GLUTAMINE PROTECTS ARTICULAR CHONDROCYTES FROM HEAT STRESS AND NO-INDUCED APOPTOSIS THROUGH INDUCTION OF HSP70

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INTRODUCTION:
In the pathogenesis of osteoarthritis (OA), various biological and chemical stress factors are believed to be involved in the onset and progression of the disorder. These include mechanical stress, heat stress and reactive oxygen species (ROS) such as nitric oxide (NO). L-Glutamine (Gln) is one of the most abundant free amino acids in the body. Under stressful situations, an administration of exogenous Gln has been shown to be beneficial for various organs (1). To clarify the functions of Gln on chondrocytes under stress, we treated primary chondrocytes with Gln and assessed its effects on cell injuries triggered by high temperature or NO. We also analyzed the expression of heat shock protein 70 (HSP70) to elucidate the mechanisms of chondroprotective effect of Gln.

METHODS:
Cultures of primary articular chondrocytes were established from rabbit joints, and treated for 12 hours with various concentrations of Gln (0-20 mM). In some experiments, cells were also treated with quercetin, a HSP70 inhibitor. Heat stress (43°C) was applied to the cells for 0 to 120 minutes. Apoptosis was induced by 0.5 mM sodium nitroprusside (SNP) dihydrate that produces NO. After stress loading, HSP70 expression was detected by Western blot analysis. Cell viability was assessed by lactate dehydrogenase (LDH) release and tetrazolium salt-based assays, while apoptosis was evaluated by Hoechst 33342 staining and TUNEL methods.

RESULTS:
Gln demonstrated dose-dependent enhancing effect on stress-mediated induction of HSP70, while in the absence of any stress HSP70 was not induced by Gln alone (Figure 1). After heating or SNP loading, chondrocytes showed severe reduction in viability, while the cytotoxic outcome was almost completely abrogated by conditioning with Gln (Figure 2A). The protective effect of Gln was significantly blocked by quercetin that effectively suppressed stress-induced HSP70 expression in chondrocytes (Figure 2B). The Gln also rendered chondrocytes unsusceptible to NO-induced apoptosis that was frequently seen in SNP-treated culture (Figure 3).

DISCUSSION:
Gln was previously shown to play a regulatory role in extracellular matrix metabolism in cartilage (2), but the effect of Gln on chondrocytes has not been fully elucidated. In this study, it was clearly demonstrated that the treatment of chondrocytes with Gln protect the cells from heat stress and NO-induced apoptosis. These chondroprotective effects of Gln may be mediated by HSP70. Previous reports showed that HSP70 expression levels in chondrocytes were correlated with histological severity in OA and overexpression of HSP70 resulted in protection of chondrocytes from various stresses (3,4). Recently, there is an increasing demand for the development of drugs or biological products that can effectively retard or stop the progression of OA. As several clinical trials demonstrated, exogenous Gln administration is simple, safe and inexpensive therapeutic modality. This study suggested that supplementation of Gln could provide a novel therapeutic approach to OA, aiming at augmentation of the protective response of chondrocytes to various stresses through induction of HSP70.

REFERENCES: