The effect of hyaluronic acid on cartilage in the immobilized rabbit knee

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Out of 30 adult rabbits, 20 had one knee immobilized with a plaster of Paris cast for 6 or 12 weeks, and 10 rabbits were used as untreated controls. Prior to immobilization, 10 knees were injected with high-molecular weight hyaluronic acid. The articular cartilage of the femoral condyles was studied by light microscopy, whereas that of the patella and tibia was analyzed biochemically. Degenerative changes of the articular cartilage similar to those seen in arthrosis were observed after 6 weeks. The intraarticular injection of hyaluronic acid did not prevent these changes; instead, the reparative processes seemed inhibited.

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Butler et al. (1970) reported that racehorses with traumatic and degenerative arthrosis obtained relief for a longer period of time if intraarticular injections of cortisone contained hyaluronate as compared with cortisone alone. Åsheim and Lindblad (1976) injected only hyaluronate in horse joints and observed a marked improvement. Since then, hyaluronate has been used in veterinary practice. Intraarticular administration of high-molecular weight hyaluronic acid has been claimed to be of some value in human arthrosis (Balazs 1974, Weiss et al. 1981, Namiki et al. 1982).

We studied biochemical and morphologic changes in rabbit knee cartilage after immobilization and after injection of hyaluronic acid.

Material and methods

Thirty adult rabbits—aged 9–10 months, weight 4.7 (4.5–5.3) kg—were used. The right hind limbs of 20 rabbits were immobilized in plaster of Paris (Scotcast) with 15° of flexion of the knee and 10° of dorsiflexion of the ankle. Totally, 0.4 mL (10 mg/mL) high-molecular weight hyaluronic acid (Healon, Pharmacia) was injected into the knee joint in 10 rabbits before immobilization. Ten untreated animals served as immobilized controls, and 10 rabbits served as normal controls without treatment. The rabbits were allowed to ambulate in standard cages. Half the rabbits were killed after 6 weeks, and the other half after 12 weeks. Cartilage for biochemical analysis was cut from the patella and the tibial plateau; from the normal control rabbits, further samples were taken from the hip and the ankle joints. Samples for histologic studies were taken from the femoral condyles. After fixation in 10 percent formalin, the samples from the femoral condyles were embedded in paraffin, sectioned, and stained with hematoxylin and eosin, and Azur B (pH 2 and 4). The biochemical analysis of glycosaminoglycans was performed by the procedure reported previously by Olsen et al. (1989) using cellulose acetate electrophoresis, optic scanning, and specific degradation (chondroitin AC and ABC-lyase, and testicular hyalurondase). Uronic acid analysis was performed with the procedure described by Blumenkrantz and Asboe-Hansen (1973) using glucuronic acid as the standard. The Student's *t*-test was used for evaluation. One normal control animal died.

Results

After 6 weeks of immobilization, the articular cartilage of the femoral condyles in the hyaluronic acid group showed advanced changes, with destruction of the superficial zone, fibrillation, and chondrocyte clusters with decreased metachromasia (Figure 2). The immobilized control knee showed chondrocyte clusters with slight destruction of the superficial zone, but increased metachromasia around the cell clusters in

Figure 1. Articular cartilage from the femoral condyles after 6 weeks of immobilization without hyaluronic acid injection. Note the superficial loss of staining intensity and the increased stainability in the territorial zone, especially around the cell clusters, as compared with the stainability in the interterritorial area. Azur B, pH 2; ×250.

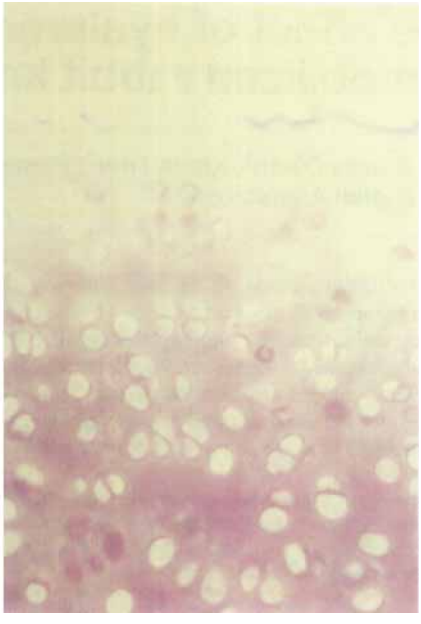
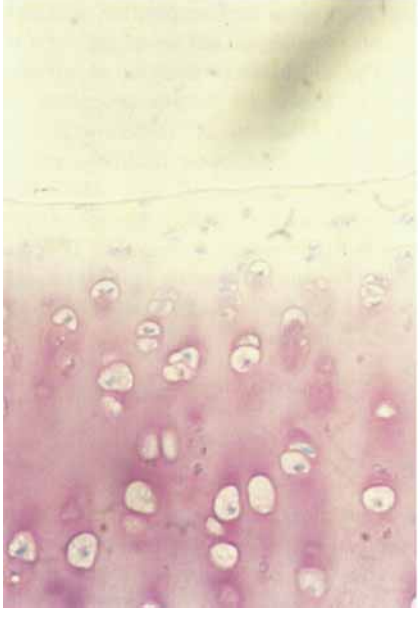


Figure 2. Articular cartilage from the femoral condyles after 6 weeks of immobilization with hyaluronic acid injection. Fibrillation and decrease in intercellular matrix superficially. Loss of normal columnar arrangement of the cells in the transitional zone and no increased stainability around the cell clusters. Azur B, pH 2; ×250 .



