Acta orthop. scand. *53*, 17–21, 1982

OSMIUM TETROXIDE IN ANTIGEN-INDUCED ARTHRITIS: FAILURE TO INHIBIT JOINT DESTRUCTION

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Antigen induced arthritis (AIA) was elicited in 28 adult rabbits using ovalbumin. All animals developed an intensive monarthritis manifested by local heat and swelling. Two, 7 and 21 days later, respectively, 0.3 ml of 1 per cent OsO_4 was injected into the arthritic knees of three groups of 9 rabbits. The rabbits were killed 1, 3 and 6 months later. No modifying influence on the severe to complete cartilage destruction was evident. One group of 8 rabbits received only OsO_4 and the cartilage of these animals remained intact after 6 months.

Key words: antigen induced arthritis; cartilage degeneration; osmium tetroxide

Accepted 22.iii.81

The antigen-induced arthritis (AIA) model introduced by Dumonde & Glynn (1962) has been studied extensively and found to mimic in many respects the immunopathology of human rheumatoid arthritis (Cooke & Jasin 1972, Cooke et al. 1972), with local persistence of antigen and immune complexes in the cartilage.

Osmium tetroxide is used in chronic arthritis in humans as an alternative to surgical synovectomy (Berglöf 1959, Boussina et al. 1974, Nissilä et al. 1977b). However, reports of harmful influences of OsO_4 on rabbit cartilage (Menkes et al. 1972, Mitchell et al. 1973) have led to warnings against its use in humans (Mitchell et al. 1973). In a previous report (Ahlberg et al. 1978) we could not confirm the adverse effects of OsO_4, using light microscopy, up to 12 weeks after injection, but on the other hand we did not find that OsO_4 offered any protection against AIA if given 4 weeks after the start of the arthritis. It was therefore felt to be worthwhile to extend these studies in two ways: one was to introduce the OsO_4 earlier in the course of the AIA and the other was to follow the animals for 6 months to detect any late cartilage damage. This paper presents further evidence for the lack of major toxicity to rabbit cartilage and also for the lack of protection against tissue destruction in AIA.

Financial support was obtained from Alfred Österlund's Foundation and Riksförbundet mot Reumatism.

MATERIAL AND METHODS

The material consisted of 36 full-grown rabbits (white New Zealand), weighing 2900 to 5050 g (mean 3690 g). They were caged under normal conditions and kept on a normal diet.

Eight rabbits were given 0.3 ml of 1 per cent OsO_4 in the right knee and 0.3 ml saline in the left knee. Three animals were killed after 1 month, 3 after 3 months and 2 after 6 months.

In the remaining 28 animals, an antigen-induced arthritis (AIA) (Dumonde & Glynn 1962) was produced as follows. Ovalbumin*, dissolved in saline to a concentration of 20 mg/ml was emulsified with an equal

* From Sigma, St. Louis, USA

0001-6470/82/010017-05 $02.50/0

volume of Freund's adjuvant**. One ml of this emulsion was injected subcutaneously into five different spots (0.2 ml in each) in the area between the scapulas. The injection was repeated after 3 weeks. Two weeks later 0.25 ml of the ovalbumin solution (5 mg) was injected into the right knee.

After 2 days 10 animals were given 0.3 ml of 1 per cent OsO_4 in the right knee and 0.3 ml saline in the left knee. Nine animals were given the same injections after 1 week and 9 after 3 weeks.

In each group 3 rabbits were killed 1 month after the intra-articular OsO_4 injection. Three rabbits were killed after 3 months and 3 or 4 after 6 months.

All animals were killed with an overdose of Nembutal® i.v. Both knee joints were removed and fixed in 10 per cent formalin. The tibia and the femur were divided in the frontal plane into two halves. These halves and the patella were decalcified in 40 per cent formic acid. The specimens were embedded in paraffin and cut into sections (5–7 μ) and the sections were stained with haematoxylin-eosin.

A grading system was devised for the microscopical changes in the articular cartilage and the subchondral bone.

Grade 0. No changes in the articular cartilage or the subchondral bone and no synovitis.

Grade 1. Synovitis of the synovial membrane but no changes in the articular cartilage or the subchondral bone.

Grade 2. Sparse areas of necrosis of the chondrocytes, flaking and/or fibrillation. Subchondral osteoblastic activity. Possibly pannus growing over the cartilage from the margins and in the area around the attachments of the cruciates. Synovitis.

Grade 3. As grade 2 but frequent death of the chondrocytes down to the calcification line (tidemark). Pannus growing over the cartilage surface from the margins, where osteophyte formation can be discerned.

Grade 4. As grade 3 but large parts of the cartilage are dead and pannus is growing from the margins, "eating" the cartilage. Occasional growth of a vessel into the columnar layer of the cartilage. Pronounced osteophyte formation and sometimes thickening of the subchondral bone.

The microscopic slides were read blind.

RESULTS

Figures 1–5 show examples of the varying degrees of joint changes observed. Repetitive

** Difco Lab., Detroit, Michigan, USA

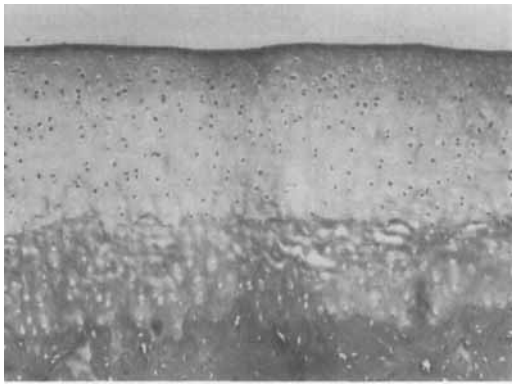

Figure 1. Normal rabbit knee cartilage from control joint, corresponding to grade 0. Objective ×6.3.

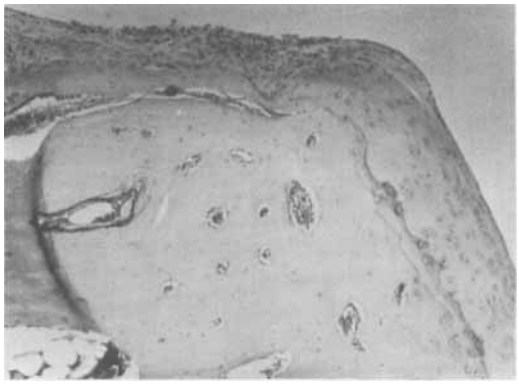

Figure 2. Grade 1 changes showing synovitis but intact cartilage and subchondral bone. Objective ×6.3.

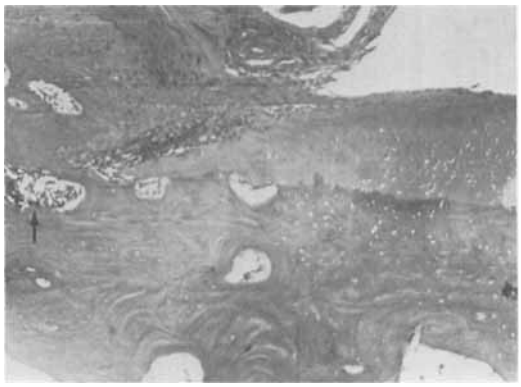

Figure 3. Grade 2 changes, with pannus, cartilage reduction and osteoblastic activity (see arrow) in the subchondral bone. Objective ×6.3.

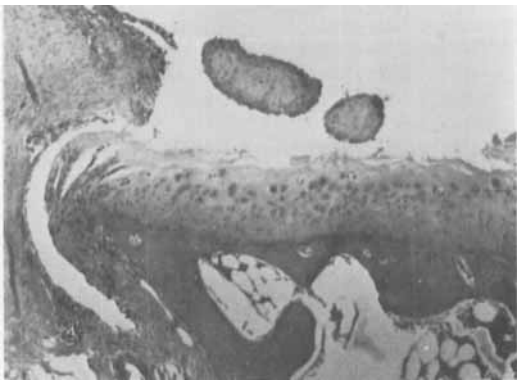

Figure 4. Grade 3 changes, with areas of cell death, fibrillation of the cartilage and osteoblastic activity in the subchondral bone. Objective ×6.3.

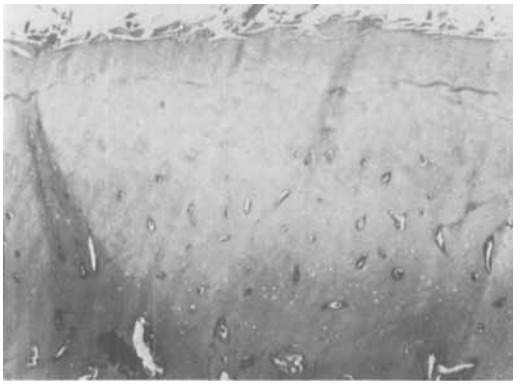

Figure 5. Grade 4 changes with wide-spread cartilage necrosis and disintegration. The subchondral bone has an increased thickness. Objective ×6.3.

examinations of the same joint preparation gave a consistent result never differing more than one grade. There was no difficulty in distinguishing grade 0 from the rest. All control (left) knees were normal with the exception of one in the group of animals that received OsO_4 3 weeks after the onset of AIA and were killed after 6 months. In this animal there was a disturbed arrangement of the chondrocytes, pannus was growing from the margins over the cartilage surface and subchondral osteoblastic activity was seen. The results of all right knee joint examinations are shown in Table 1. It is apparent that only insignificant changes were observed in the control (OsO_4) animals. All but one animal with AIA showed marked to maximal cartilage destruction. The tendency to slightly less destruction in the group injected 7 days after induction is not significant.

DISCUSSION

AIA continues to be a fruitful model for immunologically mediated arthritis (Ahlberg et al. 1978). Two recent reports from Australia (Lowther et al. 1978, Sandy et al. 1980), investigate in detail the early phase of events with regard to chondrocyte function, in particular proteoglycan content and synthesis. Evidence for marked depletion in the weight-bearing parts of the cartilage and cell death in its superficial layer, as early as 2–7 days after induction, have been presented. It was known earlier that immune complexes were located in the deep layers and persisted for long periods (Cooke et al. 1972). Similar findings have been made in human rheumatoid arthritic cartilage (Cooke et al. 1975). Thus the choice of AIA as a model for human disease seems warranted.

In our previous work (Ahlberg et al. 1978) we

Table 1. Cartilage changes 1, 3 and 6 months after the injection of 0.3 ml 1 per cent OsO_4 into the right knee of control rabbits and 2, 7 and 21 days after inducing AIA with ovalbumin. The figures 0 to 4 refer to the grading system as described in the text and in Figures 1–5. Figures within brackets are means of the group

Pretreatment	1 month	3 months	6 months
None	0, 0, 2 (0.67)	0, 1, 0 (0.33)	0, 0 (0)
AIA 2 days	2, 4, 1 (2.67)	4, 3, 4 (3.67)	4, 4, 4, 4 (4)
AIA 7 days	2, 3, 3 (2.67)	3, 3, 3 (3)	3, 0, 4 (2.33)
AIA 21 days	4, 3, 4 (3.67)	3, 4, 4 (3.67)	4, 4, 3 (3.67)

were unable to show any protective action of OsO_4 in rabbit AIA. Menkes (1972), on the other hand, had found the acid to be beneficial in carragenin arthritis in rabbits. The present data show that the inability to halt the progression of the AIA probably is not due to timing; OsO_4 given only 2 days after the antigen did not prevent the joint destruction from progressing unmodified. One explanation could be that the dose was too small. It was chosen to correspond to that used in human disease, but was probably not effective in controlling inflammation. That the early cartilage damage, which is severe and involves chondrocyte necrosis (Lowther et al. 1978, Sandy et al. 1980), still may be influenced in a favourable direction, is shown by our earlier results with ^{198}Au (Ahlberg et al. 1978), and by work with 5-fluoracil (Steinberg et al. 1971).

Our results with regard to damage to normal knee cartilage caused by OsO_4 are uniformly negative even after 6 months. Although only light microscopic examination was employed, any significant chondrocyte necrosis should have shown up. These results are at variance with those of Goldberg et al. (1976) and Mitchell et al. 1973). These authors, however, used larger volumes and doses, which might have caused increased intraarticular pressure. Menkes et al. (1972), furthermore, found young animals to be more sensitive, and arthritic cartilage to be less sensitive, to OsO_4 damage than normal adult cartilage. The tentative conclusion from this study then is that OsO_4 in doses corresponding to those used in humans probably does not cause significant joint damage.

This is in agreement with current clinical evidence (Nissilä et al. 1978) showing the results of OsO_4 treatment to be comparable to surgical synovectomy and ^{90}yttrium. A biopsy study (Nissilä et al. 1977a) in 18 patients 1–18 months after osmium injections showed only minor light microscopic differences compared with controls, although electron microscopy revealed increased amounts of cell debris. The perilacunar matrix and collagen fibres at the surface, however, appeared normal. It was not possible to determine whether this superficial cartilage damage will have any clinical consequences.

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