µg/g	0.31*	1.51**	3.16***

*Only 2 patients had concentrations measured in cartilage. **Only 5 patients had concentrations measured in synovial fluid and 3 in cartilage. ***Only 9 patients had concentrations measured in cartilage.

Table 4. Median (min–max) cartilage biochemical markers, measured on Day 21 of BAY 12-9566 therapy for efficacy-valid patients.

Biochemical Marker	Placebo, n = 11	Bay 12-9566 25 mg/day, n = 6	Bay 12-9566 100 mg/day, n = 6	Bay 12-9566 400 mg/day, n = 10
Type II collagen				
Total collagen, nmol/µg DNA	2.8 (1.8–4.3)	2.5 (2.1–3.5)	3.8* (3.6–4.6)	2.6 (2.1–3.8)
C-propeptide type II collagen, ng/µg DNA	1.42 (0.33–3.86)	2.89 (0.22–6.27)	0.94 (0.64–3.90)	2.35 (1.07–8.34)
Denatured collagen, %	6.2 (2.2–29.0)	4.3 (2.7–13.7)	4.7 (4.2–5.6)	7.8 (3.4–18.6)
COL2-3/4Cshort, pmol/µg DNA	25.7 (5.1–45.7)	21.6 (12.8–129)	38.3 (11.1–68.6)	29.8 (9.3–134)
Denatured collagen, nmol/µg DNA	0.17 (0.06–1.25)	0.13 (0.06–0.37)	0.17 (0.17–0.25)	0.21 (0.09–0.64)
Aggrecan				
846 Epitope, µg/µg DNA	0.35 (0.24–2.86)	0.44 (0.21–1.46)	2.34* (0.93–4.69)	0.78 (0.18–5.62)
Keratan sulfate epitope, µg/µg DNA	90.7 (24.1–177)	85.6 (42.0–167)	74.5 (45.8–211)	66.0 (28.7–258)

*p = 0.012 compared to placebo.

