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Effects of insulin-like growth factor-1 and dexamethasone on cytokine-challenged cartilage: relevance to post-traumatic osteoarthritis

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SUMMARY

Objective: Interleukin-1 is one of the inflammatory cytokines elevated after traumatic joint injury that plays a critical role in mediating cartilage tissue degradation, suppressing matrix biosynthesis, and inducing chondrocyte apoptosis, events associated with progression to post-traumatic osteoarthritis (PTOA). We studied the combined use of insulin-like growth factor-1 (IGF-1) and dexamethasone (Dex) to block these multiple degradative effects of cytokine challenge to articular cartilage.

Methods: Young bovine and adult human articular cartilage explants were treated with IL-1α in the presence or absence of IGF-1, Dex, or their combination. Loss of sulfated glycosaminoglycans (sGAG) and collagen were evaluated by the DMMB and hydroxyproline assays, respectively. Matrix biosynthesis was measured via radiolabel incorporation, chondrocyte gene expression by qRT-PCR, and cell viability by fluorescence staining.

Results: In young bovine cartilage, the combination of IGF-1 and Dex significantly inhibited the loss of sGAG and collagen, rescued the suppression of matrix biosynthesis, and inhibited the loss of chondrocyte viability caused by IL-1α treatment. In adult human cartilage, only IGF-1 rescued matrix biosynthesis and only Dex inhibited sGAG loss and improved cell viability. Thus, the combination of IGF-1 + Dex together showed combined beneficial effects in human cartilage.

Conclusions: Our findings suggest that the combination of IGF-1 and Dex has greater beneficial effects than either molecule alone in preventing cytokine-mediated cartilage degradation in adult human and young bovine cartilage. Our results support the use of such a combined approach as a potential treatment relevant to early cartilage degradative changes associated with joint injury.

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Introduction

Post-traumatic osteoarthritis (PTOA) refers to the development of OA as a result of traumatic joint injury. PTOA accounts for 12% of the OA population and typically affects younger individuals[1,2]. The pathogenesis following acute joint injuries (such as anterior cruciate ligament rupture and/or meniscal tear) has been reported to involve an immediate increase in synovial fluid concentrations of pro-inflammatory cytokines (e.g., IL-1[3–5], TNF-α[3–7], and IL-6[3–7]), along with increased release of cartilage matrix fragments and noticeable chondrocyte apoptosis[8,9]. While cytokine levels eventually drop to that found in chronic OA[3], their continued presence upsets the balance between anabolic and catabolic processes in cartilage, which plays an important role in the progression to PTOA[10].

An ideal pharmacological intervention to prevent or delay the onset of PTOA should address several if not all of the pathogenic responses to joint injury; such intervention should inhibit inflammatory responses, prevent cell death, prevent cartilage matrix degradation, and promote production of new matrix[11]. Recent studies have explored the potential of several therapeutic approaches: IL-1 receptor antagonists (IL-1Ra) and TNF-α blockers to inhibit selected inflammatory mediators[10,12], metalloproteinase and aggrecanase inhibitors to reduce matrix degradation[10], and

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growth factors such as BMP-7 and FGF-18 that promote cartilage matrix production and cell proliferation and counteract matrix degradation[13]. However, these studies all focused on targeting a single, specific pathway/molecule; many investigators now believe that no single compound will be sufficient to treat every aspect of OA[14], and that a combination of therapeutics will be necessary to address multiple pathogenic mechanisms.

In the current study, we take a novel approach by systematically studying the potential of a combination therapeutic to achieve both pro-anabolic and anti-catabolic effects, and at the same time, to improve cell viability. Insulin-like growth factor-1 (IGF-1) is a potent growth factor that can upregulate cartilage matrix biosynthesis and, under certain circumstances, inhibit catabolic processes stimulated by cytokines[15,16]. IGF-1 binds to the chondrocyte IGF-1 receptor and transduces signals *via* the IRS-1/PI3K/Akt pathway, which regulates protein synthesis[17]. In addition, IGF-1 provides pro-survival signals and has been shown *in vitro* to rescue chondrocyte apoptosis induced by collagen degradation[18] and by mechanical injury[19]. Dexamethasone (Dex), a potent synthetic glucocorticoid (GC), has been widely used intra-articularly to relieve inflammation for the treatment of OA and other arthritis[20]. Dex has been shown to block cytokine-induced cartilage matrix catabolism and to alleviate cytokine-induced inhibition of matrix biosynthesis in bovine cartilage *via* GC receptor-dependent pathways[21]. The ability of this combination of IGF-1 and Dex to modulate cytokine-mediated cartilage degradation and to simultaneously maintain chondrocyte viability in the face of cytokine challenge remains to be elucidated.

Using adult human knee and ankle cartilage and young bovine cartilage in an *in vitro* model system, our objectives were to quantify the effects of IGF-1, Dex and their combination on IL-1-induced degradation of aggrecan and collagen, inhibition of proteoglycan biosynthesis, and altered chondrocyte viability. Furthermore, we examined the hypothesis that the effects of IGF-1 and Dex are consequences of their direct transcriptional regulation by comparing changes at the protein level to their effects at the level of gene transcription.

