A metabolic dysfunction in early murine osteoarthritis

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SUMMARY Male mice of the STR/ORT strain spontaneously develop an osteoarthritic lesion in the medial tibial condyle. The appearance of the lesion is age-related and by about 15 months has affected almost all animals. Histochemical studies of lactate dehydrogenase and succinate dehydrogenase activities have shown that a region of inactive chondrocytes appears in the cartilage of these mice before major histological evidence of degeneration. It is suggested that this may be an early metabolic dysfunction, which subsequently leads to matrix breakdown due to the lack of essential enzyme cofactors.

Clues to the aetiology of osteoarthritis are most likely to be found in a study of the very early stages of the disease process.¹ This is best achieved in an animal model, where the development of the lesion is well defined. Male mice of the inbred STR/ORT strain consistently develop an osteoarthritic lesion in the knee joint, specifically in the medial tibial condyle.²⁻³ The lesion always occurs first close to the junction of the articular cartilage and the cruciate ligament, and subsequently spreads out across the whole condyle. The lateral condyles are not affected. There is an almost 100% incidence by the age of about 15 months. The lesion appears to be a result of an instability of the patella, which becomes displaced to the medial side; increased loads are thus transmitted through the medial condyle, and these are thought to initiate the degenerative changes.⁴

Full details of the origins of these mice, from the parent STR/1N strain in the National Institutes of Health, Bethesda, Maryland, USA, have been given.² They have also been extensively studied.³⁻⁷ In the present studies, they have been used in an attempt to define any metabolic changes which may occur in the chondrocytes before the histological appearance of degenerative changes in the articular cartilage.

Materials and methods

Male mice of the osteoarthritis-prone STR/ORT strain were used. Age-matched male mice of the normal CBA strain served as controls. The animals were killed by cervical dislocation, and their knee

joints removed and embedded in gelatin prior to freezing in *n*-hexane at -70°C .⁸ Cryostat sections were prepared at a thickness setting of $18\mu\text{m}$ in a Bright's cryostat fitted with a Jung rotary microtome and a tungsten-carbide tipped knife.⁹ The sections were picked up on coverslips and incubated for the histochemical demonstration of lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH).¹⁰ Incubation media were made up in 140 mM HEPES buffer containing 32% (w/v) polyvinyl alcohol (grade G04/140).^{11 12}

LDH media contained: 10 mg/ml sodium-DL-lactate, 2 mg/ml NAD, 3 mg/ml neotetrazolium chloride, and 0.2 mg/ml phenazine methosulphate, at a final pH of 8.0. SDH media contained 10 mg/ml sodium succinate, 3 mg/ml BPST (a new tetrazolium salt),^{13 14} and 0.2 mg/ml phenazine methosulphate, at a final pH of 8.0. Both media were saturated with oxygen-free nitrogen before use. (SI conversion: g/l=mg/ml.)

Incubations were performed at 37°C in Perspex rings placed over the sections. The rings were closed with coverslips during the incubations. After 10 minutes the rings were removed from the sections, which were allowed to drain vertically in a rack. The reactions were stopped by carefully pouring a solution of polyvinyl alcohol, at a pH of about 2, over the sections (the sections were found to disintegrate after conventional washing in water). The sections were mounted in an aqueous mountant, such as Farrant's, prior to examination.

Results

Preliminary results¹⁵ had indicated the presence of inactive chondrocytes in that region of the articular

cartilage where the osteoarthritic lesion always begins. By contrast, both LDH and SDH activities were generally evenly distributed throughout the condyles of control mice and also in the lateral condyles of the disease-prone mice. Fig. 1 shows the development of such an inactive region in 7 serial sections from a 16-week-old STR/ORT mouse, with

no apparent signs of degenerative changes in terms of surface fibrillation. The sections were alternately reacted for LDH and SDH.

In Fig. 1 (a) LDH activity is evenly distributed. Fig. 1 (b) shows SDH activity, with a small region of inactivity. This region has increased in size in Fig. 1 (c), which shows LDH activity, although one active

Fig. 1 LDH and SDH activities in serial sections of the medial tibial condyle of a 16-week-old STR/ORT mouse ($\times 340$). (a) LDH; (b) SDH; (c) LDH; (d) SDH; (e) LDH; (f) same field as in (e) but photographed with the condenser diaphragm stopped down; (g) SDH; (h) LDH.

