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Original Article

Treatment of Staphylococcal Joint Infection in the Rabbit by Administration of Systemic Antibiotics and Intra-articular Corticosteroids

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Abstract

Background/objective: Despite the present routine treatment of septic arthritis with antibacterial agents, articular damage is persistent and frequently leads to loss of joint function. The aim of this study was to assess the effect of intra-articular corticosteroids added to systemic antibiotics in the treatment of experimental staphylococcal knee joint infection in rabbits.

Methods: Thirty rabbits were injected in their knees by Staphylococcus aureus. The rabbits were divided into 3 equal groups. In group A, rabbits received no treatment. In group B, rabbits were treated with systemic antibiotics alone. Group C, received systemic antibiotics and intra-articular corticosteroids. After 16 days animals were killed and knee joint X-Ray as well as histopathological–histochemical parameters were assessed.

Results: All rabbits survived the experiment; the treated groups (B,C) had better histological–histochemical scores in comparison with the untreated group (A). Group C had significantly better scores in joint sections in comparison with group B (mean SD = 6.7 ± 2.3 v 4.0 ± 2.4; P= 0.019). Lower damage in the former group was expressed in lesser clustering of chondrocytes, proteoglycan depletion, and severity of synovitis. Radiological soft tissue scoring was significantly better in group C in comparison with group B. Three peri-articular abcesses were observed in group C but none in group B.

Conclusion: Addition of intra-articularly administered corticosteroids to antibiotic treatment of septic arthritis improved histological histochemical parameters in this experimental setting, although on account of the clinical observation of three cases with peri-articular abcesses in this group, caution is warranted in interpretation of these results.

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Introduction

Pyogenic arthritis typically occurs in young children and the elderly. Staphylococcus aureus is the most frequent pathogen[1-4] and reaches the joint through a direct penetration or via the hematogenous route, where it multiplies rapidly, in the closed joint space.[5]

Early in the twentieth century, the main treatment of septic arthritis consisted of surgical amputation.[6] Currently, the treatment of joint infections typically consists of parenteral antibiotics plus evacuation of intrasynovial exudate via arthrocentesis, arthroscopic lavage or arthrotomy.[1,2,4] However, even with appropriate treatment, started early in the disease, irreversible joint damage commonly persists.[5,7]

Smith et al have shown that with antibiotic treatment, the loss of collagen from knee cartilage of rabbits with septic arthritis was reduced by 37% (in comparison to 50% in untreated rabbits), but loss of glycosaminoglycan exceeded 50% (relatively equal to untreated rabbits).[4] Staphylococcal products, together with post-infectious inflammatory sequel, prolong cartilage destruction despite antibiotic treatment.[4,7] Breakdown of bacterial cell wall by antibiotic treatment can increase antigenic exposure, which in turn intensifies the process of joint deterioration by inducing the release of cytokines or activation of chondrocyte proteases.[5,7] Thus, controlling the immune system by corticosteroids, concomitant with appropriate antibiotic treatment, could abolish the activation of the inflamatory mediators. This approach of combined antibiotic-corticosteroid treatment has been shown to improve prognosis in acute bacterial meningitis.[5,8,9]

In this study, we analyzed the histological-histochemical effects of intra-articular corticosteroids on rabbit articular cartilage infected with Staphylococcus aureus and treated with systemic cefazolin.

Materials and Methods

Originally, we isolated the Staphylococcus species from a patient with prepatellar bursitis and checked their sensitivity to cephalosporins. Then bacterial suspension containing 2×10^4 cells/ml in normal saline was prepared.

Thirty Dutch rabbits, between 1.5 to 2.5 kg, were selected and provided with adequate amount of food and water, and kept under the standard temperature and lighting conditions. Using lateral retropatellar approach, their left knees were inoculated with 0.5 ml of Staphylococcus aureus suspension (2×10^4 cells/ml) after shaving and application of antiseptic solutions (first povidone-iodine and then alcohol). After 48h of inoculation with S.aureus, rabbits were evaluated for signs and symptoms of joint infection and all had limping, limitation of motion, swelling, hotness and tenderness. The animals were then divided into three groups; the study group (group C) included 10 animals. The second group was the control group (group B). The third group (group A) received no treatment.

Table 1: Histopathological and histochemical scoring*

	Parameters	Score
1	cellularity	
	Normal cellularity	0
	<10 % of acellular cartilage	1
	10- 50% of acellular cartilage	2
	> 50 % of acellular cartilage	3
2	Loss of matrix (erosion of the articular cartilage)	
	No loss	0
	<10 % of eroded regions	1
	10- 25 % of eroded regions	2
	> 25% of eroded regions	3
3	Clustering of chondrocytes (propagating cells in groups)	
	No clustering	0
	< 10% of chondrocytes in clusters	1
	10-25 % of chondrocytes in clusters	2
	> 25 % of chondrocytes in clusters	3
4	Adhesions (pannus)	
	No adhesions	0
	Covering only the margin of cartilage	1
	Covering < 50 % of articular surface	2
	Covering > 50 % of articular surface	3
5	Orthochromasia with Alician blue	
	Normal	0
	Slight and patchy loss of staining	1
	Moderate loss	2
	Severe loss	3

* Alician blue was used for staining acide mucopolysaccharides

Subjects of group B (n=10) were treated with cefazolin (50 mg/kg/day) for 14 days with in two divided doses intramuscularly, starting 48 hours after inducing of the infection. Group C (n=10) were treated with cefazolin as in group B, but methylprednisolone acetate (5 mg) was injected intra-articularly into the infected knees 72 hours after inducing of the infection and 24 hours after the start of antimicrobial treatment.

All rabbits' left knees were aspirated 48 hours after infection and the aspirated fluid was sent for culture. In all cases the presence of Staphylococcus aureus was documented by positive culture.

Sixteen days after the beginning of the experiment, all of the rabbits were sacrificed using an overdosage of sodium thiopentothal. Both knees were removed and fixed with 10% formalin, and then the specimens were coded for blinded assessment. AP and lateral X-Ray of samples were also taken.

Table 2: Histopathological and histochemical scoring of femoral sections and radiological soft tissue scoring.

Group	A	B	C
%Acellular cartilage	1.5 (97)	1.1 (.74)	.60 (.84)
Loss of matrix (erossion)	1.3 (.67)	.90 (.88)	.60 (.84)
Clustering	1.0 (.67)	1.1 (.32)	.50 (.71)*
Pannus	.90(.99)	.80(.79)	.70(.82)
Orthochromasia (proteoglycan depletion)	2.20 (.79)	1.40(.84)	.60(.52)*
Severity of synovitis	1.70(.67)	1.40(.52)	1.00(.00)
Total score	8.60(2.59)	6.70(2.26)	4.00(2.40)*
Radiological soft tissue swelling	1.91(.20)	1.87 (.02)	1.29 (.10)*

* P < 0.05 between group 2,3. Data shown as mean (SD).

The coded specimens were sent to pathological laboratory.

After regular fixation in buffered formalin and decalcification, the samples were cut into 5-7 µm thick slices perpendicular to the articular cartilage and the slices embedded in paraffin. The samples were stained by Hematoxylin and Eosin and Alician blue at PH= 2.5. The sections were then studied for histopathological and histochemical findings, based on cellularity of cartilage, loss of matrix, clustering of chondrocytes, adhesion and pannus formation and orthochromasia. (Table 1).

Radiological soft tissue scoring was done using the proportion of proximal tibial diameter to adjacent soft tissue diameter on AP films.

Statistics

Data were analyzed by the SPSS PC statistical software. Because of the ordinal variables and nonparametric distribution of data, groups were compared by the Kruskal-Wallis test. Values of 0.05 or less were considered as statistically significant.

Results

All animals survived up to the end of the experiment. After termination of experiment and killing of rabbits by overdosage of thiopentothal, joint capsules were opened. Pus was observed in all joints

Figure 1: Normal proteoglycan content in a control knee (non bacteria inoculated). Stained with Alician blue x 100.

Figure 2: Severe proteoglycan depletion from septic knee, group A (bacteria inoculated without any treatment). Stained with Alician blue x 100.

Figure 3: Destructed cartilage with pannus formation, group A. Stained with H & E x 100.

of group A (bacteria-inoculated rabbits without additional treatment). In this group, all joint capsules and peri-articular soft tissues and synovia were highly swollen.
In group B (bacteria-inoculated rabbits receiving antibiotic treatment alone) swelling of soft tissues was mild to moderate, significantly lower than group A. Pus was not observed in any of joints in this group but synovium was mildly hypertrophic.
In group C (bacteria-inoculated rabbits receiving antibiotic and intra-articular corticosteroid treatments) however, swelling of soft tissues around the joints was not significant and there was no synovial hypertrophy, and in three cases we observed peri-articular abscess (between skin and joint capsule) with access to joint cavity. The pathologic scoring of these three cases was worse than the other cases in this group.

Figure 1 shows an example of normal proteoglycan content in a control knee (normal, none-inoculated) and severe proteoglycan depletion of a septic knee of group A in figure 2. Figure 3 shows destruction of cartilage with pannus formation.
Table 2 shows the scoring of the femoral sections. As shown, the total scores of the antibiotic treated groups were lower in comparison with the untreated group (mean SD = 8.0 ± 2.56 v 6.70 ± 2.26; p= NS). These lower scores with antibiotic treatment is due to the lower damage expressed in lesser degrees of proteoglycan depletion and synovitis. It can be seen that group C (antibiotic + corticosteroid) has a significantly better total score in comparison with group B (antibiotic alone) mean SD = 6.70± 2.26 v 4.00± 2.40; p= 0.019.
This better score in the corticosteroid group is due to the lower damage expressed in lesser clustering of chondrocytes and proteoglycan depletion. We also observed better radiological soft tissue scoring in group C in comparison to group B.

Discussion

Despite the antibacterial treatment of septic arthritis, there is often a subsequent disabling damage to the cartilage. We tried to find a better approach to diminish the articular cartilage damage. The theoretical basis for addition of immunosuppressive agents to antibiotic treatment of septic arthritis is that the local articular damage may be secondary to activation of the host immune system and not exclusively due to bacterial destruction alone.[1,2,4-7]
Wysenbeek showed that antibiotic–corticosteroid treatment seemed to have no adverse effect. He also suggested that contraindications to local use of corticosteroids in septic arthritis, as quoted in pertinent textbooks, are unjustified as long as systemic antibiotics are combined with the corticosteroids.[10]
Smith et al. showed that addition of naproxen to antibiotic treatment, before and during induction of arthritis, decreased the loss of glycosaminoglycans and collagen from articular cartilage by 15% and 30%, respectively.[4] In this study we showed that addition of corticosteroids to antibiotic treatment of septic arthritis may have more benefits over antibiotic treatment alone. Animals with combined treatment had significantly less proteoglycan depletion and clustering of chondrocytes, and better radiological soft tissue scoring in comparison with animals treated with antibiotic alone. They also had less acellular cartilage, loss of matrix and less synovitis. This led to a significantly better radiological soft tissue scoring and histopathological-histochemical scoring in femoral sections.
Wysenbeek in his recent study on treatment of staphylococcal septic arthritis in rabbits by systemic antibiotics and intra-articular corticosteroids showed that the combined treatment with antibiotics and corticosteroids might have advantages over antibiotics alone. He used Staphylococcus epidermidis because of its lower virulence instead of highly virulent Staph. aureus, which was chosen in our study. He showed that animals with combined treatment had significantly less proteoglycan depletion and synovitis[5] which is in accordance with our

study. They also had less erosion, clustering of chondrocytes, pannus formation and enchondral bone marrow cavities.[5] Our results of peri-articular abscess in a few cases of combined treatment may be due to the use of high virulence Staph. aureus instead of Staph. epidermidis.

Stricker et al. reported the effect of bethamethasone on experimental septic arthritis. His results of reduced proteoglycan loss are in accordance with our findings. He showed that this chondroprotection was more pronounced in the group treated by systemic corticosteroids than in the group treated with local corticosteroids. However, in this study a lower dose of local corticosteroid was used, with about 50% of the potency of corticosteroid used in our experiment. This dose difference as well as prolonged action of methylprednisolone acetate, used in our study, might explain the more pronounced effect that we observed with intra-articular corticosteroids.[1]

In another study on murine septic arthritis, Sakiniene et al. added systemic corticosteroids to antibiotics. He showed that the corticosteroid-treated group had less mortality and less synovial infiltration, as observed in our study. He also showed that the course of arthritis was ameliorated because of down-regulation of T and B lymphocytes and macrophage function.[6]

Because of our clinical observation of peri-articular abscess in a few cases of combined antibiotic and corticosteroid treatment, the effect of corticosteroids in septic arthritis should be interpreted with caution. However, the histopathologic scores in the group receiving combined treatment were significantly better. There are no data showing the long term effect on permanent damage by this therapeutic approach.

We reserve the operative treatment for any complicating intra or peri-articular abscess, and also suggest further experimental study, with addition of corticosteroids to operative and antibiotic treatments of Staphylococcus aureus septic arthritis.

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