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Carbohydrate Polymers 48 (2002) 369–378

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Enhanced healing of cartilaginous injuries by glucosamine hydrochloride

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Received 15 December 2000; revised 9 June 2001; accepted 12 June 2001

Abstract

We investigated the restorative effect of orally administered glucosamine hydrochloride (GlcN) on the experimentally produced cartilaginous injuries in rabbits. A total of three holes in the left stifle joint including one in the medial trochlear ridge and two in the trochlear sulcus (proximal and distal) of articular cartilage were made surgically using a drill. For the control group, only tap water and for the glucosamine group, a water based solution of GlcN (1 g/head) was administered daily, respectively. We observed the clinical symptoms daily and the condition of the injured part was observed visually and histologically at 3 weeks after the operation. There was no difference in body weight or general conditions between the two groups. However, in the control group, the muscle weight of the biceps of the left femur was significantly reduced ($p < 0.05$). With respect to the medial trochlear injury, four out of six cases in the control group and five out of six cases in the glucosamine group were cured, respectively. With respect to the proximal and the distal holes in sulcus, only two out of six cases in the control group and five expansive out of six cases in the glucosamine group were cured. There was significant difference between the glucosamine group and the control with respect to healing of the proximal hole ($p < 0.05$) and the total points ($p < 0.05$), indicating that the artificial cartilage injuries were facilitated by GlcN. On histological examination, the injured parts were covered by fibrous connective tissues in the control, whereas in the glucosamine group, the massive proliferation of matured cartilaginous tissues was observed, and the regenerated cartilaginous tissues were surrounded by the proliferation of chondroblast cells. In the regenerated tissue, matured cartilage substrate was about to be formed. Safranin O and alcian blue stains marked significantly dense in the glucosamine group than in the control ($p < 0.01$) in injured parts as well as in non-injured joint cartilage. © 2002 Published by Elsevier Science Ltd.

Keywords: glucosamine hydrochloride; restorative effect; cartilaginous injuries

1. Introduction

Recently, glucosamine's efficacy in treatment and prevention of osteoarthritis in humans has been drawing attention (Croll and D'Este, 1980; Reichelt, Forster, Fischer, Rovati & Setnikar, 1994; Tapadinhas, Rivera & Bignamini, 1982; Vaz, 1982). Being an important connective tissue in the body, cartilage is a highly differentiated organ, which lacks blood vessels, lymph system and nerves. Among high vertebrate animals, cartilage exists particularly on the surface of the weight bearing part of the joint. Having only 2–3% of the volume of the joint, the cartilage cells are composed mostly of highly organized extracellular matrices. Cartilaginous matrix consists of approximately 70–80% of water content, 20–25% of collagen, 5–10% of proteoglycan (Maroudas, Bayliss & Venn, 1980; Muir, 1973). Collagen fiber (mainly types II, VI, IX and XI) generates a dense mesh structure and proteoglycan (mainly aglycan) that forms the shape of matrix and generates tension characteristics which has a function of generating expansive pressure by absorbing water in the tissues through osmotic pressure (Yoshihara & Shiina, 1994). Mesh structures of collagen do not have potential extensive force, therefore, the compressed aglycan in it plays quite important role to generate expansive pressure of it. This creates an ideal tissue that can stand a compressive load with minimal deformation, and supports the function of the cartilage surface to support load with sturdiness and elasticity (Hardingham & Fosang, 1992). Proteoglycan that performs this important role is composed of glycosaminoglycan and protein, while glycosaminoglycan is in turn composed mainly of extracellular matrix polymers such as hyaluronic acid, chondroitin sulfate, keratan sulfate, heparin, heparin sulfate and dermatan sulfate. As a constituent of the polymer chains, D-glucosamine or *N*-acetyl-D-glucosamine is drawing attention as an important key element. D-Gulcosamine is a monosaccharide product generated from chitin by hydrolysis and

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0144-8617/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0144-8617(01)00281-8

deacetylation and is categorized into hexosamine. The ability to synthesize glucosamine in the body declines with age. This, in turn, incapacitates the generation of proteoglycan and it is known that this incapacitation results in senile osteoarthritis (McDevitt & Muir, 1976). Setnikar, Giacchti and Zanolo (1986) reported cases in dogs where the radioactivity of intravenously injected glucosamine hydrochloride labeled with ^{14}C was detected immediately in blood plasma. According to the report, however, since glucosamine hydrochloride diffuses into various organs and tissues in connection with α,β-globulin, glucosamine released in blood decreases precipitously. Oral bioavailability of glucosamine depends on whether glucosamine passes the gastrointestinal blood barrier in an activated form. Since glucosamine is a water-soluble substance which resides in an acidic environment such as the stomach in ion form at 37°C and p*K*a of 6.91, the absorption efficiency by the stomach is poor and it is believed that most of the glucosamine is absorbed by the small intestine (Setnikar, Pauon & Revel, 1991). The study on the amount of excretion of orally administered glucosamine labeled with ^{14}C in feces was reported to be 87% (Setnikar, Giacchti & Zanolo, 1984). This glucosamine drew attention as a useful substance for prevention and treatment of osteoarthritis and its sulfate has been used as a drug for the treatment for arthritis. However, many of the reports are based only on the phenomenology of case report (Dovanti, Bignamini & Rovati, 1980; Kajimoto, Sakamoto, Takamori, Kajitani, Imanishi, Matsuo et al., 1998). Even if the improvement of the clinical symptoms is due to the administration of glucosamine, there has not been any report so far where the regeneration of cartilage and its healing process are experimentally proven.

The objective of the present paper is to evaluate experimentally the healing effect of glucosamine hydrochloride on artificial injuries of articular cartilage.

2. Materials and method

2.1. Animals

Twelve clinically healthy rabbits (Japanese Albino, six males, six females with the average age of about 12 weeks) with a body weight of approximately 2.0 kg were used. Three female and male rabbits were allocated to the glucosamine and the control groups, respectively. All rabbits were used in the experiment subsequent to the period of habituation for one week after the delivery.

2.2. Reagents

Chitin obtained from crabs shells changed into monomeric form through hydrochloric acid and the resultant D-glucosamine hydrochloride (Koyo Chemical Co. Ltd., Tokyo) was used. Purity of the D-glucosamine hydrochloride used was 99.5%. Heavy metals such as Pb and CD was less than 10 ppm. Number of normal flora was less than 300 cfu/g and coliform bacteria was negative.

2.3. Experimental design

General anesthesia was carried out with intramuscular injection of 25 mg/kg, ketamine-HCl (Ketalar injection, Sankyo, Tokyo) after sedation by subcutaneous injection of 0.1 mg/kg, medetomidine-HCl (Domittor, Meiji Confectionery, Tokyo). Rabbit's hair at the left knee joint was clipped, and disinfected with Chlorhexidine (Hibiscrub, Zeneca, Osaka, Japan) and 70% alcohol. Approaching from the lateral portion of the knee joint, an incision was made vertically on the skin from the central part of the femur toward the tibial tuberosity. The articular capsule was incised and the patella of the stifle joint was exposed completely by artificially dislocating the patella toward the lateral side. Three holes of 2 mm size in diameter and 4 mm deep were made by a hand-drill (Micro-engine D-2, Osada Medical, Tokyo) at the articular cartilage of medial trochlear one hole and trochlear sulcus (two holes) of the distal femur. Afterwards, the incision wound made during the operation was rinsed by normal saline and the articular capsule was sutured with a synthetic absorbent thread (3-0, PDS, Johnson and Johnson, USA). The subcutaneous tissues and the skin were sutured at the same time with nylon thread (USP 3-0 suture, Suprylon, Vomel, Germany). Immediately after the operation, the action of medetomidine-HCl was antagonized by 0.5 mg/kg of atipamezole-HCl (Antisedan, Meiji Confectionery, Tokyo) intramuscularly. Tranexamic acid (Ranobis, Isei, Yamagata, Japan) was administered intravenously 20 mg/kg as a hemostatic drug as well as for speedy recovery of consciousness. During the one week period after the operation, the wound surface was disinfected by povidone iodine (Isodine, Meiji confectionery, Tokyo) once a day and Oxytetracycline hydrochloride 10 mg/kg (Terramycin, Pfizer, Tokyo) was subcutaneously administered twice a day as an antibiotic to prevent infection. We describe the surgically made holes as follows: the holes in the trochlear sulcus as proximal and distal, and the one in the medial trochlear as medial.

Six rabbits (three males and three females) of the control group were given only tap water to drink freely. Six rabbits (three males and three females) of the glucosamine group had a solution of powdered glucosamine hydrochloride dissolved in tape water that was administered every day at a rate of 1 g/head/day during experimental period. Also, rabbits in the glucosamine group were able to drink the tap water after ensuring that the daily dosage of glucosamine was administered.

2.4. Macroscopic observation

Diarrhea, appetite, coat color and body weight were observed during experimental period.

At 3 weeks post-operation, the rabbits were euthanized by an overdose (80 mg/kg) intravenous injection of pentobarbital

Table 1
Effect on body weight by glucosamine administration

Experimental group	Sex	Body weight (kg)	
		Pre-operation	At the time of autopsy (after 21 days)
Control group			
1	Female	2.1	2.6
2	Female	2.6	3.2
3	Female	2.6	3.3
4	Male	2.4	2.8
5	Male	2.3	2.8
6	Male	2.0	2.4
Mean		2.3	2.9
Standard deviation		0.3	0.3
Glucosamine group			
1	Female	2.3	2.9
2	Female	2.4	3.1
3	Female	2.1	2.7
4	Male	2.6	2.9
5	Male	2.2	2.9
6	Male	2.3	2.7
Mean		2.3	2.9
Standard deviation		0.2	0.2

sodium (Nembutal, Dainippon Pharmaceutical Co., Osaka, Japan). The stifle joints were opened and were macroscopically observed at the operated site for determination of the synovial fluid contents and the healing of the injured cartilage. The degree of restoration of the experimentally produced holes at the distal femur was observed macroscopically. The degrees of restoration of the defective holes were classified into the following categories: Less than 50%: (−); 50–60%: (+); 60–80%: (++); 80–100%: (+++). In addition, for testing statistical significance, the above evaluation was assigned numbers: (−) was assigned with 0 point, (+) was assigned with 1 point, (++) was assigned with 2 points and (+++) was assigned with 3 points. The total value for each part was calculated. Quantified values were statistically processed by a Mann-Whitney's test at the p-level of < 0.05 to be considered statistically significant. The amount of the synovial fluid that remained in the articular capsule and turbidity of the liquid were also observed. When there was an increase in the synovial fluid, cellular contents in the fluid were examined by staining of fluid smear sample (Diffquick, Kokusai-Shiyaku, Kobe, Japan) and culture of the fluid was carried out using an agar culture medium (Nissui, Tokyo). The decision criteria for the observations were as follows: amount of the synovial fluid in the stifle joint was categorized into (+) for increase in the level of (−) for no change.

2.5. Muscle weight

The left and right lateral great muscles and the biceps of the femur were separated and sampled after euthanesia, and their fresh weights were taken, and the muscle weight ratio (%) was calculated by comparing the operated side with the non-operated side. The weight ratio (%) was examined by a student's *t*-test at the p-level of < 0.05 being to be considered statistically significant.

2.6. Microscopic observation

The recovered left femur was fixed by a 10% neutral buffered formaldehyde water solution. After the fixation, the operated stifle joint was trimmed to a thickness of 5 mm and decalcified for a day with shaken in 5% formic acid solution. After decalcification, the tissue was soaked for neutralization in the 5% sodium sulfate solution for a day, and then was washed for approximately 10 h under running water. After applying the usual method of embedding paraffin, the tissue was sliced by a microtome into 5 μm slices. Staining was carried out using the hematoxylin/eosin double staining method. By carrying out Safranin O stains with the purpose of staining proteoglycan, and Alcian blue stains with the purpose of staining glycosaminoglycan, we observed the difference between restored substances at the injured parts between groups.

2.7. Image analysis

The 200 times magnified images of restored parts, articular cartilage and growing zone stained with Safranin O stains and Alcian blue stains were taken into the computer by Photograb 300 version 1.0 (Macintosh Software, Fujifilm, Tokyo) and the images digitized by Adobe Photoshop 3.0 (Macintosh Software, Adobe System, Tokyo). Then, the proportion of the pixel number that is accounted for by the desired hue in the total of 120,000 pixels (random sampling of 20,000 pixels at six locations) was calculated through the image processing technique. The obtained values were tested by student's *t*-test and were considered statistically significant at the p-level below 0.05.

3. Result

3.1. Macroscopic findings

Table 1 shows the change in body weight before and after the operation. Both groups showed an increase in weight postoperatively. However, there was no remarkable increase in the body weight in the glucosamine group. In addition, there was no animal in both groups where abnormal change was observed in their general condition. In this experiment, the weights were distributed from 2.1 to 2.6 kg. As a result of this, the amount of glucosamine administered to the animals ranged from 370 to 476 mg/kg. The average value was approximately 400 mg/kg.

The surgically created artificial injuries in the stifle joint were shown in Fig. 1a. Fig. 1b shows the stifile joint at

