Inhibitory Effect of Cephalothin on Matrix Metalloproteinase Activity around Loose Hip Prostheses

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Matrix metalloproteinases (MMPs) are key enzymes in pathological collagen degradation in aseptic loosening of total hip arthroplasty (THA) prostheses. They are involved in the cascade of degradation of the matrix surrounding the prostheses (19, 25, 26).

In the study described here cephalothin [monosodium (6R,7R)-acetoxymethyl-8-oxo-7-[2-(2-thienyl)acetamido]-5-thiazabicyclo[4.2.0.]oct-2-ene-2-carboxylate], tetracycline, doxycycline, and gentamicin were tested for their eventual inhibitory effects on MMP activity by means of a DNP-S (dinitrophenyl-Pro–Gln–Gly–Ile–Ala–Gly–Gln–D-Arg) peptide degradation assay. Samples were obtained from periprosthetic tissue around loose THA prostheses.

Eleven samples were obtained from interface tissues between the prostheses and bone of patients requiring revisions for aseptic loosening of the acetabular component of THA. Six were women and five were men (mean age, 63.0 years; age range, 41 to 81 years). Eight THAs were originally performed to treat primary osteoarthritis, two were performed to treat secondary osteoarthritis caused by congenital dislocation of the hip joint, and one was performed to treat secondary post-traumatic osteoarthritis. Revisions were performed a mean 4.5 years (range, 1.0 to 9.0 years) after the primary operation. Two THAs had been fixed with methylmethacrylate bone cement and nine were cementless. Control samples (n = 8) comprised noninflammatory knee synovial tissue obtained from suprapatellar recesses during knee arthroscopy performed to examine meniscus injury. These knees were not clinically inflamed and had no signs of osteoarthritis. The mean age of the eight control patients was 34.3 years (age range, 20 to 46 years); half were female.

Control and test samples were divided into 0.2- to 1.5-g pieces and were kept in airtight vinyl bags at −40°C until tissue extraction. These tissue samples were minced into small pieces and were homogenized with the ULTRA-TURRAX T25 (Janke & Kunkel GmbH & Co. KG, IKA Laboratory Technology, Staufen, Germany) in an ice bath after the addition of a neutral salt extraction buffer (50 mM Tris-HCl, 10 mM CaCl₂, 2 M KCl [pH 7.5], 1.9 g of tissue per 1 ml of buffer). After a 1-h incubation at 40°C, homogenates were centrifuged at 7.5 × 10⁴ g for 60 min at 4°C. The supernatants were dialyzed against an MMP buffer (50 mM Tris-HCl, 5 mM CaCl₂, 10 mM ZnCl₂, 0.2 M NaCl [pH 7.5]) before the assay (25).

Collagen- and gelatinolytic activities were measured by reverse-phase high-performance liquid chromatography (HPLC) with a synthetic DNP-S substrate. The assay has been validated in detail in previous reports describing this method (6, 13, 22). Collagen and denatured collagen (i.e., gelatin) contain Gly-X-Y repeat sequences, where X is often proline and Y is either hydroxyproline or hydroxylysine. Synthetic DNP-S substrate is degraded by both collagenases and gelatinases, because it contains a typical cleavage site for such enzymes in its glycinelisocelulose bond. Undegraded DNP-S substrate and the DNP-containing degradation product DNP-P (dinitrophenyl-Pro–Gln–Gly) can be easily separated by HPLC (6, 13, 22). Then, 30 μl of tissue extract was mixed with 5 μl of 10 mM DNP-S (Sigma, St. Louis, Mo.) and 10 μl of 50 mM Tris-HCl-0.2 M NaCl–5 mM CaCl₂ (pH 7.5) (TNC buffer). After the addition of 5 μl of each of the antibiotics or TNC buffer, the samples

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were incubated for 4 h at 37°C. The final concentrations were 10, 100, and 1,000 µg/ml for cephalothin; 5, 50, and 500 µg/ml for tetracycline and doxycycline; and 0.5, 5, and 50 µg/ml for gentamicin. The median value (for the three concentrations of antibiotics used) was selected on the basis of the concentration in blood found to be clinically effective. The reaction was stopped by the addition of 400 µl of 30% acetonitrile in 0.1% trifluoroacetic acid. The DNP-S substrate was separated from its two degradation products, DNP-P and Ile–Ala–Gly–Gln–d-Arg, at ambient temperature by HPLC (LKB HPLC pump model 2150 and LKB LCC model 2252; Pharmacia LKB Biotechnology, Uppsala, Sweden) equipped with a 0.46-mm-by-25-cm, 218TP C18 reverse-phase column (5-µm particle size; Vydac). The flow rate was 1 ml/min, and the sample volume was 10 µl. The column effluent was continuously monitored at 380 nm with a variable-wavelength monitor (LKB model 2151, Pharmacia LKB Biotechnology) and the wavelength was recorded with an on-line printer (Recording Integrator, LKB model 2220; Pharmacia LKB Biotechnology). The degradation of DNP-S substrate was expressed as (DNP-P/DNP-S) × 100 (percent). The negative control was 30 µl of TNC buffer.

