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

QIU Bo邱波*, LIU Shi-qing刘世清 and PENG Hao彭昊

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***

Chin J Traumatol 2008; 11(5):293-296

Osteoarthritis (OA) is one of the most common arthropathy. Traumatic OA resulted from the injury or operation of the joints is commonly seen. It is recognized that nitric oxide (NO) generated enzymatically by nitric oxide synthase (NOS) plays a vital role during the development of OA. Intra-articular injection of sodium hyaluronate (SH) has been reported to have a positive effect on the treatment of OA.[1-3] However, the mechanisms of such beneficial effect of SH on OA have not yet been fully established. To further clarify the effect of SH on OA, we investigated the mechanisms of SH on traumatic OA in this study. The expression of inducible NOS (iNOS) in the synovium and NO content in synovial fluid were assessed in experimentally induced traumatic OA rabbits with or without intra-articular injection of SH.

Department of Orthopedics, Renmin Hospital of Wuhan University, Wuhan 430060, China (Qiu B, Liu SQ and Peng H)

*Corresponding author: Tel: 86-27-88041911 ext 2263, E-mail: qb19712003@yahoo.com.cn

This work was supported by the project of Science and Technology of Hubei Province (2003AA301C11).

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

Chinese Journal of Traumatology 2008; 11(5):293-296

Shanghai Sangon Biological Engineering Technology and Service Company. Primers used for rabbit glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and iNOS were as follows. GAPDH (444bp)[4] sense, 5'- ATC ACT GCC ACC CAG AAG AC -3'antisense, 5'- ATG AGG TCC ACC ACC CTG TT -3'. iNOS (262bp)[5] sense, 5'-CGC CCT TCC GCA GTT CT-3'; antisense, 5'-TCC AGG 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) μmol/L vs. (152.17 ± 15.69) μmol/L, t=2.55, P=0.023).

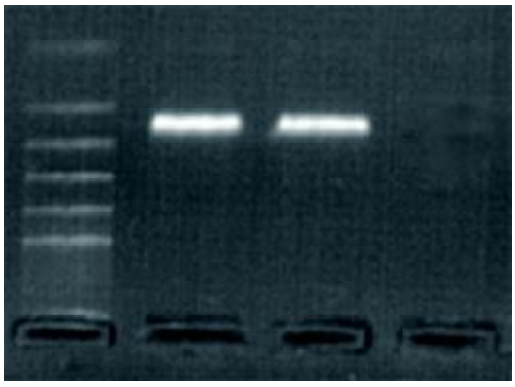

Fig.1. An electrophoregram of GAPDH. M:PCR marker, from downside to upside, the size is 1543bp,994bp, 697bp, 515bp, 377bp and 237bp, respectively. 1: the synovium of experimental group. 2: the synovium of control group. 3: negative control.

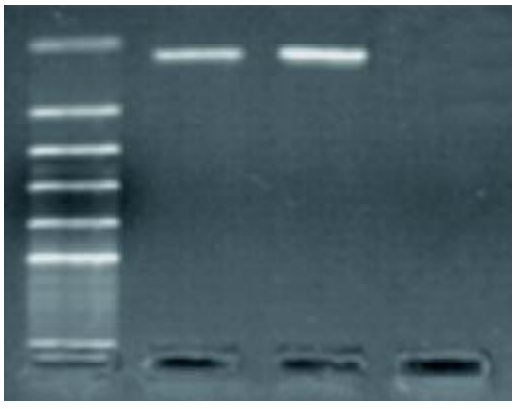

Fig.2. An electrophoregram of iNOS shows the effect of SH on iNOS mRNA expression.

DISCUSSION

NO is an inorganic, gaseous free radical. NO is produced in large amounts by chondrocytes and synoviocytes. Chondrocytes and synovium cells are known to produce a large amount of NO when they are