

Hyperthermia for the treatment of articular cartilage with osteoarthritis

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Abstract

Osteoarthritis (OA) is one of the most frequent musculoskeletal disorders in the elderly population. OA is characterised by a gradual loss of extracellular matrix in the articular cartilage of joints. OA can only be managed by artificial joint replacement when joint destruction becomes severe. Therefore, it is preferable to administer conservative therapy that is easy, simple and effective in inhibiting OA progression at the early stage. Heat shock protein 70 (Hsp70) has a protective effect on the cartilage and inhibits the apoptosis of chondrocytes. Heat stimulation by microwave to the joints can increase Hsp70 expression in chondrocytes, and at the same time, Hsp70 expression partially enhances matrix metabolism of the cartilage. These findings suggest that hyperthermia can be positively applied to the treatment of OA. Hyperthermia is therefore expected to be an inexpensive and less-invasive conservative therapy for OA.

Keywords: *hyperthermia, osteoarthritis, cartilage, chondrocyte, Hsp70*

Introduction

Osteoarthritis (OA) is one of the most frequent musculoskeletal disorders in the elderly population. OA is characterised by a gradual loss of extracellular matrix in the articular cartilage of joints. Since the joint function is severely impaired, personal and social activities of patients with OA are limited. Because the articular cartilage, which is the matured hyaline cartilage, does not have blood vessels, the cells required to repair damaged cartilage are not provided. Chondrocytes themselves have limitations in their proliferative potential and repair capacity, therefore, when OA progresses and the articular cartilage degenerates, it becomes difficult to treat. Intra-articular injection of hyaluronan has been applied as a conservative therapy for OA [1], but its

effect on advanced OA is limited. Orally administered supplements such as glucosamine and chondroitin sulphate reduce the pain due to OA, and inhibit the narrowing of the joint-space width [2, 3]. However, they cannot change the natural course of OA or greatly interrupt its progression. When OA has progressed, the wide range of cartilage tissue should be repaired. Therefore, at present, surgical procedures for injuries of localised articular cartilage (e.g. autologous transplantations of the articular cartilage [4, 5] and chondrocytes [6]) cannot be applied for OA. OA can only be managed by artificial joint replacement when joint destruction becomes severe. However, this is associated with some problems including the invasiveness, and high cost of the procedure and also the long-term prognosis.

Therefore, it would be preferable to develop a conservative therapy that is easy, simple and can also effectively inhibit the progression of OA at the early stage.

Thermotherapy is widely used for musculoskeletal disorders as a physical therapy in clinical settings [7]. Hyperthermia with hot packs, paraffin baths, ultra-short waves and microwaves are generally performed as well as therapeutic exercise for joint diseases such as OA and rheumatoid arthritis. There are two types of effects of hyperthermia on soft tissues: local and distant effects. The distant effects are mainly the heat transfer by blood flow and biological reaction via the autonomic nervous system. On the other hand, the local effects include an increase of elasticity of collagen fibres by heat [8], pain relief due to increasing the pain threshold [9], decreasing muscle tension by reducing electrical pulses in the muscular spindle [10], increase of local blood flow [11], and acceleration of tissue metabolism. Hyperthermia, which is applied to the rehabilitation for musculoskeletal disorders, is generally thought to be an adjunctive therapy. The local effects of hyperthermia such as pain control and reduction of muscle tension are utilised in a range of joint motion exercise. However, the intensity and duration of heat stimulation used for hyperthermia are empirically determined, and its effect has not been scientifically proven. In addition, the direct effect of heat on metabolism and repair of the articular cartilage, which is the mainly affected tissue of OA, is unknown.

This article investigated the role of a stress protein, heat shock protein (Hsp) 70, in chondrocytes, and the effect of heat on the articular cartilage. Furthermore, the application of hyperthermia for articular cartilage with OA is also herein considered.

