INTRODUCTION: Septic arthritis remains a severe disease in the 21st century, involving rapid cartilage destruction despite the availability of improved antimicrobial agents. The most common bacterium in this condition is gram-positive Staphylococcus aureus (SA), a relevant gram-negative one is Pseudomonas aeruginosa (PA). SA is described to cause matrix degradation, but the precise signalling pathways are not fully understood. There is evidence for reactive oxygen species (ROS), respectively radicals, being involved in the bacterial-induced cartilage degradation, since upregulation of iNOS was demonstrated [1]. In the present study we investigate the effect of bacterial supernatant on articular cartilage explant matrix destruction. Moreover, we assess the effect of superoxide dismutase (SOD), a potent antioxidant [2], to evaluate the involvement of reactive oxygen species (ROS) in this condition.

METHODS: Bovine articular cartilage explants (3 mm diam. x 1mm thickness) were obtained from the femoropatellar groove from 18- to 26-months old cattle and cultured in DMEM (+10% FBS, vitamin C and E, proline, antibiotics) for 2 days to allow equilibration. The following 24 h of culture were +/- supernatants of Pseudomonas aeruginosa (PA; 1:100 dilution) or Staphylococcus aureus (SA; 1:100 dilution) and +/- superoxide dismutase (SOD) mimetic (Mn(III)-porphyrin, 2.5µM, Alexis Biochemicals, Cat-No: ALX-430-070). Then, media was changed and cartilage explants were cultured without PA, SA but +/- SOD the following 3 days (Fig. 1). GAG loss was analyzed as GAG content of the medium by DMMB assay. Medium was determined for Nitrite, the stable endproduct of NO, using the Griess reagent. Biosynthetic activity was analyzed on day 4 by radiolabel incorporation of [³⁵S]-proline and [³⁵S]-sulfate in a separate experiment chondrocytes were killed by repetitive freezing/thawing of the explants. In the following these explants were stimulated according to the experimental design listed above and GAG loss was determined. In additional investigations with different dilutions (1:10 – 1:1000) of the bacterial supernatant chondrocyte viability was detected using the FDA-EB assay (red-green-assay). Statistical analysis of data was made using the Students t-test.

RESULTS: Both, PA and SA supernatant, caused a massive increase in loss of GAG to the media relative to control 24h after intervention (Fig. 2). GAG loss in the following 3 days after stimulation was distinct, but still significantly elevated. In presence of SOD the bacterial supernatant-induced loss of GAG was reduced in significant extent after 24h. However, at day 4 the presence of SOD had significant effects only for PA stimulated cartilage explants. NO content of the medium was increased after bacterial supernatant stimulation. Again, SOD decreased the bacterial induced effects at both time points (data not shown). Biosynthetic activity showed different results for PA or SA stimulation and proline or sulfate incorporation, respectively. SA caused significant increase in sulfate incorporation, reduced back to control level in presence of SOD (Fig. 3). In contrast sulfate incorporation was decreased by PA stimulation. SOD was able to elevate incorporation rate in the PA group, but this was not significant. Proline incorporation was significantly decreased for both, PA and SA stimulation, with more pronounced effect for PA supernatant. Presence of antioxidant SOD inhibited these decreasing effects.

A separate experiment using freeze-thaw-killed cartilage was performed to test whether matrix degrading activity was induced by bacterial supernatant, or if the supernatants themselves already contain proteolytic enzymes. Loss of GAG within the first 24h of stimulation tended to similar results as in the main experiment, but in the following 3 days after intervention GAG loss was less distinct (Fig. 4). In contrast to the viable tissue SOD showed no inhibiting effects on GAG loss at both time points.

Chondrocyte viability was significantly decreased only after stimulation with supernatant concentration 1:10 for 48h (data not shown).

DISCUSSION: The observed increase in loss of GAG and decrease in biosynthesis are typical degenerative changes of cartilage tissue in septic arthritis [3]. The bacterial supernatant induced effects were inhibited in presence of antioxidant SOD, suggesting a ROS-dependent pathway being involved. Matrix destruction was a combined effect of enzyme activity in the supernatant itself and induction of proteases in the cartilage. This is proved by our finding, that avital cartilage showed no further increase in GAG loss in the following 3 days after bacterial stimulation. In addition, the protective effect of SOD requires a viable tissue. This report gives insight in signalling pathways of cartilage matrix destruction during bacterial arthritis. One might suggest that matrix destruction is ROS-dependent under the given conditions.


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