THE IN VITRO EFFECTS OF DEHYDROEPIANDROSTERONE ON HUMAN OSTEOARTHRITIC CHONDROCYTES

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INTRODUCTION
Metalloproteinasises (MMPs) are produced by chondrocytes and increased in OA. The increase in MMPs activities appears to correspond to the disruption of the collagenous network. The activities of the MMPs are controlled by the specific inhibitor TIMP. In OA, the TIMP level does not increase as much as the MMPs level. The resulting excess of active MMPs over TIMP may contribute to cartilage degradation. Interleukin-1 (IL-1) enhances enzyme synthesis and activation of a number of enzyme systems in the cartilage including latent MMPs. The stimulation of MMPs by IL-1 is thought to be important in catabolic events in OA. Dehydroepiandrosterone (DHEA) is a highly lipophilic 17-ketosteroid, produced primarily by adrenal gland and testis. Circulating levels of DHEA decline with age (1) and low levels of DHEA can be related to the inflammatory arthritis (2). In this in vitro study, the effects of DHEA on the osteoarthritic chondrocytes were determined. We evaluated the changes of phenotype and expression of MMPs and TIMP of osteoarthritic chondrocytes exposed to DHEA.

METHODS
Chondrocytes Isolation and Cultures: Chondrocytes were isolated by enzymatic digestion of degenerated articular cartilage from patients undergoing total knee arthroplasty. Isolated cells were cultured in the alginate beads in complete medium for 7 days and treated with DHEA (0, 10, 50, and 100 µM) and further incubated for 3 days.

IL-1β Treatment: Sixteen hours before treatments, complete medium was replaced by serum-free medium. Chondrocytes in alginate beads were then treated with 100 pg/ml of rhIL-1β (Calbiochem, CA) along with DHEA (0, 10, 50, and 100 µM) for 3 days.

Semiquantitative RT-PCR: Total RNA was isolated with RNeasy Mini Kit (Qiagen, Germany) for evaluating the expression of genes of interests by RT-PCR. All RT-PCR analyses were normalized using glyceraldehydes phosphate dehydrogenase (GAPDH).

RESULTS
A 3 days exposure of human chondrocytes in the alginate beads to DHEA at concentrations of 10, 50, and 100 µM resulted in changes of gene expressions. DHEA induced up-regulation of type II collagen gene expression and down-regulations of type I, MMP1 and MMP3. When 100 µM of DHEA was added, the gene expression of type II collagen was increased to 146% of control (0 µM of DHEA) and that of type I collagen was decreased to 28% of control. A marked down-regulation of MMP1 and MMP3 was observed with DHEA. When 100 µM of DHEA was added, the expressions of MMP1 and MMP3 decreased to 56% and 56% of control, respectively. No detectable changes in TIMP1 gene expression were observed between the groups with or without DHEA (Fig 1).

Exogenously added rhIL-1 stimulated the expression of MMP3 gene in osteoarthritic chondrocyte. The rhIL-1 induced up-regulated gene expression of MMP3 was down-regulated by DHEA. As the concentration of DHEA increased, the decrease of IL-1 induced MMP3 gene expression became marked. When 100 µM of DHEA was added, the MMP3 gene expression decreased to 146% of control (no IL-1), whereas IL-1 induced MMP3 gene expression was 200% of control (Fig 2).

DISCUSSION
In this in vitro study, exogenously added DHEA induced up-regulation of type II collagen gene expression and down-regulations of type I, MMP1 and MMP3 genes in human osteoarthritic chondrocytes. And the rhIL-1 induced increased MMP3 gene expression was down-regulated by DHEA. Cartilage degeneration is associated with the imbalance between the synthesis and degradation of matrix components. One way to activate or inactivate the degradative pathway can be through the signal transduction pathway that leads to net production of matrix components. Our experiments demonstrated that DHEA could stimulate the production and inhibit the degradation of the major components of extracellular matrix of human osteoarthritic chondrocytes in the alginate beads. DHEA was also effective to inhibit cytokine-induced MMP production. DHEA appeared to able be an effective agent to treat the cartilage degenerative disease by increasing type II synthesis and decreasing MMP production.