The Effect of Exogenous Glucosamine Hydrochloride on the Proteoglycan Concentration of the Articular Disc of the Rabbit Temporomandibular Joint

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Aims: *To test the effect of glucosamine hydrochloride (glucosamine-HCl) on the proteoglycan (PG) concentration of the articular disc of non-arthritic temporomandibular joints (TMJs) in rabbits.* ***Methods:*** *Twenty-four of 48 New Zealand white 10- to 12-week-old male rabbits (2.2 kg average) were injected with the irritant chymopapain in the knee joint. Both groups of 24 rabbits were divided into 3 groups of 8 animals. The rabbits were fed a control diet or a diet supplemented with glucosamine-HCl incorporated at a level to provide 20 mg/kg (approximating the recommended dose 500 mg/tid/70 kg man) or 100 mg/kg, for 8 weeks. Sulfated glycosaminoglycans (GAGs) were assayed in protease K digests of the TMJ articular disc by dimethylmethylene blue method to quantify PG concentration. The groups were compared with 2-way analysis of variance.* ***Results:*** *Glucosamine-HCl did not cause a significant change in the PG concentration of the TMJ articular disc ($P > .8$). There was also no detectable effect of chymopapain injection to the knee joint on the TMJ ($P > .07$) and no interaction between glucosamine-HCl treatment and chymopapain injection ($P > .3$).* ***Conclusion:*** *Glucosamine-HCl has no effect on PG concentration of the articular disc of non-arthritic TMJ in rabbits.* J OROFAC PAIN 2003;17:251–253.

Key words: glucosamine-HCl, glycosaminoglycans, proteoglycan, rabbit, temporomandibular joint

Glucosamine is an aminomonosaccharide and the precursor of the disaccharide repeats of the sulfated glycosaminoglycans (GAGs) including chondroitin sulfate, dermantan sulfate, keratan sulfate, and heparan sulfate, which are elements of proteoglycans (PGs). It is also the precursor of the unsulfated GAG, hyaluronan, which forms a critical element of the matrix of all connective tissues. Chondroitin sulfate, keratan sulfate, and hyaluronan are the major GAGs in cartilage. In vitro glucosamine inhibits protease activity and stimulates GAG synthesis in chondrocytes.[1] It has a special tropism for cartilage and is incorporated by chondrocytes into PGs.[1,2] In conjunction with the collagen fibrillar network, which provides the tensile strength, the PG molecules provide the shape and reversible resistance to compression of the cartilage matrix. Decreased PG content in cartilage was found closely correlated with osteoarthritic changes in joints.[3] This makes glucosamine an important molecule in determining the health of cartilage.

The effects of glucosamine on cartilage, metabolism, pain, and inflammation have promoted its use as a slow-acting drug for the

Table 1 The Effect of Glucosamine-HCl on Proteoglycan (PG) Concentration in Male Rabbits

Treatment	n	Mean*	SEM
Chymopapain-injected			
Control	8	7.18	0.57
Low-dose glucosamine	8	6.54	0.37
High-dose glucosamine	8	6.36	0.14
Non-injected			
Control	8	7.09	0.61
Low-dose glucosamine	8	7.90	0.87
High-dose glucosamine	8	7.48	0.38

*μg PG/mg wet weight.

treatment of osteoarthritis.[1,4,5] Glucosamine products are marketed as nutritional supplements for osteoarthritis in the United States, mostly as sulfate or hydrochloride salt, or less frequently as N-acetyl glucosamine. The most popular forms of glucosamine, the sulfate and hydrochloride salts, have some differences in regard to purity, sodium content, and equivalent dosages. For example, 2,608 mg glucosamine sulfate, which has 47.8% bio-reactive glucosamine and 20% sodium, is equal to 1,500 mg of the typical glucosamine hydrochloride (glucosamine-HCl) formulation, which has 81.3% bio-reactive glucosamine with no sodium additive.[2] Although most of the studies in Europe have been conducted with glucosamine sulfate because it is under patent protection, there is no scientific evidence to show any of the forms to be superior to another. There is some discussion as to whether sulfate may have its own independent effect in some patients.[6] Glucosamine products (glucosamine-HCl in combination with chondroitin sulfate) are also reported to be beneficial in the treatment of temporomandibular joint (TMJ) disorders.[7,8] However, their biochemical effect on TMJ connective tissues is not documented. Glucosamine HCl was chosen for this study because it is being used in ongoing clinical trials of knee osteoarthritis in the United States and is readily available over the counter. The objective of this pilot study was to test the effect of glucosamine-HCl on PG concentration of the articular disc of non-arthritic TMJs in growing rabbits.