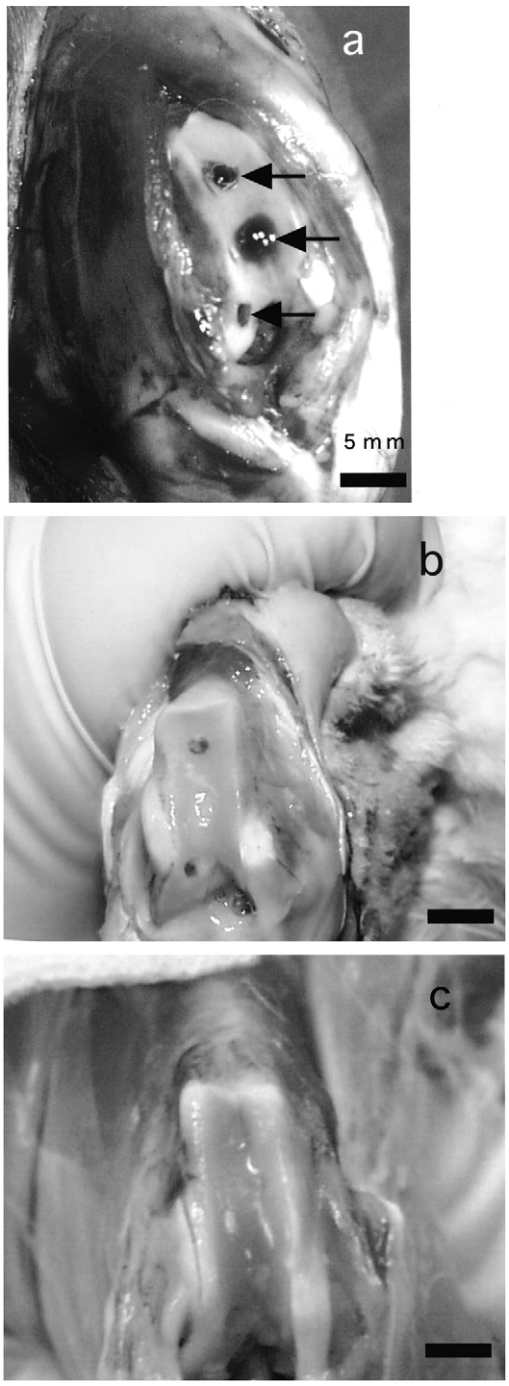


Fig. 1. Surgically created articular cartilage injuries and macroscopic findings of the operated sites at 3 weeks after the operation. (a) Three holes of the size 2 mm in diameter and 4 mm in depth were made by a hand-drill in the knee joint of the distal femur bone. From top to bottom, we called proximal hole (trochlear sulcus), distal hole (trochlear sulcus) and medial hole (medial trochlea). (b) The findings of rabbits in the control group at 3 weeks after the operation. It shows that the medial and the proximal are not healed. (c) The findings of rabbits in the glucosamine group at 3 weeks after the operation. Three holes were perfectly healed.

3 weeks after surgery in the control group. Two holes (the proximal and the distal) were not healed completely. Fig. 1c shows the findings of the glucosamine group 3 weeks after the operation where are all the three injured parts perfectly healed. From the results of the degrees of healing (Table 2) at the medial holes, four out of six cases showed (+++) in the control group and five out of six cases in the glucosamine group. For 80–100% healing the proximal and the distal holes, five out of six cases (83%) in the glucosamine group showed 80–100% healing (+++), but only two out of six (33%) in the control group showed (+++). This result was quantified using numerical assignment (Table 3). Although there was no significance seen (Mann-Whitney's U test) in injured parts of the medial and the distal holes, we found a significant healing for the proximal holes ($p < 0.05$) and for the total points ($p < 0.05$) of the glucosamine group.

In the control group, one case showed an increase in synovial fluid production (Table 1). The bacterial culture of sampled synovial fluid and smear findings did not detect bacteria. In no case of the glucosamine group was seen an increase in the amount of synovial fluid.

Table 4 shows the comparison between the weight of the biceps of the femur and that of the lateral great muscle. As a result of the significance test, a clear decrease in the weight of the muscle was observed in the control group in comparison with that in the glucosamine group. In particular, the weight of the biceps of the femur showed a significant decrease ($p < 0.05$). However, with respect to the weight of the lateral great muscle, no significant difference was found.

3.2. Histological findings

In the control group (Fig. 2a), the injured parts showed normal cartilage, a lack of bone trabecula and permeation of macrophage, neutrophils and lymphocytes, and the bone crest surface showed permeation of osteoclast (Fig. 3a). Hyperplasia of capillary vessels and proliferation of fibroblast, and proliferation of fibrous connective tissues were found in the injured parts, and new generation of cartilage tissues was found in its most medial part (Fig. 3c). On the other hand, matured cartilage tissues were massively proliferated in the glucosamine group (Fig. 2b). They were surrounded by the proliferation of undifferentiated blast cells (fibroblast cartilage cells), while the tissue image of almost forming matured cartilage substrates was observed (Fig. 3d). Other than these, permeation of osteoclast found in the control group was not observed in the bone trabecula (Fig. 3b). Among the normal articular cartilage layer, in the glucosamine group the structure of cartilage cells looked more like the columnar alignment of the growing zone, and the number of cartilage cells was seen to have increased as a whole when compared with the control group. In addition, the hue of the cartilage substrate had a dark bluish color, showing a possible increase in

Table 2
Macroscopic findings of the operated sites at 3 weeks after the operation

Experimental group	Sex	Degree of healing[a]			Exudation in the joint[b]
		Medial trochlear ridge	Trochlear sulcus (proximal)	Trochlear sulcus (distal)	
Control group					
1	Female	++	+	+++	−
2	Female	+++	+++	++	++
3	Female	+++	+	++	−
4	Male	+++	+	+	−
5	Male	+++	+	++	−
6	Male	+	+++	+++	−
Glucosamine group					
1	Female	+++	+++	+++	−
2	Female	+++	+++	+++	−
3	Female	+++	+++	++	−
4	Male	+++	+++	+++	−
5	Male	+	++	+++	−
6	Male	+++	+++	+++	−

[a] −: Less than 50% healing; +: 50–60% healing; ++: 60–80% healing; +++: 80–100% healing.
[b] −: None; +: moderate; ++: severe.

the number of matrix content, when compared with that of control group. When compared within the growing zone, the thickness of the cells in the supplementary zone appeared thicker with more cells in the glucosamine than in the control group.

