Combination Treatment of Physiological Concentrations of Glucosamine and Chondroitin Sulfate can Modulate Inflammatory Cytokine and Chemokine Production in Osteoarthritic Chondrocytes

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Introduction:
Osteoarthritis (OA) is the most common form of arthritis and is the major cause of pain and disability in people 65 and older. It is estimated that 20 million people in the United States suffer from OA and will increase to 40 million by 2030. The predominant symptom and complaint of OA patients is pain. However, the etiology of OA and the causes of OA knee pain remains unknown and unresolved. Presently, OA patients are treated with acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDs), intra-articular corticosteroids, or Hylaurotan injections and opiates. None of these treatments are totally effective in reducing OA pain, have adverse affects and do not stop the progression of the disease. Clinical trials using the nutraceutical compounds individually have shown that they are effective in relieving pain in OA patients. Recently, a large clinical trial sponsored by the National Institute of Health (NIH) reported that the combination of GLN and CS had efficacy in reducing pain scores in a subset of patients with moderate and severe OA knee pain. The mechanisms behind this apparent efficacy are as yet unclear. Available in vitro mechanistic studies are limited by concentrations of the nutraceuticals used which are too high and the use of animal tissue instead of human cartilage. Previously we showed that combinations of GLN and CS were effective in depressing expression of pain receptors and NO release especially at the highest biologically relevant concentrations (0.3 µg/ml GLN + 62 µg/ml CS) and 5GC (3 µg/ml GLN + 2 µg/ml CS) on human OA chondrocytes in vitro [1]. Increasing evidence shows that inflammatory cells in the joint maybe involved in promoting pain and inflammation. To further elucidate their mechanism of action and the role of these molecules and the ability of OA chondrocytes to promote inflammatory cells to the joint, we determined the effects of GLN and CS in combination, at concentrations attainable in vivo and lower than those reported in vitro on the production of inflammatory cytokines and chemokines on control or interleukin-1beta (IL-1β) stimulated human OA chondrocyte cultures.

Materials and Methods:
We took advantage of the recent pharmacokinetic information of GLN and CS concentrations in plasma and synovial fluid [2] and conducted studies with 3 different combinations of GLN (hydrochloride form) and low molecular weight CS (the form used in the NIH study) using human OA chondrocytes isolated from cartilage obtained after total joint arthroplasty (TKA). The concentrations used were in the percentage of 56% (GLN) to 44% (CS) which is the typical ratio in commercial combination of the nutraceuticals. Human chondrocyte cultures: Human (n=3) OA chondrocytes (ages: 56, 59 & 76) were isolated from articular cartilage obtained from TKA at HFHS. Chondrocytes were liberated from the cartilage slices with an overnight digestion at 37°C in DMEM bacterial collagenase. Following overnight digestion, chondrocytes were filtered through a cell strainer. Chondrocytes were then washed with DMEM alone, counted and seeded (2 ml/well) on 12 well flat bottomed plates at a density of 0.5x10⁶ cells/ml. Cells were cultured in 2 ml of DMEM supplemented with 50 µg/ml ascorbic acid and 10% FCS for 48h. The chondrocytes were then serum starved for 24h and treated with either of the following: Control (Cult, DMEM alone), 5 ng/ml IL-1β, 0.03µg/ml GLN + 0.02µg/ml CS (0.05GC), 0.3µg/ml GLN + 0.2µg/ml CS (0.5GC), 3µg/ml GLN + 2µg/ml CS (5GC), IL-1 + 0.5µGC, IL-1 + 0.5GC or IL-1 + 5GC. Cells and conditioned media were collected after 48h.

Cytokine and Chemokine Array Profiling: We used a commercial human proteome profile antibody array (ARY005, R&D, MN) to characterize 36 human cytokine and chemokines in our OA chondrocyte conditioned media. These arrays allow for rapid and much more economical profiling of inflammatory cytokines and chemokines than conventional ELISAs. Relative protein levels can be quantitated by densitometry. Conditioned media (500µl) was analyzed according to the manufacturer’s protocol. Arrays were exposed to X-ray film for 1 minute and images were scanned for analysis.

Results:
Cytokine and Chemokine Array Profiling

Conditioned media (500 µl) from OA chondrocytes that were exposed to 5GC (Fig. 1), IL-1β or IL-1β +5GC (Fig.2) were used for cytokine/chemokine profiling.

Treatment of OA chondrocytes with 5GC decreased the amount of protein from 6 cytokines/chemokines (CD54, IL-1RA, IL-17, IL-27, RANTES/CCL5) (Fig 7. ). It did not stimulate the production or increased any other cytokine. All of the 6 cytokine/chemokines that were downregulated have been implicated in inflammatory processes and trafficking of inflammatory cells. IL-17 has been shown to stimulate inflammation and MMP-13 in OA. IL-27 has been shown to mediate T-cell trafficking in rheumatoid arthritis. RANTES/CCL5 is a potent chemoattractant for leukocytes and has been shown to be expressed in osteoarthritis. In IL-1β stimulated OA chondrocytes, 5GC treatment also downregulated 5 different cytokines/chemokines (G-CSF, GRO-α, CD54, IL-17E, IL-23) (Fig 2).

Fig.2
Interestingly, 5GC increased the production of IL-16 and IL-12p70. IL-16 has been shown to be anti-inflammatory in RA109. This data suggests that 5GC can decrease inflammatory cytokines and chemokines that can promote trafficking of immune cells to the joint and therefore modulate inflammation and pain mediators in OA patients.

Discussion:
Cytokine array profiling revealed that the combination of GLN and CS can modulate inflammatory factors involved in inflammatory cell trafficking to the joint at relevant concentration on human OA chondrocytes in vitro. We previously identified possible pain related pathways in OA chondrocytes that may be responsiveness to these subset of inflammatory factors [1]. These cytokines can also act in autocrine manner to the chondrocytes or paracrine to synovial cells and subchondral bone which are also thought to be involved in joint pain. The downregulation of these inflammatory factors could reduce pain by preventing continual secretion of inflammatory factors and cells into the joint. To our knowledge this is the first study to show that physiological concentrations of GLN and CS can modulate inflammatory cytokine/chemokines production by OA chondrocytes and this will allow further studies into the these biochemical and signaling pathways that are associated with OA knee pain.

Reference: