# CARTILAGE CHANGES IN RATS INDUCED BY PAPAIN AND THE INFLUENCE OF TREATMENT WITH N-ACETYLGLUCOSAMINE

by J. GREVENSTEIN*, I. MICHIELS*, M. ARENS-CORELL** and E. STOFFT***

**Cartilage damage in the knee joints of rats was induced with a single intraarticular injection of 10 mg papain. Such damage was indicated by a decrease in the content of glycosaminoglycans and histologically detectable alterations in the surface of patellar cartilage. Daily treatment of the rats with N-acetylglucosamine by gavage led to an increase of the glycosaminoglycans of the cartilage and of incorporation of the radioactive precursor of glycosaminoglycan synthesis, 3H-glucosamine hydrochloride, in comparison to control animals treated with saline. The incidence of pathological alterations of the cartilage in histological samples was reduced.**

**Keywords** : cartilage ; papain ; treatment ; N-acetylglucosamine.
**Mots-clés** : cartilage ; papaïne ; traitement ; glucosaminoglycans.

## SAMENVATTING

*J. GREVENSTEIN, I. MICHIELS, M. ARENS-CORELL en E. STOFFT. Kraakbeendegeneratie in rattenknieën geïnduceerd door papaine en invloed van behandeling met N-acetylglucosamine.*

Door éénmalige injectie van 10 mg papaine in rattenknieën werd een kraakbeendegeneratie geïnduceerd, welke op basis van glucosaminedosering en histologisch werd geobjectiveerd. Door dagelijkse toediening van n-acetylglucosamine kon een stijging van het glycosaminglycaangehalte in het kraakbeen worden waargenomen. In vergelijking met de controlegroep steeg ook de incorporatie van de radioactieve precursor, het 3H-glucosamine hydrochloride. Histologisch viel de met N-acetylglucosamine behandelde groep op door een duidelijk lagere frequentie van anatomo-pathologische kraakbeenveranderingen.

## RÉSUMÉ

*J. GREVENSTEIN, I. MICHIELS, M. ARENS-CORELL et E. STOFFT. Influence de la N-glucosamine acétylique sur la dégénérescence cartilagineuse du genou du rat, provoquée par l'injection de papaïne.*

L'injection de 10 mg de papaïne dans le genou du rat, provoque une dégénérescence cartilagineuse, concrétisée d'une part par la baisse des glucosaminoglycans et d'autre part par des altérations histologiques.
L'administration journalière de N-glucosamine acétylique aux rats aboutit à une augmentation des glucosaminoglycans du cartilage et à une croissance de l'incorporation du précurseur radioactif de la synthèse des glycosaminoclycans, le H-Glucosamine hydrochloride en comparaison avec les animaux traités avec une solution saline isotonique. L'incidence des altérations du cartilage dans les échantillons histologiques s'est trouvée réduite.

* Orthopädische Universitätsklinik, Mainz.
** Steigerwald Darmstadt.
*** Anatomisches Institut der Johannes Gutenberg Universität, Mainz (Germany).

## INTRODUCTION

Degenerative joint disease is a common phenomenon in human beings and vertebrates, with increased prevalence in old age. Therapy is mainly restricted to the treatment of symptoms with nonsteroidal antiinflammatory drugs. Efforts have been made to arrest or retard the progression of cartilage degeneration with drugs which stimulate collagen or proteoglycan synthesis or inhibit their enzymatic breakdown. In the preclinical evaluation of the efficacy of such substances they are tested in animal models of degenerative joint disease.

N-acetylglucosamine (GlcNAc) was tested for its incorporation into cartilage glycosaminoglycans and may have antidegenerative potential. This has been verified in a model of experimentally-induced cartilage damage in rats after intraarticular injection of papain (4, 7, 10). Papain catalyzes the hydrolysis of peptide chains in cartilage proteins, affecting primarily the protein cores of proteoglycans and later even the highly resistent collagen chains of cartilage matrix (2), thus mimicking the loss of matrix occurring in human osteoarthrosis. In guinea pigs these changes heal spontaneously (7). In contrast, osteoarthritic cartilage lesions in man are considered functionally irreversible in spite of findings of degenerative processes (8).