3.3. Image analysis

The results of the image analysis of the special staining samples were shown in Tables 5–7. After carrying out image analysis of various restored parts (Table 5), the non-injured articular cartilages (Table 6) and growing zone (Table 7) with the Alcian blue stains (Fig. 2c,d), the glucosamine group showed highly significant values in restored parts and the articular cartilages. However there was no significance in the growing zone. Carrying out same image analysis of various parts on the Safranin O stains (Fig. 2e,f, Tables 5–7), the glucosamine group

Table 3
Macroscopic findings of the operated sites at 3 weeks after the operation (There are significant difference at the proximal points ($P = 0.045$) and the total points ($P = 0.026$))

Experimental group	Sex	Degree of healing[a]			Total points
		Medial trochlear ridge	Trochlear sulcus (proximal)	Trochlear sulcus (distal)	
Control group					
1	Female	2	1	3	6
2	Female	3	3	2	8
3	Female	3	1	2	6
4	Male	3	1	1	5
5	Male	3	1	3	7
6	Male	1	3	3	7
Mean		2.5	1.7	2.3	6.5
Standard deviation		0.8	1.0	0.8	1.0
Glucosamine group					
1	Female	3	3	3	9
2	Female	3	3	3	9
3	Female	3	3	2	8
4	Male	3	3	3	9
5	Male	1	2	3	6
6	Male	3	3	3	9
Mean		2.7	2.8	2.8	8.3
Standard deviation		0.8	0.4	0.4	1.2
P[b]		0.68	0.045	0.211	0.026

[a] 0 point: Less than 50% healing; 1 point: 50~60% healing; 2 points: 60–80% healing; 3 points: 80~100% healing.
[b] Statistical analysis was performed between control and glucosamine groups on each operated area and total points.

Table 4
Effect of glucosamine on the muscle weights of the femur (There are significant difference at the muscle weight ratio of biceps of the femur muscle $P = 0.0196$)

Rabbit number	Control group		Glucosamine group	
	Lateral great muscle	Biceps of the femur muscle	Lateral great muscle	Biceps of the femur muscle
1	95.4[a]	96.7	100.5	106.2
2	91.2	91.2	87.6	102.2
3	93.7	95.8	97.7	100.6
4	92.2	89.1	94.8	96.4
5	94.5	99.1	101.3	100.3
6	97.2	99.1	99.1	100.1
Mean ± S.D.	94.0 ± 2.2	95.2 ± 4.2	96.8 ± 5.1	101.0 ± 3.2
	$P = 0.115$			
		$P = 0.0196$		

[a] Muscle weight ratio was calculated by following formula: muscle weight ratio = operate side muscle weight/non-operate side muscle weight.

showed higher values in the restored part of articular cartilage and in the growing zone.

