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Anti-inflammatory effects of continuous passive motion on meniscal fibrocartilage

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Abstract

Motion-based therapies have been applied to promote healing of arthritic joints. The goal of the current study was to determine the early molecular events that are responsible for the beneficial actions of motion-based therapies on meniscal fibrocartilage. Rabbit knees with Antigen-Induced-Arthritis (AIA) were exposed to continuous passive motion (CPM) for 24 or 48 h and compared to immobilized knees. The menisci were harvested and glycosaminoglycans (GAG), interleukin-1β (IL-1β), matrix metalloproteinase-1 (MMP-1), cyclooxygenase-2 (COX-2), and interleukin-10 (IL-10) were determined by histochemical analysis.

Within 24 h, immobilized knees exhibited marked GAG degradation. The expression of proinflammatory mediators MMP-1, COX-2, and IL-1β was notably increased within 24 h and continued to increase during the next 24 h in immobilized knees. Knees subjected to CPM revealed a rapid and sustained decrease in GAG degradation and the expression of all proinflammatory mediators during the entire period of CPM treatment. More importantly, CPM induced synthesis of the anti-inflammatory cytokine IL-10. The results demonstrate that mechanical signals generated by CPM exert potent anti-inflammatory signals on meniscal fibrochondrocytes. Furthermore, these studies explain the molecular basis of the beneficial effects of CPM observed on articular cartilage and suggest that CPM suppresses the inflammatory process of arthritis more efficiently than immobilization.

Keywords: Rheumatoid arthritis; Interleukin-1; Continuous passive motion; Meniscus; Fibrocartilage

Introduction

The meniscal fibrocartilage of the knee joint is important for load bearing, load distribution, shock absorption, and stability of the knee [5]. The functions of the menisci are facilitated by proteoglycans and collagen type I and type II [4,5]. Proteoglycans enable the matrix to absorb shock, whereas collagens provide the rigidity [4]. Joints afflicted by rheumatoid arthritis (RA) exhibit meniscal matrix degradation [11]. Evidences suggest that RA is initiated by a sustained antigenic stimulus that induces perpetual activation of inflammatory and resident cells of the joint and results in the production of proinflammatory mediators that initiates progressive erosion of cartilage [21]. Interleukin-1 (IL-1), a major mediator, induces proinflammatory mediators, cyclooxygenase-2 (COX-2), and matrix metalloproteinases (MMPs) and triggers the pathologies of the cartilage and fibrocartilage associated with RA [1,13]. Both

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chondrocytes and fibrochondrocytes from inflamed joints are known to produce proinflammatory mediators in response to IL-1 in vitro [1,18] and in vivo [11].

The beneficial effects of CPM on the healing of articular tissue are well recognized [10,16,17]. However, to date, little is known about the molecular mechanisms responsible for the actions of motion-based therapies on the cartilage or the fibrocartilage of the joint. In light of the fact that motion can exert beneficial effects on the arthritic cartilage of the joints, it was our hypothesis that (i) CPM promotes healing by inhibiting the expression of proinflammatory mediators in the meniscus, as compared to immobilization, which leads to cartilage degradation via induction of proinflammatory mediators, and (ii) the effects of mechanical signals are rapid and can be observed within 24–48 h. Therefore, in this report we have examined the early biochemical events induced by mobilization or immobilization on meniscus from an AIA joint to understand the mechanisms of actions of motion-based therapies in vivo.

Material and methods

Reagents

Anti-COX-2, anti-MMP-1, and anti-IL-1β immunoglobulins were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and their respective fluorescein isothiocyanate (FITC) labeled secondary antibodies were purchased from Jackson Immuno Research (West Grove, PA). Monoclonal rat anti-mouse IL-10 antibody and FITC labeled monoclonal mouse anti-rat IgG were purchased from BD Pharmingen (San Diego, CA). Protein blocking agent was from Thermo Electron Corp. (Pittsburgh, PA) and mounting solution Vectashield from Vector Labs (Burlingame, CA). All other reagents were purchased from Sigma-Aldrich (Saint Louis, MO).

