OSTEOARTHRITIS
and
CARTILAGE

Effect of high molecular weight hyaluronan on cartilage degeneration in a rabbit model of osteoarthritis

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Summary

The effects of high molecular weight hyaluronan (HA) on cartilage degeneration were investigated in a partial menisectomy model of osteoarthritis (OA) in the rabbit knee. This study compared HA80 (0.8×10^6 Da, 1%), HA190 (1.9×10^6 Da, 0.01–1%) and saline. HA (0.1 ml/kg) or saline were injected intra-articularly twice a week immediately after surgery. Degenerative changes in femoral and tibial cartilages were graded histopathologically 2 and 4 weeks after surgery. Two weeks after surgery, HA190, only when used at a 1% concentration, resulted in a dramatic inhibition of cartilage degeneration in both the femoral condyle and the tibial plateau ($P < 0.01$). Two weeks after surgery, the protection against cartilage degeneration was significantly ($P < 0.05$) greater with HA190 than with HA80. Four weeks after surgery, only the femoral cartilage degeneration was significantly and similarly inhibited with HA190 ($P < 0.01$) and HA80 ($P < 0.05$). Scanning electron micrographs of femoral cartilage showed that cartilage degeneration was less severe with HA190 than with saline. These results might suggest that, in the rabbit model, intra-articular administration of higher molecular weight HA is more effective than lower molecular weight HA in inhibiting cartilage degeneration in early OA.

Key words: Hyaluronan, Osteoarthritis, Animal model, Cartilage.

Introduction

Osteoarthritis (OA) is a common joint disease, characterized by degenerative changes in articular cartilage and reactive proliferation of bone and cartilage around the joint. Recent findings indicate that OA is not a 'wear and tear' process, but rather a cell-mediated active process that may result from the inappropriate response of chondrocytes to catabolic and anabolic stimuli. Changes in some matrix molecules of articular cartilage reportedly occur even in early OA [1, 2].

Hyaluronan (HA), a high molecular weight polymer of glucosamine and glucuronic acid residues, is one of the key components of the articular cartilage matrix. A tertiary macromolecular complex of aggrecan, link protein, and HA endow articular cartilage with many specific mechanical properties, including viscoelasticity. HA is also a major component of the synovial fluid, which supplies nutrients to the articular cartilage and lubricates the joints, thereby minimizing friction on the surface of the articular cartilage. Moreover, HA is believed to modulate the function of various types of cells, including articular chondrocytes.

In advanced OA, a significant decrease in HA concentration and molecular size in the synovial fluid has been documented [3, 4]. Because a quantitative and qualitative decrease in the synovial fluid HA is believed to accelerate cartilage destruction in OA, intra-articular HA injection therapy has been widely used in the treatment of OA. Many clinical investigations on HA [5–11] reported symptomatic improvement in OA patients.

The effects of HA on OA have been extensively studied in animal models. Toyoshima [12] described the effect of HA on cartilage degeneration after synovectomy in rabbits. Kitoh *et al.* [13] described

the effect of HA on cartilage degeneration induced by papain injection. Wigren *et al.* [14] documented the effect of HA on cartilage degeneration induced by joint immobilization. Abatangelo *et al.* [15] and Schiavinato *et al.* [16] studied the effect of HA on cartilage degeneration induced by resection of the anterior cruciate ligament of the knee joint. A marked inhibitory effect of HA on cartilage degeneration was observed in all these studies.

Several HA preparations with different molecular weights have been developed and used for intra-articular injection therapy of OA. The molecular weight of commercially available HA ranges from 8×10^5–7×10^6 Da (8×10^5 Da for Artz® from Seikagaku Kogyo Co. Ltd. and Hyalgan® from Fidia Co. Ltd., 7×10^6 Da for Synvisc® from Biomatrix Co. Ltd.). The molecular weight of HA in human synovial fluid ranges from 1.6×10^6–10.9×10^6 Da [3]. Most human and animal studies used a low molecular weight HA (less than 1×10^6) obtained from rooster combs. Few, if any, studies used HA derived from other sources [17]. The present study investigated the effects of a high molecular weight HA (HA190:1.9×10^6 Da), derived from a fermentation process using *Streptococcus equi*. Its efficacy is compared to that of a rooster comb HA (HA80) in an experimental rabbit model of OA.

Materials and methods

ANIMALS AND SURGERY

A total of 72 male New Zealand White rabbits, weighing 2–3 kg, were subjected to the section of the fibular collateral and sesamoid ligaments of the left knee joint and to the resection of a 3–4 mm segment (approximately 30–40%) of the lateral meniscus, according to the method reported by Colombo *et al.* [18]. Sixty (10 groups of six) were treated with HA and 12 (two groups of six), treated with saline were used as controls. Sham operations, where ligaments were resected and the joint space exposed without subsequent partial meniscectomy, were performed on 12 additional animals (two groups of six), used as surgical controls. The basic design of the study and the assignment of animals for treatment and sacrifice are summarized in Table I.

Surgical interventions were performed under sodium pentobarbital anesthesia and in sterile conditions. Animals were housed and maintained in accordance with the NIH guidelines. At the time of death, animals were exsanguinated by carotid dissection while under deep sodium pentobarbital anesthesia.

HYALURONAN AND INTRA-ARTICULAR TREATMENT

HA190 was obtained from Roussel Uclaf Japan Co. Ltd. (Tokyo); it was produced by fermentation using the lactobacillus *Streptococcus equi* and then purified by ultrafiltration and ethanol precipitation. HA190 was an amorphous white powder with an average molecular weight of 1.9×10^6 Da. HA190 was soluble in water, and a 1% solution in physiological saline had a neutral pH (6.8–7.8) and a high viscosity (23.7–29.7 dl/g at 30°C) [19]. HA80 was obtained from Seikagaku Kogyo Co. Ltd. (Tokyo); it was extracted from rooster combs and had an average molecular weight of 0.8×10^6 Da.

HA190 was dissolved in physiological saline at concentrations of 0.01, 0.3, 0.6 or 1% (w/v). HA80 was dissolved in a 1% physiological saline (w/v). The dose injected each time was 0.1 ml/kg of body weight. HA or saline were injected intra-articularly twice a week, immediately after surgery until sacrifice at 2 or 4 weeks.

HISTOPATHOLOGICAL METHODS

After sacrifice, the femoral condyle and the tibial plateau were resected and immediately fixed in 10% formalin buffer (pH 7.4) containing 0.5% of cetylpyridinium chloride. The samples were decalcified in 0.5% EDTA (pH 7.4) and embedded in paraffin. To avoid sampling bias, tissue sections were always collected from the same anatomical site on the condyle and plateau (Fig. 1). The femoral condyle was cut into coronal sections in the plane posterior to the attachment point of the popliteus tendon. The tibial plateau was cut into coronal sections in the plane anterior to the attachment point of the meniscofemoral ligament. Sections were stained with hematoxylin and eosin (H&E) for general morphology or Safranin-O (SO) for proteoglycan staining. All sections were read blindly by one observer. Cartilage degeneration was analyzed using the scoring system developed by Colombo *et al.* [18] with minor changes (i.e. bone cysts and osteochondrophytes were excluded, and fissures and fibrillations were combined). The following eight parameters were graded: loss of superficial layer, erosion of cartilage, fibrillation and/or fissures, loss of stainable proteoglycan, disorganization of chondrocytes, loss of chondrocytes, cluster formation and exposure of subchondral bone. Each item was graded from 1–4 (Table II). The sum of the values of each item was regarded as a global histological score. According to the report of Colombo *et al.* [18], the model induced a lateral knee joint OA but degenerative cartilage changes developed in neither the medial femoral condyle nor medial tibial plateau. Therefore, only the

Table I.
Experimental procedures and animal groups

Group	Treatment	Duration	Concentration (%)	N
HA treated animals				
	HA190, biweekly	2 weeks (four injections)	0.01	6
			0.3	6
			0.6	6
			1.0	6
		4 weeks (eight injections)	0.01	6
			0.3	6
			0.6	6
			1.0	6
	HA80, biweekly	2 weeks (four injections)	1.0	6
		4 weeks (eight injections)	1.0	6
Treatment controls				
	Physical saline	2 weeks (four injections)		6
		4 weeks (eight injections)		6
Total				72

cartilage of the lateral femoral condyle and of the lateral tibial plateau were histologically examined.

SCANNING ELECTRON MICROSCOPY (SEM)

Two representative cartilage specimens from sham operated and saline groups and 1% HA190 treatment groups were examined by SEM. The
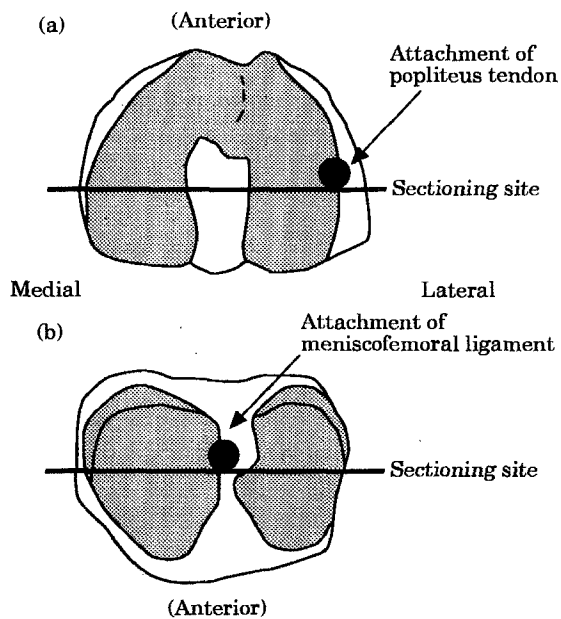

FIG. 1. Histopathologically examined sites of the femoral condyle and tibial plateau. (a) Femoral condyle was cut into coronal sections in the plane just posterior of the attachment of popliteus tendon. (b) Tibial plateau was cut into coronal sections in the plane just anterior of the attachment of meniscofemoral ligament.

articular cartilage was trimmed and its surface washed with physiological saline. The samples were fixed in 2.5% glutaraldehyde and then in 1% osmic acid. After dehydration in ethanol solutions of increasing concentrations, the samples were treated with isoamyl acetate and subjected to critical point drying, using an HCP-2 (Hitachi Co. Ltd., Tokyo). After vapor deposition of gold using an ion sputter (JFC-1100, Nihon Denshi Co. Ltd., Osaka), the samples were examined with SEM (JSM-840, Nihon Denshi).

STATISTICAL ANALYSIS

Histological scores were analyzed by the F-test. If they were found to be of equal variance, the data were further analyzed by Wilcoxon's rank sum test. Differences were considered significant if $P \leqslant 0.05$.

Results

GROSS PATHOLOGY

The resection of only two ligaments did not induce gross or histological OA changes.

Two weeks after surgery, the saline treated animals showed gross cartilage erosion of the lateral femoral condyle and of the lateral tibial plateau, whereas the medial condyle and the plateau cartilage showed no changes. The surface area of erosions tended to be smaller in the 1% HA190 and HA80 treatment groups than in the saline treated group (figure not shown).

Table II.
Method for histopathological evaluation of cartilage degeneration.