Anatomy of articular cartilage

Articular cartilage possesses a zonal structure that includes the superficial, middle, and deep zones, each with a distinct cellular phenotype and matrix composition (Figure 1). Chondrocytes decrease in number from the superficial zone to the deep zone and increase in size. Articular cartilage contains 2% chondrocytes and the extracellular matrix. The extracellular matrix is composed of approximately 20% collagen (primarily Type II collagen), approximately 10% proteoglycan, and 70% water. Collagen forms a meshwork structure in the cartilage matrix to maintain its shape. Collagen orientation changes in the different layers of articular cartilage. Proteoglycan is negatively charged and has large capacity to retain and maintain water in the cartilage matrix, which contributes to the viscoelasticity

necessary for the cartilage to function as a shock absorber. Chondrocytes are the only cell type of the articular cartilage. They produce collagen and proteoglycan, and metabolise the extracellular matrix. Chondrocytes themselves release growth factors and cytokines, and these factors regulate metabolism of the cartilage matrix by autocrine and paracrine mechanisms. Adult human cartilage is avascular, thus, there is no external cell supply to compensate for cell loss caused by necrosis, apoptosis, or other cellular mechanisms.

Etiology of OA

OA is a progressive disease that induces degeneration of the articular cartilage, subsequent reparative proliferation of the bone, and secondary synovitis. The radiological changes are characterised by a combination of bony proliferation, such as osteophyte formation and sclerosis of subchondral bone, and joint space narrowing which corresponds to degeneration of cartilage (Figure 2). Histologically, the surface of articular cartilage becomes irregular and reveals fibrillation and clefts. The cartilage thickness gradually decreases, and finally cartilage disappears and eburnated subchondral bone is exposed. The laminar structure of articular chondrocytes is completely destroyed, and cluster formation is often observed in chondrocytes during the early phase (Figure 3). The pathological basis of OA has not yet been clarified, but it is thought that its onset and progression are related to biological or

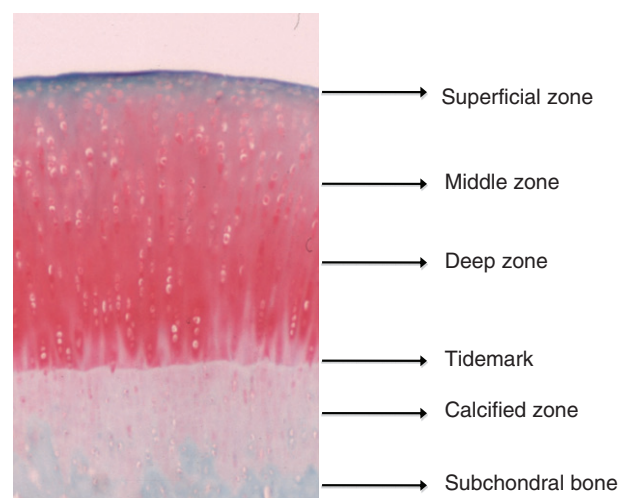


Figure 1. Structure of cartilage. The main difference observed between the chondrocytes in hyaline cartilage is their morphological variation between the zones. Chondrocytes in the superficial zone are flattened and elongated, whereas the cells in the middle zone appear rounded and those in the deep zone have an ellipsoid morphology.

chemical stress as well as non-physiological mechanical stress (Figure 4) [12]. If the mechanical stress increases to a level excessively higher than the physiological levels, the cartilage matrix is impaired and OA could occur. This process of disease progression is proven by the fact that (1) cartilage degeneration in OA starts from the weight-bearing area [13], and (2) many OA-related animal models are prepared by making mechanical changes to the joints [14, 15]. Locally produced humoral factors, e.g. inflammatory cytokines (IL-1, IL-6, IL-17 and TNF- α) and



Figure 2. Osteoarthritis of the knee. The knee joint is the most common site of osteoarthritis. The anteroposterior radiograph reveals the valgus deformity of the joint, joint space narrowing, osteophyte formation and sclerosis of subchondral bone. In knee osteoarthritis, medial femorotibial alterations often predominate.

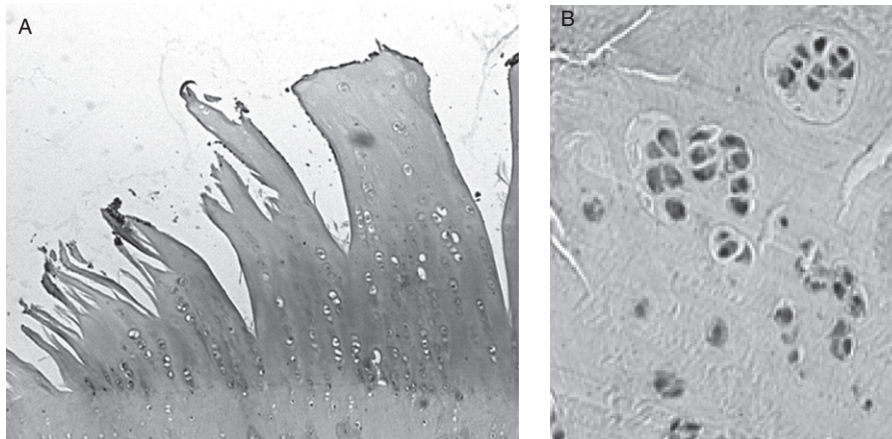


Figure 3. Histological features of cartilage in osteoarthritis. Osteoarthritic cartilage demonstrates severe fibrillation leading to cracking that extends into the tidemark. There is an obvious decrease in the numbers of chondrocytes in the late phase of the disease (A), while cluster formations, corresponding to chondrocyte proliferation, are sometimes observed to be adjacent to the area of fibrillation in the early phase (B).

metalloproteinases (MMP-1, MMP-3, MMP-8, MMP-13 and aggrecanases), are thought to be important, in addition to mechanical stress [16–19]. Chondrocytes undergo apoptosis in response to stress, and apoptotic chondrocytes are frequently found in OA cartilage. Therefore, apoptosis has attracted the attention of researchers as a potential etiological factor in the progression of OA [20–24]. D’Kima et al. demonstrated caspase inhibitors to reduce the severity of cartilage lesions in experimental OA.

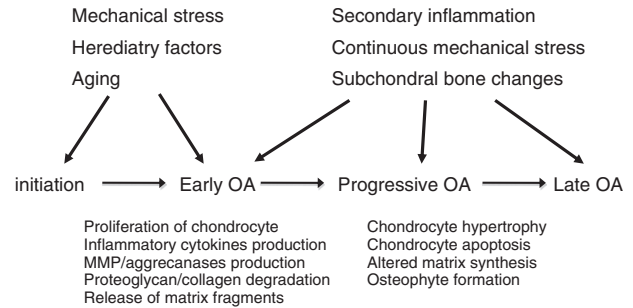


Figure 4. A schematic drawing of the events involved in the initiation and progression of osteoarthritis. Potential causative factors are listed above and the cellular and morphological changes are listed below [12]. Articular cartilage is an important component of the joint, and it is always exposed to pressure produced by weight bearing and muscle contraction. If the mechanical stress increased to a level excessively higher than the physiological levels, the cartilage matrix will be impaired and osteoarthritis could occur. In addition to mechanical stress, its onset and progression could be induced by inflammatory cytokines produced by chondrocytes in cartilage or the synovial tissue in the joint. Inflammatory cytokines contribute to the dysregulation of the chondrocyte function. The association of increased production of metalloproteinases induced by inflammatory cytokines with cartilage damage has been established. The apoptosis of chondrocytes caused by various types of stress has attracted the attention of researchers as an important etiological factor in OA progression.

Caspases are activated during apoptosis, thus suggesting that apoptosis of chondrocytes may be a potential target for therapeutic intervention in OA [25].

Current clinical application of hyperthermia to OA

In a systemic review of the literature on the thermal modalities in OA, evidence exists that an increase in the temperature of the joint may provide the short-term relief of joint pain, although treatment with hot packs did not demonstrate any significant beneficial effect when used to treat OA [26]. This may be due to several reasons. Firstly, superficially applied treatments such as hot packs heat only skin and subcutaneous tissues, while deep heating apparatus such as ultrasound may increase temperature by 4–5 °C at depths of 8 cm [26]. Secondly, the few published studies in this area tend to have serious methodological limitations to accurately distinguish the treatment effects from placebo effect. Thirdly, thermal therapy is usually practiced for only a short time. Heat-generating sheet used on knee OA 6 hrs per day at the longest is reported to be effective for alleviating pain, and improving both stiffness and gait impairment [27].