Induction of antigen-induced arthritis

All protocols were approved by the Institutional Animal Care Committee at the University of Pittsburgh and University of Toronto. Male New Zealand white rabbits (18–20 weeks old) were sensitized with 5 mg of Bovine Serum Albumin (BSA) in 0.5 ml saline emulsified with 0.5 ml of Freund's Complete Adjuvant. After 20 days, hypersensitivity to BSA was examined by subcutaneous injection of 0.1 mg BSA in 0.5 ml saline in 0.5 ml Freund's Incomplete Adjuvant. Five days later, rabbits exhibiting hypersensitivity reactions were anesthetized, right knees shaved, and 2.5 mg BSA in 0.5 ml saline was injected intra-articularly to induce AIA (10).

Immobilization or CPM treatment of the knees

Following intra-articular injection, the right knee of the rabbits ($n = 5$ rabbits/group for each time point) was immediately placed on a CPM device kindly provided by Orthomotion Inc, Pickering, Ontario, Canada. The angle of flexion of the joint was 70° with movement between 40° and 110° at a rate of 45 s per cycle. For immobilization, the right knee of rabbits ($n = 5$/group for each time point) was wrapped with bandages immediately after intra-articular injection with BSA. In both groups, the left limbs of the rabbits were not subjected to any treatment. To examine the early molecular events induced by motion, the rabbit knees were immobilized or exposed to CPM for 24 or 48 h and the rabbits were sacrificed to harvest the tissue.

Tissue preparation and immunohistochemical analysis

After harvesting, menisci were cleaned with saline and fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 5 μm thickness. The GAG content was analyzed by 1.5% safranin-O staining [9]. During examination, each meniscus was divided into two parts: the outer 25% zone (zone A), and the inner 75% fibrocartilage (zone B).

For immunohistochemistry, sections were deparaffinized, hydrated, and treated with 0.1 M sodium citrate buffer-pH 6.0 at 70 °C for antigen retrieval. Subsequently, sections were blocked in 5% pre-immune serum diluted in Protein Blocking Agent and incubated in 1:400 dilution of primary antibodies at 4 °C overnight. Thereafter, sections were washed with phosphate buffered saline (PBS) containing 1% BSA and 0.02% Tween-20 and incubated with 1:200 dilution of FITC-conjugated secondary antibody for 1 h. The primary antibodies used were goat polyclonal anti-mouse IL-1β, goat polyclonal anti-human MMP-1, and goat polyclonal anti-rat COX-2. Secondary antibodies for the above primary antibodies were FITC conjugated donkey anti-goat IgG. To detect IL-10, monoclonal rat anti-mouse IL-10 antibody and FITC-conjugated monoclonal mouse anti-rat IgG1 were used. The slides were washed 5 times with PBS at each step, mounted with Vectashield and observed under UV light in an Zeiss Axioplan-2 epifluorescence microscope equipped with Axiovision image capturing software. The control slides were also stained with secondary antibody alone to assure specificity of primary antibodies.

Data analysis

Sections obtained from knees subjected to CPM or immobilization in each group ($n = 5$) were analyzed after histochemical and immunofluorescence staining. In all sections four 500 μm^2 areas were enumerated for COX-2, MMP-1, IL-1, and IL-10 positive cells and presented as mean ± error of the mean. The statistical significance was calculated by students t test and considered significant at a value of $p = 0.05$. The analysis of slides was carried out by two investigators, one blinded to rabbit group assignments and the other was aware of the rabbit group assignments. The data collected by both investigators was found to be similar in all cases.

Results

Menisci from joints afflicted with AIA exhibit lesser GAG loss following CPM treatment

Safranin-O stained cross sections of menisci from healthy knees exhibited the presence of GAGs in zones A and B (Fig. 1A). The immobilized knees revealed that 48% of zone A and 26% of the total zone B lacked GAGs within 24 h as compared to the sections from healthy knees (Fig. 1A and B). Further immobilization induced greater reduction in GAGs, i.e., 37% of the zone A and 26% of the zone B exhibited loss of GAGs after 48 h (Fig. 1D). This degradation in Zone B was localized in the central area of the meniscus (Fig. 1B and D). On the contrary, exposure of the AIA knee to CPM for the initial 24 h resulted in a 12% reduction of GAGs in zone A and 6% reduction of GAGs in zone B as compared to healthy controls (Fig. 1C). Menisci from knees subjected to CPM showed a sustained retention of GAGs, in comparison to immobilized menisci. Only an 8% reduction of GAGs in zone A and a 3% reduction of GAGs in zone B was observed after 48 h exposure to CPM (Fig. 1E). However, some matrix disorganization was