THE ROLE OF GAP JUNCTION FOR INFLAMMATORY CYTOKINES IN FIBROBLAST-LIKE SYNOVIOCYTES OF JOINT DISEASE

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Purpose: To investigate whether connexin43 (Cx43) gene silencing in fibroblast-like synoviocytes (FLSs) of rat in vitro by RNA interference enhances inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6.

Methods: Small interfering RNA (siRNA) duplexes targeting the rat Cx43 gene were synthesized. In order to investigate whether interference of Cx43 gene expression affect the inflammatory cytokine, the cells were transfected with 50nM of siRNA 48hr before stimulation with lipopolysaccharide (LPS/0.1 μg/ml) for 6hr. After LPS stimulation, total RNA were extracted from the cells. The expression levels of Cx43, TNF-α, IL-1β, and IL-6 mRNA were analyzed by quantitative real-time PCR.

Results: After stimulation with LPS to FLSs, the expression of Cx43, TNF-α, IL-1β, and IL-6 mRNA were stimulated, respectively, compared to the cells without LPS (Fig 1). When transfection with Cx43 siRNA was performed, Cx43, TNF-α, IL-1β, and IL-6 mRNA expression were markedly reduced to 25.7±1.2, 56.1±9.3, 40.8±8.7, and 18.5±8.2 (%), respectively, compared to the cells which were transfected of non specific siRNA (Fig 1).

Conclusions: Gap junctions are specialized connections between cells in a tissue, and each gap junction consists of a number of individual hexagonal channels, each formed by 6 molecules of a structural protein known as connexin. Several recent studies have shown that intercellular communication facilitated by gap junctions may play an important role in the early development of osteoarthritis (OA). We found that synovial tissue from patients with OA has been found to have 4 times the number of gap junctions as that from unaffected patients, and also showed that the level of Cx43 which was the main protein in gap junction protein was 50% higher in osteoarthritis synovial tissue than in control synovium. In the pathogenesis of OA, various inflammatory cytokines produced by synovium leads to destruction of joints. In particular, proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 are known as important mediators in OA. Therefore, to suppress the expression of TNF-α, IL-1β, and IL-6 in joints in vivo, we expected an effective and less invasive conservative therapy of joint disease such as OA. In this study, we elucidated that the Cx43 mRNA levels highly expressed into FLSs by stimulation of LPS, and that the expression of all of those genes in Cx43 siRNA transfected FLSs was down-regulated. These results suggest that targeted down-regulation of Cx43 gene by siRNA may have the potential of the treatment for inflammatory joints.
found mainly in the extracellular matrix of soft connective tissues. In synovial fluid, HA fulfills lubricating functions, as well as serving as a shock absorber to protect joints. The development of inflammatory processes, such as osteoarthritis, implies the reduction in HA molecular weight (MW), which is essential to fulfill specific HA physiological and therapeutic effects. Its intraarticular administration implies cartilage protection and pharmacological effects, mainly due to its role in inflammatory mediators. The aim of this study was to determine the in vitro differential effects of low (64 kDa), medium (419 kDa) and high (1500 kDa) MW HA on fibroblast cultures under inflammatory conditions induced by lipopolysaccharide (LPS).

Methods: Human fibroblasts were cultured in triplicate and under different experimental conditions: not treated with HA and not subjected to inflammatory induction, treated with HA, subjected to inflammatory induction, treated with HA and subjected to inflammatory induction. LPS (10 μg/ml) was added to the cultures to establish inflammatory conditions. Cells were treated with different MW HA solutions (64, 419 and 1500 kDa; 1 mg/ml) to evaluate their anti-inflammatory effects. The response to inflammation and the influence of HA were compared among the experimental groups using cell viability, lipid peroxidation, cellular antioxidant status and protein oxidation.

Results: The induction of inflammation on fibroblast cultures caused inhibition of cell growth and cell death, increase of lipid peroxidation evaluated by the analysis of malondialdehyde (MDA), decrease of reduced glutathione (GSH) and superoxide dismutase (SOD) levels, and rise of protein oxidation by evaluating carbonyl content. The treatment with HA from different MW showed beneficial effects, particularly when high MW HA (1500 kDa) is present in the sample, being MDA levels (Figure 1A) and carbonyl content (Figure 1B) the parameters that more significantly were reduced and, therefore, more clearly showed the decrease in inflammatory damage with HA treatment.

**Figure 1.** Effect of hyaluronan treatment as a function of its molecular weight (kDa) in MDA (A) and carbonyl content (B) in human fibroblasts stimulated by LPS (Mean = SEM; n=3). Statistically significant differences are also shown (*compared to control group; †compared to stress factor (p < 0.05)).

