DIETARY SUPPLEMENTATION WITH CHONDROITIN SULFATE AND GLUCOSAMINE HCL DOES NOT SLOW DOWN ARTICULAR CARTILAGE EROSION IN BIGLYCAN/FIBROMODULIN DEFICIENT MICE

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Introduction

Recently, the biglycan/fibromodulin double-deficient mouse was shown to develop successively and progressively gait impairment, ectopic tendon ossification and severe premature knee OA (Ameye et al. 2002). Weak tendons were suggested to cause OA in this mouse because gait problems, as well as structurally and mechanically compromised tendons were present before OA starts to develop.

Currently, there is no recognized gold standard animal model of OA for testing the chondro-protective effects of new therapeutic agents. The biglycan/fibromodulin double-deficient mouse present several of the key characteristics required to become such a model: it develops knee OA spontaneously, early (between one and 2 months), rapidly (complete cartilage erosion between 3 and 6 months) and reproducibly.

In order to validate this new animal model of OA as a tool to assess the *in vivo* chondro-protective effects of new therapeutic agents, the double deficient mice were orally supplemented with oral glucosamine HCl and chondroitin sulfate. Glucosamine and chondroitin sulfate were chosen due to their reported chondro-protective effects. Glucosamine sulfate has been shown to prevent radiographic changes in joint space width narrowing in two 3-year-long human clinical trials (Reginster et al. 2001, Pavelka et al. 2002) whereas chondroitin sulfate has been shown to slow down cartilage degradation in chemically induced animal models of OA (Uebelhart et al 1998a, Omata et al. 1999) and was also suggested to have chondro-protective effects in human clinical trials based on radiographs and on urinary, blood and synovial biomarkers (Ronca et al. 1998, Uebelhart et al 1998b, 2004). In addition, a 2% dietary supplementaton with glucosamine HCl, chondroitin sulfate and manganese ascorbate was shown to slow down the destruction of articular cartilage in rabbits with transected anterior and posterior cruciate ligaments (Lippiello et al. 2000). Based on that study, a slightly higher dose with the same ratio of glucosamine HCl and chondroitin sulfate was used here.

Abstract

Aim of the study: The effects of oral glucosamine HCl/chondroitin sulfate on preservation of articular cartilage in the biglycan/fibromodulin double-deficient mouse (DKO) were studied to determine if this new animal model of osteoarthritis (OA) would be suitable to test the in vivo efficacy of structure modifying compounds.

Results: Supplementation with glucosamine/chondroitin sulfate did not slow down cartilage erosion in DKO. Articular cartilage was similarly eroded below the tidemark in both DKO groups while being intact in WT groups. This observation was confirmed by measurements of Coll2-1 and C2C biomarkers. The mean serum concentrations of Coll2-1 and C2C in control WT were significantly lower than in control DKO (see Table) but no differences were found between treated and control groups except for higher coll2-1 levels found in WT control group at day 81 (245±40 vs 204±37 nM, p=0.007) and in DKO control group at day 95 (362±60 vs 293±55, p=0.007).

Conclusions: While glucosamine and chondroitin sulfate have been shown to slow down cartilage erosion in surgically induced OA in rabbits, this supplementation failed to show any benefit in the DKO. The higher serum levels of coll2-1 in DKO vs WT suggest that this new biomarker, which is specific for a peptide of the α-helical region of type II collagen, could be useful to monitor collagen degradation in joint diseases.