The effect of oral treatment with GlcNAc on papain-induced degenerative changes in rat knee joints was studied with respect to its influence on the glycosaminoglycan content, the incorporation of a radiolabelled precorsor in these molecules and cartilage histology.

## MATERIALS AND METHODS

Male Sprague-Dawley rats from Interfauna, Süddeutsche Versuchstierfarm, Tuttlingen, Germany, weighing about 150 g at the beginning of the experiment, were kept in groups of 5 each in Macrolon cages at 24 ± 1°C with a 12-light/dark cycle. The animals had free access to tap water from Macrolon bottles and received standard chow from Altromin, Lage/Lippe, Germany, ad libitum.

Cartilage damage was induced by a single intraarticular injection of 0.1 ml of a 10% (w/v) solution of papain (Serva, Heidelberg, Germany) into the left knee joint. The right knee, serving as an intraindividual control, was injected with an equal volume of 0.9% NaCl.

Two sets of experiments were carried out. Animals of set 1 were randomly assigned to 2 groups of 15 animals each. The group 1 animals serving as controls received 5 ml 0.9% NaCl/kg b.w. daily by gavage, whereas the animals of group 2 were treated with 85 mg GlcNAc/kg b.w. daily by gavage for 23 days. In experiment set two, 5 groups with 5 animals each were compared. Animals of group 1, as controls received NaCl, whereas animals of groups 2 through 4 were given N-acetylglucosamine in daily oral doses of 10, 100 and 1000 mg/kg b.w., respectively. Rats of group 5 were injected 3 times/wk intraarticularly with 100 mg N-acetylglucosamine/kg b.w. into both knee joints. The animals of experiment set 1 were sacrificed after 23 days. The knee joints were excised and trimmed of muscles and tendons. The joints of 10 animals from each group were further processed for measurement of their glycosaminoglycan content. From the joints of 5 animals per group, histologic sections of femora, tibiae and patellae were made.

In experimental set 2, which lasted 34 days, the rats received an intravenous injection of 370 kBq of H-glucosamine hydrochloride (GlcN.HCl) with trace amounts of the nonlabelled compound which is a precursor for the synthesis of glycosaminoglycans. The joints were removed as described above, and glycosaminoglycans extracted. The glycosaminoglycans were solubilized from the knee joints by 22h incubation in 50-mM potassium phosphate buffer pH 6.5 with 2-mM N-acetylcysteine and 2-mM $Na_2$ EDTA and 10-mg papain per sample at 65°C. The hydrolysate was used for the determination of the content of glycosaminoglycans in experiment 1 as described below. Further purification of glycosaminoglycans was performed in experiment 2 by subsequent precipitations with a 10% solution of acetylpyridiniumchloride for 2 h at 38°C and twice with ethanol overnight in the refrigerator after dissolving the pellets obtained by 30-min centrifugation at 15,000 g. The pellets of the last precipitation were dried at 80°C for 6 h, weighed and burned in a

Packard TriCarb sample oxidizer to obtain tritium-labelled water, the activity of which was measured after addition of Permafluor (Packard) in a Packard TriCarb liquid-scintillation counter. Values of activity were determined as dpm after correction using the channel relation of an external standard.

The glycosaminoglycans were determined with the colorimetric method of Farndale *et al.* (3) : 2.5 ml of the color reagent, containing dimethyl methylene blue (Serva, Heidelberg, Germany) were added to 1 ml of the sample, mixed, and absorbance was measured immediately in a photometer at 535 nm. Chondroitin sulphate at concentrations from 1 to 100 µg/ml was used for calibration.

## RESULTS

In the first set of experiments, the glycosaminoglycan content of the left, papain-damaged knee joints was higher than in the right in both groups. Rats receiving 85 mg GlcNAc/kg b.w. daily p.o. had higher content of glycosaminoglycans in both knee joints than saline-treated control animals (table I). The differences were not statistically significant.

