Influence of sodium hyaluronate on iNOS expression in synovium and NO content in synovial fluid of rabbits with traumatic osteoarthritis

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Objective: To observe the influence of intra-articular injection of sodium hyaluronate (SH) on the expression of inducible nitric oxide synthase (iNOS) in the synovium and nitric oxide (NO) content in synovial fluid of rabbits with traumatic osteoarthritis (OA).

Methods: Sixteen white rabbits underwent unilateral anterior cruciate ligament transection and were randomly divided into 2 groups 5 weeks after the operation. Rabbits in the experimental group received intra-articular injection of 0.3 ml of 1% SH, once a week for 5 weeks. Animals in the control group were treated under the same conditions using physiological saline. All the animals were sacrificed at the 10th week after surgery. The mRNA expression of iNOS in the synovium was analyzed using reverse transcription-polymerase chain reaction. The content of NO in the synovial fluid was assayed.

Results: The level of iNOS expression of the synovium in the experimental group was lower than that in control group (0.47 ± 0.09 vs. 0.65 ± 0.12, t = 3.45, P < 0.01). Compared with control group, the content of NO decreased significantly in synovial fluid of SH injection group (134.11 µmol/L ± 12.47 µmol/L vs. 152.17 µmol/L ± 15.69 µmol/L, t = 2.55, P < 0.05).

Conclusions: SH significantly decreases the content of NO in the synovial fluid of rabbits with traumatic OA. SH may exert the effect on synovial fluid NO level as a result of the suppression of iNOS expression in the synovium. It may be one of the mechanisms of the therapeutic effect of SH on early traumatic OA.

Key words: Osteoarthritis; Nitric oxide; Synovial fluid

METHODS

Experimental animals

Sixteen white rabbits weighing 2.4-2.8 kg were used in this study. All animals provided by the Experimental Animal Centre of Medical College of Wuhan University were anesthetized intravenously with ketamine hydrochloride (1.0 mg/kg). The animals received unilateral anterior cruciate ligament transection (ACLT). Rabbits were divided into 2 groups randomly 5 weeks after operation. Each group had 8 rabbits. Experimental group received 0.3 ml of 1% SH by intra-articular injection, once a week for 5 weeks. The control group was treated under the same condition using physiological saline. After surgery, the animals were housed individually in stainless-steel cages without any immobilization and maintained under the same environmental condition. All animals were killed 10 weeks after surgery.

Reverse transcription-polymerase chain reaction (RT-PCR) assay

Primers used in this study were synthesized by
Shanghai Sangon Biological Engineering Technology and Service Company. Primers used for rabbit glycer-aldehydes-3-phosphate dehydrogenase (GAPDH) and iNOS were as follows. GAPDH (444bp)\(^4\) sense, 5'-ATG ACT GCC ACC CAG AAG AC-3' antisense, 5'-ATG AGG TCC ACC ACC CTG TT-3'. iNOS (262bp)\(^6\) sense, 5'-CGC CCT TCC GCA GTT CT-3'; antisense, 5'-TCCAGG AGG ACA TGC AGC AC-3'.

The synovium closed to the position of degenerative cartilage was harvested. The synovium tissue was powdered in liquid nitrogen by hand milling. Total RNA extraction was performed according to the instruction of Trizol Reagent (Invitrogen Co. USA). Reverse transcription and polymerase chain reaction were undertaken according to the literature described previously.\(^6\) Amplification consisted of 45 seconds at 95\(^°\)C, 45 seconds at 57\(^°\)C (GAPDH) and 65\(^°\)C (iNOS) for annealing, 45 sec at 72\(^°\)C for extension. Thirty cycles and 35 cycles of amplification were performed for GAPDH and iNOS respectively. Electrophoresis of the PCR products on a 1.5% agarose gel with 0.5 pg/ml of ethidium bromide was performed to evaluate amplification and size of generated fragments. French VL analysis system was used to scan the RT-PCR agarose gel. GAPDH was used to verify that equal amount of RNA was added to the reaction. The band intensities of gene expression were reported as the ratio of iNOS to GAPDH on the expression quantities.

Assay of NO content in the joint fluid

NO is chemically active and can be converted into nitrite (NO\(_2^-\)) and nitrate (NO\(_3^-\)) quickly in vivo. So the total concentration of NO\(_2^-\) and NO\(_3^-\) can exactly indicate the level of NO.

The synovial fluids were aspirated from the knee joints and stored at -80\(^°\)C until analysis. The synovial fluid of the knee joint was collected and then analyzed according to the instruction of the NO detection kit (Nanjing Jiancheng Bioengineering Institute, China) for NO\(_2^-\) and NO\(_3^-\) determination. The method used nitrate reductase to determine the NO level of the synovial fluids.