The results were expressed as means ± standard deviations. The MMP activities of the interface tissue samples were compared with those of the noninflammatory knee joint synovial samples by unpaired t test. The effect of the dose of cephalothin, tetracycline, doxycycline, or gentamicin on DNP-P degradation was evaluated by analysis of variance. Fisher’s probability by the least significant difference post hoc test was used to analyze differences between groups.

High-performance liquid chromatograms displayed DNP-S as a single peak at a retention time of approximately 12.1 min. When this substrate was incubated with tissue extract from a loose THA, DNP-S was partially degraded to Ile–Ala–Gly–Gln–d-Arg (not seen in the chromatogram) and DNP-P, which was eluted before the DNP-S peak at a retention time of approximately 11.8 min; this was followed by DNP-S at 12.1 min. MMP activity was markedly elevated in the interface tissue extract (52.9% ± 19.7%) compared with that in noninflammatory synovial tissues from the knee joint (2.7% ± 1.9%; P < 0.001). Samples from patients 7, 8, and 11, which showed high MMP activity, were pretreated with antibiotics before the DNP-S degradation assay. After pretreatment with cephalothin, MMP activity was not decreased by cephalothin at 10 µg/ml (52.6% ± 15.8%; P = 0.3442), but was decreased significantly by cephalothin at 100 µg/ml (31.9% ± 8.8%; P = 0.0072) and 1,000 µg/ml (13.6% ± 0.1%; P = 0.0001). This inhibition was dose dependent (P = 0.0349; see also Table 1) and was not explained by trivial reasons such as pH (data not shown). In addition, 500 µg of tetracycline per ml inhibited MMP activity (45.2% ± 8.5% inhibition; P = 0.0412) (Table 1). Doxycycline and gentamicin did not inhibit the MMP activity of the interface tissue extract (Table 1).

MMPs can be controlled by the rate of synthesis of the proenzymes and their activators and inhibitors (28). TIMPs are endogenous and specific MMP inhibitors, but α1-macroglobulin can also inhibit MMPs (28). Until recently, chemical inhibition of the excessive MMP activity associated with various diseases was considered to be difficult, especially in vivo (5). However, recent studies have shown that tetracycline, chemically modified tetracyclines, and antracycline antibiotics can noncompetitively inhibit MMPs via Ca2+ and Zn2+ chelation (12, 28, 30). Differences in the sensitivities of various members of the MMP family to both natural and chemical inhibitors do exist (5, 10, 24, 28). For example, tetracyclines preferably inhibit polymorphonuclear neutrophil leukocyte-derived MMPs, whereas TIMPs are more effective against fibroblast-derived MMPs (2).

The present study demonstrated that tissue extracts from periprosthetic THA tissues show highly elevated MMP activity compared with that of noninflammatory knee joint synovial tissues and that this elevated MMP activity is significantly inhibited by cephalothin in a dose-dependent manner. The present findings indicate that cephalothin, a β-lactam antibiotic, can directly inhibit MMP activity in diseased human tissue when it is administered at therapeutically effective concentrations. Our findings support the results of recent studies showing that cephalothin can inhibit human uterine MMP by a nonantibacterial mechanism (21). Furthermore, recent studies have shown that a monocyclic β-lactam inhibitor can efficiently inhibit human leukocyte elastase (9). Although tetracycline and doxycycline preferentially inhibit MMP-8 (neutrophil-type collagenase) (1, 5, 8, 11, 30), in the present study tetracycline and doxycycline did not, however, markedly inhibit MMP activity in periprosthetic THA tissues. This may be explained by the MMP species profile, as described in previous reports (12, 25, 26). Immunohistochemical studies of MMPs around loose THA prostheses have revealed (i) that the major cellular components are monocytes/macrophages, fibroblasts, and endothelial cells and (ii) that they produce MMP-1 (fibroblast-type collagenase), MMP-2 (fibroblast-type gelatinase), MMP-3 (stromelysin-1), and MMP-9 (92-kDa gelatinase), but not polymorphonuclear leukocyte MMP-8 (26). Taken together, these results suggest that the collageno- and gelatinolytic enzymes produced in periprosthetic tissues around loose THA prostheses are derived from cellular sources expressing MMP-1, MMP-3, MMP-2, and MMP-9, but not MMP-8 (25). Therefore, tetracycline and doxycycline could not be expected to efficiently inhibit the MMPs present in samples from loose THA prostheses.

In summary, cephalothin is one of the cephalosporin family of antibiotics effective against Staphylococcus aureus, Streptococcus...
The effects of MMPs in the loosening of THAs. The present study suggests that cephalexin has potential for use in clinical applications, with the aim of inhibiting the unfavorable effects of MMPs in the loosening of THAs.

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