Table I. — Glycosaminoglycan content of rat knee joints after intraarticular injection of papain

| group | right knee joint | left knee joint |
|---|---|---|
| 1 | 760 ± 228 | 790 ± 272 |
| 2 | 800 ± 205 | 870 ± 198 |

Rats received a single intraarticular injection of papain into the left knee joint and were afterwards treated with 0.9% NaCl (group 1 : control) or 85 mg GlcNAc/kg b.w. (group 2) for 23 days. The glycosaminoglycan content of the knee joints was determined photometrically with chondroitin sulfate as standard substance. Mean values and standard deviations for 10 animals in each group are given in µg chondroitin sulfate/joint.

In the second set of experiments, the incorporation of $^{3}$H-GlcN into glycosaminoglycans in the knee joints, as measured by the specific activity of the glycosaminoglycans, was higher in rats treated with 10, 100 and 1000 mg GlcNAc/kg b.w. daily p.o. than in saline-treated controls after papain injections, and only slightly less in comparison with untreated animals (table II). Untreated animals showed the highest values ; control animals, receiving saline after the papain injection, the lowest. Whereas the glycosaminoglycan content of the right knee joints was similar in all three groups which were treated with N-acetylglucosamine, the two lower dosages of the compound led to higher values for the left damaged knee joint than the largest dosage.

Table II. — Incorporation of $^{3}$H-GlcN into the glycosaminoglycans in rat knee joints after intraarticulation of papain

| group | right knee joint | left knee joint |
|---|---|---|
| 1 | 106 ± 32,5 | 77 ± 42,1 |
| 2 | 151 ± 23,5 | 167 ± 40,9 |
| 3 | 155 ± 50,8 | 160 ± 14,9 |
| 4 | 154 ± 47,6 | 119 ± 19,1 |
| 5 | 204 ± 17,6 | 190 ± 19,8 |

After single intraarticular injection of papain into the left knee joint, rats were treated with saline (group 1) or 10, 100 and 1000 mg GlcNAc/kg b.w. daily p.o. (groups 2, 3 and 4, respectively). The results were compared to values from untreated animals (no papain, no GlcNAc, group 5). The 3H-GlcN HCl was injected i.v. 24 h prior to sacrifice. The radioactivity of the glycosaminoglycans in the knee joints was determined and is given as the mean specific activity in dmp$^{3}$H/mg glycosaminoglycans and the standard deviation for 5 animals in each group.

Fissures and roughening of the cartilage surface were seen more frequently in histological samples of the left patellae than of the right (fig. 1 and 2) in controls receiving saline in the first set of experiments. Occasionally, slight damage was also observed in the right knee joint. In animals treated with N-acetylglucosamine, no such lesions occurred in the three left specimens, whereas in the control group, all five patellae showed superficial damage (table III).

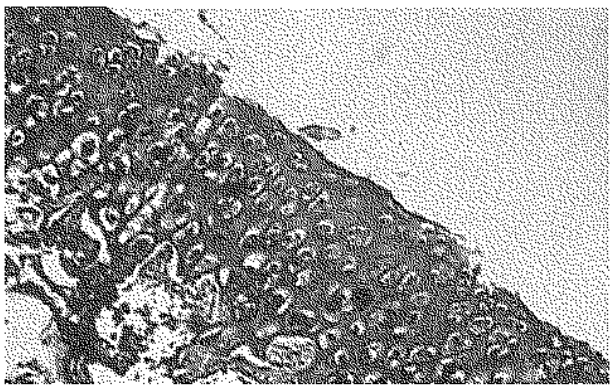

*Fig. 1.* — Histological section of a normal rat patella. The patella of the right knee joint of a rat 23 days after a single intraarticular injection of papain into the left knee joint was fixed in Bouin, decalcified with EDTA and embedded in Paraplast, and 0.8-mm slices were stained with HE and PAS. 400 × magnification.

