OSTEOARTHRITIS and CARTILAGE

SHORT COMMUNICATION

Blotchy mice: a model of osteoarthritis associated with a metabolic defect

Introduction/Summary

Blotchy mice have alterations in collagen and elastin cross-linking which cause widespread pathology including emphysema [1], aortic aneurysms [2], abnormal hair coat and osteoarthritis [3]. We investigated the progression of osteoarthritis (OA) over 10 months to discern whether heterozygous Blotchy mice could provide a predictive model of OA. At 2-4 months of age, unaffected (wild type) mice had Mankin scores [4] of 0.01 ± 0.01 (mean ± S.E.M.) whereas heterozygous Blotchy mice had scores of 4.9 ± 1.1, $P=0.0001$ (unpaired nonparametric test), indicative of OA. At 10 months of age, controls had scores of 1.7 ± 0.6, whereas heterozygous Blotchy mice had scores of 8.8 ± 1.1, $P=0.002$.

Methods

Heterozygous female Atp7aM~b~°/C57BL16JEi Blotchy mice (Jackson Labs, Bar Harbor ME, U.S.A.) are an intermediate group between a lethal homo/hemizygous group and normal mice [1, 5]. Hemizygous males and homozygous females have defective elastin in the aorta and usually die from rupture of aortic aneurysms at approximately 6 months of age [2]. Silberberg has previously investigated osteoarthrosis in hemizygous males [3].

We chose to investigate heterozygous female Atp7aM~b~°/C57BL16JEi Blotchy mice, because they would not be prone to rupture of aortic aneurysms before the end of the 10 month study. Age-matched homozygous female C57BL16JEi mice were used as controls. Animals were housed in groups of three per cage and were given ad libitum access to de-ionized water laboratory rodent diet #5001 (PMI Feeds, Inc. Richmond IN, U.S.A.). At appropriate intervals, animals were euthanized by carbon dioxide exposure and both knee joints were harvested for histopathology.

Histopathology was performed on knees from control and Blotchy mice fixed in 10% formalin in phosphate buffered saline (PBS) (pH 7.4) for 48 h at 5°C, and decalcified in SurgiPath™ Decalcifier I (Surgipath Medical Industries, Inc. Richmond IL, U.S.A.) for 5 days. The knees were rinsed in running water for 1 h and embedded in paraffin. Frontal sections (5 μm) were cut at 100 μm intervals and stained with Safranin O, Weigert's hematoxylin and Fast green.

Results/Discussion

Cartilage lesions on the femoral–tibial joint were evaluated according to the Mankin criteria [4] and are represented in Fig. 1. Control animals ($N=14$) had Mankin scores of 0.01 ± 0.01 (mean ± S.E.M.) at 2–4 months of age, and revealed minimal histopathological changes at 10 months of age ($N=12$) with scores of 1.7 ± 0.6. In contrast, young Blotchy mice, 2–4 months of age ($N=14$), had a mean of 4.9 ± 1.1, $P=0.0001$, compared with age-matched controls (unpaired nonparametric test) and had evidence of structural and cellular correlates of minimal to moderate osteoarthritis. At 10 months of age, Blotchy mice ($N=8$) had a mean of 8.8 ± 1.1, $P=0.002$ compared with age-matched controls. These changes increased in severity with age, and included loss of integrity of the surface of the weight bearing regions of the joint. Cellular changes, including cloning and cell loss were also found. Reduction in Safranin O staining indicated minimal to moderate loss of proteoglycan in the cartilage (Fig. 2). These results confirm that the osteoarthritic lesions seen in Blotchy mice are progressive with age.
Nonreducible pyridinoline cross-links in collagen were analyzed in the cartilage of 2-month-old Blotchy (N=3) and control (N=3) mice to determine the ratio of pyridinoline:collagen cross-links. Cartilage was peeled from the tibial plateau after 30 min of 5% formic acid decalcification. The cartilage samples were hydrolyzed for subsequent analysis of hydroxyproline content by Pico-Tag reverse-phase HPLC [8]. Pyridinoline cross-links were quantitated by ELISA as described [9] and expressed as nanomoles pyridinoline per nanomoles of collagen type II. Results from control cartilage samples (N=3), gave a mean of 18.7 nmol pyridinoline/nmol collagen compared with 16.7 for the heterozygous Blotchy mice. This decrease in cross-links observed in the cartilage of Blotchy mice gave a result intermediate to that observed with the hemizygous males and control mice. Mechanic et al. [6] have previously shown a 59% decrease in lung, and a 39% decrease in tail tendon collagen cross-links of male hemizygous Blotchy mice when compared with age matched controls. The involvement of tendon indicates that instability may be superimposed upon the decreased cross-linking of the cartilage collagen in the progression of OA, and highlights the systemic nature of the disease.