Conclusions: These outcomes confirm the antioxidant and anti-inflammatory properties of HA and point out the dependency of these properties in HA MW, supporting the pharmacological beneficial effects of HA treatment. So the use of HA as viscosupplementation device implies the presence in the arthritic joint of a compound that works as a target for reactive species generated during inflammatory processes, protecting cell structures when HA is of high MW.

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**ISOLATION AND CHARACTERIZATION OF NATURAL ANTIBODIES AGAINST ENDOGENOUS MEDIATORS FROM THE PERIPHERAL BLOOD OF PATIENTS WITH OSTEOARTHRITIS OF THE KNEE AND HEALTHY INDIVIDUALS**

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**Purpose:** kOA is a group of chronic, painful, disabling conditions affecting synovial joints of 18% population. Development technologies that permit assessment of potentially disease-modifying agents of vascularization and inflammation are the current approach to the management of kOA. The analysis of the OA-associated antigen-antibody systems in the normal peripheral blood have new approach to the patient at risk for or with newly diagnosed kOA. Serological evidence of the presence NA to EM is an early future in kOA and not restricted to patients with end-stage disease undergoing joint replacement surgery. An understanding of the properties of NA, which characterizes their activity in relation to self-antigens, is important for binding capacity, functional activities, immune recognition. Prognosis of the typical features of NA to EM renaissance opens a new area of kOA diagnostics and therapeutics. To characterize the functional properties of affinity-isolated NA to EM from the peripheral blood of healthy individuals and patients with OA of the knee joint: focusing on isotype, affinity, specificity, and serum expression.

**Methods:** Affinity chromatography yielded NA to self-antigens (revealed upon ion-exchange Chromatography on QAE Sephadex) from both healthy individuals and kOA patients. The affinities of NA to EM were determined by using surface plasm resonance (SPR) technology (BIACORE AB, Upsala, Sweden). The reactivity of affinity-purified NA was assessed using Immunoblot and Cross-blot. Immunoassay kit was developed for the measure the expression NA to EM in the peripheral blood sera. The study group consisted of 50 kOA patients who were classified according to Kellgren&Lawrence. The control group consisted of 50 age- and sex-matched healthy individuals. The patients and control had no associated organic disease and exhibited no evidence of autoimmune disease.

**Results:** Affinity chromatography yielded two (IgM, IgG) isotypes of EM-specific NA from the sera of healthy individuals and kOA patients. EM-IgA was not useful for the presence kOA. Purified NA to EM displayed the expected characteristics and was functionally fully active. Low affinity M-IgM was predominantly isotype of Ig which present in healthy individuals: 55%BK, 45%AII, 42%VEGF. Deficiency in the sera EM-IgM isposes to development expression of high affinity EM-IgG in kOA patients. Serum BK-IgM levels varied widely in healthy individuals, but was highly dependent on the concentration of IgM in the serum (r=0.85; p<0.0005). The secondary immune response is characterized by the rapid production of high affinity EM-IgG. Affinity-purified EM-IgG from the sera of kOA patients showed the prevalence: 85%BK, 55%AII, 65%VEGF. Serum BK-IgG was detectable in 85% of kOA patients, with 100% specificity for kOA. NA to BK and AII showed no cross-reactivity to other structurally similar EM. High affinity BK-IgG antibodies produced in kOA patients and showed high serum levels.

**Conclusions:** In our Unit, a serum expression profiling study identified 3 self-antigens specifically expressed in kOA patients, which were identified by affinity chromatography. High-affinity, BK-IgG has demonstrated a direct role for BK in kOA development. We detected and identified BK-IgG in kOA patients, therefore probably involved in the pathogenesis and/or progression of KOA. The isolated IgG fractions of patients suffering from kOA had higher anti-BK reactivities than those detected in normal individuals. BK-IgG demonstrated the presence of this structure in 85% of newly diagnosed kOA patients and suggested that the presence of NA and/or circulating BK protein in the sera of kOA patients may have clinical utility. Identification of novel broadly cross-reactive kOA-neutralizing NA to EM in the sera has major implications for the development of treatment, angiogenesis inhibitors, and tools to study mechanisms.

### Intervertebral Disc

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**LUMBAR DEGENERATIVE DISC DISEASE PROGRESSION IS INFLUENCED BY GENETIC FACTORS: THE UK TWIN SPINE STUDY**

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**Purpose:** Cross-sectional studies have shown that lumbar degenerative disc