	+1	+2	+3	+4
Loss of superficial layer	<Slight	Moderate	Focally severe	Extensively severe
Erosion of cartilage	<Detectable	Moderate	Focally severe	Extensively severe
Fibrillation and/or fissures	<Noticeable (<1 very small)	Moderate (1 small)	Marked (2 small or 1 medium)	Extensive (3 small, 2 medium or 1 large)
Loss of proteoglycan	<Paler stain than control	Moderate loss of safraninophilia	Marked loss of safraninophilia	Total loss of safraninophilia
Disorganization of chondrocytes	Noticeable	Moderate, with some loss of columns	Marked loss of columns	No recognizable organization
Loss of chondrocytes	<Noticeable decrease in cells	Moderate decrease cells	Marked decrease in cells	Very extensive decrease in cells
Exposure of subchondral bone	<Focal exposure of bone	Moderate exposure of bone	Fairly extensive exposure of bone	Very extensive exposure of bone
Cluster formation	<3–4 small, or 1–2 medium	5–6 small, 3–4 medium or 1–2 large	7 or more medium or 5–6 large	7 or more small, 5–6 medium or 3–4 large

HISTOPATHOLOGY

Two weeks after surgery, the cartilage from the lateral femoral condyle and from the lateral tibial plateau of saline-treated animals showed degenerative changes, including: loss of the superficial layer, erosion, fibrillation and/or fissures, loss of stainable proteoglycan, disorganization of chondrocytes, loss of chondrocytes and cluster formation [Fig. 3(a) and (b)]. Exposure of subchondral bone was only rarely seen and the areas were quite small. Four weeks after surgery, the degenerative changes clearly progressed in the cartilage of the lateral compartment, while no change was shown in the medial compartment (Fig. 2).

Two weeks after surgery, cartilage degeneration was mild in the 1% HA190 treatment group, compared to the saline-treated control group, i.e. fibrillation of the femoral cartilage was limited and the tibial cartilage was nearly unchanged [Fig. 3(a) and (b)]. Four weeks after surgery, cartilage lesions in the 1% HA190 group were less severe than in the saline-treated control group [Fig. 3(c) and (d)]. Two and 4 weeks after surgery, degenerative changes of both the tibial and femoral cartilage were observed in the HA80 treated groups. The lesions of the HA80 group were less severe than in the control group, but more severe than in the 1% HA190 group.

GLOBAL HISTOPATHOLOGICAL SCORE

Two and 4 weeks after surgery, the mean global score of the femoral condylar cartilage was

significantly lower ($P < 0.01$) in the 1% HA190 treatment group than in the saline-treated group (Fig. 4). Two weeks after surgery the mean global score of the tibial plateau cartilage was also significantly lower ($P < 0.01$) in the 1% HA190 treatment group than in the saline-treated group. However, the difference between the two groups was not statistically significant 4 weeks after surgery. The mean global score of the femoral condylar cartilage of the animals treated with HA80 and killed 4 weeks after surgery was significantly lower than in the saline group ($P < 0.05$). The mean global scores of the cartilage from both the femoral condyle and the tibial plateau of animals killed 2 weeks after surgery were significantly lower ($P < 0.05$) in the 1% HA190 treatment group than in the HA80 treatment group (Fig. 4).

The protective effect of HA190 on cartilage was dose and site-dependent. When compared with the saline groups, the mean global score of the femoral condylar cartilage, both at 2 and 4 weeks, was significantly lower ($P < 0.01$) with a HA190 only with 1%. An exception was found for 0.6% HA190 at 4 weeks in the samples of the femoral condylar cartilage. With respect to the tibial plateau, a statistically significant decrease in the mean global score was only found with 1% HA190 and only at 2 weeks; lower doses of HA190 had no significant effect (Table III).

ITEMS OF THE HISTOPATHOLOGICAL SCORE

The effect of HA on the various items of the

histopathological scores is summarized in Table IV. With exception of subchondral bone exposure, each item of the score had a high grade in femoral and tibial cartilage from saline treated animals killed at 2 weeks. When compared with the saline group, three items of the femoral cartilage score ($P < 0.05$) and five items of the tibial cartilage score ($P < 0.01$) were significantly decreased with 1% HA190, 2 weeks after surgery (Table IV). In neither the femoral nor in the tibial cartilage, were the items significantly modified with HA80.

Four weeks after surgery, four items of the femoral cartilage score ($P < 0.05$) and one item of the tibial cartilage score (disorganization of chondrocytes, $P < 0.01$) were significantly lower in the 1% HA190 treatment group than in the saline group. (Table IV). In the HA80 treatment group, only two items of the femoral cartilage score were reduced, compared to the saline treated control group 4 weeks after surgery ($P < 0.05$), and items of the tibial cartilage score were unmodified.

SEM

Two weeks after surgery, the surface of the articular cartilage of animals from the control group was smooth and showed no evidence of fibrous structure, such as collagen fibers. In contrast, the cartilage surface of saline-treated animals was roughened with many large fissures, exposing collagen fibers of varying sizes in both the superficial and deeper layers. Such degenerative changes in the cartilage were also observed in the 1% HA190 treatment group, but the changes were less severe than in the saline-treated control group (Fig. 5).

Discussion

The present study examined the effect of HA injections on the course of experimental OA induced by the sectioning of the fibular collateral and sesamoid ligaments and the partial removing of the lateral meniscus in knee joints of adult rabbits. Significant destructive cartilage changes, quite similar to those observed in human OA, were found relatively early after surgery. Severe lesions developed within 4 weeks after surgery in the saline-treated control group. No obvious
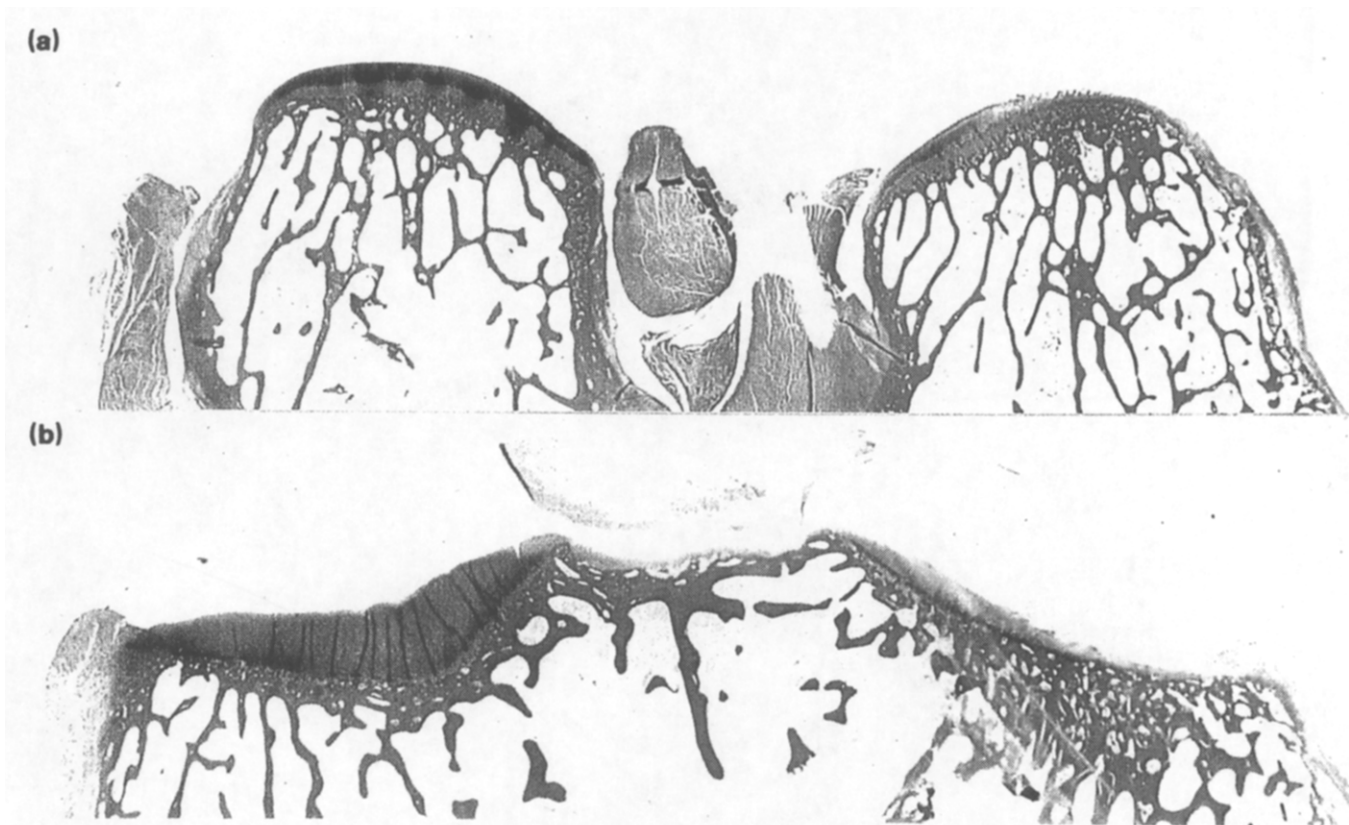

FIG. 2. Safranin-O-staining of the femoral condyl and tibial plateau from the knee joint of the rabbits with OA (4 weeks after surgery). (a) femoral condyle; (b) tibial plateau; left, medial; right, lateral.
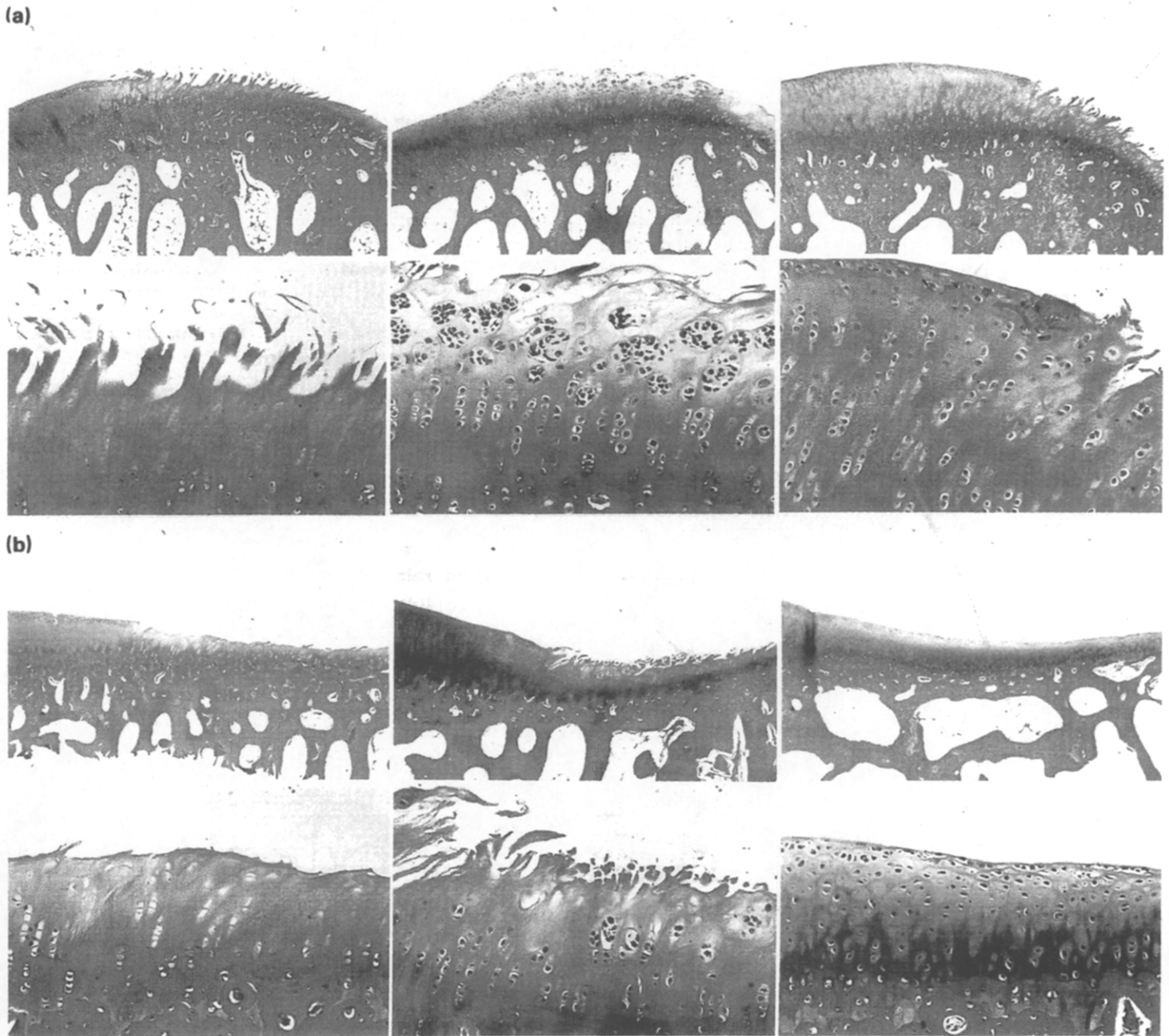
(a)

(b)

FIG. 3. (a) Safranin-O-stained femoral condyle at 2 weeks after surgery. Left column, saline-treated control; middle column, 1% HA80 (MW: 0.8×10^6 Da) treatment; right column, 1% HA190 (MW: 1.9×10^6 Da) treatment. Upper row, original magnification ×10; lower row, original magnification ×50. Eight histological parameters (loss of superficial layer, erosion of cartilage, fibrillation and/or fissures, loss of proteoglycan, disorganization of chondrocytes, loss of chondrocytes, exposure of subchondral bone, cluster formation) were evaluated according to the scoring system described in Materials and Methods. Scores for above eight parameters of three groups are judged as followed. Saline-treated: +4, +4, +4, +4, +4, +4, +1, +2. HA80-treated: +3, +2, +2, +4, +3, +2, +1, +2. HA190-treated: +2, +2, +2, +2, +2, +2, +1, +1.
(b) Safranin-O-stained tibial plateau at 2 weeks after surgery. Left column, saline-treated control; middle column, 1% HA80 (MW: 0.8×10^6 Da) treatment; right column, 1% HA190 (MW: 1.9×10^6 Da) treatment. Upper row, original magnification ×10; lower row, original magnification ×50. Eight histological parameters (loss of superficial layer, erosion of cartilage, fibrillation and/or fissures, loss of proteoglycan, disorganization of chondrocytes, loss of chondrocytes, exposure of subchondral bone, cluster formation) were evaluated according to the scoring system described in Materials and Methods. Scores for above eight parameters of three groups are judged as followed. Saline-treated: +2, +3, +2, +4, +4, +4, +1, +2. HA80-treated: +3, +3, +3, +4, +4, +4, +1, +1. HA190-treated: +1, +1, +1, +1, +1, +1, +1, +1.
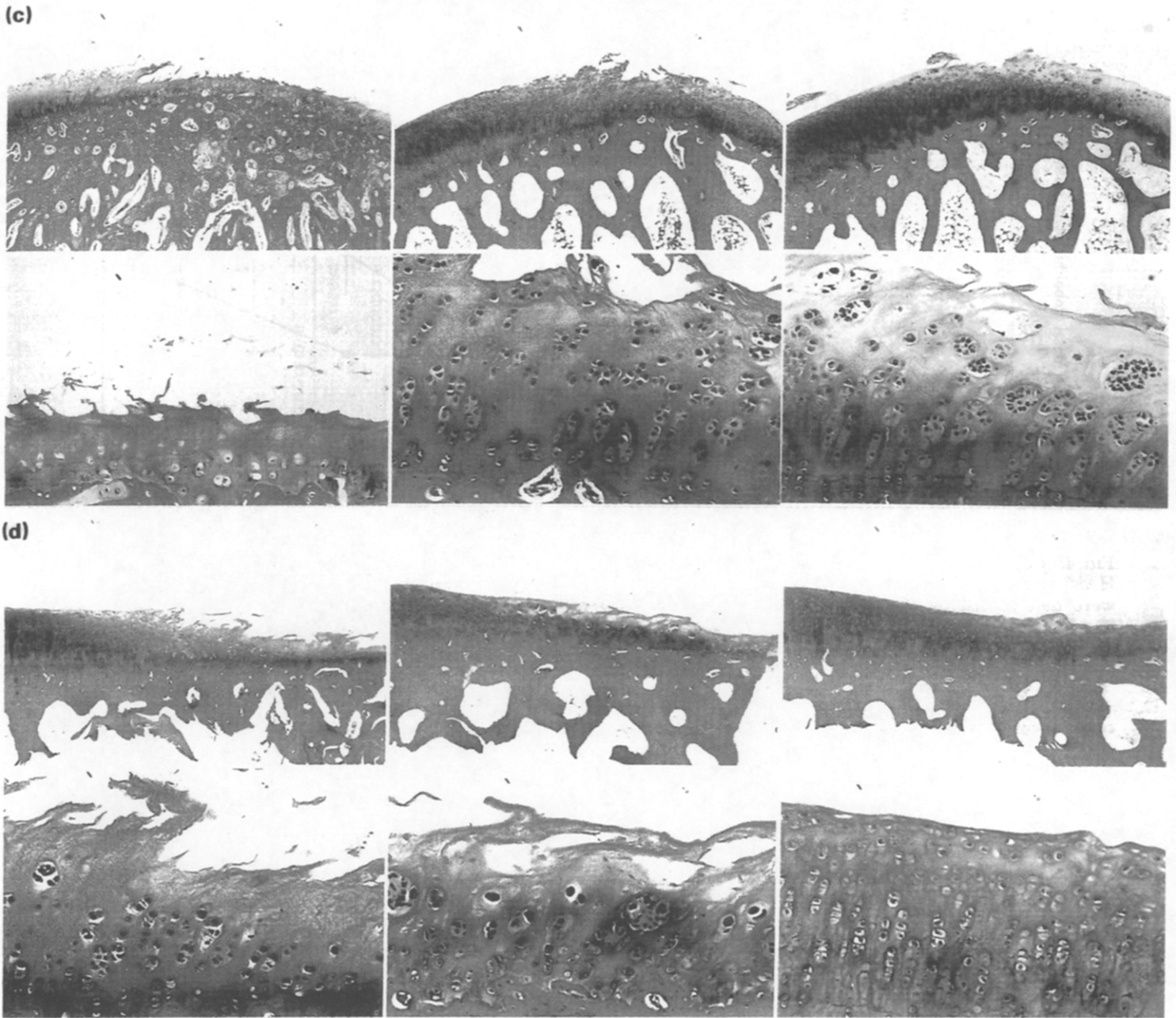
(c)

(d)

FIG. 3. (c) Safranin-O-stained femoral condyle at 4 weeks after surgery. Left column, saline-treated control; middle column, 1% HA80, (MW: 0.8×10^6 Da) treatment; right column, 1% HA190 (MW: 1.9×10^8 Da) treatment. Upper row, original magnification ×10; lower row original magnification ×50. Eight histological parameters (loss of superficial layer, erosion of cartilage, fibrillation and/or fissures, loss of proteoglycan, disorganization of chondrocytes, loss of chondrocytes, exposure of subchondral bone, cluster formation) were evaluated according to scoring system described in the Materials and Methods. Scores for above eight parameters of three groups are judged as followed. Saline-treated: +4, +4, +4, +4, +4, +4, +1, +4. HA80-treated: +3, +3, +3, +3, +3, +2, +1, +3. HA190-treated: +1, +1, +1, +2, +2, +1, +1, +2.

(d) Safranin-O-stained tibial plateau at 4 weeks after surgery. Left column, saline-treated control; middle column, 1% HA80 (MW: 0.8×10^6 Da) treatment; right column, 1% HA190 (MW: 1.9×10^6 Da) treatment. Upper row, original magnification ×10; lower row, original magnification ×50. Eight histological parameters (loss of superficial layer, erosion of cartilage, fibrillation and/or fissures, loss of proteoglycan, disorganization of chrondrocytes, loss of chondrocytes, exposure of subchondral bone, cluster formation) were evaluated according to the scoring system described in Materials and Methods. Scores for above eight parameters of three groups are judged as followed. Saline-treated: +4, +4, +4, +4, +4, +3, +1, +4. HA80-treated: +2, +3, +2, +2, +2, +1, +1, +1. HA190-treated: +1, +1, +1, +2, +1, +1, +1, +1.
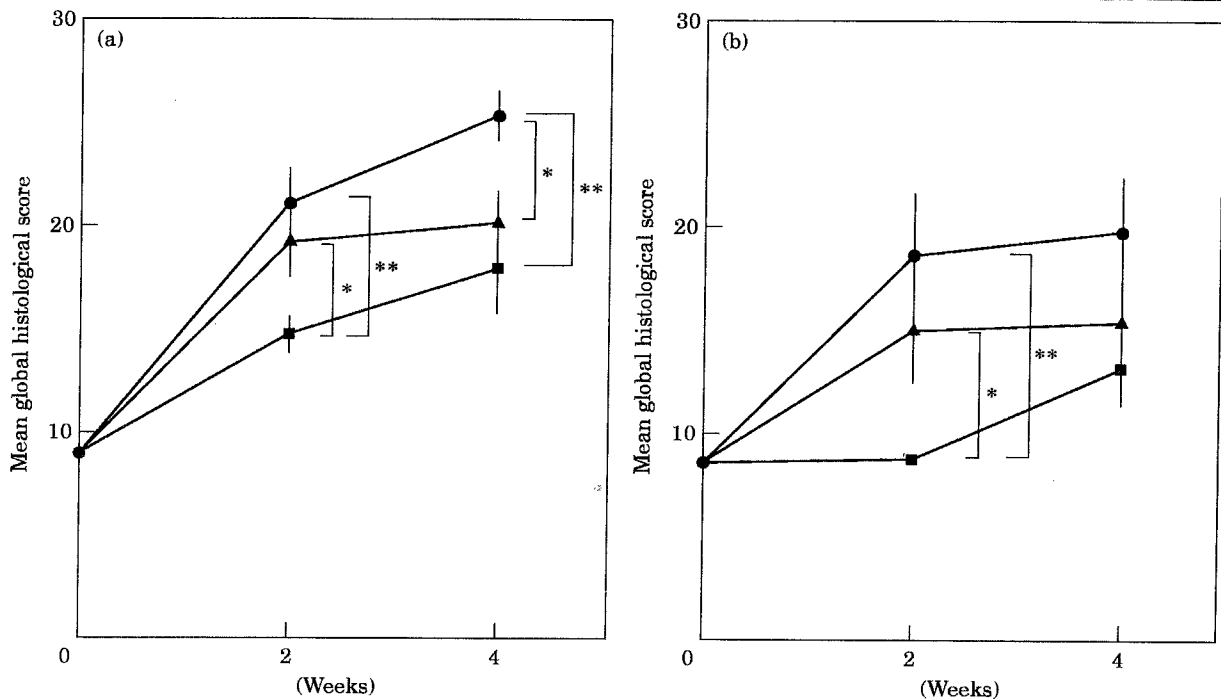
FIG. 4. Global histopathological scores after intra-articular HA or saline injection. One percent solution of HA190 and HA80 was used. Values represent averages of six rabbits and standard deviation. Significance of intergroup differences were determined by the Wilcoxon's rank sum test. (a) femur; (b) tibia; ● = control; ▲ = HA80 (MW 0.8×10^6 Da); ■ = HA190 (MW 1.9×10^6 Da). $*P < 0.05$, $**P < 0.01$.

osteochondrophytes were seen during the observation period of this experiment.

The results using histological grading showed a significant inhibition of degenerative changes in the cartilage by 1% HA190 treatment in an early stage. In the 1% HA190 treatment group, inhibition of cartilage degenerative changes was obvious in both the femoral condyle and the tibial plateau 2 weeks after surgery. However, such effects were less marked 4 weeks after surgery in the tibial plateau (Fig. 4). These results indicate that repeated intra-articular administration of HA190 at a concentration of 1%, has a preventive effect on the development of early cartilage degenerative changes. No evidence for osteochondrophyte formation was presently seen and a longer follow-up period study could be necessary to determine whether HA190 can prevent osteophyte formation.