Statistical analysis

The data were expressed as \(\bar{x} \pm s\). Using a commercial software SPSS10.0, statistical analysis was performed by Student’s t test and statistical significance was defined as \(P<0.05\).

RESULTS

In the synovium, iNOS mRNA expression of the experimental group (0.47 ± 0.09) was significantly lower than that of the control group (0.65 ± 0.12) (\(t=3.45, P=0.004\)) (Figs.1-2).

A significant decrease of NO level in synovial fluid was detected in SH treated group compared with control group (134.11 ± 12.47) \(\mu\)mol/L vs. (152.17 ± 15.69) \(\mu\)mol/L, \(t=2.55, P=0.023\).

Fig.1. An electrophoregram of GAPDH. M:PCR marker, from downside to upside, the size is 1543bp, 994bp, 697bp, 515bp, 377bp and 237bp, respectively. 1: the synovium of experimental group. 2: the synovium of control group. 3: negative control.

Fig.2. An electrophoregram of iNOS shows the effect of SH on iNOS mRNA expression.

DISCUSSION

NO is an inorganic, gaseous free radical. NO is produced in large amounts by chondrocytes and synoviocytes. Chondrocytes and synoviocytes are known to produce a large amount of NO when they are...
stimulated by proinflammatory cytokines. In contrast to normal cartilage, OA cartilage spontaneously produces NO. A high-level of NO has been detected in the synovial joint fluid and serum of the patients with OA. Increasing evidence indicates that NO may contribute to the pathophysiology of OA. It has been demonstrated that high local concentration of NO may exert detrimental effects on chondrocyte functions including the inhibition of collagen and proteoglycan synthesis, the activity of metalloproteinases, the decreased expression of interleukin-1 (IL-1) receptor antagonist, the inhibition of chondrocyte proliferation, the induction of apoptosis, and so on. NO is involved in the pathogenesis of OA. Therefore, the role of NO located in the joints on pathogenesis of OA have attracted the attention of researchers.

None of the synovium samples examined from normal joints demonstrated detectable amounts of iNOS. A high-level of iNOS expression in cartilages of OA has been detected. Large amounts of NO can be produced by iNOS catalysis. Strong iNOS expression is observed in the synovial lining layer and subsynovium. NO produced by the constitutive isoform of nitric oxide synthase (cNOS) is a key regulator of homeostasis, whereas the generation of NO by the iNOS plays an important role in inflammation, host defense response, and tissue repair. Cytokines such as IL-1 and tumor necrosis factor (TNF), which induce iNOS production in chondrocytes and synovium, have been implicated in the destruction of cartilage in OA. iNOS is calcium independent and, once activated, produces large amounts of NO. In the present study, iNOS was observed to be expressed strongly in the synovium and NO in the joint fluid was detected at high levels in the control group. The results indicate that NO is one of the mechanisms of degeneration of cartilage and plays an important role in the progression of OA. These findings make us thinking that NO in synovial fluid is a potent mediator in cartilage damage in OA.

Intra-articular injection of SH has been used in OA treatment to prevent degeneration of articular cartilage. Several studies have reported that SH has beneficial effects on cartilage during OA development. Exogenous SH has been used as a lubricative agent, but it is also known to delay the degradation of cartilage by inhibiting the release of proteoglycan from articular cartilage. SH has anti-inflammatory effects and down-regulates the expression of MMP-3 and IL-1 beta in the synovium. Guarda et al. have observed that injection of SH caused significant improvement in the main clinical symptoms. Among patients who received SH injection, those who reached a good outcome showed the lowest basal levels of NO. Kobayashi et al. have reported that the treatment of OA with intra-articular injection of SH led to a significant reduction of NO level in the joint fluid. Takahashi et al. reported that SH exerted the inhibitory effect on NO level in the synovial fluid as a result of the suppression of NO production in the menisci and synovium in patients with OA. SH does not show definite effect on iNOS expression in cartilage of OA. Our results showed that the level of iNOS expression in the synovia among rabbits treated with SH intra-articular injection was significantly lower than that in control group and SH had inhibitory effect on NO production of the synovium. We also observed that the level of NO decreased significantly in synovial fluid of SH injection group. It might be related to the down-regulation effect of SH on iNOS of the synovium.

The present study provides the evidence that intra-articular injection of SH significantly decreases the NO level in synovial joint fluid in the ACLT rabbit OA model. Exogenous SH treatment has an inhibiting effect on iNOS expression in the synovium. We conclude that low NO levels in synovial fluid are related to the suppressive effects of SH on iNOS expression of the synovium. It may be one of the mechanisms of the therapeutic effect of SH on traumatic OA.

REFERENCES


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