GLUCOSAMINE INHIBITS FORMATION OF ADVANCED GLYcation ENDPRODUCTS IN HUMAN ARTICULAR CARTILAGE: A MECHANISM FOR CHONDROPROTECTION?

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Introduction:

Osteoarthritis (OA), one of the most common diseases of the elderly, is characterized by the progressive destruction of articular cartilage, ultimately leading to impaired joint motion and pain [1]. Age is the main risk factor for the development of OA and data are accumulating that suggest that the age-related accumulation of Advanced Glycation Endproducts (AGEs) is one of the molecular mechanisms by which aging contributes to the development of OA. AGEs, which are formed by the spontaneous reaction between sugars and amine-groups in proteins (in lysine, hydroxylysine and arginine residues), adversely affect cartilage stiffness, brittleness and repair capacity [2-4], thereby making the tissue more prone to damage, hence OA. Inhibition of AGE formation may be an interesting new approach to prevent the development of OA and suppress its progression.

Glucosamine is a dietary supplement that receives much attention, because of its claimed chondro-protective and analgesic effects. The possible molecular mechanism may be that the amine-group of glucosamine competes with proteins for the nonenzymatic reaction with reducing sugars, thus inhibiting the formation of AGEs and thereby interfering with the progression of OA. The aim of the present study was to examine the effect of glucosamine on the formation of AGEs in articular cartilage collagen.

Methods:

Normal human articular cartilage (male humeral head, 56 years, obtained at autopsy; n = 6 per condition) was depleted of proteoglycans by sequential treatment with ABC, chondroitinase ABC, trypsin and hyaluronidase (each 24 hrs at 37°C). Subsequently, cartilage was washed and incubated in the presence or absence of 50 mM D(-)-ribose (Sigma R-7500) in sodium phosphate buffer (0.2 M; pH 8.0) for 4 days at 37°C, to induce AGE formation. In addition, cartilage was incubated with ribose (see above; 50 mM) together with D(+)-glucosamine (0.05 to 1.0 M; Sigma G-4875), to assess the ability of glucosamine to inhibit ribose-induced AGE formation. After incubation, cartilage was washed and solubilized with papain. AGE-fluorescence (ex 360 nm, em 460 nm) and absorption (at 340 nm) were measured in the papain digest. In addition, pentosidine and amino acid modification were measured by HPLC after acid hydrolysis (6 N HCl, 20 hrs, 110°C) of the papain digest [5]. Fluorescence, absorption and amino acid modification were normalized to the collagen content of the cartilage, assuming 300 Hyp residues per triple helical collagen molecule.

Results:

Incubation with ribose resulted in a statistically significant 23 fold increase in cartilage collagen AGE levels (measured as AGE-fluorescence, absorption, pentosidine formation and loss of AGE-sensitive amino acids; see figure 1A to 1D). Glucosamine dose-dependently inhibited AGE formation. This inhibitory effect was most prominent for pentosidine: at equimolar concentrations glucosamine completely prevented ribose-induced pentosidine formation.

Discussion:

Accumulation of AGEs occurs in articular cartilage with age, which leads to impairment of the tissue’s mechanical properties and repair capacity. The current data demonstrate that glucosamine, a popular dietary supplement with possible beneficial effects for OA patients, can inhibit the formation of these AGEs. Currently, studies are in progress to investigate the effect of glucosamine on the functional consequences of articular cartilage AGEs.

References:


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Figure 1. Effect of glucosamine on ribose-induced AGE formation. * and # indicate P < 0.05 versus control (no additions) and 50 mM ribose, without glucosamine, respectively. See text for details.

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