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OSTEOARTHRITIS and CARTILAGE

Intra-articular injection of collagenase induces experimental osteoarthritis in mature rabbits

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Summary

Objective: The induction of osteoarthritis-like changes by intra-articular injections of collagenase in the knee joint of mature rabbits was examined.

Methods: Collagenase (0.5, 1.0 or 2.0 mg) was intra-articularly injected twice into the right knee, and the cartilage and synovia was histologically examined at 6 weeks after the initiation of collagenase injections. In addition, 1 mg of collagenase was intra-articularly injected twice into rabbits, and histological examinations of the cartilage and synovia were performed at various time points. In other experiments, articular cartilage was digested in 5 ml of 0.4 mg/ml collagenase *in vitro*, and biochemical analyses of the cartilage were performed.

Results: The degeneration of the cartilage and synovia were found to be dependent on the dose of collagenase. The cartilage degeneration of the femoral condyle and tibial plateau was more severe at the lateral side than at the medial side. The degeneration of the cartilage progressed, whereas the degeneration of the synovia lessened with time. In the biochemical analyses of the digested cartilage *in vitro*, the proportion of water increased, and the dry weight of the collected cartilage, the amounts of hydroxyproline and sulfated glycosaminoglycan decreased with the digesting time.

Conclusion: These results suggest that collagenase injected intra-articularly digests cartilage directly and stimulates an inflammatory reaction of joint tissues at an early stage, and then cartilage degeneration proceeds. This experimental osteoarthritis is a useful animal model, since the cartilage degeneration is similar to the corresponding lesion in human osteoarthritis, and it is conveniently induced by a dose of collagenase lower than that of papain used, within a short period.

Key words: Experimental osteoarthritis, Collagenase, Rabbit.

Introduction

Osteoarthritis (OA) is a chronic disease involving cartilage degeneration and pain, and it frequently occurs in the knee joint. Abnormal mechanical stress and chemical factors which are produced from joint tissues are involved in the progression of OA. A larger amount of collagenase was detected in OA cartilage than in normal cartilage [1–3]. Cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF), which are secreted from inflammatory cells and synovial cells of OA and rheumatoid arthritis patients, stimulate the production of proteolytic enzymes such as collagenase and stromelysin, and the release of proteoglycans from cartilage [4, 5]. These reports suggest that chemical factors are related to the progression of the destruction of articular cartilage, which is composed mainly of collagen, proteoglycan and other minor matrix components. Hence, we examined in this study whether experimental OA can be induced by the injection of collagenase as an enzymatic factor.

Various kinds of experimental OA have been induced in the knee joint of several species of animals [6], and these models were used to investigate the pathogenesis of OA and to examine the effect of anti-OA reagents. These models were designed to be similar to spontaneous OA, and to be reproducible. OA-like changes were observed to be induced by the transection of the meniscus and/or ligaments [7–9], and by the intra-articular injection of a chemical substance such as papain [10–12] or collagenase [13]. The surgical procedures to make an OA model are complicated and the induction of cartilage degeneration takes a longer time compared with the intra-articular injection of chemical substances. The intra-articular injection can be conveniently performed. Several studies have used the injection of a chemical substance to induce OA-like changes. Most of these studies used papain; reports of OA

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models induced by collagenase injection are few. A large amount of papain is needed for the induction of OA-like changes [10–12], and the mechanism of cartilage degeneration by papain injection is unclear. OA induced by collagenase injections has been examined in mice [13], but not in rabbits in detail. For the induction of OA-like changes by intra-articular injection and for the testing of intra-articular treatments using anti-OA reagents, the animal used must have a sufficient joint space to perform the intra-articular injection easily and precisely. In this present study, therefore, we used rabbits as an experimental model of OA induced by the intra-articular injection of collagenase.

Materials and Methods

MATERIALS

Collagenase (*Clostridium histolyticum*, type II) was obtained from Sigma (St. Louis, MO, U.S.A.). The enzyme activity of collagenase for collagen digestion was 456 U/mg solid. This collagenase was dissolved in saline and filtrated with a 0.22 μm membrane, and the solution was used for the intra-articular injection. Inactivated collagenase was prepared with treatment of the collagenase solution at 100°C for 10 min.

ANIMALS

Healthy Japanese white adult male rabbits (weighing 2.6–3.2 kg; Tokyo Laboratory Animals, Tokyo, Japan) were used. Animals were housed individually and maintained in accordance with the NIH guidelines. No restriction of movement was enforced during the test period.

Table I
Histological evaluation methods of articular cartilage

I. Articular cartilage
(1) Structure (11 grades)
(2) Cell
(a) Tangential zone (3 grades)
(b) Transitional and radial zone (11 grades)
(3) Safranin-O staining (5 grades)
(4) Tidemark (4 grades)
(5) Punnus formation (4 grades)
II. Synovial tissue
(1) Synovial lining layer
(a) Hyperplasia of synovial lining cells (4 grades)
(b) Hypertrophy of synovial lining layer (4 grades)
(c) Infiltration of inflammatry cells (4 grades)
(2) Subsynovial tissue
(a) Proliferation of granulation tissue (4 grades)
(b) Vascularization (4 grades)
(c) Infiltration of inflammatory cells (4 grades)

INDUCTION OF EXPERIMENTAL OA

Animals were anesthetized with an intra-muscular injection of 2 mg/kg xylazine hydrochloride (Bayer, Tokyo) and 45 mg/kg

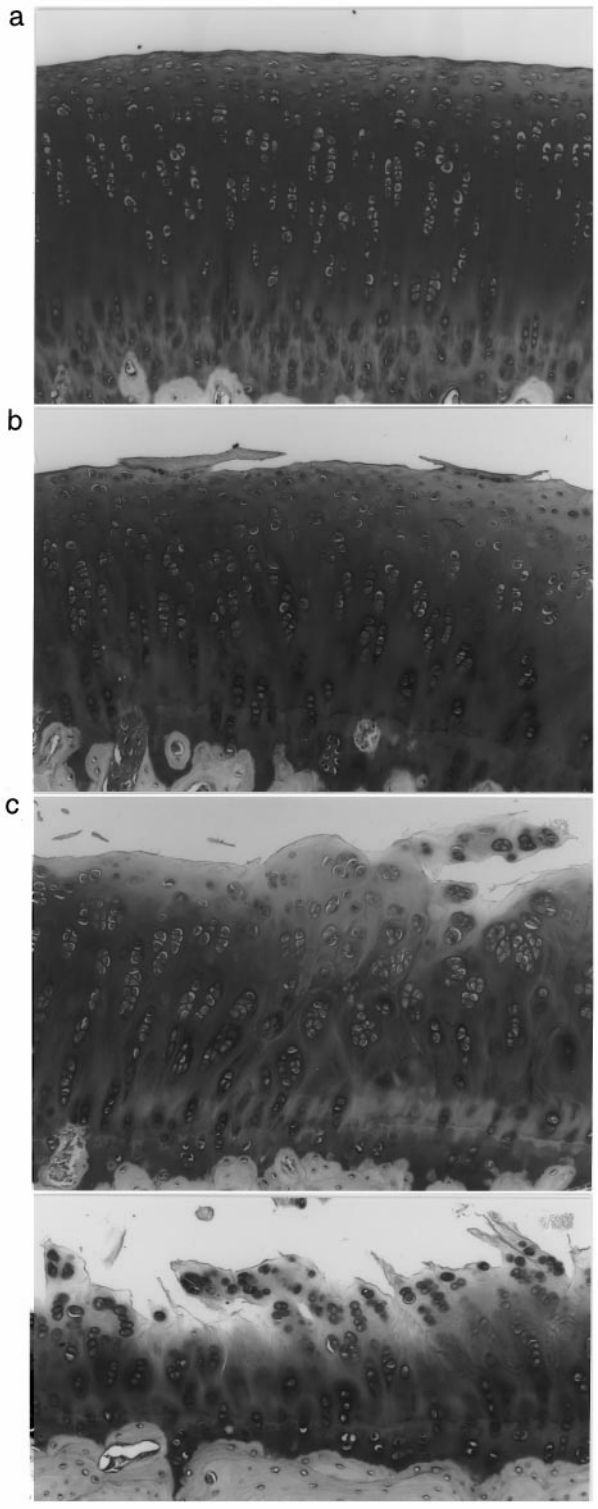

FIG. 1. (*caption facing page*)

Table II
Histological evaluation scores of articular cartilage and synovial tissue with light microscopy, and the changes with the dose of collagenase

	Saline ($N = 3$)	0.5 mg ($N = 3$)	1.0 mg ($N = 3$)	2.0 mg ($N = 3$)
Femoral condyle				
Lateral side	2.3 ± 0.6	9.3 ± 3.5	15.0 ± 3.0	19.3 ± 2.1
Medial side	1.7 ± 0.6	7.0 ± 3.0	13.3 ± 1.5	15.0 ± 1.0
Tibial plateau				
Lateral side	2.7 ± 0.6	12.0 ± 1.0	16.0 ± 1.0	17.3 ± 2.1
Medial side	2.3 ± 0.6	9.3 ± 2.5	11.3 ± 1.5	12.3 ± 0.6
Synovial tissue	1.0 ± 1.0	3.0 ± 1.0	4.7 ± 1.5	7.3 ± 0.6

Each data represents the mean ± S.D. ($N = 3$). Each dose of collagenase was injected twice (at day 1 and day 4) into the right knee joint.

ketamine hydrochloride (Sankyo, Tokyo, Japan). After the right knee joint was shaved and sterilized, 0.5 ml collagenase solution or saline as a control ($N = 3$, each group) was intra-articularly injected into the right knee joint. The injection was performed twice, at day 1 and day 4, in accord with the method of papain injection [11, 12]. In the test of the dose of collagenase, 0.5, 1.0 or 2.0 mg collagenase or saline was injected twice ($N = 3$, each group), and the animals were dissected at 6 weeks after the initiation of the injections. In the test of the serial changes after the injection of collagenase solution, 1.0 mg collagenase was injected with the same conditions as those used in the experiment described above, and three animals were dissected at 1, 2, 4 and 6 weeks after the initiation of the injections. To determine the effect of the inactivated collagenase on the cartilage degradation, 1.0 mg collagenase or inactivated collagenase was injected twice into three rabbits in each group, and the animals were dissected at 6 weeks after the initiation of the injections.