Table 1 Inactive LDH/SDH regions in osteoarthritis-prone mice of various ages

Age (weeks)	Osteoarthritis grade*	Inactive LDH/SDH region
2½	0	NO
3	0	NO
5	0	NO
7	0	NO
8	0	NO
9	0	NO
9	0	YES
9	0	YES
10	0	NO
11	1	YES
12	0	NO
12	2	YES
12	2	YES
13	0	YES
13	0	NO
13	2	YES
14	1	YES
15	0	NO
16	0	YES
16	1	YES
16	1	YES
17	2	YES
17	1	YES
17	1	YES
18	1	YES
19	3	YES
20	0	YES
23	2	YES
28	2	YES

*Arbitrary grading based on: 0 = normal; 1 = slight fibrillation; 2 = more extensive fibrillation; 3 = chunks of cartilage lost; 4 = complete loss of cartilage down to subchondral bone.

cell remains. The inactive region spreads further across the condyle in subsequent sections. Fig. 1 (f) shows the same field as in Fig. 1 (e), but photographed with the condenser diaphragm stopped down. It shows quite clearly that cells are still present in the inactive region. This effect has been observed several times in the cartilage from the medial tibial condyle of STR/ORT mice, but never in cartilage from the lateral condyle of the same animal, or from age-matched control mice. Table 1 gives some data on the appearance of this effect with age and degree of severity of osteoarthritis.

The findings in the medial tibial cartilage can therefore be summarised as follows: (i) all animals with some degree of osteoarthritis had an inactive region; (ii) some animals with no apparent osteoarthritis (grade 0) also had an inactive region; (iii) inactive regions were not observed in animals under 9 weeks of age.

Discussion

The first histological change which is seen to occur in the STR/ORT mice is a loss of staining in the deeper zones of the cartilage, due to a loss of mucopolysaccharide.³ The affected region of cartilage then

becomes progressively acellular, and fibrillations appear in the cartilage surface. The results presented here suggest that there is a time in the early stages of this process, perhaps before cell death occurs, when the chondrocytes have lost some of their enzyme activity. Fig. 1 (e) shows a large completely inactive region in an otherwise active population of chondrocytes. Fig. 1 (f) shows that this inactive region still contains cells in their lacunae. It is tempting to suggest that this enzyme depletion is a very early metabolic change, which precedes any histological change, though this has yet to be confirmed. Other enzymes have not yet been tested, so it is not known to what extent this inactivity applies to other reactions. However, a depletion of LDH alone could account for matrix degeneration, as is indicated in Fig. 2.

Articular cartilage has a glycolytic rate comparable to that of other tissues, but its oxygen uptake is very low.^{16 17} Chondrocytes therefore depend largely on anaerobic respiration for the reoxidation of NADH generated during glycolysis, that is, on the lactate dehydrogenase reaction. Apart from its requirement in glycolysis, NAD is also specifically required as a coenzyme for 2 oxidative reactions involved in the biosynthesis of UDP sugars needed for glycosaminoglycan (GAG) production, namely, UDP glucose dehydrogenase and UDP glucose epimerase. The key role of lactate dehydrogenase in this sequence now becomes clear. If this enzyme is lacking, NADH will build up, and glycolysis, and glycosaminoglycan

Fig. 2 Interrelationships between glycolysis and UDP sugar metabolism, showing the dependence of glycosaminoglycan (GAG) biosynthesis on a supply of NAD generated by lactate dehydrogenase (LDH).

biosynthesis, will cease. In this context it is of interest that the intra-articular injection of iodoacetate, a potent inhibitor of glycolysis, has been shown to induce an experimental osteoarthritis in hens.¹⁸ Furthermore, it has been shown that NADH is a potent inhibitor of the UDP glucose epimerase reaction,¹⁹ so even if there should be sufficient NAD available in the cell this reaction would still be affected owing to the inhibitory action of the accumulated NADH. One may then postulate that the reduced biosynthetic capability of the chondrocytes will lead to matrix weakness, and breakdown under even slightly increased stresses. Repair of such early degenerative changes would be impaired, and osteoarthritis would gradually develop.

Recently, and independently, a similar focal loss of oxidative enzyme activity has been reported to occur in the knee joint cartilage of osteoarthritic mice of the C57B1 strain.²⁰ This loss of activity extended to lactate dehydrogenase, succinate dehydrogenase, NADH diaphorase, glucose 6 phosphate dehydrogenase, NADPH diaphorase, cytochrome oxidase, and UDP glucose dehydrogenase, and seems to represent a far-reaching metabolic disturbance.

Studies are at present in progress to assess the viability of the chondrocytes in the inactive regions in the light and electron microscope.

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