The Effects of Hyaluronic acid using the Combination Treatment of Ultrasound Sonophoresis and Microbubbles Cavitation in a Monolayer Culture Model of Rabbit Chondrocyte

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INTRODUCTION
Hyaluronic acid (HA), a high molecular weight polymer of glucosamine and glucuronic acid residues, is one of the key components of articular cartilage matrix. A marked inhibitory effect of HA on cartilage degeneration was observed in several studies. In addition, Ultrasound (US) treatment emerged as one of the alternatives in the management of cartilage damage because it improved the repair of articular cartilage and also shown efficacy in cartilage restoration. Furthermore, Microbubbles (MB), echo-contrast materials enhance the echogenicity of US and have been clinically used for diagnosis in current medical fields, also could enhance US mediated gene delivery, drug delivery, and mechanical and sonochemical bioeffects of US. The purpose of this study is to investigate the in vitro effect of one therapeutic HA preparation using the combination treatment of US sonophoresis and MB cavitation on IL-1b induced apoptosis of OA chondrocytes.

METHODS:
Articular chondrocytes were isolated from the knee and hip and shoulder joints of rabbits. The chondrocytes were maintained in monolayer culture. Hyaluronic acid (Suvenyl® Chugai co., 40 μg/ml) was added with or without Microbubbles (Sonazoid®, Sankyo co., 0.05%). Then US sonophoresis (probe size 8mm, Frequency 3100kHz, Voltage 20V, duty rate 20%, duration time 60sec) was performed. After 1 hour, IL-1b (10ng/ml) was added to the culture medium without rinse for a further 24 hours. The effect of HA with the combination treatment of US and MB was analysed by assessment of cell viability and TUNEL staining, by assessment of the expression levels of Col2a1, MMP-13, by immunohistochemistry of Type 2 collagen. Statistical analysis was performed by Tukey-Kramer. P<0.05 was considered to be statistically significant.

RESULTS:
Viable cell amount was significantly decreased at 48 hours after IL-1b induced apoptosis. In contrast, at 72 hours after treatment viable cell amount was increased by HA and additional combination treatment of US and MB (Fig 1). Moreover these treatment could reduce the TUNEL-positive cells compared with IL-1b induced apoptosis with the other treatment group (Fig 2).

The expression of Col2a1 was significantly down-regulated by IL-1b. HA and additional combination treatment of US and MB could inhibit the down-regulation by IL-1b (Fig 3A). In contrast, the expression of MMP-13 was significantly up-regulated by IL-1b, and was inhibited by HA and additional US and MB treatment (Fig 3B).

Immunohistochemical analysis of Type 2 collagen revealed that HA and additional combination treatment of US and MB could effectively inhibit the IL-1b induced down-regulation of Type 2 collagen protein level.

DISCUSSION:
The principal finding of this study is that the combination treatment of HA and US and MB significantly inhibited the chondrocyte apoptosis induced by IL-1b. This display of anti-apoptotic effect suggests that the combination treatment of US and MB may offer additional benefits in reducing the cartilage degeneration more than the treatment only of HA. So there is a possibility of promoting the therapeutic effect of HA by intraarticular injection to the osteoarthritis of the Knee.