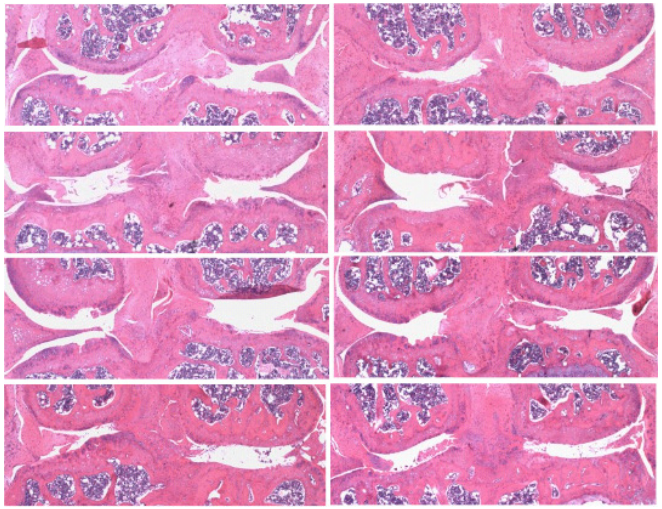
Material and methods

Two groups of 15 wild-type (WT) mice and 2 groups of 15 biglycan/fibromodulin double-deficient mice (DKO) were fed from weaning (day 21) with a regular diet, supplemented or not with 1.2% chondroitin sulfate and 1.5% glucosamine HCl. Serum levels of the C2C (i.e Coll 2 3/4 C Long, IBEX, Montréal, Canada) and coll2-1 type II collagen biomarkers were determined at day 66 and 141 and at day 49, 81, 95 and 141, respectively. Mice were sacrificed at day 141 and preservation of the articular cartilage in the knees was assessed by histology.

Results

Body weight gains and final body weights were not statistically different between treated and untreated groups. Supplementation with glucosamine HCl and chondroitin sulfate did not slow down cartilage erosion in DKO. Articular cartilage was similarly eroded below the tidemark in both DKO groups (see Figure) while being intact in WT groups (data not shown). Safranin O staining did not demonstrate any difference either between treated and untreated DKO groups (data not shown), suggesting that the OA-induced glycosaminoglycan loss observed in the DKO was not reduced by the glucosamine and chondroitin sulfate supplementation.

Figure 1: Representative coronal sections of knees from four 141-day-old untreated DKO (left column) and four treated DKO of the same age (right column) displaying no important differences between the 2 groups.



The lack of chondro-protective effects was confirmed by measurements of Coll2-1 and C2C biomarkers. The mean serum concentrations of Coll2-1 and C2C in control WT were significantly lower than in control DKO (see Table) but no differences were found between treated and control groups except for higher coll2-1 levels found in WT control group at day 81 (245±40 vs 204±37 nM, p=0.007) and in DKO control group at day 95 (362±60 vs 293±55, p=0.007). The DKO/WT ratio for each biomarker remained approximatively constant with time and were of ≈1.15 for coll2.1 and ≈1.63 for C2C.

Table 1: Serum levels (mean±SD) of Coll2-1 and C2C biomarkers in control WT and DKO groups

	Coll2-1 (nM)			C2C (ng/ml)		
	Ctl WT	Ctl DKO	p	Ctl WT	Ctl DKO	p
Day 49	200±30	222±31	0.06	/a	/a	/a
Day 66	/a	/a	/a	32±16	52±14	0.001
Day 81	245±40	281±61	0.06	/a	/a	/a
Day 95	305±32	362±60	0.005	/a	/a	/a
Day 141	264±24	303±35	0.008	30±16	50±26	0.03

Discussion

While glucosamine and chondroitin sulfate incorporated together at 2% in the diet of rabbits with surgically induced OA have been shown to slow down cartilage erosion (Lippiello et al. 2000) , the same supplementation incorporated at 2.7% in the diet of biglycan/fibromodulin deficient mice failed to show any chondro-protective effects. This suggests that the biglycan/fibromodulin double-deficient mouse is either an inadequate animal model of OA to test the efficacy of structure modifying compounds due to its lack of response to dietary or pharmacological interventions or that the glucosamine HCl and chondroitin sulfate dietary supplementation used here was not potent enough to slow down the rapidly progressing OA which characterizes this animal model.

For the first time, analyses of 2 type II collagen biomarkers demonstrate a higher collagen catabolism in the biglycan/fibromodulin double-deficient mouse, compared to WT. Two different biomarkers were used: the C2C biomarker, which recognizes the carboxy terminal neoepitope of the 3-quarter length piece of type II collagen generated by collagenase cleavage (Chu et al. 2002), and the newly developed coll2-1 biomarker , which is specific for a peptide of the α-helical region of type II collagen (Henrotin et al. 2004). Our data suggest that both biomarkers could be useful to monitor collagen degradation in joint diseases. The higher DKO/WT ratios observed for C2C compared to coll2-1 at different ages suggests that the 2 biomarkers could give complementary rather than redundant information on type II collagen catabolism.

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