Expression of Hsp70 in OA chondrocytes

Heat shock protein 70 (Hsp70) is a member of a family of highly conserved proteins which are synthesised in cells after stress loading, and protects cells from various types of stress. A study using spontaneous OA mice showed that expression of several HSPs is increased in the articular cartilage from the early stage of OA [28]. In addition, studies using clinical specimens report that Hsp70 expression levels in chondrocytes are correlated with the histological severity of OA [29, 30]. It is not clear what induces the expression of Hsp70 in OA chondrocytes, but the non-physiological mechanical stress involved in OA induces Hsp70 in chondrocytes in vitro [31, 32].

Roles of Hsp70 in chondrocytes

The roles of Hsp70 in chondrocytes were investigated by Hsp70 gene transduction in several studies. Cartilage metabolism is accelerated in chondrocyte-like cells in vitro by adenovirus vector-mediated gene transduction of Hsp70 [33], and chondrocytes are protected from cytotoxic stress [34]. In addition, transduced Hsp70 gene in articular chondrocytes dramatically inhibits apoptosis of chondrocytes

induced by nitric oxide (NO) [35]. The inhibition of apoptosis by Hsp70 may not be based on its influence on the level of cytosolic cytochrome C released from NO-stimulated mitochondria, but on the inhibition of caspase 3 activation [35]. Glutamine and MG132, which induce Hsp70, protect chondrocytes from cytotoxic stress when they are added to cultured chondrocytes [36, 37], and reduce degeneration of the articular cartilage when they are administered intra-articularly to experimental OA rats [38]. Glutamine and MG132 might produce effects other than that via Hsp70 on chondrocytes, but the direct effect of Hsp70 on the articular cartilage in vivo was investigated by Grossin et al. [39]. They reported that cartilage degeneration induced by mono-iodoacetate was inhibited by Hsp70 gene transduction into the articular cartilage of the patellae [39]. Future studies should investigate the therapeutic effects of Hsp70 in spontaneous and injury-induced OA models, in addition to drug-induced cartilage degeneration.

These studies indicate that Hsp70 has a protective effect on the cartilage and inhibits apoptosis of chondrocytes. Induction of Hsp70 may be useful for slowing the progression of OA (Figure 5). OA progresses even though Hsp70 expression increases in human OA possibly because (1) the expression level of Hsp70 is insufficient in comparison to the stress added to chondrocytes, and (2) the cytoprotective action of Hsp70 did not function properly.

Effect of heat stimulation on cultured chondrocytes

Hojo et al. applied heat stimulation to cultured chondrocytes, and evaluated the proteoglycan metabolism [40]. Proteoglycan metabolism is increased at 39 °C and 41 °C, and decreased at 43 °C. The metabolism at 43 °C decreases further when the duration of heat stimulation is prolonged [40]. This suggests that consideration of duration as well as temperature of heat stimulation is required in the clinical setting. A narrow therapeutic range of temperature seems to pose problems in clinical settings since the effect of hyperthermia shows a significant variation in response to small differences in the applied temperature. There is joint fluid in the joints in vivo and there is also free water in the extracellular matrices of the articular cartilage. Therefore, it is not possible for the cartilage in the deep areas to have a higher temperature than the skin due to microwaves that are clinically used with outputs that do not burn the skin. Furthermore, the abovementioned free water should be dispersed when differences in the temperature occur, so the possibility of a localised high temperature is low.