GROSS OBSERVATIONS

The appearance of the knee joint was observed and the weights of the animals were measured twice a week. At the dissection, the pooling and properties of synovial fluid, the medial and lateral side of the femoral condyle and tibial plateau, and the synovia were observed.

HISTOLOGICAL EXAMINATIONS

The lateral and medial sides of the femoral condyle and tibial plateau were fixed with 10% neutral buffered formalin (pH 7.4) and decalcified with 20% EDTA. The decalcified femur and tibia were embedded in paraffin, and 5 μm microsections of them were prepared and stained with hematoxylin and eosin (H&E) and with safranin O. The weight-bearing regions of cartilage were evaluated by scoring in accordance with the evaluation criteria of Yoshimi *et al*. [9] (Table I), which are a modification of those described by Mankin *et al*. [14]. The highest score, which is 32, indicates the most severe degeneration of cartilage. For the examination of synovial tissue, the articular capsule was fixed in the manner described above and embedded in paraffin. The microsections thus prepared were stained with H&E, and the synovial surface-layer and the subsynovial tissue were evaluated by scoring in accordance with the criteria of Yoshimi *et al*. [9] (Table I). The highest score, which is 18, indicates the most severe degeneration of synovial tissue. The preparations were objectively evaluated using only the designated number of the animal subject.

COLLAGENASE DIGESTION OF FEMORAL CONDYLE *IN VITRO* AND BIOCHEMICAL ANALYSES

Collagenase was dissolved in Tris–NaCl–$CaCl_2$ buffer (pH 7.4), and prepared to 0.4 mg/ml. The femoral condyle was soaked in 5 ml collagenase solution, and incubated for 1, 3 or 6 h at 37°C. After the termination of the incubation, the lateral side

FIG. 1. Photomicrographs of the cartilage (safranin-O staining) of the lateral femoral condyle from rabbits which were injected with saline (a), 0.5 (b), 1.0 (c) or 2.0 mg (d) of collagenase. (a) The cartilage surface is smooth. (b) The cartilage surface is irregular, and a slight reduction of safranin-O staining is seen in the transitional and radial zones. (c) Clefts or loss of cartilage extending to the transitional zone, cell cloning and a moderate reduction of safranin-O staining in the transitional and radial zones is evident. (d) The loss of cartilage extends to the radial zone. Cell cloning and a severe reduction of safranin-O staining are apparent in the radial zones.

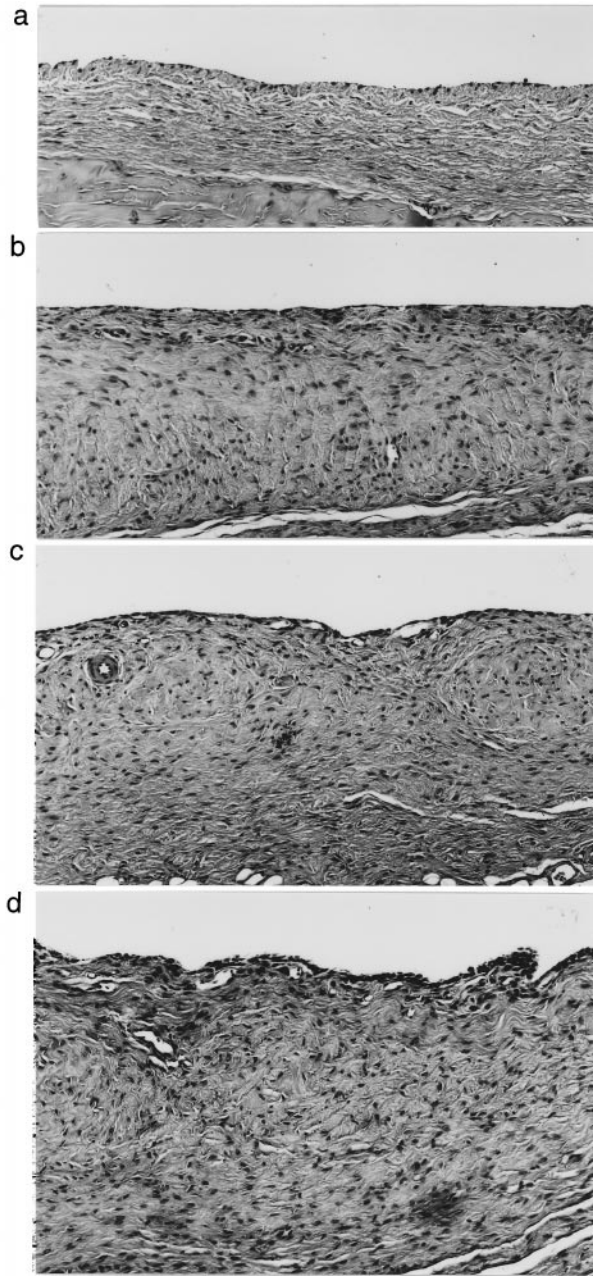

FIG. 2. Photomicrographs of the synovia (H&E staining) from rabbits which were injected with saline (a), 0.5 (b), 1.0 (c) or 2.0 mg (d) of collagenase. (a) The synovia in the saline group is the same as normal synovia. (b) Slight hyperplasia of the lining cells and hypertrophy of the synovial and subsynovial tissue are seen. These aspects of degeneration were worse with the higher doses of collagenase (c, d).