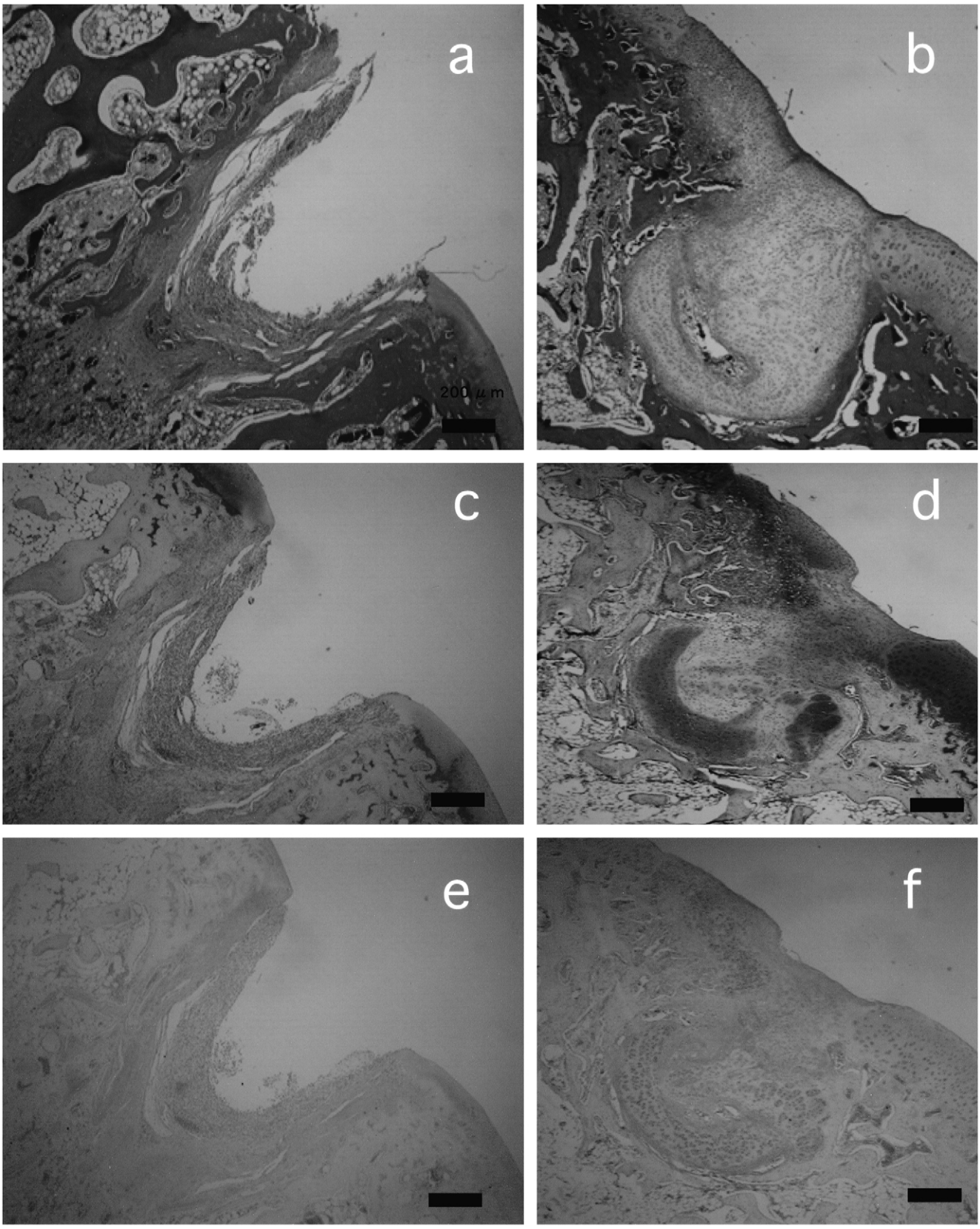
4. Discussion

The present study proved that oral administration of glucosamine hydrochloride of 1 g/head (400 mg/kg on the average) facilitate healing of artificially created cartilage injuries in rabbits. Particularly, the restoration of the injuries on the trochlear sulcus was remarkable. We conducted this experiment under maximum dose of glucosamine that we could give to the rabbits. However, this dose of glucosamine hydrochloride did not cause side effects such as diarrhea, appetite loss and weight loss. In human, the efficiency of glucosamine against arthritis was reported when its oral administration of 1.0–1.5 g/head. Regarding the dose of glucosamine, further study is necessary.

The injuries on medial trochlea caused almost no physical stimulus at the injured part when the knee joint was in motion. On the other hand, since the patella's pressure stimulus impinges on the injuries of the trochlear sulcus constantly, the result seems to have highlighted the healing effect of the glucosamine group in comparison with the control group. The difference between both of the groups is apparent in terms of the histological findings. The effects of glucosamine were summarized as follows. (1) Glucosamine increases the number of cartilage cells even in the normal cartilage. (2) With respect to the injured part, the stimulus on it was much stronger and proliferated cartilage blast cells in a massive manner. (3) The injured bone trabecula in the control group was not treated within 3 weeks and permeation into osteoclast was observed. However, in the glucosamine group perfect restoration has completed and no osteoclast was observed. (4) From the special staining sample of matrix, there was an overwhelming increase in the content by the amount of proteoglycan and that of glycosaminoglycan in the glucosamine group. The amount of synthesized proteoglycan of cartilage cells in a reported in vitro experiment increased/decreased depending on the amount of glucosamine administration. Glucosamine is known to facilitate biosynthesis of collagen (Theodosaikis, Adderly & Fox, 1997).

There are many papers reporting the usefulness of a large amount of orally administered glucosamine in improving the symptoms of osteoarthritis (Dovanti et al., 1980; Kajimoto et al., 1998). Karzel and Domenjoz (1971) speculated that administration of externally generated glucosamine directly transform it into *N*-acetylglucosamin-6-phosphoric acid and then into hyaluronic acid or keratan sulfate (Karzel and Domenjoz, 1971). Although it was only speculation, the present experiment was able to verify these ideas in the in vivo experiment. However, there is no direct evidence on whether glucosamine hydrochloride is used for the transformation of hyaluronic acid, chondroitin sulfate etc. that are important among glycosaminoglycans in composing

Fig. 2. Histological findings at 3 weeks after the operation (lower magnification images) (a) In the control rabbits, the holes were covered by connective tissues and there was a slight regeneration of cartilage in the most medial surface (HE staining). (b) In the glucosamine, the regeneration of massive cartilage was observed and the restored of the bone crest was also observed. (c) In the Safranin O staining, the injured site of the control showed no positive Safranin O staining image. The strength of staining inside the cartilage of the non-injured parts is poor in comparison with it of the glucosamine group. (d) Safranin O staining image of rabbits in the glucosamine group of the same parts of the body. There was a clear positive Safranin O staining image at the injured parts inside the regenerated cartilage. The degree of staining inside the non-injured cartilage shows a clear increase in comparison with rabbits in the control group. (e) Alcian blue staining image of rabbits in the control group of the same parts of the body. There is a small positive part in the injured parts. The degree of staining inside non-injured cartilage is poorer in comparison with rabbits in the glucosamine group. (f) Alcian blue staining image of rabbits in the glucosamine group of the same parts of the body. There was a clear positive part at the injured parts inside the regenerated cartilage. The degree of staining inside the non-injured cartilage shows a clear increase in comparison with rabbits in the control group.


