Acta orthop. scand. 48, 263–265, 1977

THE EFFECT OF OSTEOTOMY AND CARTILAGE DAMAGE ON MITOTIC ACTIVITY

An Experimental Study in Rabbits

O. JOHNELL & H. TELHAG

Department of Orthopaedic Surgery, Malmö General Hospital (University of Lund), Malmö, Sweden.

In 15 rabbits, osteotomy and osteotomy including cartilage damage were performed. With autoradiography (^{3}H-thymidine) it was shown that only one knee in the osteotomy group had labeled chondrocytes in the tibial cartilage. In the knees with articular damage, labeled chondrocytes were found in the femur as well, which could be the result of a factor liberated from the damaged cartilage, a factor stimulating mitotic activity.

Key words: cartilage; mitosis of chondrocytes; autoradiography; chalones

Accepted 27.i.77

Mitotic division of chondrocytes has never been demonstrated with any certainty in normal adult joint cartilage from animals or human beings. When a well-defined zone with calcified cartilage has been formed basally in articular cartilage and when "tidemark" has developed, the chondrocytes cease to divide (Mankin 1968, 1970, Hulth et al. 1972, Telhag 1972). Previous investigations have shown that local traumatization of articular cartilage gives rise to local repair. The chondrocytes around the defect recover their ability to take up ^{3}H-thymidine, i.e. to divide (De Palma et al. 1966). Havdrup et al. (1975) have shown that when the patella is scored chondrocytes in the femur and the tibia can also take up tritiated thymidine. In the same investigation they found that after arthrotomy of the rabbit's knee without cartilage damage some chondrocytes were labeled with tritiated thymidine.

The aim of the present investigation was to determine the effect of osteotomy of the tibia and cartilage damage without arthrotomy and compare this with pure osteotomy of the tibia.

MATERIAL AND METHODS

Fifteen full-grown rabbits were used (gray Silver). The animals were divided into three groups. All the animals were operated upon under intravenous Nembutal anesthesia (Abbott), under sterile conditions. In group 1, a medial incision was made in the right knee over the medial tibial condyle and from there an osteotomy of the tibia was made about ½ cm from the proximal end of the tibia, by sawing the condyle halfway to the lateral side (Figure 1). The left knee joint served as a control. In

Financial support was obtained from Svenska Läkarsällskapet, Herman Järnhardts Stiftelse and Riksförbundet mot Reumatism.

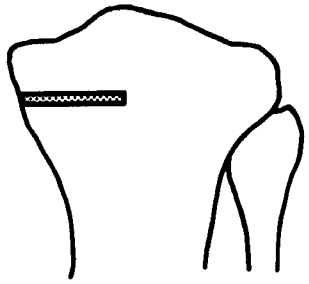

Figure 1. Osteotomy in the right knee.

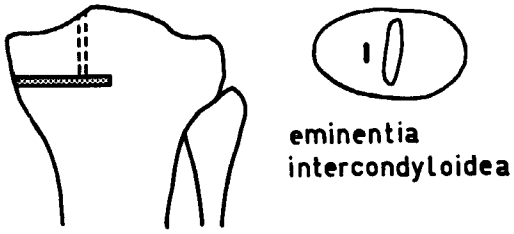

Figure 2. Osteotomy and cartilage damage in the left knee.

group 2, the same procedure was carried out as in group 1 in the left knee, but after making the osteotomy the articular cartilage was damaged for a length of 2 mm through the osteotomy line with a dentist's drill. Precautions were taken not to damage the femoral condyle (Figure 2). At no time was the synovial membrane cut. The right knee served as a control. In group 3, the right knee was operated upon as in group 1 and the left as in group 2.

Fourteen days after the operation, the animals were killed by an i.v. overdose of Nembutal. Six hours before sacrifice 40 μCi ^{3}H-thymidine was injected into each knee joint. X-rays were then taken to identify the osteotomy and to find out whether the epiphyseal line was closed. Both knee joints were removed and fixed in 10 per cent formalin. The tibia and the femur were dissected free and treated separately. With a circular saw, the tibia and the femur were divided into two halves in the frontal plane. These halves were then decalcified in 40 per cent formic acid. The specimens were embedded in paraffin and cut into sections (4–7 μ). The sections were stained with hematoxylin eosin, according to van Gieson and with toluidine blue. Autoradiograms of routine histological specimens from both knees were prepared according to the dipping method with Ilford K2 liquid emulsion. After exposure of the specimens for 3 weeks, the autoradiograms were developed in Gevaert X-ray developer G 230 and fixed in Gevaert X-ray fixer G 305. The preparations were stained through the emulsion with Mayer's hematoxylin.

RESULTS

Histological examination of specimens from two animals in group 3 revealed small signs of the epiphyseal line but the animals had a well-defined layer of calcified cartilage and a "tidemark" could be found.

In group 1, where osteotomy was performed in the right knee, two labeled chondrocytes were found in one knee in the tibia. However, no labeled chondrocytes could be found in the other right knee joints or in the left unoperated knee. The articular cartilage showed no signs of degeneration, such as death of the chondrocytes, flaking, or fibrillation. No clusters were seen. At the margins, proliferation of cells in and near the periosteum could be seen and in addition osteoblastic activity with new bone formation.

In group 2, where osteotomy was performed and the cartilage damaged, two operated left tibias showed 1–3 labeled chondrocytes and two left femurs 1–8. The labeled chondrocytes in the tibia and femur were from the same knee. No labeling of the chondrocytes was found in the right unoperated knee joints. At the margins, the same picture was seen as in group 1.

In group 3, three left tibias showed 1–4 labeled chondrocytes and one left femur showed two. In the same group, one right tibia showed one labeled chondrocyte and one femur showed two. These labeled chondrocytes were from different knees.

Histologically, a few clusters were found around the damaged cartilage. Around the defect, there was fibrillation of the cartilage as well as necrosis of the chondrocytes close to the injury. The defect was filled with fibrous tissue. No labeled chondrocytes were found in the vincinity of the cartilage defect.

DISCUSSION

Previous investigators have shown that the ability of the chondrocytes to take up ^{3}H-thymidine, i.e., to divide, decreases with advancing age and when a well-defined zone with calcified cartilage can be found and when "tidemark" has developed, the chondrocytes cease to divide (Mankin 1968). Such tidemarks were found in all the joint cartilages in the present investigation, although residues of the epiphyseal line were found in two animals. All the epiphyseal lines were roentgenographically closed.

In a previous investigation, Hjertqvist & Lemperg (1971) produced an osteochondral defect in the femoral head in rabbits. They could not find any degenerative cartilage changes outside the defect after 20 weeks. However, after 40–52 weeks degeneration of the cartilage was seen.

Reimann (1973) performed osteotomy combined with displacement of the tibia condyles in rabbits, and the animals were killed 12 weeks later. The histological examination showed degenerative cartilage changes, but no signs of osteophytes.

In this investigation, the articular cartilage did not reveal any changes after 2 weeks, except for local changes around the cartilage injury. This is in agreement with the findings of Wigren & Olerud (1973) who removed a part of the medial femoral condyle and fixed it back with a metal screw.

Havdrup et al. (1975) have shown that arthrotomy after scoring of the patella gave mitotic activity not only in the patella but also in the tibia and the femur in the same knee, suggesting that a factor stimulating mitotic activity had been released. In the articular cartilage of the contralateral knee, where only arthrotomy was performed, some chondrocytes were labeled with ^{3}H-thymidine. In an earlier investigation, Telhag (1973) could not find any mitotic activity after arthrotomy alone. In this study we found sparse labeled chondrocytes also in the femur, which supports the theory that when the cartilage is damaged, a factor is liberated, which stimulates mitotic activity. This factor might be a proteolytic enzyme from the injured cartilage, a factor that has a chalone effect, i.e., an effect resulting in reducing the concentration of the cell specific inhibitors of the mitotic activity in normal adult joint cartilage.

REFERENCES

De Palma, A. F., McKeever, C. D. & Subin, D. K. (1966) Process of repair of articular cartilage demonstrated by histology and autoradiography with tritiated thymidine. *Clin. Orthop.* **48**, 229–242.

Havdrup, T., Hulth, A. & Telhag, H. (1975) Scattered mitoses in mature joint cartilage in rabbits after local trauma. *Clin. Orthop.* **113**, 246–248.

Hjertqvist, S. & Lemperg, R. (1971) Histological, autoradiographic and microchemical studies of spontaneously healing osteochondral articular defects in adult rabbits. *Calcif. Tiss. Res.* **8**, 54–72.

Hulth, A., Lindberg, L. & Telhag, H. (1972) Mitosis in human osteoarthritic cartilage. *Clin. Orthop.* **84**, 197–199.

Mankin, H. J. (1968) The effect of aging on articular cartilage. *N.Y. Acad. Med. Bull.* **44**, 545–552.

Mankin, H. J. (1970) The articular cartilage: a review. AAOS Instructional Course Lectures Vol. XIX, 204–224.

Reimann, I. (1973) Experimental osteoarthritis of the knee in rabbit induced by alteration of the load-bearing. *Acta orthop. scand.* **44**, 496–504.

Telhag, H. (1972) Mitosis of chondrocytes in experimental "osteoarthritis" in rabbits. *Clin. Orthop.* **86**, 224–229.

Telhag, H. (1973) DNA-synthesis in degenerated and normal joint cartilage in fullgrown rabbits. *Acta orthop. scand.* **44**, 604–610.

Wigren, A. & Olerud, S. (1973) Reincorporation of an avascular articular surface bearing fragment. *Upsal. J. med. Sci.*, Suppl. 14.

Correspondence to: Hans Telhag, M.D., Department of Orthopedic Surgery, General Hospital, 21401 Malmö, Sweden.