was used for the histological examination, and the medial side was used for the biochemical analyses of cartilage. The histological examination was performed according to the previously described method. The articular cartilage for biochemical analyses was collected from the definite area of the medial side, and freeze-dried after measuring the

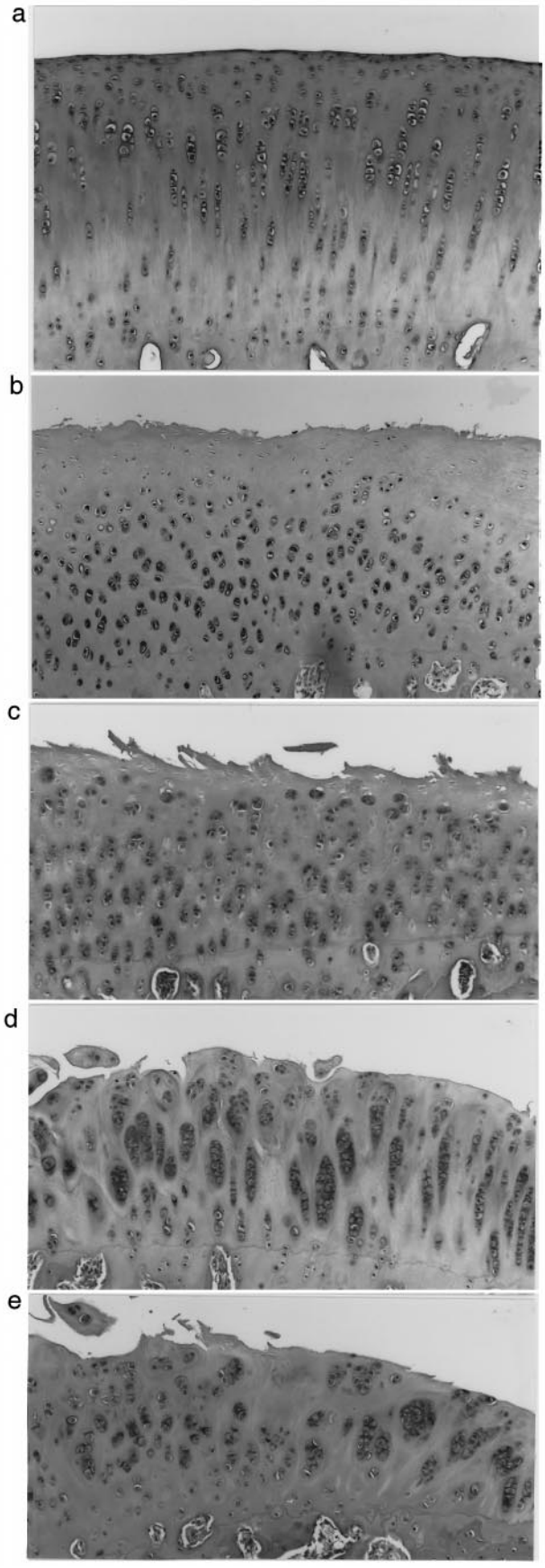

FIG. 3. (*caption facing page*)

Table III
Histological evaluation scores of articular cartilage and synovial tissue with light microscopy, and the time-related changes

	Control ($N = 3$)	1 week ($N = 3$)	2 weeks ($N = 3$)	4 weeks ($N = 3$)	6 weeks ($N = 3$)
Femoral condyle					
Laferal side	1.7 ± 0.6	10.0 ± 1.0	11.0 ± 1.7	15.3 ± 4.0	17.3 ± 2.5
Medial side	1.7 ± 1.2	7.3 ± 1.5	9.7 ± 0.6	13.7 ± 2.1	13.7 ± 1.5
Tibial plateau					
Lateral side	1.7 ± 0.6	10.0 ± 1.7	12.0 ± 4.6	14.7 ± 1.5	16.0 ± 1.0
Medial side	2.0 ± 1.0	9.3 ± 1.5	10.0 ± 2.6	11.3 ± 1.2	12.0 ± 1.0
Synovial tissue	0.3 ± 0.6	8.3 ± 1.2	6.3 ± 0.6	5.0 ± 1.0	3.3 ± 0.6

Each data represents the mean ± S.D. ($N = 3$). One milligram of collaganase was injected twice (at day 1 and day 4) into the right knee joint.

wet weight. The dry weight of the collected cartilage was measured, and the proportion of water was calculated. The amounts of sulfated glycosaminoglycan and hydroxyproline were measured according to the method of Farndale *et al*. [15] and Woessner *et al*. [16], respectively.