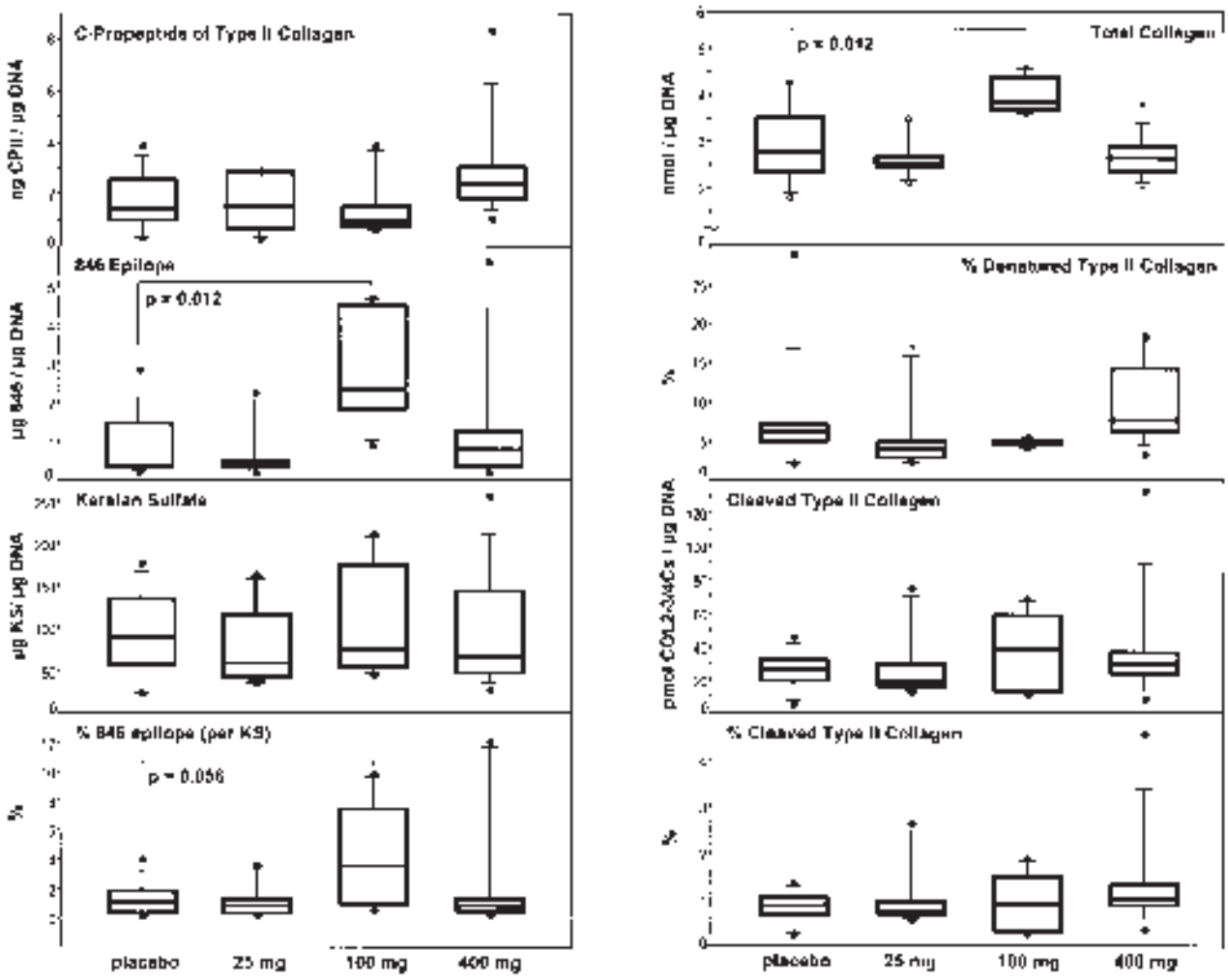

Figure 1. Analyses of immunochemical measures of aggrecan and type II collagen in articular cartilage of patients treated with and without of BAY 12-9566 once daily. The upper and lower margins of the boxes represent the 75th and 25th percentiles, respectively; upper and lower whiskers represent 90th and 10th percentiles. Outliers are shown as single experimental points. Human proteoglycan standards were fetal for 846 epitope and adult for keratan sulfate (KS) epitope.

observed at higher doses. Compared to placebo, type II collagen cleavage and denaturation were not significantly different at the different dosages. However, a significantly higher collagen content was observed for BAY 12-9566 at the 100 mg dose level compared to placebo ($p < 0.012$). At 400 mg there was a trend toward an increase in denaturation of type II collagen and an increased synthesis of this molecule (CPII content), but these changes were not significant.

DISCUSSION

This pilot investigation had a small sample size, but did show that BAY 12-9566 penetrated the cartilage and synovial fluid of patients with OA and that a 3 week course is associated with some differences in cartilage matrix composition that suggest alterations in matrix turnover. Although the majority of results were not statistically significant, we believe these data provide evidence that treatment with an MMP inhibitor can produce detectable changes in articular cartilage. Others have shown in a similar kind of study that oral doxycycline reduces collagenase and gelatinase activities in extracts of human OA articular cartilage[15]. This kind of experimental design may be of value in future studies designed to investigate direct effects of MMP inhibitor therapy on degenerate articular cartilage.

The damage to articular cartilage in OA is thought to be largely due to the excessive activity of MMP[3-8]. Direct evidence for this has been obtained for aggrecan[7] and type II collagen[8] cleavage. BAY12-9566 (in the 10–100 nM range) has been shown to inhibit the interleukin 1α induced degradation of proteoglycan and collagen cleavage by collagenase in cultures of equine articular cartilage[9]. The high level of BAY 12-9566 binding to human blood proteins might have limited its penetration into cartilage. The concentrations in joint fluid were similar to the blood levels and noticeably much higher than cartilage levels. The concentrations reached in articular cartilage were, however, in the 500–5000 nM range according to dose, and therefore, based on equine studies, the inhibitor reached concentrations high enough to inhibit cartilage proteolysis. We examined the cleavage of type II collagen by collagenase using the COL2-3/4C$_{Short}$ epitope and measured its denaturation using the COL2-3/4m epitope. These analyses failed to reveal any significant reduction in collagen cleavage over the 3 week treatment period, in contrast to *in vitro* studies[9]. There was, however, increased content of total type II collagen in the 100 mg treatment group.

Excessive cartilage degradation in OA is accompanied by increased type II collagen[13] and aggrecan[3,6] synthesis, most likely indicating an attempt at articular cartilage repair. Although collagen synthesis (C-propeptide) was not altered, the 846 epitope, which is present on the chondroitin sulfate chains of the largest and most intact aggrecan molecules[14], was also increased in the 100 mg treatment group. This suggests that aggrecan with a characteristic of undegraded, newly synthesized molecules may be increased by this dose. The 846 epitope is most highly concentrated in fetal cartilages and is virtually absent in healthy adult cartilage[16]. Its content is increased with aggrecan synthesis in OA cartilage[14]. Its presence, therefore, is thought to be related to the synthesis of aggrecan.

We conclude that the MMP inhibitor BAY 12-9566 was well tolerated by patients with OA during a 3 week course of once daily oral administration. BAY 12-9566 was detected in both articular cartilage and synovial fluid from these patients in concentrations sufficient to arrest cartilage degradation in culture. Moreover, administration of BAY 12-9566 was associated with some dose-dependent differences in articular cartilage matrix content suggestive of enhanced matrix assembly. There was, however, no evidence of a dose related effect on the variables we analyzed. This may be in part because of the small sample size in these studies. Whether MMP inhibitors of this kind can effectively reduce cartilage degeneration *in situ* or promote repair must await future studies with larger groups and longer treatment periods, examining not only biochemical markers of matrix turnover, but also measures of cartilage structure, such as joint space narrowing on radiographs or cartilage volume on magnetic resonance image scans. It is with great difficulty that we draw conclusions from such a study regarding matrix turnover and the inherent variability in cartilage composition and damage in patients of this kind.

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