The Blotchy mouse, AtpvaM°b~° has a blotchy gene with a semi-dominant mutation in the ATPase Cu 2+ transporting alpha polypeptide gene [10], at the mottled locus on the X chromosome. The ATP driven copper pumps are believed to be responsible for the regulation of cellular copper, by pumping excess copper out of the cell [10]. The blotchy gene defect results in altered copper absorption and distribution in different organs [5]. Initial copper uptake by intestinal epithelial cells is normal [11], although copper release is decreased [12].

Fibroblasts from hemizygous Blotchy mice accumulate high-levels of copper (500%) and exhibit markedly decreased levels of active lysyl oxidase (45%) compared with the normal C57B10 fibroblasts [13]. The mutation in the ATP-driven copper pumps may affect delivery of copper to sites of cellular utilization [12], and therefore, lead to decreased complexing of copper with copper dependent enzymes, such as LO. This would explain the apparent paradox in the concurrent elevation of intracellular copper, with a deficiency in copper dependent enzymes. Thus, LO deficiency can occur despite the low copper requirement (1:1 ratio), and large intracellular stores of copper.

The tension-resistant, collagen fibrillar network, provides a cohesive framework which contains the hydrostatic pressure of highly charged proteogly-
cans and allows binding of smaller proteoglycans [14]. The tensile strength of collagen, and hence of cartilage, is dependent on intermolecular cross-linking. Blotchy mice have a deficiency of collagen cross-links, despite normal synthesis of collagen precursors [6]. Cross-linking of collagen may not only be important for optimum function of connective tissue in vivo but may also be the principal mechanism of regulating the rate of collagen catabolism by mammalian collagenase [7]. The collagen matrix is, therefore, prone to breakdown following normal loading, and normal levels of matrix metalloproteinases and other catabolic enzymes. Proteoglycans are thus lost from the cartilage because they are no longer entrapped by the collagen framework.

It is postulated that the alterations in matrix cross-linking may lead to changes in the physical properties of cartilage. One can further speculate cellular–matrix interactions are primary effectors in promoting enzymatic challenge. This paradigm is supported by joint instability models of OA that result from altered mechanical forces in cartilage. Cartilage collagen defects, in addition to instability resulting from decreased tendon cross-linking, may be acting in unison in this model of OA.

Blotchy mice develop early onset OA with severe changes attributed to a deficiency of the active cuproenzyme LO. The disease is progressive and affects articular cartilage. Heterozygous female Blotchy mice can, therefore, be used as a model of OA, for assessment of agents which may impede cartilage breakdown, or those which stimulate cartilage repair.

References


SONYA S. GLASSON, OLGA V. TRUBETSKOY, PATRICIA M. HARLAN, ANTHONY E. CHAVARRIA, HOWARD B. HAIMES AND PABLO A. JIMENEZ

*Department of Biological Sciences, OsteoArthritis Sciences Inc., One Kendall Square, Bldg. 200, Cambridge, Massachusetts 02139, U.S.A.*