DETECTION OF ANTI-COLLAGENASE ANTIBODY IN SERUM

The serum was collected at the dissection, and anti-collagenase antibody was examined by an enzyme-linked immunosorbent assay. First, 100 µl collagenase solution (100 µg/ml) was applied to a 96-well plate, and incubated at 4°C overnight. After washing and blocking, 100 µl diluted serum from the collagenase injected or from normal rabbits was added, and the mixture was incubated for 2 h at room temperature. After washing, peroxidase-conjugate anti-rabbit Ig G (1:1000, Organon Teknika, West Chester, PA, U.S.A.) was added, followed by incubation for 1 h at room temperature. After washing, 100 µl ABTS substrate/hydrogen peroxide (Vector, Burlingame, CA, U.S.A.) was added, and the absorbance at 405 nm was measured.

FIG. 3. Photomicrographs of the cartilage (H&E staining) of the lateral femoral condyle from non-treated rabbits (a), and 1 (b), 2 (c), 4 (d) and 6 weeks (e) after the initiation of collagenase injections. (a) The normal cartilage surface is smooth. (b) The surface layer of the cartilage is lost, and chondrocytes in the transitional zone have disappeared. (c) Cleft in the transitional zone and moderate hypercellularity are seen in the transitional and radial zones. (d) Cleft and loss of cartilage in the transitional zone, and moderate cell cloning in the transitional and radial zones are evident. (e) The loss of cartilage extends to the transitional and radial zones. Cell cloning of chondrocytes is apparent in the transitional and radial zones.

Results

THE CHANGES WITH THE DOSE OF COLLAGENASE

Gross observations

In the groups of collagenase-injected rabbits, the body weight decreased in the first week after the injections, and gradually increased after that. In the saline group, the weights did not decrease. The swelling and reddening of the knee joint were the most severe at 1 week after the initiation of collagenase injections, and gradually lessened after that. The swelling and reddening increased in severity with the dose. These changes were not observed in the saline group. At the dissection 6 weeks after the initiation of injections, pools of synovial fluid, which was clear and light yellowish, were found in the 2.0 mg collagenase group, but were not found in the saline, 0.5 and 1.0 mg collagenase groups. Hypertrophy of the synovial tissue was observed in all animals of the collagenase-injected groups, and its degree was dependent on the dose of collagenase. The cartilage degeneration increased in severity with the dose, and there was even abrasion of the cartilage layer in the 2.0 mg group. These changes were not observed in the saline group.

Histological examinations

The cartilage of the lateral and medial sides of the femoral condyle and tibial plateau and the synovial tissues were examined by light microscopy. The cartilage degeneration at the weight-bearing regions was more severe with the higher doses of collagenase in all areas examined. The cartilage layer of the saline group was not degenerated [Fig. 1(a)]. In the 0.5 mg group, the irregularity of cartilage surfaces, the disappearance of surface-layer cells, slight diffuse

