EFFECTS OF PRETREATMENT WITH GLUCOSAMINE ON MECHANICALLY TRAUMATIZED CARTILAGE EXPLANTS

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Introduction: In recent clinical trials, the hexosamine glucosamine sulfate (GS), which is classified as a symptom-modifying agent for osteoarthritis (OA)[1], has been found to significantly reduce the progression of joint-space narrowing in patients with knee OA[2]. While this drug is also suggested to be a disease-modifying agent, its mechanism of action is currently unclear. Cartilage explants are frequently used in studies investigating the mechanism of GS. Most explant studies use catabolic factors, such as interluekin-1 (IL-1), to produce a rapid aggrecanase-mediated degradation of cartilage[3]. Excessive mechanical loading has also been associated with the initiation of posttraumatic OA. Numerous studies have shown that mechanical loads can generate matrix damage and cell death in cartilage explants[5,6]. The extent of matrix damage and cell death has also been found to be dependent on the rate of mechanical loading[7]. This study indicated that relatively more matrix damage for high-rate loading is contrasted with more cell death in low-rate experiments. In the current study, the effect of GS treatment was investigated in an explant model using different rates of loading experiments in an attempt to isolate the mechanism of its potential action on mechanically traumatized tissue.

Methods: Four bovine forelegs were obtained from a local abattoir within 6 hours of slaughter. The forelegs were cleaned and skinned prior to opening the knee joint under a laminar flow hood. 72 articular cartilage disks (6-mm dia.) were removed from the underlying bone of the metacarpal surfaces. The weight of approximately 2 cartilage explants/well was recorded immediately after separation. 36 explants were placed in DMEM:F12 media, while the remaining 36 were placed in media with 2.5 mg/ml GS. All explants were allowed to equilibrate for 2 days in a humidity-controlled incubator. Media samples were collected daily to determine proteoglycan (PG) and nitric oxide (NO) released from the explants. Prior to mechanical tests, the explants were equally divided into 6 groups: non-impacted without GS, low-rate of loading without GS, high-rate of loading without GS, non-impacted with GS, low-rate of loading with GS, and high-rate of loading with GS. All wells were weighed prior to loading. The test specimens were loaded in unconfined compression between two highly polished stainless steel plates using a servo-hydraulic testing machine. The low- and high-rate of loading experiments applied a peak of 30 MPa in 1s and 50ms, respectively. Post-impact, the explants were washed in media and returned to the incubator. One day after loading, the weight of each well was again recorded. Cell viability was evaluated in 6 explants from each group. Six 0.5mm thick sections from each explant were stained with Calcein AM and Ethidium Homodimer. The sections were viewed under a fluorescent microscope at 100X. Full-thickness digital photos were taken of each specimen. A blind observer (MK) manually counted the number of live and dead cells using image software. The percentage of dead cells was determined (# dead cells / # total cells). The data were analyzed using ANOVA with SNK post-hoc tests. Statistical significance was indicated at $p<0.05$.

Results: There was no difference between the peak load in the without GS group (n=18) and the GS-treated group (n=18). The peak load was 841.6 ± 15.5N without GS and 836.3 ± 17.9N with GS. However, there was a significant decrease in compression from 0.32 ± 0.04mm in the without GS group to 0.29 ± 0.04mm in the GS-treated group. After 2 days of incubation in media, the increase in weight between groups was not significantly different being 10.4 ± 8.5% for the group without GS and 14.9 ± 6.4% for the GS-treated group. One day after impact, the weight change in both the low- and high-rate impact groups was significantly greater than the controls (Fig. 1). Importantly, following high-rate loading the GS-treated explants gained significantly less weight than explants without GS (Fig. 1). The amount of PG released to the media after one day for non-impacted controls was higher for the GS-treated group than for the without-GS group (Fig. 2). After either a low- or high-rate loading, the amount of PG released to the media increased for the group without GS. Conversely, PG released to the media did not change following mechanical loading for the GS-treated group (Fig. 2). There was no statistical difference in percentage of dead cells between controls with and without GS (Table1). However, all impact groups had significantly more cell death than non-impacted controls. There was significantly more cell death following a low-rate of loading than after a high-rate of loading with and without GS. Furthermore, the presence of GS did not affect the amount of cell death following low- or high-rates of loading. The amount of NO released to the media for without-GS and GS-treated control groups was 4.98 ± 1.92 μM and 1.35 ± 0.89 μM, respectively. Impaction did not change the amount of NO released to the media in either the without GS or GS-treated groups.

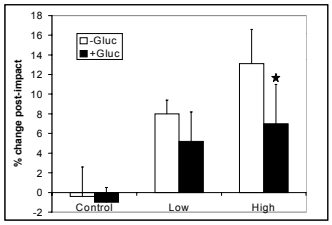

Figure 1. Change in water content at 1 day post-impact with and without GS

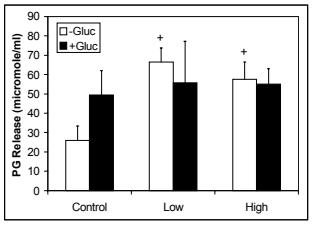

Figure 2. PG release at 1 day post-impact with and without GS

+ increase from controls without GS
★less than high-rate without GS

Table 1. Percentage cell death

Group	% Mean ± SD	
Non-impacted without GS	1.19 ± 0.83	
Low-rate loading without GS	41.28 ± 9.11	+
High-rate loading without GS	25.59 ± 11.78	+ ★
Non-impacted with GS	0.12 ± 0.29	
Low-rate loading with GS	40.47 ± 10.20	+
High-rate loading with GS	28.75 ± 9.15	+ ★

+ greater than controls
★ less than low-impacted with and without GS

Discussion: The results of this study suggest that while pre-treating cartilage explants with GS does not affect the amount of cell death from a mechanical insult, it does help minimize the degree of damage to the tissue matrix. This effect may be directly related to the action of GS on chondrocytes. Interestingly, non-impacted GS-treated explants displayed a higher PG release to the media than without-GS explants. This may relate to the documented increase in PG synthesis resulting from treatment with GS[4]. It also has been suggested that increased levels of PG result in a higher tissue stiffness[8,9]. An increase in tissue stiffness has previously been documented following pre-treatment of explants with manosamine, another hexosamine[10]. The reduction in tissue compressive strain for the GS group may be due to increased stiffness and may explain the reduction in tissue matrix damage in this group. On the other hand, an increase in the PG synthesis with GS pre-treatment can also increase the solid volume fraction of the "biphasic" cartilage. For a high-rate of loading compression, an increase in the solid fraction will lead to a reduction of the solid phase tensile stresses to reduce the extent of matrix damage according to biphasic analysis. While the current study specifically addressed the effect of GS pre-treatment on the resistance of cartilage to impact injury using a tissue explant model, the results may have future utility in the clinical setting. For example, some rationale may exist for athletes to supplement their diets with GS prior to engaging in sporting events. This notion, of course, will be dependent on the results of future experimental and clinical data with this nutriceutical.

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References: [1] Altman et al., OA & Cart 1996 [2] Reginster et al., Arth Rheum 1999 (suppl) [3] Sandy et al., Biochem J 1998 [4] Mims et al., 46th ORS, 2000 [5] Jeffery et al., Arch Bioch Bioph, 1995 [6] Kim et al., J Rhem, 2000 [7] Ewers et al., 46th ORS, 2000 [8] Jurvelin et al., Eng in Med, 1988 [9] Mizrahi et al, Biorheo 1986 [10] Patwari et al., 46th ORS, 2000