a
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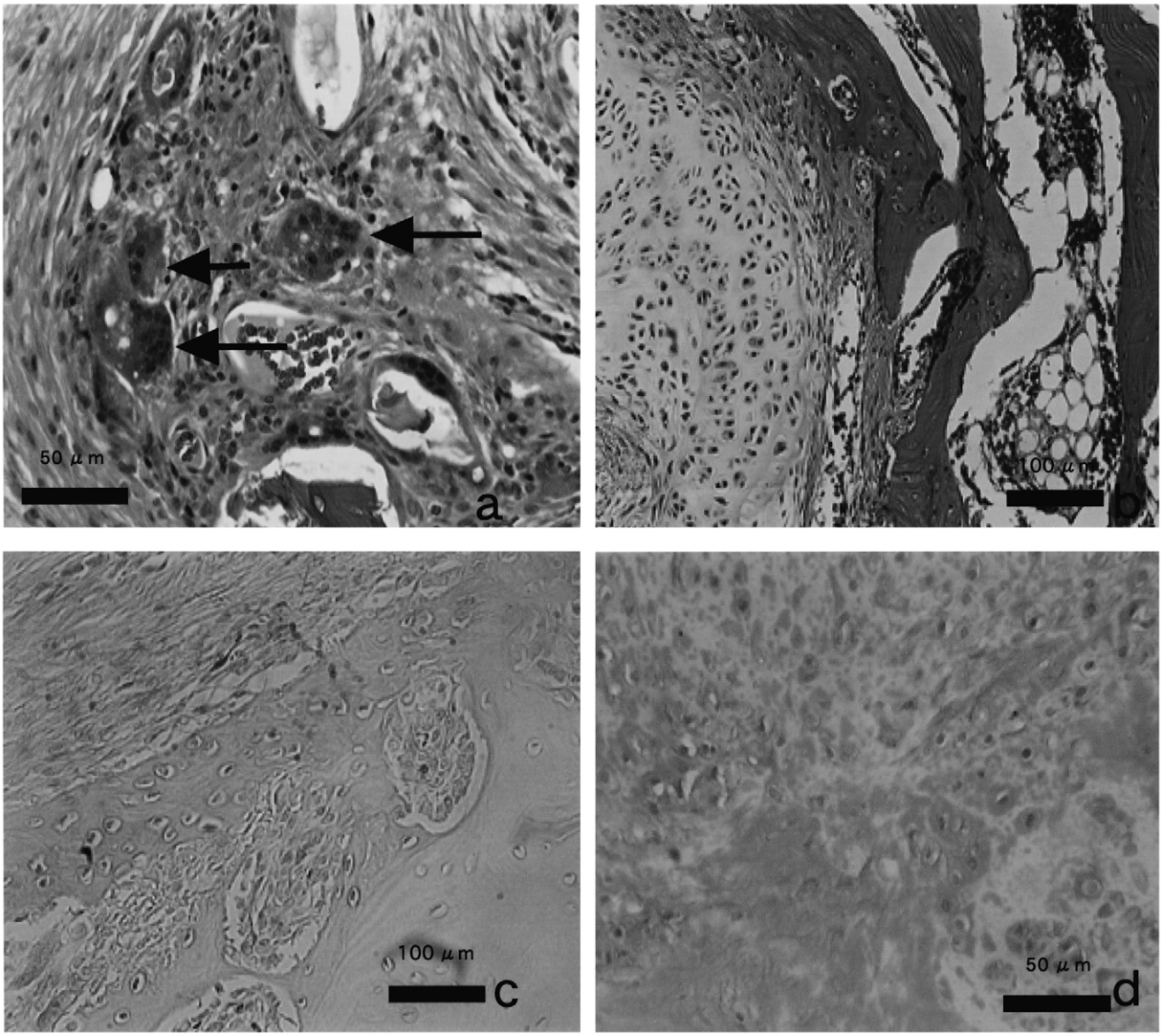

Fig. 3. Histological findings 3 weeks after the operation (higher magnification images) (a) The restored part of the bone trabecula of rabbits in the control group. Permeation was found in inflammation cells and osteoclast (marked with arrowheads). (b) The restored parts of the bone trabecula of rabbits in the glucosamine group. (c) The parts restored from the injuries in rabbits of the control group. A thin layer of regenerated cartilage tissue is observed in the connective tissue layer immediately beneath the surface of the injured parts. (d) The parts restored from the injuries in rabbits of the glucosamine group. A massive proliferation of cartilage blast cells is observed.

cartilage. This requires further study in the future. With respect to the facilitation of restoration of the bone crest and prevention of atrophy of muscles, the anti-inflammatory effect of glucosamine as well as an increase of matrix should be taken into account. Glucosamine is already known to have anti-inflammatory activity (Setnikar et al., 1991) without potential dependence on cyclooxygenase, which is different from regular anti-inflammatory agents, as well as the action of free radical's scavenger (Bucci, 1994). These effects are considered to be responsible for the restorative effect of the bones and the pain-reducing effect that lead to the maintenance of movement function. There was no significant difference between both groups in the lateral great muscle. The fact that there was a significant difference detected between the groups in the biceps of the femur seems to point to the possibility that it is related to the movement of the muscle. The former has the role of extending the knee joint and the latter has the role of flexing the joint. When flexing the knee joint, the patella is strongly pressed by the joint sulcus. This causes stitches. Therefore, when restoration of the injuries was delayed, the knee joint was extended in order to alleviate the pain. It was believed that disuse atrophy occurred due to non-use of flexing biceps of the femur.

With regard to the regeneration of cartilage, Mitchell and Shepard (1976) reported that the tissues observed one year

Table 5
Effect of glucosamine on matrix formation at the restored areas by the image analysis of special staining speciments (Total pixels 120,000 (random sampling of 20,000 pixels at 6 locations) were calculated through the image processing technique)

	Safranin O staining		Alcian blue staining	
Group	Control group	Glucosamine group	Control group	Glucosamine group
1	129.72[a]	173.85	149.94	158.43
2	129.85	175.34	149.15	158.06
3	130.49	175.68	149.85	161.34
4	131.45	179.26	153.08	159.94
5	130.90	179.70	152.69	159.85
6	131.22	174.72	147.58	160.88
Mean	130.61	176.43	150.38	159.75
p	< 0.0001		< 0.0001	

[a] Pixels: The 200 times magnified images of restored parts with Safranin O stains and Alcian blue stains were taken into the computer Photograb 300 and the images digitized by using Adobe Photoshop.

Table 6
Effects of glucosamine on matrix formation at the normal cartilage of trochlear sulcus of femur by the image analysis of special staining speciments (Pixels: The 200 times magnified images of restored parts with Safranin O stains and Alcian blue stains were taken into the computer Photograb ab-300 and the images digitized by using Adobe Photoshop. Total pixels 120,000 pixels (random sampling of 20,000 pixels at 6 locations) were calculated through the image processing technique)

	Safranin O staining		Alcian blue staining	
Group	Control group	Glucosamine group	Control group	Glucosamine group
1	152.30	202.19	148.67	167.17
2	153.93	202.40	149.25	174.51
3	154.28	202.85	150.24	172.94
4	151.98	201.76	154.26	172.25
5	154.53	200.20	154.20	167.84
6	149.34	199.23	152.35	173.55
Mean	152.73	201.44	151.50	171.38
p	< 0.0001		< 0.0001	

Table 7
Effects of glucosamine on matrix formation at the growing zone by the image analysis of special staining speciments (Pixels: The 200 times magnified images of restored parts with Safranin O stains and Alcian blue stains were taken into the computer Photograb ab-300 and the images digitized by using Adobe Photoshop. Total pixels 120,000 pixels (random sampling of 20,000 pixels at 6 locations) were calculated through the image processing technique)

	Safranin O staining		Alcian blue staining	
Group	Control group	Glucosamine group	Control group	Glucosamine group
1	184.90	193.98	167.59	170.72
2	187.62	195.84	169.84	172.51
3	193.85	198.29	171.74	174.52
4	186.59	197.19	175.72	174.44
5	183.02	197.44	175.25	172.63
6	180.39	198.10	173.96	174.56
Mean	186.06	196.81	172.35	173.23
p	0.0003		0.56	

after the injury showed the characteristics of fibrous cartilage-like tissues rather than cartilage-like tissues. In addition, Furukawa, David, Koide, Melvin and Glimcher (1980) reported that there was a large amount of variance in the hexosamine content of the restored cartilage (galactosamine/glucosamine). According to the report, although the restored tissues resemble hyaline cartilage due eventually to the reduction in proteoglycan, complete healing cannot be attained because of the mixture of fibrous cartilage and hyaline cartilage. Since the present experiment did not investigate on collagen content in the restored tissue, it is impossible to decide the restored tissue will be mature hyaline cartilage or not. In the future study, a longer period of observation and an analysis of regenerated cartilage are necessary.

As a model of restoration of the knee joint, the present experiment made it possible to acquire useful information without difficulty and appropriate evaluation of administered substances. From the result of the present experiment, we can conclude that glucosamine facilitates healing of mechanical injuries on cartilage and that glucosamine increases the amount of glycosaminoglycan and that of proteoglycan. Since glucosamine increased glycosaminoglycan and proteoglycan evn in the normal tissues, we speculate that glucosamine may be useful for the prevention of articular cartilage injury due to aging and for players and athletes who excessively use their joints. While glucosamine has been drawing attention recently as oral agents that can improve pathology of osteoarthritis, the present paper is the first-ever report, we know of, which proved its effect experimentally. Systemically, glucosamine would be used effectively for synthesis of extracellular matrix polymers.

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