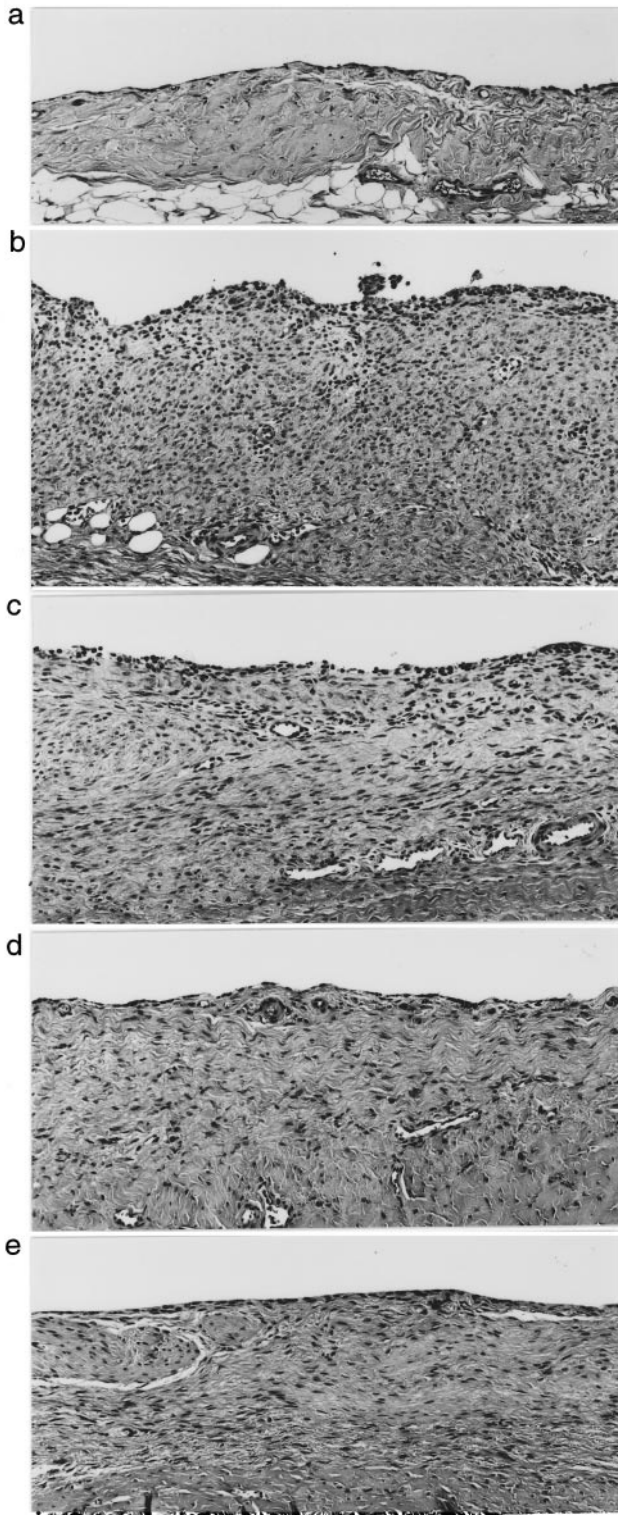

FIG. 4. Photomicrographs of the synovia (H&E staining) from a non-treated rabbit (a), 1 (b), 2 (c), 4 (d) and 6 weeks (e) after the initiation of collagenase injection. Hypertrophy of synovial and subsynovial tissue and infiltration of inflammatory cells are the most severe at 1 week after the initiation of collagenase injections. These degenerations of synovial and subsynovial tissue became weaker with time.

cell growth in the transitional and radial zones, and slightly reduced stainability with safranin O were observed [Fig. 1(b)]. In the 1.0 mg group, a cleft or loss in the transitional or radial zones, cell cloning in the transitional and radial zones, and moderately reduced stainability with safranin O were observed [Fig. 1(c)]. In the 2.0 mg group, loss in the radial zone, cell cloning in the radial zone, and extensively reduced stainability with safranin O were observed [Fig. 1(d)]. The hypertrophy of cartilage layer was seen at the non-weight-bearing regions, and the osteophytes were seen at the margins of the tibia plateau in the collagenase-injected groups. The average scores for the above findings at the weight-bearing regions using the evaluation criteria are shown in Table II. The cartilage scores became higher with the dose of collagenase. The cartilage degeneration at the lateral side of the femur and tibia in each collagenase-injected group was more severe than that at the medial side.

The changes of the synovial tissues, the hyperplasia of synovial lining cells, the hypertrophy of synovial lining layer and subsynovial tissue, and the infiltration of inflammatory cells such as neutrophiles became more severe with the dose of collagenase, though the degree of these changes were mild [Fig. 2(b)–(d)]. The average score of synovial tissue also increased with the dose of collagenase (Table II).

THE TIME-RELATED CHANGES

Gross observations

The weight changes and appearances of the knee joints were similar to the results described in the previous section. Pooling of the synovial fluid was found in the dissection groups at 1 and 2 weeks after the initiation of injections. The hypertrophy of synovial tissue was the most severe at 1 week, and hyperemia was observed in some animals. The hypertrophy of the synovial tissue lessened with time. In contrast, the cartilage degeneration increased in severity with the passage of time.

Histological examinations

The cartilage degeneration at the weight-bearing regions progressed with time on both sides of the femoral condyle and tibial plateau. At one week after the initiation of injections [Fig. 3(b)], irregularity of the cartilage surfaces, the disappearance of surface-layer cells, slight diffuse

cell growth in the transitional and radial zones, and slightly reduced stainability with safranin O were observed, and the cartilage degeneration further progressed with time [Fig. 3(b)–(e)]. The hypertrophy of cartilage layer was seen at the non-weight-bearing regions from 2 weeks after the initiation of injections, and the osteophytes were seen at the margins of tibia plateau at 4 and 6 weeks after the initiation of injections. These findings at the weight-bearing regions were evaluated, and the average scores are shown in Table III. The scores of the cartilage of the femur and tibia were higher with time, and the scores of the lateral side were higher than those of the medial side.

The degeneration of synovial tissues was the most severe at 1 week after the initiation of injections, and the moderate infiltration of inflammatory cells and hypertrophy of the synovial lining layer and subsynovial tissues were also observed at that time. These changes lessened after 1 week, and weak inflammatory responses remained at 6 weeks [Fig. 4(b)–(e), Table III].

EFFECT OF THE INACTIVATED COLLAGENASE INJECTION

Gross observations

In the 1.0 mg collagenase group, the weight changes, appearance of the knee joints, and the findings of synovial fluid, synovial tissue and cartilage at the dissection were the same as in the 1.0 mg group described above. In contrast, the body weights and all gross findings in the rabbits that received inactivated collagenase injections were similar to those in the saline group described above. In this examination, anti-collagenase antibody in the serum was measured, and was not detected in either group.

Histological examinations

The degeneration of the cartilage and synovial tissue in the collagenase injection group was to the same degree as that observed in the 1.0 mg group in the previous experiment [Fig. 1(c), 2(c)]. These degenerations were not observed in the inactivated collagenase injection group, and the histological aspects were the same as those of the previously described saline group [Fig. 1(a), 2(a)]. The results of the evaluations are shown in Table IV. The average scores of the inactivated collagenase group were as low as those of the saline group given in Table II.

