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Influence of trypsin on the regeneration of hyaline articular cartilage

A purely chondral lesion was inflicted at the medial or lateral femoral condyle of the knee joints of 54 adult male rabbits in order to study hyaline cartilage regeneration. The experimental group was given a sequential intraarticular instillation of trypsin and autologous blood, the control groups were given trypsin or blood or nothing. Only the experimental group (trypsin and autologous blood) showed hyaline cartilage regeneration in 2/3 of the cases examined. Apparently neither the healthy cartilage nor the synovial membrane suffered damage from the trypsin injection.

A possible mechanism of cartilage regrowth may be the inhibition of chalones by the fermentative effect of trypsin, which stimulates the aggregation of thrombocytes and fibrin from the injected blood on the chondral lesion, thus initiating the regeneration of hyaline cartilage.

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Recent studies (Zucker-Franklin & Rosenberg 1976, 1977) have shown that with trypsin an *in vitro* as well as *in vivo* aggregation of thrombocytes to the collagen fibers of the hyaline articular cartilage may be achieved. Havdrup (1977, 1979, 1982) managed to prove by autoradiography that intraarticularly injected trypsin or papain, stimulates chondrocyte mitoses in animal experiments on healthy cartilage.

Our study aimed to find out if this mechanism could lead to hyaline cartilage regeneration in larger, purely chondral defects.

Material and methods

Fifty-four adult male rabbits, mean weight 4000 g, were used. The animals were kept in wire cages on dry feed and water.

Under intravenous nembutal anesthesia, an arthrotomy was carried out in the right knee joint by a lateral, parapatellar incision. A 5 × 1.5 mm chondral longitudinal defect of the medial or lateral femoral condyle was made. The joint was then rinsed with Ringer solution, followed by precise fascia and skin suture. There was no postoperative fixation of the knee-joint. The rabbits were divided into four groups (one experimental and three control):

Group 1 was injected at 1 week with 5 mg trypsin in 1 ml isotonic solvent intraarticularly into the arthrotomized joint; 1 week later, 1 ml of their own fresh blood was injected intraarticularly.

Group 2 was given only trypsin without subsequent blood injection.

Group 3 was injected with 1 ml fresh autologous blood 2 weeks after arthrotomy

Group 4 had no instillation at all after arthrotomy.

Four weeks after arthrotomy the animals were killed by an intravenous dose of nembutal. The femoral condyles, the patellas and the synovial membranes were removed and preserved in 10 per cent formalin solution. The tissues removed were embedded in paraffin. The histologic sections were then stained with hematoxylin-eosin or Alcian blue, and evaluated. (Table 1).

Table 1. Histologic effect of trypsin

Group	Trypsin	Aut. blood	No. of animals	Cartilage regeneration		
				Good	Medium	Poor
1	+	+	12	4	4	4
2	+	0	9	0	1	8
3	0	+	8	0	0	8
4	0	0	12	0	0	12

Results

In none of the rabbits was a toxic effect of trypsin observed. Seven animals died during anes-

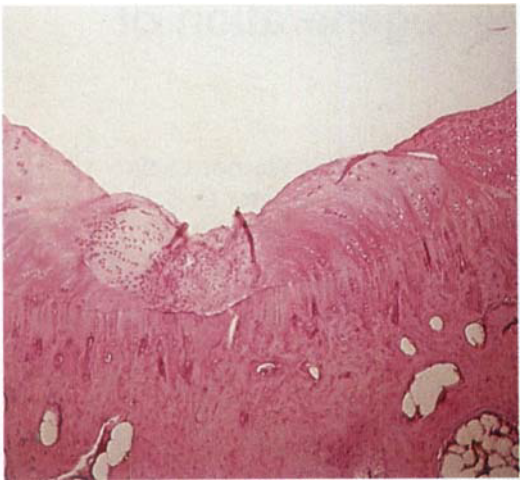

Figure 1. Group 1, 28 days after surgery. Refill of defect by dense chondroid cell tissue, good result. (HE, ×100).

