THE in vivo EFFECTS OF DEHYDROEPIANDROSTERONE (DHEA) IN THE DEVELOPMENT OF OSTEOARTHROSIS

*Jo, H; Ahn, HJ; Seong, SC; Kim, HJ; Jeong, MY; ++*Lee MC
++Seoul National University College of Medicine, Seoul, Korea

Introduction

Osteoarthritis is characterized by the destruction of articular cartilage which results from degradation and loss of proteoglycans and collagens mediated by metalloproteinases (MMPs). These catabolic enzymes are controlled by the cytokines such as IL-1β. In addition to the catabolic enzymes, tissue inhibitors of MMPs (TIMPs) also plays important roles. The imbalance between MMPs and TIMPs is a well known cause of the progression of osteoarthritis. We revealed that the in vitro effects of DHEA on the human osteoarthritic chondrocytes [1]. The purpose of this study is to investigate the in vivo effects of dehydroyepiandrosteron (DHEA) in the development of osteoarthritis using experimentally induced animal models.

Methods

Animals: Sixteen New Zealand White Rabbits were underwent bilateral anterior cruciate ligament transection (ACLT). The right knees of the rabbits received intra-articular injections of 0.3ml DHEA beginning four weeks after ACLT, once a week for five weeks. The contralateral, left knees were injected with same amount of vehicle solution of DHEA and used as controls. All animals were sacrificed nine weeks after ACLT and assessed by gross morphology using India Ink application. Then, animals were divided into two groups; DHEA injected group (n=8) for histomorphometric evaluation, and control group 2 (n=8) for gene expression assays.

Gross morphology: The femoral condyles of all animals were photographed using a high resolution digital camera equipped with a close-up micro lens after application of India Ink. The gross morphological changes were evaluated with previously reported criteria [2]: gr 1, intact surface; gr 2 minimal fibrillation; gr 3, overt fibrillation, and gr 4, erosion (further divided 4a, b, c according to the eroded length).

Histomorphometry: Three equally separated sections were obtained from each medial condyle and stained with Safranin O/fast green. Histological images were obtained with a color image analysis system consisting of microscope attached to a high resolution video camera, and a computer with an image analysis system. Processed images were visualized on a high resolution color monitor. A customized image analysis software was developed to measure the cartilage thickness, area, and surface roughness. Each image (3 from 1 medial condyle) was further divided into three equal parts along the sagittal contour of the medial femoral condyle (one from weat-bearing area of the femorobial joint surface and two from anterior and posterior to it, respectively) and a 5mm long image was obtained in each part at 25X magnification. Three parameters were calculated as previously reported [2].

Gene expression assays: The experiments are performed triplicate. Total RNA was extracted from the cartilage harvested from the femur and tibia of each animal. RT-PCR was carried out for type II collagen, TIMP-1, MMP-1, 3, IL-1β and normalized to GAPDH.

Statistics: Paired t-test was used and significance was set p<0.05.

Results

Gross morphology: Nearly all the femoral condyles nine weeks after ACLT showed osteoarthritic changes. However, the severity was much less in DHEA injected groups than in the control groups (Fig. 1).

Histomorphometry: In control groups, the cartilage thickness and area was significantly decreased when compared with that in DHEA groups (p<0.05; Fig 2a, b). The surface roughness normalized to the thickness was significantly lower in the DHEA groups than in the control groups (p<0.05; Fig 2c).

Gene expression assays: RT-PCR revealed that the expression of MMP-1, 3, and IL-1β was increased in the control groups when compared with in the DHEA groups, while the expression of type II collagen and TIMP-1 was prominently increased in DHEA groups (Fig 3).

Discussion

This study shows that DHEA has inhibitory effects on the cartilage damage evidenced by gross morphology and histomorphometric measurements and on the gene expression of MMP-1, 3 which are well known to be important catabolic enzymes in the development of osteoarthritis as well as on the expression of IL-1β, a pivotal cytokine in the initiation and progression of osteoarthritis. Furthermore, the expression of type II collagen and TIMP-1 was increased by DHEA treatment, which means DHEA has not only anti-catabolic effects but also anabolic effects.

References