COLLAGENASE DIGESTION OF FEMORAL CONDYLE *IN VITRO*

Histological examinations

Degenerative changes were not observed in the non-digested cartilage [Fig. 5(a)]. The cartilage digested for 1 h showed a severe reduction of safranin O stainability in the transitional zone, though the structure of the cartilage layer was maintained [Fig. 5(b)]. The loss of cartilage in the transitional zone and a severe reduction of safranin O stainability in the radial zone were observed in the cartilage digested for 3 h [Fig. 5(c)]. With the digestion for 6 h, the cartilage layer was almost lost [Fig. 5(d)], and articular cartilage could not be collected.

Table IV
Histological evaluation scores of articular cartilage and synovial tissue of rabbits injected with collagenase or inactivated collagenase

	Collagenase ($N = 3$)	Inactivated collagenase ($N = 3$)
Femoral condyle		
Lateral side	15.7 ± 2.3	2.7 ± 0.6
Medial side	12.3 ± 2.3	2.7 ± 0.6
Tibial plateau		
Lateral side	15.7 ± 2.1	3.3 ± 0.6
Medial side	11.7 ± 0.6	3.0 ± 1.0
Synovial tissue	4.3 ± 0.6	0.7 ± 0.6

Each data represents the mean ± S.D. ($N = 3$). One milligram collagenase or inactivated collagenase was injected twice (at day 1 and day 4) into the right knee joint.

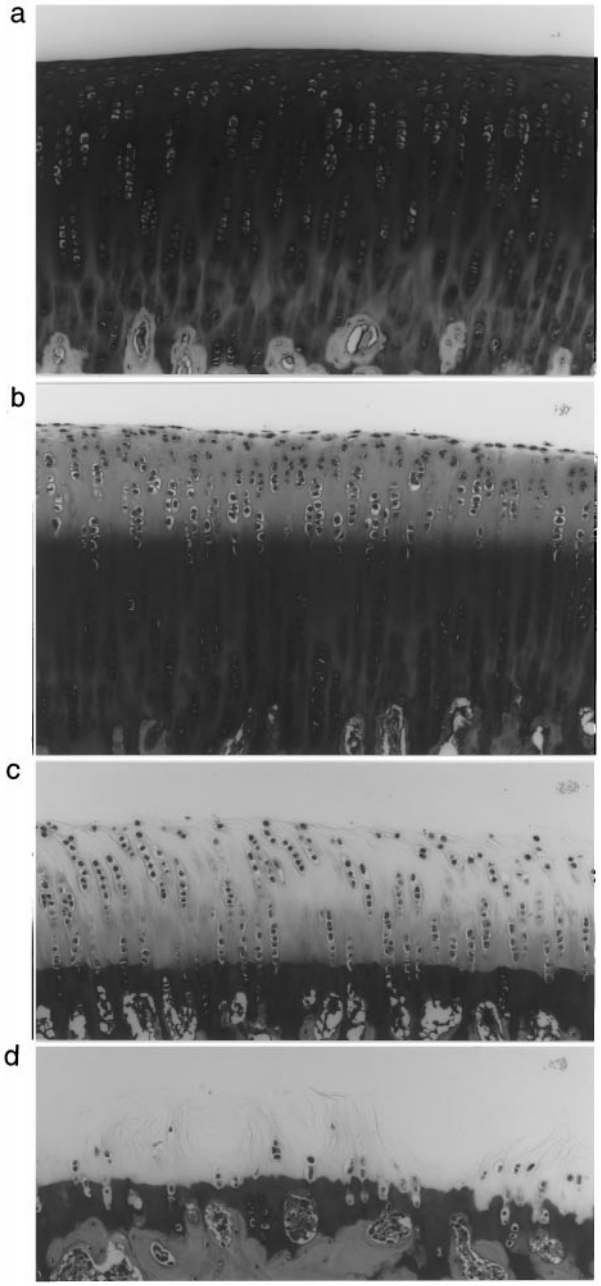

FIG. 5. Photomicrographs of the cartilage (safranin-O staining) from femoral condyles which were non-treated (a), or treated with collagenase for 1 (b), 3 (c) or 6 h (d) *in vitro*. (a) The normal cartilage. (b) Although the structure of cartilage is maintained, a severe reduction of safranin-O staining in the transitional zone is evident. (c) Loss of cartilage in the transitional zone, and a severe reduction of safranin-O staining in the radial zone are observed. (d) All of the cartilage layer is lost, whereas the calcified zone remains.

Biochemical analyses

The results of the biochemical analyses are shown in Table V. The dry weight of cartilage collected from the femoral condyle decreased with the time of the collagenase digestion. The proportion of water increased with the digestion time. The contents of sulfated glycosaminoglycan and hydroxyproline in the cartilage decreased with the digestion time.