At a concentration of 1%, HA80 also significantly inhibited the development of cartilage degenerative changes, but the effect was only obvious 4 weeks after surgery and only for the femoral condyle. Two weeks after surgery, the global score of HA80 treated animals was not significantly different from that of the saline treated group (Fig. 4). The HA80 treatment did not result in a significant inhibition of degenerative changes in the cartilage of the tibial plateau, either

2 or 4 weeks after surgery. Prevention of cartilage degeneration at 2 weeks was clearly better with HA190 than HA80, in both the femoral condyle and the tibial plateau. However, no significant difference between the two HA preparations was observed at 4 weeks. These data indicate that the superiority of HA190 over HA80 in the inhibition of cartilage degenerative lesions is only obvious in the early stage of the experimental OA. The global score was generally not significantly reduced by HA190 at concentrations lower than 1%. In this study, 0.1 ml/kg HA190 at 1% concentration was the minimum effective dose for the prevention of cartilage degeneration.

SEM observation revealed that animals of the saline-treated group developed superficial defects of the cartilage, fibrillation, fissures and erosion (Fig. 5). These SEM findings are in agreement with the histopathological findings and underscore the efficacy of high molecular weight HA to inhibit the development of degenerative changes in this experimental OA model.

The mechanisms by which the cartilage degeneration is prevented by HA are still unknown. The effect of the variations in the molecular weight of HA on cartilage metabolism is thus poorly understood. Shimazu *et al.* [20] reported that HA inhibition of proteoglycan release by chondrocyte

Table III.

Histopathological scores after intra-articular injection of HA190 of different concentrations

	2 Weeks					4 Weeks				
	Saline	Concentration of HA-190				Saline	Concentration of HA-190			
		0.01%	0.3%	0.6%	1%		0.01%	0.3%	0.6%	1%
Femur										
Global score	22.5 ± 3.3	21.8 ± 4.2	22.0 ± 4.2	17.8 ± 4.4	15.0 ± 1.4**	25.7 ± 2.4	23.8 ± 3.1	23.3 ± 3.5	20.0 ± 5.0*	18.0 ± 4.2**
Loss of superficial layer	3.0	2.7	3.0	2.3	2.0	3.5	3.0	2.8	2.5	2.7
Erosion of cartilage	2.8	2.8	3.2	2.3	2.0	3.8	3.8	3.7	3.0	2.3*
Fibrillation and/or fissures	3.0	2.7	3.0	2.3	2.0	3.7	3.3	2.8	2.7	2.5*
Loss of proteoglycan	3.7	3.5	3.5	2.8	2.5	3.8	3.8	3.7	3.7	3.2
Disorganization of chondrocytes	3.3	3.5	3.5	3.0	2.2*	3.8	3.2	3.5	2.8*	2.7*
Loss of chondrocytes	3.2	3.3	2.3	2.0	1.8*	3.3	3.2	3.0	2.5*	1.8*
Exposure of subchondral bone	1.0	1.0	1.0	1.0	1.0*	1.0	1.0	1.0	1.0	1.0
Cluster formation	2.5	2.3	2.5	2.0	1.5	2.7	2.5	2.8	1.8	1.8
Tibia										
Global score	19.0 ± 5.4	18.7 ± 5.3	19.3 ± 5.7	17.2 ± 3.3	8.7 ± 1.0**	20.2 ± 5.2	22.0 ± 3.5	21.0 ± 5.1	19.2 ± 4.1	14.2 ± 3.3
Loss of superficial layer	2.2	2.2	2.7	2.2	1.0	2.5	2.5	2.7	3.0	1.8
Erosion of cartilage	2.5	2.3	2.8	2.2	1.0**	2.5	3.3	3.0	2.3	1.7
Fibrillation and/or fissures	2.3	2.7	2.7	2.2	1.0**	2.2	3.0	2.8	2.7	1.5
Loss of proteoglycan	3.2	3.0	2.8	3.2	1.3**	3.5	3.7	3.5	3.3	2.7
Disorganization of chondrocytes	3.0	2.8	3.2	2.7	1.0**	3.7	3.5	3.0	3.0	2.0**
Loss of chondrocytes	2.7	2.7	2.7	2.3	1.0**	2.2	2.3	2.7	2.2	1.3
Exposure of subchondral bone	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Cluster formation	2.2	2.0	1.5	1.7	1.3	2.7	2.7	2.3	1.7	2.3

