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The influence of doxycycline on articular cartilage in experimental osteoarthrosis induced by iodoacetate

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The experiment was performed on 36 Wistar rats. On the first day of the experiment iodoacetate was administered to the left posterior knee joint of the 18 rats which composed Group I. The second group of 18 rats received additionally doxycycline (doxy) through the gastric tube in doses comparable with those of doxycycline used in humans. The experiment lasted 21 days. The animals were sacrificed after 7, 14 and 21 days in groups of 6 rats each. In sections stained with Safranin 0 semiquantitative histochemical intensity tests were performed on articular cartilage glycosaminoglycans (GAG) using a four-point scale (0–3). In the first group examined destructive lesions in the articular cartilage and weak reactivity on GAG were noted at all stages of the experiment. The intensity of GAG staining was higher in the second group after 14 and especially after 21 days, which may suggest a protective action of doxy on articular cartilage.

Key words: osteoarthrosis, mono-iodoacetate, doxycycline

INTRODUCTION

Osteoarthrosis (OA) is a degenerative disease of the joints, the earliest changes being characterised by cartilage GAG degradation, inhibition of their synthesis and impairment of the structure of the collagen fibres [1]. One of the main factors mediated in the cartilage destruction is increased activity of matrix metalloproteinases (MMP) [2–5]. Inhibition of MMP activity and/ /or their synthesis would be a mode of casual OA therapy [6, 7]. Tetracycline antibiotics and their semi- synthetic forms (doxy and monocycline) significantly inhibit MMP activity. Doxy was found to reduce the intensity of experimental OA in animals [7, 8], although the protective action of doxy was not effective in other investigations [9]. The aim of this study was, then, to assess the influence of doxy on articular cartilage GAGs in an experimental model of OA induced by intraarticular injection of mono-iodoacetate (MIA).

MATERIAL AND METHODS

The experiment was carried out on 36 female rats of the Wistar strain divided into 2 groups. In Group I (18 rats) 3.0 mg of MIA in 0.1 ml 0.9% NaCl were injected under chloroform anaesthesia into the left knee joint. In Group II (18 rats) MIA was injected in the same manner and over the following days a solution of doxy was administered by gastric tube in a dose of 3 mg/1000 mg body weight daily, which was equivalent to the doses given to humans. After 7, 14 and 21 days 6 rats from both groups were sacrificed by intramuscular injection of Rometar and Narkamon.

For the histological and histochemical assessment the knee joints were immediately disarticulated, opened through a lateral incision, fixed in 10% buffered formalin and, after decalcification in EDTA, embedded in paraffin. Sections of 6 μm thick were

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Table 1. Morphological changes in the cartilages of rats with experimental OA treated by doxy

Type	Control knees			Group I After MIA injection			Group II MIA and doxy		
Days	7	14	21	7	14	21	7	14	21
Score of histological changes	0	0	0	2.6	2.8	2.1	2.7	2.4	1.8
Score of GAGs in cartilage/matrix	2.3	2.5	2.7	0.3	0.7	0.5	0.5	0.6	1.6*
Score GAGs in chondrocyte	2.6	2.8	2.8	0.6	1.2	1.6	0.7	1.3	2.2**

*I/II $p < 0.01$; **I/II $p < 0.001$

cut perpendicular to the surface and stained with haematoxylin and eosin and Safranin 0 — fast green (S). The histological changes were graded according to severity by points ranging from 0 (normal cartilage) to 3 (the most marked defects). GAG reaction was scored on a scale of 0–3 where 0 = negative staining, 1 = weak positive, 2 = positive, 3 = strong positive reaction. The mean index (score) for each joint and mean group score were calculated from the sum of successive fields of vision. The results were analysed using the statistical program Statgraphics.

RESULTS

The articular cartilage of the right knee in the control group was histologically intact. GAG staining was positive in all layers of cartilage matrix and in the majority of chondrocytes on days 7, 14 and 21. In the first group of rats with intra-articular MIA injection varying degrees of damage to the articular cartilages were observed in all the experimental periods. On day 7 the cartilages were generally thinner. There were numerous fissures, fibrillations and defects on the cartilage surface with concomitant small horizontal ruptures. A considerable focal or more diffuse decrease in GAG staining was observed in the cartilage as a whole.

At day 14 after injection of MIA the microscopic changes were similar. Focal and diffuse depletion of GAG staining was seen in almost all cases but reparative activity could be observed in the form of chondrocytes in clones, which showed increased reactions of GAG in the pericellular matrix. In the synovial membrane a slight infiltration of mononuclear cells was seen.

On day 21 the cartilages revealed a wide spectrum of alterations in structure, cellular composition and GAG content. Apart from areas of severe cartilage damage, reparative activity could be observed in the form of chondrocyte proliferation in clones and clusters, fibrous connective tissue pannus, especially on the periphery of the cartilage, or osteophyte-like changes.

In the second experimental group of rats similar qualitative microscopic changes were observed but with slightly less intensity, especially on the 14th and 21st days of the experiment. These changes were manifest in the somewhat greater intensity of S staining in the cartilage matrix and in the vicinity of the chondrocytes.

The results of the semiquantitative morphometric intensity assessment of the histological and histochemical stainings are shown in Table 1.

DISCUSSION

MIA-induced experimental OA can reproduce OA lesions as observed in humans. The method is easy to perform and provides an opportunity of observing a complete sequence of morphological changes from joint destruction to reparative processes in a relatively short time. It also provides a useful model by which to evaluate the therapeutic influence of chondroprotective drugs [10]. In our study the intensity of histological and histochemical changes was similar in rats of Group I and Group II after 7 and 14 days of experiment. After 21 days the intensity of GAG staining in the rats of Group II, those treated with doxy, was more pronounced both in the cartilage matrix and the chondrocytes. The results of our study suggest that doxy may have some protective action upon cartilage degradation but at a rather late stage. The results of the study with respect to morphological bases must be interpreted with care. In the light of current concepts concerning pathogenesis, some agents which inhibit MMP release can inhibit the development of OA [6–8]. Alternatively, the more pronounced histochemical staining of GAG on 21 day of our study may be related to stimulation of chondrocyte proliferation in the course of cartilage repair. Biochemical tests which are currently being carried out may elucidate this problem.

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