Materials and methods

Bovine cartilage harvest and culture

Cartilage disks were harvested from the femoropatellar grooves of 1–2-week-old calves (obtained from Research 87, Boylston, MA). Explants were harvested within 8 h after animal death and a total of 15 joints from 14 animals were used. Briefly, a 3-mm dermal punch was used to core full-thickness cartilage cylinders, and the top 1-mm disk containing intact superficial zone was obtained with a blade. For each experiment, disks from different treatment groups were matched for anatomic location along the joint surface. Explant disks were then equilibrated in serum-free medium: low glucose Dulbecco's Modified Eagle Medium (DMEM); 1 g/L (Corning Cellgro, Manassas, VA) supplemented with 10 mM HEPES buffer (Gibco, Grand Island, NY), 0.1 mM nonessential amino acids (Sigma Aldrich, St. Louis, MO), 0.4 mM proline (Sigma), 20 μg/ml ascorbic acid (Sigma), 100 units/ml penicillin G, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B (Sigma) for 2–3 days (5% CO_2; 37°C). Serum-free conditions were chosen to distinguish the specific effects of exogenous IGF-1 from the unknown concentrations of endogenous growth factors that may be present in serum.

Adult human cartilage harvest and culture

Cartilage from adult human knee and ankle joints was obtained postmortem from the Gift of Hope Organ and Tissue Donor Network (Itasca, IL). All procedures were approved by the Rush University Medical Center Institutional Review Board (ORA Number: 08082803-IRB01-AM01) and the Committee on the Use of Humans as Experimental Subjects at MIT. At the time of donor tissue harvest, the joint surfaces were scored by an experienced forensic pathologist using the modified Collins grading system[22]. 20 joints from 13 donors were used in this study (an ankle/knee pair was obtained from one donor, and ankle pairs were obtained from six donors). 14 ankle joints (Collin's grade 1) were from eight donors, age 64–76 years old (see Supplementary Table S1 for enumeration of joints). Six knee joints (Collin's grade 0–2) were from six donors, age 19–66 years old. Full-thickness (~1–2 mm) cartilage disks cored with a 3-mm punch were harvested from the talar domes of ankles and the tibial plateau or distal femur of knees. Explants were harvested within 24–36 h after death of donors and only unfibrillated cartilage was used. Joint surfaces excluded from the study were the visibly roughened surfaces where the superficial layer had clearly lost its smooth architecture and the collagen appeared to be fibrillated. In order to be consistent with a previous study using human tissue[23], human explants were equilibrated for 2–3 days in high glucose DMEM (4.5 g/L; Corning Cellgro, Manassas, VA) containing the same supplements as in the bovine culture medium. To determine the effects of glucose concentration on cartilage response, a separate control study was performed using explants from three additional knee joints and six additional ankle joints (Table S1, Fig. S5) cultured in low glucose DMEM with otherwise identical supplements.

Dose responses of IGF-1 and Dex in the presence of IL-1

Many studies of cartilage explants treated with IL-1 have been reported utilizing IL-1 concentrations ranging from 0.05 to 100 ng/ml[15,24]. Based on our own preliminary dose–response study of sGAG loss vs IL-1α (R&D Systems, Minneapolis, MN) at 1, 2, 5, and 10 ng/ml) with bovine cartilage, we chose an IL-1α concentration of 1 ng/ml for all the present experiments, representing a moderately aggressive cytokine treatment. We then tested the effects of Dex (Sigma) alone (at 10 nM, 100 nM and 1 μM) and IGF-1 (R&D Systems) alone (at 10, 100 and 300 ng/ml) on sGAG loss and proteoglycan biosynthesis in bovine cartilage treated with 1 ng/ml IL-1α. Based on the results of these dose response studies [Fig. 1(A and B)], we chose concentrations of 100 nM Dex and 100 ng/ml IGF-1, both alone and in combination, for all subsequent tests of sGAG loss, biosynthesis, gene expression and cell viability.

Biosynthesis, sGAG, and biochemical analysis

Cartilage disks were radiolabeled with 5 μCi/ml ^{35}S-sulfate (Perkin-Elmer, Norwalk, CT) for 36–48 h. When terminated, disks were washed in PBS, weighed and digested with proteinase K (Roche, Indianapolis, MN). Radiolabel incorporation was measured using a liquid scintillation counter (PerkinElmer), and normalized to DNA for bovine explants (measured *via* Hoechst 33258 dye-binding assay[25]), and to wet weight for human explants. The DMMB dye-binding assay[26] and hydroxyproline assay[27] was used to determine the sGAG content and collagen content, respectively, of digested cartilage explants and medium. The sGAG and collagen content data were expressed as a percentage of the total sGAG or collagen, respectively.

Cell viability

Slices (100–200 μm thick) were cut with a scalpel from the center of cartilage disks[28] (see Supplementary Figure S1 for slice orientation) and incubated for 2–3 min in the dark in PBS containing fluorescein diacetate (FDA; 4 μg/ml) and propidium iodide (PI; 40 μg/ml) (both from Sigma), for viable and non-viable cell

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