RESEARCH ARTICLE

Experimental Study on Protective Role of NSAID on Articular Cartilage Destruction in Septic Arthritis

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Abstract

Background: Surgical drainage and antibiotic therapy are the cornerstones of treatment protocols in septic arthritis; however, in some circumstances, the diagnosis and initiation of treatment may be retarded by slow disease progression or the time when the patient's condition precludes early surgery. Therefore, it is beneficial to find ways to reduce the amount of articular injury. This study aimed to evaluate the effects of nonsteroidal anti-inflammatory drugs (NSAIDs) on the prevention of articular cartilage damage in an animal model of staphylococcal septic arthritis.

Methods: Knee joints of 40 rabbits were infected by the intra-articular injection of 10^5 colony-forming units of Staphylococcus aureus. Subsequently, they were categorized into four groups. The first (i.e., control group) and second groups were treated with a placebo and intramuscular injection of Ceftriaxone, respectively. Moreover, the third and fourth groups were treated with Naproxen alone and a combination of Ceftriaxone and Naproxen, respectively. All medications were started 24 h after the inoculation of microorganisms into the knee joint and continued for 3 days. Following that, the cartilage was evaluated using the International Cartilage Repair Society (ICRS) Visual Histological Assessment Scale.

Results: The group treated with the combination of Ceftriaxone and Naproxen obtained better results in terms of cell viability in tibial side cartilage and surface in both tibial and femoral cartilages (*P<0.0125*), compared to the group treated with antibiotics alone.

Conclusion: According to the results, in case of septic arthritis, the early administration of NSAID in conjunction with an appropriate systemic antibiotic may decrease further articular cartilage damage that is evoked by an infection.

Level of evidence: III

Keywords: Articular cartilage, International cartilage repair society, Joint, Nonsteroidal anti-inflammatory drugs, Septic arthritis

Introduction

The currently accepted treatment for the management of septic arthritis is the combination of antibiotic therapy with joint drainage and irrigation (1). Even with early antibiotic therapy and surgical debridement, cartilage damage will continue after the resolution of infection (2).

Chondrocytes play a major role in the production of collagen and proteoglycan, which are the cornerstones of the cartilage character in neutralizing pressure in the joint (3, 4). The role of inflammatory mediators is well recognized in the pathogenesis of the articular cartilage damage by the production of destructive enzymes in septic arthritis (1, 5).

In 1991, Vignon et al. investigated the effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on human osteoarthritic cartilage. They reported that the use of specific NSAIDs might minimize cartilage damage in patients with osteoarthritis as a result of inflammation

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and cytokine activation (6). With this background in mind, it is hypothesized that NSAIDs may minimize the damage to the cartilage in the setting of septic arthritis by attenuating the inflammation.

Materials and Methods

Totally, 40 male Dutch rabbits weighing between 1.7 and 2.3 kg were purchased from the animal laboratory of Shiraz University of Medical Sciences, Shiraz, Iran. The rabbits were placed in separated cages at a room temperature of about 25±2°c and 12/12 h light/dark cycle. The animals had free access to food and water throughout the experiment. All procedures conformed to the guidelines for the care and handling of animals prepared by the Iranian Ministry of Health and Medical Education and were in accordance with the international conventions on animal experimentation. The protocol of this animal study was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran.According to standard protocols, all animals were anesthetized using the intramuscular injection of 2% Xylazine hydrochloride (8 mg/kg) and 5% Ketamine hydrochloride (10 mg/kg). The procedures were performed in the operating room of the animal laboratory of Shiraz Medical School affiliated to Shiraz University of Medical Sciences, Shiraz, Iran, under a sterile condition. To induce septic arthritis, the right knee of each rabbit was inoculated with 10^5 colony-forming units of Ceftriaxone-sensitive strain of Staphylococcus aureus that was achieved by optical densitometry using a 25-gauge needle through the patellar ligament (2, 5, 7).

All rabbits were observed closely for any signs of joint redness, hotness, discharge, and limping to be sure that septic arthritis has been induced. The diagnosis was confirmed by joint aspiration and culture as well as the comparison of infected knee joint with the intact side at the time of rabbit sacrifice.