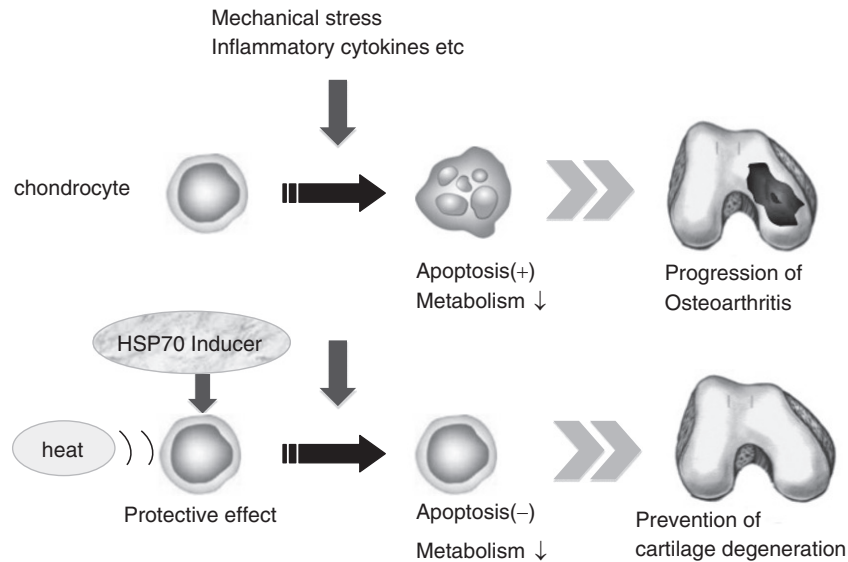


Figure 5. Strategy for applying hyperthermia to the treatment of OA. Hsp70-induction in chondrocytes by heat stimulation and/or HSP inducer, such as glutamine, MG132, GGA and curcumin can have a therapeutic effect on OA by inhibiting chondrocyte apoptosis as well as increasing the cartilage metabolism.

The activity of chondrocytes decreased by heat stimulation at 43 °C can be controlled by adding glutamine that induces Hsp70 [34].

In vivo effect of hyperthermia on the articular cartilage in an animal model

Conversive heat, such as ultrasound and microwaves which are converted into heat energy and reach the deep region, should be used to apply heat stimulation to the deep-lying articular cartilage in joints. Tonomura et al. applied heat stimulation to the knee joints of rabbits for 20 minutes using a clinically available 2.45-GHz microwave applicator, and observed the temperature in joints increase as the intensity of heat stimulation by microwave gets higher [41]. They also reported the joint temperature of the rabbits to reach approximately 40 °C at an intensity of 40W, and that the expression of proteoglycan and type II collagen in the articular cartilage was observed to remarkably increase at this intensity. At the same time, Hsp70 has been confirmed to accumulate in the chondrocytes [41]. In that study, the pre-treatment of joints with quercetin that inhibits expression of Hsp70 reduced the effect of heat stimulation by microwaves on increasing the expression of proteoglycan, but its effect on type II collagen was not observed to change [41]. Consequently, heat stimulation by microwave to the joints can increase matrix metabolism of the cartilage partially via Hsp70 expression. However, since there are large species differences in regard

to the distance from the stimulation site to joints and the amount of subcutaneous fat and muscle between human and rabbits, it is therefore necessary to investigate optimal conditions of heat stimulation in humans. In addition, further studies on the effects of heat stimulation on the catabolic factors or cell viability of chondrocyte should be performed.

Conclusions

The number of studies is small, but they demonstrate the effect of Hsp70 and hyperthermia that induces Hsp70 on protecting chondrocytes and increasing metabolism of the articular cartilage. This suggests that hyperthermia can be positively applied to OA treatments. However, the effect of hyperthermia on OA has not been sufficiently investigated in clinical studies [42], and the associated mechanism is still unknown. To effectively perform thermotherapy to the articular cartilage, it is necessary to determine the optimal intensity and duration of heat stimulation in humans. Since thermo-tolerance and intracellular accumulation of Hsp70 are observed after heat stimulation [43], the interval of heat stimulation should also be studied. Hsp70 inducers, such as geranylgeranylacetone (GGA) [44] and curcumin [45], as well as glutamine [37] and MG132 [36] could be applied to treat OA. Hsp70 inducers or hyperthermia may provide an inexpensive and less invasive conservative therapy for OA.

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