The Relationship between Temperature and Lubricin Expression

Sekiguchi, A; +Kishimoto, K N; Hatta, T; Okuno, H; Oyanagi, T; Ozawa, H; Itoi, E
+Department of Orthopaedic Surgery, Tohoku University, Sendai, Japan
kishimoto@med.tohoku.ac.jp

INTRODUCTION
Balneotherapy is one of the most common non-pharmacological approaches for joint diseases, such as rheumatoid arthritis and osteoarthritis. It is widely known that bathing in warm water improves the symptoms of joint diseases. However, the mechanisms underlying this empirical knowledge are not yet well understood.

Lubricin, also referred to as superficial zone protein or proteoglycan 4, plays an important role in the lubrication of articular cartilage. The lubricin is thought to protect articular surface. So far, the effect of temperature on the lubricin expression in articular cartilage has not been elucidated.

In the present study, we conducted both in vitro and in vivo experiments. ATDC5 cells cultured under various temperature in the existence of TGF-β1 were used in vitro experiments. Normal rat knee cartilage was subjected to in vivo experiments after animal was immersed in warm or cool water bath.

MATERIALS AND METHODS

Cell culture:
ATDC5, A murine chondroprogenitor cell line was obtained from RIKEN cell bank (Tsukuba, Japan) and maintained in the 1:1 mixture of DMEM and Ham’s F-12 (Invitrogen) supplemented with 5% fetal bovine serum (FBS: Invitrogen) and antibiotics. A 10 μl drop containing 3 x 10⁴ cells was placed in the center of a well in 12 well-plates, and then cultured with 1 ml of DMEM/F12 (1:1) supplemented with 1% FBS, 0.2% BSA, 50 μg/ml ascorbic acid and insulin transferrin selenium (ITS+ Premix: BD) for 72 hours. These cells were then cultured with the medium added to 10 ng/ml of recombinant human (rh) TGF-β1 (R&D), for the purpose of induction of lubricin expression. After 72-hour exposure of rhTGF-β1, these cells were cultured under low temperature condition (38°C) for 1 hour, 8 hours, 24 hours, 48 hours or low temperature condition (36°C) for XX hours. Cells cultured under 37°C were served as control.

Animals:
Hind limbs of adult female Sprague-Dawley rats were soaked in the bath set at 40°C as control for the purpose of induction of lubricin expression in Fig. 1, B: control group. At the same time, control group was soaked in water bath set at 25°C as control group. Warmer bath (40°C) was served as control for the purpose of induction of lubricin expression in Fig. 2, A: control group. Cooler bath (25°C) was served as control for the purpose of induction of lubricin expression in Fig. 3, A: control group.

Quantitative RT-PCR:
Total RNA was extracted by Trizole reagent (Invitrogen) and cleaned up by RNeasy Mini kit (QiAGEN). Single stranded cDNA was synthesized using High-Capacity cDNA Archive Kit (Applied Biosystems). Quantitative real-time PCR assay was carried out using Power SYBER Green PCR Master Mix (Applied Biosystems) on the ABI StepOne plus (Applied Biosystems) to assess the expression of lubricin mRNA. Ct values of lubricin (Ct[lubricin]) were standardized by that of GAPDH (Ct[GAPDH]). Results were shown as -ΔΔCt=- (Ct[lubricin] -Ct[GAPDH]).

RESULTS

In vitro:
Lubricin mRNA expression in ATDC5 cells treated with TGF-β1 cells did not show significant difference between high (38°C), low (36°C) and normal (37°C) temperature condition. (Fig. 2)

DISCUSSION

Our in vitro experiments demonstrated that lubricin mRNA expression in the chondrocytes stimulated by TGF-β1 was not altered by the temperature. Decreased expression of lubricin showed by bathing in warm and cool water bath might be due to the reason other than the temperature. We used normal rats in this study. Experiments with joint disease model are required to elucidate the effect of balneotherapy on lubricin expression in the cartilage.

SIGNIFICANCE

This is the first report showing the relationship between temperature and lubricin expression. Lubricin expression in chondrocytes was not altered by the temperature in vitro and remains high in vivo experiment.