INHIBITION OF NITRIC OXIDE CAN AMELIORATE APOPTOSIS AND MODULATE MATRIX PROTEIN GENE EXPRESSION IN BACTERIA INFECTED CHONDROCYTE IN VITRO

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Introduction Septic arthritis is still a serious medical problem that adequate treatment can not prevent the prolonged destruction of articular cartilage. In our previous study, we have demonstrated that bacteria infected chondrocytes are susceptible to infection and undergo apoptosis.[1] Because the apoptotic process can not be abolished by bacterial removal using antibiotic alone, a reasonable explanation is that other factors, co-expressed in or induced by bacteria, participate in the pathogenesis of chondrocyte apoptosis.

Nitric oxide has been implicated as a mediator of inflammatory arthritis. In bacterial cell wall fragment induced arthritis model, the arthritis can be modulated by inhibition of nitric oxide synthase [2]. However, the role of nitric oxide in the induction of apoptosis in bacteria-infected chondrocyte has not been reported. The purpose of this study is to investigate the hypothesis that inhibition of nitric oxide synthase could also ameliorate the extent of apoptosis and modulate matrix protein gene expression in bacteria-infected chondrocyte.

Methods Human articular chondrocytes were isolated and cultivated in Dulbecco's modified Eagle medium (DMEM) with 10% fetal calf serum until confluent. Monolayer cultures in 100 mm petri dish were infected by Staphylococcus aureus ATCC29213 as described previously. [1] There were five groups in the study. In the control group, chondrocytes were not infected and were maintained in DMEM until harvest. In the infection group, chondrocytes were infected by bacteria without any treatment. In the pretreatment group, cells were pretreated with a nitric oxide inhibitor, No-nitro-L-arginine methyl ester (L-NAME, 1 mM), 2 hours prior to infection. In the pretreatment/vancomycin group, vancomycin (1 mg/ml) was added 2 hours after infection to the cells pretreated with L-NAME. In the after-treatment group, both L-NAME and vancomycin were added 2 hours after infection. All groups were harvested 24 hours after the induction of infection. Extent of apoptosis was characterized by registering the hypodiploid nuclei using a flow cytometric analysis [1]. The production of nitrite was represented by the nitrite levels using a spectrophotometric method based on the Griess reaction. The mRNA expression levels of type II collagen, aggrecan, inducible nitric oxide synthase, matrix metalloproteinase II (MMP-2), and β-actin were analyzed by reverse transcription polymerase chain reaction (RT-PCR).

Results In the control group, the nitrite levels were 9.67 nM and the extent of apoptosis was 1.03%. In the infection group, both the nitrite levels and the extent of apoptosis were significantly increased to 72 nM and 52.88%, respectively. In chondrocytes pretreated with L-NAME, the nitric oxide production was significantly inhibited (14 nM) and the apoptosis was decreased to 18.5%. In the pretreatment/vancomycin group, the nitrite levels and the extent of apoptosis were also significantly decreased as compared to the infection group (14.83 nM & 13.05%, respectively). In the after-treatment group, the nitrite levels and the extent of apoptosis remained high (46 nM and 40.5%, respectively). (Figure 1) The increased production of nitric oxide after bacterial infection was associated with the induction of the iNOS gene and L-NAME (1mM) could not completely abolish the iNOS gene expression by bacterial infection. (Figure 2) As compared to the control group, the mRNA expression levels of type II collagen was decreased to 81% (infection), 88.5% (pretreatment), 76.5% (pretreatment/vancomycin), and 64.8% (after-treatment). Relative to the control group, the mRNA expression levels of aggrecan were changed to 64% (infection), 144±22% (pretreatment), 103±29% (pretreatment/vancomycin), and 107±20% (after-treatment). The mRNA expression levels of MMP-2 in the bacteria-infected chondrocytes were increased in an average about 1.9 folds as compared to the control group.

Discussion Even though the general responses to the current therapeutic regimen of septic arthritis are good, the post-infectious arthropathy remains to be a serious medical condition. In experimental model of septic arthritis, 40% of the glycosaminoglycans can be lost from the articular cartilage within 48 hours of joint infection, and by three weeks 50% of the collagen [3]. In our previous study, we had shown that the bacteria-infected chondrocytes were subjected to apoptosis. We had also found that the addition of bactericidal antibiotic to the bacteria-infected chondrocytes could not abolish the triggered apoptotic processes. [1]

In joint with inflammation and infection, chondrocytes release high concentration of nitric oxide that inhibits the synthesis of aggrecan and enhances the catabolism of aggrecan and type II collagen through the activation of MMP. Nitric oxide has been reported as the primary inducer of chondrocyte apoptosis. Bacterial toxins such as staphylococcal proteoglycan releasing factor and lipopolysaccharide are also known stimulators for nitric oxide synthesis in chondrocytes. In this study, bacterial infection increased the release of nitric oxide through the upregulation of nitric oxide synthase gene. Pretreatment of chondrocytes by nitric oxide synthase inhibitor decreased the nitric oxide levels and the extent of apoptosis in bacteria-infected chondrocytes. In contrast, the amount of nitric oxide release and the extent of apoptosis could only be partially decreased when the NOS inhibitor was added 2 hours after the induction of infection. The inhibition of NOS could not only ameliorate the extent of apoptosis but also could the lessen the inhibition of type II collagen and aggrecan mRNA expression by bacterial infection. However, our results also demonstrated that the upregulated NOS and MMP-2 mRNA by bacterial infection could not be abolished by the NOS inhibitor. In conclusion, the results of this study support the hypothesis that nitric oxide has an important role in the pathogenesis of chondrocyte apoptosis in bacterial infection. Modulation of nitric oxide levels could partially ameliorate the extent of apoptosis and reestablish the cartilage matrix synthesis. However, more studies to investigate the detailed mechanisms responsible for the sustained destruction of articular cartilage in post-infectious arthropathy are still in need.

Fig 1. Nitrite levels and the extent of apoptosis in bacteria-infected chondrocytes.

Fig 2. Illustrated RT-PCR results. C:control; 1: pretreatment with L-NAME; 2: pretreatment/vancomycin; 3:after-treatment; 4: infection.

Reference

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