Discussion

In this study, we produced a model of OA in rabbits by the intra-articular injection of collagenase. In an experimental OA model induced by papain, 4–12 mg of papain was intra-articularly injected two or three times in rabbit knee [10–12]. In the present study, the OA-like changes were induced by the intra-articular injection of 0.5–2.0 mg collagenase, and were dependent on the dose. The injection of 1.0 mg collagenase was sufficient to induce OA-like changes for 6 weeks. The effective dose of collagenase to induce the OA-like changes in cartilage was lower than that of papain, perhaps because collagenase has a higher substrate specificity to collagen (which is a main component in cartilage) than does papain.

With regard to the mechanism of cartilage degeneration by collagenase, van der Kraan *et al*. [13] stated that the collagenase did not directly damage articular cartilage, but damaged mainly the joint structure such as tendons, ligaments and menisci, and secondarily destructed articular cartilage. In the present study, the destruction of the cartilage superlayer and a reduction of safranin O stainability were observed in the early stage, and the collagenase digestion of articular cartilage *in vitro* resulted in the breakdown of the cartilage layer. The collagenase used in this study was thought to directly digest the collagen in the cartilage and induce the primary degeneration of articular cartilage.

In the biochemical analyses of articular cartilage digested with collagenase *in vitro*, the dry weight and the amount of hydroxyproline in the collected cartilage gradually decreased, the amount of sulfated glycosaminoglycan rapidly decreased and the proportion of water increased with the time of collagenase digestion. These biochemical changes reflected the histochemical appearance of the cartilage, e.g., the safranin O stainability rapidly reduced before the destruction of cartilage structure. Thus, the tight structure of cartilage is thought to become loose with the digestion of collagen in the cartilage, and a rapid outflow of cartilage matrix components such as proteoglycans and cartilage degeneration is thought to proceed in the early stage *in vivo*. However, the destruction of cartilage *in vitro* was much faster than that *in vivo*. Because few negative factors such as collagenase inhibitors are

Table V
Effect of collagenase digestion of femoral condyle on the dry weight of cartilage, water content and total amount of sulfated-glycosaminoglycan and of hydroxyproline

Digestion time (h)	Dry weight (mg)	Water content (%[1])	Sulfated-GAG (μg[2])	Hydroxyproline μg[2])
0	3.7 ± 0.2	69.2 ± 1.0	402 ± 51	306 ± 35
1	3.1 ± 0.5	80.3 ± 1.2	170 ± 48	257 ± 53
3	1.6 ± 0.2	85.5 ± 1.7	37 ± 11	132 ± 18
6[3]	N.D.	N.D.	N.D.	N.D.

Mean ± S.D. ($N = 5$), GAG glycosaminoglycan, N.D. not done, (1) the proportion of water in the wet weight of cartilage; (2) total amount of sulfated-GAG and hydroxyproline in the collected cartilage; (3) articular cartilage could not be collected from the femoral condyles which were digested for 6 h.

present in the experiment *in vitro*, whereas there are several factors inhibiting collagenase activity *in vivo*.

The cartilage degeneration at the lateral side of the femur and tibia was greater than that at the medial side in the rabbits in the present study. van der Kraan *et al*. [13] showed that the cartilage degeneration at the medial side was greater than that at the lateral side of the experimental OA induced by collagenase in mice. Conversely, Yoshimi *et al*. [9] reported that after the resection of the anterior cruciate ligament in rabbits, the greater degree of cartilage degeneration occurred at the lateral side rather than the medial side. We suspect that this difference may be attributable to the species differences in weight loading, which are due to the anatomical structure of the knee joint.

In the present study, 1 week after the initiation of collagenase injections, the inflammatory reaction of the knee joint, the retention of synovial fluid, hypertrophy of synovial tissue and infiltration of inflammatory cells were the most severe. However, the inflammatory responses in the synovial tissue lessened with time, and they weakly remained at 6 weeks after the initiation of injections, and pannus formation in cartilage was negligible. Inflammatory cells in synovial tissue or synovial fluid produce cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (TNF), which stimulate the production of collagenases in chondrocytes and synovial cells. The inflammatory reaction in joints is thought to be associated with the degeneration of cartilage in the early stage. A collagenase injection into the knee joint is thought to not only directly destroy the cartilage, but also cause the inflammatory reaction of synovial tissues and accelerate cartilage degeneration. In the present study, anti-collagenase antibody was not detected in the serum. Therefore, the inflammatory response in synovial tissue was not related to the immunological reaction. The inactivated collagenase did not induce cartilage degeneration. This result suggested that chemical substances such as endotoxins other than collagenase were not directly related to the cartilage degeneration.

In this study, we showed that the intra-articular injections of collagenase induced OA-like changes in the articular cartilage. The cartilage degeneration in this model is thought to be associated with the direct digestion of collagen in cartilage and the inflammatory reaction in the joint tissue. In addition, mechanical stress may subsequently enhance the cartilage degradation. Hypertrophy of the cartilage layer was seen at the non-weight-bearing regions from the early stage, and the osteophytes were seen at the margins of the tibia plateau in the late stage. The OA-like changes of articular cartilage in the present model were similar to human OA and were induced by a convenient method, within a short period, and at a low dose. Thus, this OA model will be useful for the investigation of the pathogenesis of OA and the assays of anti-OA reagents.

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