ADAMTS-1 KNOCKOUT MICE DO NOT EXHIBIT ABNORMALITIES IN AGGREGAN TURNOVER IN-VIVO OR IN-VITRO

INTRODUCTION:

Turnover of aggregan, the predominant proteoglycan of cartilage, is important in both normal and pathological processes. During development, the aggregan-rich cartilaginous growth plates are resorbed and replaced with bone. In arthritis, accelerated breakdown of aggregan is a critical early event in the progressive cartilage destruction that is a hallmark of the disease. It is believed that both physiological and disease induced aggregan turnover occurs principally through proteolysis of the core protein. Two predominant families of enzymes have been implicated in aggregan proteolysis in vivo, the matrix metalloproteinases (MMPs) and the aggreganases (defined by their ability to cleave specific glutamyl bonds in the C-terminus and interglobular domain of aggregan). Three members of the ADAMTS family of proteinases, ADAMTS-1, -4 and -5, have been shown to cleave aggregan in vitro where specific antibody recognition of active sites, are expressed in cartilage, and are upregulated in states of increased aggregan catabolism (reviewed in 1). The role of aggreganases versus MMPs in normal and pathological aggregan turnover remains an important area of investigation. Furthermore, the relative importance of ADAMTS-1, -4 & -5 in aggregan degradation in different tissues and in health and disease has not been resolved. In this study we have evaluated aggregan catabolism in growth-plate and articular cartilage of ADAMTS-1 knockout mice compared with wild-type littersmates.

METHODS:

Using an exon 2 deletion construct and homologous recombination, ADAMTS-1 null mice were developed at the Monash Institute of Reproduction and Development in accordance with their institutional animal ethics regulations. Homozygous null (KO) and wild type (WT) mice were raised on inbred backgrounds as described (2). Femoral heads were incubated in DMEM containing 10% FCS for 3 days, then washed and cultured individually for 3-7 days in 0.4ml serum free DMEM + 10 M retinoic acid (RA) or 10ng/ml rhIL-1 alpha (IL-1). At the termination of culture, femoral heads were blotted dry, weighed, and extracted with 4 M GuHCI containing proteinase inhibitors. The proteoglycan content of the femoral and tibial growth plates and articular cartilage, in WT versus KO mice, were evaluated in 6 µ serial sections stained with H&E or toluidine blue/fast green, respectively.

To investigate stimulated aggregan catabolism in WT versus KO mice, intact femoral heads including articular cartilage and growth plate, were harvested for tissue culture as described (2). Femoral heads were blotted dry, washed and cultured for 3 days in 0.4ml serum free DMEM + 10 M retinoic acid (RA) or 10ng/ml rhIL-1 alpha (IL-1). At the termination of culture, femoral heads were blotted dry, weighed, and extracted with 4 M GuHCI containing proteinase inhibitors. The proteoglycan content of the culture medium, cartilage extract and papain digested residue were measured as sulfated glycosaminoglycan (GAG) (4). Differences in GAG release associated with treatment and genotype were analysed using ANOVA, with P<0.05 considered significant. Aggrecan metabolites in the medium and extracts were separated by SDS-PAGE, and analysed by Western blotting with antibodies recognizing G1; the aggrecanase- and MMP-generated neoepitopes ..EGE and ..PEN, respectively, and chondroitin-4 -6 sulphate. In addition, link protein, collagenase cleaved type II collagen neoepitope, and MMP-3 & -13 were evaluated by Western blotting with specific antibodies. Duplicate femoral heads were embedded in OCT and central 6 µ sections stained with toluidine blue/fast green, or ..EGE or ..PEN immunolocalised.

RESULTS:

ADAMTS-1 KO mice had renal abnormalities and females were subfertile as reported previously (3). KO mice were smaller than WT (weight = 10.3 ± 0.5 versus 12.8 ± 0.4 g; P = 0.0005). No differences were observed between WT and KO mice in growth plate morphology, architecture or cellular arrangement. Similarly, no difference in proteoglycan content (toluidine blue staining) was observed in the articular or growth plate cartilages. This was confirmed in isolated femoral heads where no difference in total GAG content of WT versus KO mice was noted (26.7-27.2 µGAG/mg wet weight). Furthermore, the extent of toluidine blue staining in the metaphyseal calcified cartilage trabeculae was similar in both genotypes.

There was no difference in basal GAG release from WT versus KO femoral head cartilage at either day 3 (Fig 1) or 7 (not shown). GAG release increased significantly (P < 0.0001) in response to RA (56-58% release) and IL-1 (57-64% release), with no significant difference between genotypes at either day 3 (Fig 1) or day 7 (not shown). The GAG release induced by RA and IL-1 was associated with an increase in aggrecanase-generated G1-EGE metabolites in both WT and KO mice (Fig 2). MMP-generated G1-PEN metabolites were increased in RA, but not IL-1 treated cultures of both WT and KO mice.

Figure 1: GAG release (% total ± SEM) from WT and KO femoral head cartilage cultured for 3 days ± 10^5 M RA or 10ng/ml IL.

Figure 2: Western blot analysis of extracts of WT and KO femoral heads cartilage cultured for 3 days ± 10^5 M RA or 10ng/ml IL.

No differences between WT and KO mice were observed in Western blot analysis for aggrecan G1, chondroitin-4 -6 sulphate, link protein, MMP-3,-13 or collagenases-cleaved type II collagen.

There was a significant reduction in toluidine blue staining in both the articular and non-calcified growth plate cartilage in RA and IL-1 treated femoral heads. In articular cartilage but not growth plate, this coincided with increased G1-EGE (but not G1-PEN) in both RA and IL-1 stimulated femoral heads. In contrast, increased G1-PEN, particularly present in RA stimulated cultures, was primarily in the growth plate cartilage. No difference was detected in the GAG loss or the staining intensity or distribution of aggrecan neoepitopes in WT versus KO mice.

DISCUSSION:

Although ADAMTS-1 is highly expressed in the metaphysis and is upregulated by parathyroid hormone (4), we were unable to detect any abnormalities in growth plate histomorphology in KO mice. Similarly, although ADAMTS-1 is expressed in articular cartilage (5), we could not demonstrate a significant role for this enzyme in basal or stimulated aggregcan catabolism in vitro. These results are consistent with studies where aggrecanase activity was not reduced in vitro with an ADAMTS-1 specific antibody (6). The lack of a cartilage specific phenotype in ADAMTS-1 KO mice may be due to compensation by other aggrecanases, however in preliminary analyses, we have detected no differences in ADAMTS-4 protein in WT versus KO cartilage. Double and triple KO of ADAMTS-1, -4, -5, are likely to provide more definitive information on the role of these important proteinases.


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