After the presentation of septic arthritis signs, the rabbits were categorized into four groups of 10. The first group (control group) was treated with a placebo (i.e., intramuscular normal saline). The second group was treated with intramuscular Ceftriaxone 40 mg/kg for 3 days. Moreover, all rabbits in the third group were treated with Naproxen sodium solution (600 mg/ 250 cc of water) in drinking water for 3 days. This dosage of Naproxen sodium had been shown to be effective against human diseases (5). The last group was treated with both Ceftriaxone and Naproxen sodium in the same manner, dosage, and duration (2, 5, 7). All medications were started at 24 h after bacterial injection when limping was started. All cultures sent before the starting of medications were positive for Staphylococcus aureus. The medication was given to the rabbits every morning at 8 am. The rabbits were caged together in separated groups. The cages were large enough in order not to limit the activity of rabbits. Since cartilage destruction was the most within the first 48-72 h, the treatment duration was only 3 days to evaluate the effect of Naproxen sodium in the first 3 days (1, 2).

After 3 days, the rabbits were sacrificed by the administration of high doses of Pentothal (200 mg/ kg). Through skin incision on the joint, the knee joint containing tibial and femoral condyles was extracted totally. Proper sections were prepared by an experienced pathologist from the distal femur and proximal tibia containing two complete condyles and joint surfaces. The pathologist was not aware of the treatment groups and the histological assessments were performed blindly. The sections were fixed in 10% Formalin for 1 week and decalcified for 2 weeks. Furthermore, the sections were stained with Safranin-O as well as Hematoxylin and Eosin staining procedures. The stained sections were microscopically assessed according to the International Cartilage Repair Society (ICRS) Visual Histological Assessment Scale (8).

The ICRS scaling is based on six parameters. Considering P-value less than 0.001, the Kruskal-Wallis test was performed to evaluate the distribution of each of these parameters between all four groups. Subsequently, each group was compared with the control group, and the antibiotic alone group was compared with antibiotic + NSAID group using the Mann-Whitney test as a post hoc test. A *P-value* less than 0.0125 was considered statistically significant (9).

Results

The results obtained from this study revealed that the matrix was hyaline in all four groups. Subchondral bone was normal; however, there was only a subchondral hemorrhage that was not mentioned in the ICRS classification. Cartilage mineralization was also normal in all four groups. The results were summarized in tables 1 to 3.

Table 1. Comparison of tibial and femoral matrix variables between each paired groups

Group		*P-value*
Tibial matrix	Control Antibiotic	1.000
	Control *NSAID	1.000
	Control Antibiotic + NSAID	1.000
	Antibiotic Antibiotic + NSAID	1.000
Femoral matrix	Control Antibiotic	1.000
	Control NSAID	1.000
	Control Antibiotic + NSAID	1.000
	Antibiotic Antibiotic + NSAID	1.000

* Nonsteroidal anti-inflammatory drugs

Table 2. Comparison of tibial and femoral subchondral bone variables between each paired groups

	Group	*P-value*
Tibial subchondral bone	Control Antibiotic	1.000
	Control *NSAID	1.000
	Control Antibiotic + NSAID	1.000
	Antibiotic Antibiotic + NSAID	1.000
Femoral subchondral bone	Control Antibiotic	1.000
	Control NSAID	1.000
	Control Antibiotic + NSAID	1.000
	Antibiotic Antibiotic + NSAID	1.000

* Nonsteroidal anti-inflammatory drugs

Table 3. Comparison of tibial and femoral cartilage mineralization variables between each paired groups

	Group	*P-value*
Tibial cartilage mineralization	Control Antibiotic	1.000
	Control *NSAID	1.000
	Control Antibiotic + NSAID	1.000
	Antibiotic Antibiotic + NSAID	1.000
Femoral cartilage mineralization	Control Antibiotic	1.000
	Control NSAID	1.000
	Control Antibiotic + NSAID	1.000
	Antibiotic Antibiotic + NSAID	1.000

* Nonsteroidal anti-inflammatory drugs

On the other hand, there was a significant difference between the tibial and femoral components regarding surface as well as cell viability and distribution. Accordingly, each group was compared with the control group. The results were summarized in tables 4 to 6.

Furthermore, these two groups were compared since the primary aim of this study was to determine whether antibiotic + NSAID therapy, compared to antibiotics alone, could decrease joint destruction or not. The observed differences were significant in terms of tibial cell viability, tibial joint surface, and femoral joint surface (tables 4 to 6).