Values represent averages of six rabbits and standard deviation. Significance of intergroup differences were determined by the Wilcoxon's rank sum test. (*$P < 0.05$, **$P < 0.01$.)
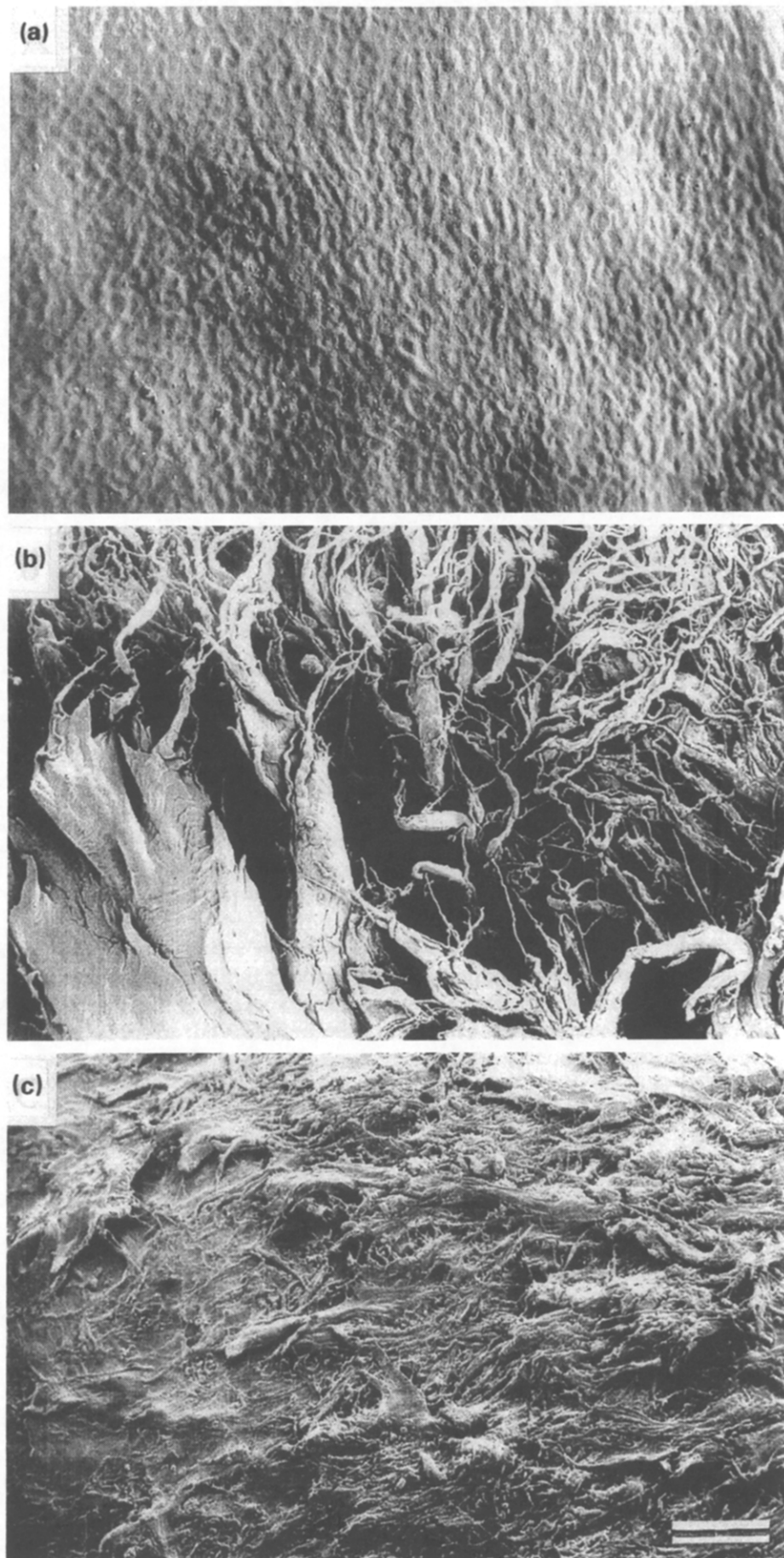
FIG. 5. Scanning electron micrograph of the articular plane of the femoral condyle at 2 weeks after surgical procedures. (A) normal joint group; (B) saline-treated control group; (C), 1% HA190-treated group. (Bar = 100 μm).

Table IV.
Histopathological scores after intra-articular HA or saline injection

	2 Weeks			4 Weeks		
	Saline	HA-80	HA-190	Saline	HA-80	HA-190
Femur						
Loss of superficial layer	3.0	2.7	2.0	3.5	2.8	2.7
Erosion of cartilage	2.8	2.2	2.0	3.8	3.0	2.3*
Fibrillation and/or fissures	3.0	2.7	2.0	3.7	2.7*	2.5*
Loss of proteoglycan	3.7	3.5	2.5*	3.8	3.7	3.2
Disorganization of chondrocytes	3.3	3.0	2.2*	3.8	3.2	2.7*
Loss of chondrocytes	3.2	2.3	1.8*	3.3	2.2*	1.8*
Exposure of subchondral bone	1.0	1.0	1.0	1.0	1.0	1.0
Cluster formation	2.5	2.0	1.5	2.7	2.3	1.8
Tibia						
Loss of superficial layer	2.2	1.7	1.0	2.5	1.7	1.8
Erosion of cartilage	2.5	1.8	1.0**	2.5	2.2	1.7
Fibrillation and/or fissures	2.3	2.0	1.0**	2.2	2.2	1.5
Loss of proteoglycan	3.2	3.0	1.3**	3.5	3.0	2.7
Disorganization of chondrocytes	3.0	2.8	1.0**	3.7	2.7	2.0**
Loss of chondrocytes	2.7	2.3	1.0**	2.2	1.8	1.3
Exposure of subchondral bone	1.0	1.0	1.0	1.0	1.0	1.0
Cluster formation	2.2	1.5	1.3	2.7	2.0	2.3

One percent solution of HA190 and HA80 was used. Values represent averages of six rabbits. Significance of inter-group differences were determined by the Wilcoxon's rank sum test. (*$P < 0.05$, **$P < 0.01$.)

culture was dependent upon the concentration and molecular size of the molecule. The results of studies on the clearance of proteins from joint cavity vary. Sliwinski *et al.* [21] reported that radiolabeled immunoglobulins of various sizes were cleared from synovial fluid independently of their molecular weight. However, Sato *et al.* [22] found that when two types of ^{14}C-labeled HA (molecular weight: 0.9×10^6 Da and 1.8×10^6 Da) were injected into the joints of rabbits, the retention of the HA in the articular cavity after the injection was dependent upon its molecular size.

Destruction of cartilage matrix in OA is known to be caused by a variety of proteinases [23, 24]. The matrix metalloproteinases (MMPs), which are specifically controlled by tissue inhibitors of metalloproteinases (TIMPs), are the most likely candidates [25, 26]. Yasui *et al.* [27] found that HA induced TIMP-1 production by bovine chondrocytes, and that the effect was dependent on the molecular size of HA. It was reported by Sakamoto *et al.* [28] that, due to tissue fibrillation, HA could penetrate into the deep layer of OA cartilage and could have a direct access to chondrocyte membranes. Thus, HA might affect the catabolic activity of chondrocytes through TIMP production. The presently observed superiority of HA190 over HA80 in inhibiting cartilage degeneration could be explained by the differences observed *in vitro* in relation with the molecular weight of HA.

The molecular size-dependent effects of HA on the development of early degenerative OA changes observed in the present study suggest that higher molecular weight HA is clinically efficacious in the treatment of incipient OA.

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