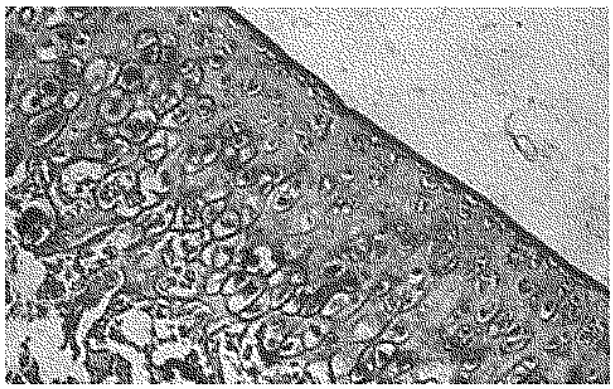

*Fig. 2.* — Histological section of a papain-damaged rat patella. The left patella of a rat 23 days after a single intraarticular injection of papain into the left knee joint was fixed in Bouin, decalcified with EDTA and embedded in Paraplast, after which 0.8-mm slices were stained with HE and PAS. 400 × magnification.

Table III. — Frequency of histological changes in the cartilage surface of rat patellae after intraarticular injection of papain

| group cartilage | number of specimen | | surface of patellar | | | |
|---|---|---|---|---|---|---|
| | right | left | intact | | damaged | |
| | | | right | left | right | left |
| 1 5 | 5 | 5 | 4 | 0 | 1 | |
| 2 0 | 4 | 3 | 3 | 3 | 1 | |

Histological samples of the patellae of rats experimentally damaged by a single intraarticular injection of papain into the left knee were examined for fissures and roughening of the cartilage surface. The animals received daily oral treatment with saline (group 1 : control) or 85 mg GlcNAc/kg b.w. for 23 days.

## DISCUSSION

Intraarticular injection of papain induces cartilage damage in joints as demonstrated by Sokoloff (1956). Papain destroys the core proteins of the proteoglycans, the link proteins stabilizing proteoglycan aggregates and the collagen chains. Inflammatory reactions induced by the products of these breakdown reactions may additionally lead to secondary destruction of the cartilage by mediators such as Interleukin-1 produced in the synovial cells and in the chondrocytes. As a reaction to the loss of matrix, chondrocytes produce increased amounts of proteoglycans (1).

Preliminary experiments demonstrated the incorporation of exogenously supplied GlcNAc into glycosaminoglycans as the basis of a possible beneficial effect on cartilage degradation. In fact, GlcNAc-treated rats showed an increased glycosaminoglycan content in the knee joints as compared to saline-treated controls after matrix depletion by papain. It is not likely that orally administered saline had an effect on this parameter. Therefore, the controls show the spontaneous development of the cartilage damage. This also holds true for the rate of incorporation of the precursor into the glycosaminoglycans. The increased specific activity of the glycosaminoglycans in GlcNAc-treated animals in comparison to controls adds further to the arguments for a favorable influence of the substance on experimentally damaged cartilage. Nevertheless, the joints of papain-treated animals receiving GlcNAc contained slightly lower concentrations of glycosaminoglycans than those of undamaged and untreated rats. By supplying the knee joints with an increased amount of GlcNAc, which serves as a precursor for the synthesis of glycosaminoglycans, GlcNAc

probably accelerates the repair of matrix damage induced by the action of papain. An increased requirement of precursors for the synthesis of glycosaminoglycans is also to be expected in osteoarthritic cartilage (1), where chondrocytes initially show higher rates of proteoglycan synthesis as a reaction to the matrix depletion.

GlcNAc may also have a protective effect on cartilage, partially preventing the initial enzymatic matrix depletion. Further possibilities to be considered in future experiments are effects on inflammatory reactions of the synovia or regulatory effects on the synthetic activity of the chondrocytes. The determination of qualitative changes in the cartilage matrix following papain damage and N-acetylglucosamine treatment could help to elucidate the mechanism by which the effects we observed were induced and their significance for cartilage function and the course of cartilage degeneration. The effect of N-acetylglucosamine in other experimental models of degenerative joint disease would be a further step in defining the therapeutic value of the substance.

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J. GREVENSTEIN
Orthopädische Klinik und Poliklinik
der Johannes Gutenberg Universität Mainz
Langenbeckstr. 1
1-6500 Mainz (Germany)