Discussion

Destruction of articular cartilage in infection-induced arthritis is mainly due to the activation of inflammatory enzymes which in addition to the antibacterial activity will degrade the proteoglycan and cellular contents of cartilage. Surgical drainage and antibiotic administration are cornerstones of treatment in septic arthritis. Although this protocol is usually successful, the affected cartilage is usually deteriorated over time, and eventually osteoarthritis would be the final outcome. As a result, it is beneficial to find approaches to modify the usual treatment protocol with more emphasis on cartilage protection in slowing the long-term sequelae of bacterial involvement of joints.

The primary aim of this experimental study was to assess the possibility of the chondroprotective effect of NSAIDs on the antibiotic-based treatment of septic arthritis. The results obtained from this study revealed that the combination of Naproxen sodium and antibiotic in the treatment of septic arthritis would have a significant protective effect on chondrocytes viability and preservation of articular surface, compared to the utilization of antibiotics alone in the early phase of the disease.

Sticker et al. showed that combined antibiotic and corticosteroid therapy would have a better chondroprotective effect; however, there were some major considerations about safety and long term consumption of corticosteroids in humans (2).

In a study conducted by Smith et al., the effects of Naproxen and antibiotics were investigated on septic arthritis. The results indicated that this combination was especially successful in the preservation of collagen and proteoglycan in tibial cartilage. However, the aforementioned study investigated the late effect of this combination (5). They started Naproxen Sodium 2 days

Table 4. Comparison of tibial and femoral surface variables between each paired groups

Group		*P-value*
Tibial surface	Control Antibiotic	.012
	Control *NSAID	.067
	Control Antibiotic + NSAID	<.001
	Antibiotic Antibiotic + NSAID	.012
Femoral surface	Control Antibiotic	.012
	Control NSAID	.067
	Control Antibiotic + NSAID	<.001
	Antibiotic Antibiotic + NSAID	.012

* Nonsteroidal anti-inflammatory drugs

Table 5. Comparison of tibial and femoral cell distribution variables between each paired groups

Group		*P-value*
Tibial cell distribution	Control Antibiotic	.001
	Control *NSAID	.118
	Control Antibiotic + NSAID	<.001
	Antibiotic Antibiotic + NSAID	. 17
Femoral cell distribution	Control Antibiotic	.006
	Control NSAID	1.000
	Control Antibiotic + NSAID	.001
	Antibiotic Antibiotic + NSAID	.189

* Nonsteroidal anti-inflammatory drugs

Table 6. Comparison of tibial and femoral cell viability variables between each paired groups

Group		*P-value*
Tibial cell viability	Control Antibiotic	.003
	Control *NSAID	.060
	Control Antibiotic + NSAID	<.001
	Antibiotic Antibiotic + NSAID	.012
Femoral cell viability	Control Antibiotic	.003
	Control NSAID	.051
	Control Antibiotic + NSAID	<.001
	Antibiotic Antibiotic + NSAID	.342

* Nonsteroidal anti-inflammatory drugs

before the inoculation of microbial agents to ensure the achievement of effective serum level of Naproxen Sodium (5). Since this pattern departs from real clinical scenarios, simultaneous administration of antibiotic and NSAID was tried one day after the inoculation of Staphylococcus aureus, which would be more compatible with true clinical practice.

Regarding the role of chondrocytes in the production of collagen and proteoglycan, higher chondrocyte viability in the group treated by antibiotic and NSAIDs (i.e., Naproxen Sodium) is in accordance with the results of a study performed by Smith et al. in which they revealed higher levels of proteoglycan and collagen in rabbits treated with Naproxen Sodium and antibiotic after 3 and 7 weeks (4, 5).

In this study, cell viability in the tibial component improved with the addition of Naproxen Sodium to the antibiotic treatment; nonetheless, there was no significant change in cell viability of the femoral component. This result is also in line with the findings of a previous study performed by Smith et al; however, it is unclear why NSAID has no effect on femoral cell viability in both studies (5).

The surface of the cartilage has more exposure to inflammatory mediators in the early phase, and it was shown that the addition of NSAID could protect the articular surface. Cell distribution in both tibial and femoral components had no significant changes in antibiotic + NSAID and antibiotic alone groups although

this factor was significant when either of the above group is compared with the control group.
Short-time treatment is among the limitations of this study. Therefore, further studies are recommended to be conducted with longer treatments for the better evaluation of changes in deep cellular layers of cartilage using antibiotic + NSAID. In addition, local and systemic inflammatory markers were not measured to identify the involved mechanisms in this study.

According to the results of this study, it can be concluded that early and simultaneous administration of antibiotic and NSAID with anti-inflammatory dosage may decrease cartilage damage after the development of septic arthritis.

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