Figures 3 and 4. Articular cartilage from the femoral condyles treated without (left) and with hyaluronic acid (right) after 12 weeks of immobilization. Increased stainability around the cell clusters and in the interterritorial area is seen in both samples. Azur B, pH 2; ×250.

Table 1. Changes in the patella and the tibial plateau cartilage due to immobilization for 6 and 12 weeks, with and without hyaluronic acid injection, and articular cartilage from various joints of normal control rabbits. Uronic acid nmol/mg dried, defatted tissue. Dermatan sulfate, hyaluronic acid, chondroitin sulfate, and keratan sulfate as percentages of the total glycosaminoglycan content. Mean, *SEM*

	Hyaluronic acid inj.	Uronic acid		Dermatan sulfate		Hyaluronic acid		Chondroitin sulfate		Keratan sulfate	
Patella (n 5)											
6 weeks	+	89	*15* [a]	7.3	*2*	19	*4* [a]	66	*4* [a]	8.3	*2*
	–	121	*9*	5.7	*1*	13	*2*	75	*3*	5.7	*1*
12 weeks	+	136	*12*	6.1	*2*	12	*1*	71	*3*	8.8	*2*
	–	126	*18*	4.9	*3*	13	*3*	75	*3*	7.6	*1*
Tibial plateau (n 5)											
6 weeks	+	135	*19*	5.1	*1*	11	*2*	67	*3*	17	*3*
	–	178	*15* [a]	3.0	*1*	9.8	*2*	80	*2* [b]	7.3	*2*
12 weeks	+	134	*12*	9.6	*1*	11	*2*	66	*3*	14	*3*
	–	120	18	4.4	*1*	11	*2*	73	*3*	12	*4*
Normal cartilage (n 9)											
femoral head		139	*14*	3.2	*1*	11	*2*	77	*2*	10	*1*
patella		145	*10*	3.9	*1*	12	*2*	73	*2*	11	*2*
tibial plateau		122	*11*	5.3	*1*	12	*2*	70	*2*	13	*2*
talus		61	*12*	11	*3*	13	*1*	63	*4*	13	*3*

[a] $P < 0.05$, [b] $P < 0.01$.

the middle zone (Figure 1). The articular cartilage of the femoral condyles in the remaining animals of the 6-week group appeared normal. After 12 weeks of immobilization, the articular cartilage of the immobilized joints treated with hyaluronic acid was abnormal in 3 out of 5 animals; there were cluster formation, thinning of the cartilage and rounded chondrocytes of the superficial zone, but only slight disintegration of the cartilage (Figure 4). The immobilized controls had similar changes in 4 of 5 cases. (Figure 3).

Biochemical analysis

After 6 weeks of immobilization, the patellar cartilage of knees treated with hyaluronic acid showed a reduced uronic acid content, a decrease in chondroitin sulfate, and an increase in hyaluronic acid (Table 1). In the immobilized control knees, the tibial cartilage showed an increase in uronic acid and an increase in the chondroitin sulfate fraction. After 12 weeks of immobilization, there were only slight biochemical differences between treated/untreated immobilized and normal control animals (Table 1).

In samples of the ankles from normal control rabbits, the talus cartilage showed a twofold decrease in uronic acid content and a twofold increase in the dermatan sulfate fraction.

Discussion

Our investigation confirms the morphologic study by Wigren and Wik (1974). A single intraarticular dose of high-molecular hyaluronic acid could not diminish or inhibit the effect of immobilization on the articular cartilage of normal rabbits joints. Further, the increases in uronic acid and chondroitin sulfate, as seen in the tibial plateau of immobilized control knees after 6 weeks, were inhibited in the hyaluronic acid treated group (Table 1). In the patellar cartilage of the immobilized knee treated with hyaluronic acid, we found degenerative changes accompanied by a sharp drop in uronic acid and chondroitin sulfate, and an increase in hyaluronic acid (Table 1). The more normal appearance of the cartilage, morphologically (Figures 3 and 4) and biochemically (Table 1), after 12 weeks of immobilization may be explained by the fact that plaster of Paris fixation is not rigid enough to inhibit movements of the joint. Wigren and Wik (1974) found no increase in the morphologic changes in the articular cartilage from 8 to 12 weeks of immobilization. Similar observations of the time course of degenerative cartilage changes after immobilization have been made by Evans et al. (1960) and by Friedman and Finsterbush (1973).

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