EFFECTS OF HEAT STIMULATION VIA MICROWAVE APPLICATOR ON CARTILAGE MATRIX GENE AND HSP70 EXPRESSION IN THE RABBIT KNEE JOINT

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Introduction: Osteoarthritis (OA) is a progressive disorder primarily characterized by articular cartilage degeneration. Thermotherapy is widely used as a part of physical therapy for OA in clinical settings. Heat stimulation on the local soft tissues has been shown to alleviate pain by increasing pain threshold; increase the elasticity of collagen fibers; relax muscles by reducing extension sensitivity of the muscular spindles; increase local blood flow; and accelerate metabolic activities in the local tissue (1,2). Some in vitro studies reveal that excessive heat stimulation (48ºC, 10 min) induces apoptosis of chondrocytes and inhibits proteoglycan synthesis, but appropriate heat stimulation (39 or 41ºC, 15 or 30 min) positively affects cell viability and proteoglycan metabolism of articular chondrocytes (3). However, no in vivo studies have closely investigated the effects of thermotherapy on articular cartilage, the main target of OA.

To investigate the usefulness and potential of thermotherapy as a conservative therapy for OA, we applied heat stimulation to rabbit knee joints using a microwave (MW) applicator and investigated the expression of extracellular matrix genes and HSP70 in the articular cartilage.

Materials and Methods: Heat stimulation was applied to the knee joints of Japanese white rabbits for 20 min using a MW applicator (2.45-GHz, 20, 40, 60 or 80 W) (n = 4 for each group). While the knee was flexed, heat stimulation was applied from above the patella. Temperatures in joint capsule were measured immediately after heat stimulation. After 8-72 hrs, the articular cartilage was removed from the knee joints and proteins and total RNA were extracted. Rabbits receiving no heat stimulation served as controls in each experiment (n = 4). The expression of HSP70 was confirmed by real-time PCR and western blotting. The expression of proteoglycan core protein (PG) and type II collagen (Col II) was quantified using real-time PCR to assess cartilage matrix metabolism. In some experiments, 600 μM of quercetin (HSP inhibitor) with 300 μl PBS was injected into the left knee of rabbits before MW irradiation. As a control, 300 μl of PBS was injected into the left knee of rabbits.

Results: The intraarticular temperature after heating was 35.5 ± 0.51ºC with 0 W, 39.1 ± 0.42ºC with 20 W, 40.9 ± 0.14ºC with 40 W, 42.6 ± 1.13ºC with 60 W and 45.9 ± 4.03ºC with 80 W, respectively. Since 80 W of heat stimulation caused obvious skin damage such as redness and ulceration in all cases, the following experiments were conducted by applying 0, 20, 40 or 60 W of MW. Compared to controls, HSP70 expression increased at both mRNA and protein levels in an intensity-dependent manner when more than 40 W of heat stimulation was applied. With 20 W of heat stimulation, HSP70 expression was comparable to that in controls (Figure 1). The expression of PG and Col II mRNA was higher in the rabbits receiving more than 20 W than in controls. The highest expression of PG and Col II mRNA was observed with 40 W of heat stimulation (Figure 2). Heat stimulation of the knee joints using the MW applicator induced a stress response and increased the expression of matrix genes in the articular cartilage with different time-courses. When quercetin was used to inhibit the induction of HSP70 expression, the increases in PG mRNA following heat stimulation was suppressed, but not in Col II mRNA (Figure 3).

Discussion: The present study showed that external MW application increased intraarticular temperature and reached the articular cartilage, and that appropriate heat stimulation increased the expression of PG and Col II mRNA in the articular cartilage. This increased expression of matrix genes might be partially mediated by HSP70. Investigation of optimal heat stimulation for protecting the articular cartilage in vivo and ascertainment of therapeutic effects using animal OA models should be required in future studies.


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