Materials and Methods

This pilot study was a part of a larger study examining the effect of glucosamine-HCl on normal and osteoarthritic knee joints in growing rabbits.[9] Forty-eight New Zealand white 10- to 12-week-old male rabbits (2.2 kg average) were fed a control diet of Purina Rabbit High Fiber Chow for 1 week. Then under anesthesia, 24 of the rabbits were injected in a shaved left knee with 1.5 mg chymopapain in 0.2 mL of phosphate-buffered saline with cysteine and ethylenediaminetetraacetic acid (EDTA).[10] For both the chymopapain-injected and the non-injected rabbits, there were 3 groups of 8 animals each. The rabbits were fed the control diet or a diet supplemented with glucosamine-HCl incorporated at a level to provide 20 mg/kg (approximating the recommended dose of 500 mg/tid/70 kg man) or 100 mg/kg, for 8 weeks. The amount of diet per animal was based on the average weight of the group, and was periodically adjusted to the increased weight of the growing animals. The animals were fed once a day in the morning with free access to water, and were allowed free movement. At the end of 8 weeks, the animals were anesthetized and euthanized. The articular disc of 1 TMJ of each animal was randomly selected and dissected, the wet weight obtained, and the disc digested with 1 mg/mL proteinase K in 100 mM sodium phosphate, 50 mM EDTA, pH 7.4. Sulfated GAGs were assayed with dimethylmethylene blue, using chondroitin-4-sulfate as a standard.[11] Chymopapain-injected rabbits and non-injected controls, and the low- and high-dose glucosamine-HCl treated groups, were compared with 2-way analysis of variance (ANOVA). Values of $P < .05$ were considered to reflect statistical significance.

Results

All diets were well tolerated by the rabbits. There were no differences in the initial or final weights of the groups, and all rabbits gained about 40% of their initial weight by the end of the 8 weeks. On visual inspection, there was no macroscopic surface irregularity observed on the articular surface of the mandibular condyle or the articular eminence in any of the TMJs in any group. Chymopapain-injected and non-injected treatment groups were compared with 2-way ANOVA. Glucosamine-HCl treatment did not cause a significant change in the PG concentration of the TMJ articular discs ($P > .8$). There was also no detectable effect of chymopapain injection to the knee joint on the TMJ after the 8 weeks of treatment ($P > .07$), and no interaction between glucosamine treatment and chymopapain injection ($P > .3$). Table 1 shows the means and SEMs for sulfated GAGs in the TMJ articular disc of the different groups of animals.

Discussion

This pilot study was part of a larger study examining the effect of glucosamine-HCl on normal and osteoarthritic knee joints where the disease was produced by mild proteolytic damage in growing rabbits. The rabbit is accepted as an appropriate animal model for TMJ studies as it provides an anatomic, histologic, biochemical, and functional analog for the human TMJ.[12] Osteoarthritis in knee joints was induced by chymopapain injection to left knee joints in each animal. With chymopapain-induced damage, it is not yet clear if chymopapain has a systemic effect on other joints. This study found no statistically significant changes in the PG concentration of the TMJ articular disc that could be associated with chymopapain injection to the knee joint even though there was evidence for this in the contralateral knee. In these same rabbits, glucosamine-HCl enhances PG replacement ($P < .05$) of the initial 35% loss caused by chymopapain injection into rabbit knee joints, but has no effect on the non-arthritic knee joint.[9] Our findings of no effect of glucosamine-HCl on articular cartilage in a non-arthritic TMJ of rabbits parallel other studies reporting no effect of glucosamine-HCl on a normal joint.[9,13] This may be because, in the normal joint where energy and glucosamine precursors such as glutamine are not in short supply, glucosamine is not the limiting factor in proteoglycan synthesis.[9] These agents exert their effect by intensifying cellular responses of chondrocytes under adverse environmental conditions and do not alter normal cartilage metabolism.[14] The same may be true for glucosamine sulfate since no changes in cartilage histology were found in safety studies,[2] although detailed studies on normal joints have not been reported.

This study supports the contention that in a normal joint, glucosamine is not the limiting factor in PG synthesis.[9] It is important to further investigate if there is any biochemical effect of glucosamine on cartilage maintenance in the arthritic TMJ, possibly by the use of the same model with chymopapain-induced arthritis.

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