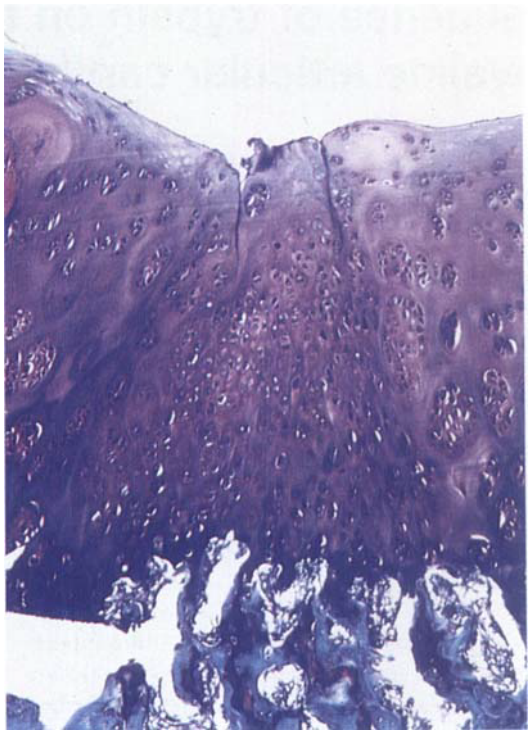

Figure 2. Group 1, 28 days after surgery. Restoration of defect by dense hyaline chondral cell tissue, sporadic capsule formations on surface and edges, subchondral space not opened, good result. (Alcian blue, ×100).

thesia or heart puncture to collect blood, or had to be killed because of automutilation. In six knee joints histologic evaluation was impossible: there were four infections, one defect reaching the subchondral bone was too deep, and one sample did not allow clear histologic identification of the defect.

No irritation of the synovial membrane was observed macroscopically. Compared to the findings after surgery, most of the defects were superficial and could hardly be recognized; there was no distinct macroscopic difference between the groups.

Only in Group 1 (trypsin + blood) had histologic hyaline cartilage regrowth into the chondral defect taken place, in 8 out of 12 animals (Figures 1, 2). In these cases the defect was filled with dense hyaline chondroid tissue, with the partial formation of clusters.

Apart from one animal in Group 2 (treated with trypsin only, 7 days after surgery), no signs of regeneration were observed in any animal in any of the three control groups.

The histologic examination of the patellar cartilage gave no indication of damage to the cartilaginous tissue by the injected trypsin. Histologic evaluation of the stratum synoviale of the articular capsule showed no signs of inflammation; there was no difference between the experimental group and the control groups.

Discussion

In our experiment we observed hyaline cell regeneration only in the group treated with trypsin and blood. This regeneration could not be achieved by trypsin or blood alone, nor in a control group without any treatment. The regrowth, observed macroscopically in a few cases in these groups, histologically consisted of necrotic or synovial tissue.

The fact that hyaline cartilage regeneration normally does not occur is generally explained by avascularity of the cartilage tissue (Otte 1966, Mankin 1982). However, after opening the subchondral space with connection to the vascular system only fibrocartilage regeneration is observed (Mitchell & Sheppard 1976). This fact and the observation that intra-artic-

ular injection of blood does not induce hyaline regeneration (Puhl et al. 1971), as the results of our control Group 3 prove, might indicate that there are other reasons than avascularity for the absence of hyaline cartilage repair. Regeneration of any tissue starts with blood coagulation; during this process platelets will aggregate and release their granule constituents. Later on the neutrophils, together with fairly large numbers of monocytes, appear as the dominant cells in the process of wound healing (Ross 1976). Unlike the wound healing process in connective tissue, in cartilage there seems to be an inhibition at one or more levels of this process. We cannot determine exactly the point of action of this inhibition; the experiments of Zucker-Franklin & Rosenberg (1976, 1977) showed on the ultrastructural level the forming of bridges between platelet membranes and collagen fibrils in various connective tissues, with the exception of articular cartilage where these bridges could be seen only after pretreatment with proteolytic enzymes. This observation suggests that proteoglycans may inhibit the wound healing process in cartilage, as our results confirm. If the inhibition of platelet-activity, macrophages or other blood elements is the essential mechanism, this must be proven by further experiments.

We do not believe the inhibition of mitotic activity of hyaline cartilage cells by chalones is the decisive factor for missing cartilage repair, as we found no signs of regeneration in the group treated only with trypsin and not with blood.

The hyaline repair of a purely chondral defect in our experimental group does not allow any clinical conclusion. Long-term results have to be awaited. No judgment can be made on the proteoglycan content or quality of the regrown chondral tissue. There was no damage to the healthy articular cartilage or synovial membrane by the single injection of trypsin or blood, or both.

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