

# Gross Morphology Analysis of Osteoarthritis in Rabbits

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## Abstract

The aim of this study was to evaluate the effect of glucosamine sulfate on morphological changes investigated on the articular cartilage of knee joint during development of osteoarthritis in rabbits following collagenase-induced osteoarthritis. A total of 32 New Zealand White male and female rabbits were used in this study. All animals were kept in standard conditions at Research Institute of Livestock Production in Nitra. Animals were divided into 4 groups: healthy control group, osteoarthritic untreated group and two experimental groups. After collagenase-induced osteoarthritis daily oral administration of glucosamine sulfate (80 mg.kg<sup>-1</sup> and 160mg.kg<sup>-1</sup> of live weight) was performed. At the end of the eight week, the rabbits were sacrificed and their both right and left knees with proximal femur and distal tibia were harvested. Gross morphology of knee joint was tested. Basal statistical indicators were calculated from obtained data using Statgraphics software and analysis of variance. Morphological changes in osteoarthritic group without treatment were seen on both, medial and lateral region, but markedly on medial condyle. Articular cartilage was characterized by a rough and hypertrophic appearance with severe erosions. The severity of cartilage damage was generally lower in glucosamine treated groups in comparison with osteoarthritis group. The gross morphological examination of healthy control revealed very little or no changes. Gross morphological grading of cartilage damage showed significantly lower extent of damage in glucosamine treated groups.

**Keywords:** glucosamine sulfate, gross morphology, knee joint, osteoarthritis, rabbit

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## 1. Introduction

Osteoarthritis is a slowly progressive degeneration of articular cartilage, which is manifested mainly in supporting joints such as hip, knee, lumbar spine and the joints of the fingers below the upper limb in older people [1]. Osteoarthritis is considered a degenerative illness, but it is a metabolically active process, in which pro-inflammatory cytokines are involved. Secondary inflammatory changes arise and clinical synovitis may occur in the synovial membrane [2]. Osteoarthritis affects not only the articular

cartilage, but all periarticular structures, including subchondral bone, synovial membrane, joint capsule, ligaments and adjacent muscles [3]. The cartilage damage are emerging cracks, scratches and ulceration and cartilage is thinner [4]. Osteoarthritis is a disease that includes both destructive and the reparative processes in the cartilage. These can be activated by various biochemical or mechanical damage of the joint

## 2. Materials and methods

Monitoring the effects of chondroprotectives on articular cartilage during the development of osteoarthritis in rabbits after enzymatic induction of osteoarthritis was performed in one experiment. Laboratory animals were kept under standard conditions in an approved experimental facility of

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Animal Production Research Centre Nitra. Male and female New Zealand white rabbits of average live weight  $2500 \pm 100$  g were used in the experiment. Animals were divided into 4 groups: healthy control group, osteoarthritic untreated group and two experimental groups. After collagenase-induced osteoarthritis, daily oral administration of glucosamine sulfate ( $80 \text{ mg} \cdot \text{kg}^{-1}$  GS1 group and  $160 \text{ mg} \cdot \text{kg}^{-1}$  GS2 group of live weight) was performed. At the end of the eight week, the rabbits were sacrificed and their both right and left knees with proximal femur and distal tibia were harvested. The parameter tested was gross morphology. Basal statistical indicators were calculated from obtained data using Statgraphics software and analysis of variance. The treatment was prophylactic - test substances were applied from the first day of the experiment. Treatment lasted a total of 60 days. Original glucosamine sulfate with a molecular weight of 456.42 (Rottapharm, Ltd) was used. The solutions were prepared fresh before each administration to animals. Glucosamine sulfate was administered orally to rabbits through the probe a daily basis.



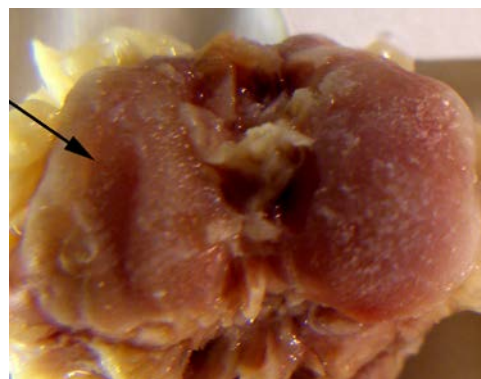
**Figure 1.** Lateral and medial femoral condyle in the healthy control group

Comparing the differences between the control groups and experimental groups, we observed statistical evidence ( $p < 0.05$ ). The severity of damage of the articular cartilage surface was generally lower in the groups treated with glucosamine sulphate compared to osteoarthritic group. We can conclude that the severity of damage to the articular cartilage during development of osteoarthritis in the knee joints of rabbits proportionally decreased with increasing doses of glucosamine sulfate. Persiani et al., [5]

Osteoarthritis was induced by applying 0.5 ml of collagenase ( $1.5 \text{ mg} \cdot \text{ml}^{-1}$ ) (Sigma-Aldrich Chemie GmbH) in saline intraarticular injection into the right and left knee joint of rabbits. Water for injection purposes in healthy control animals and untreated osteoarthritic control animals was used. After completion of the experiment, rabbits were subjected to euthanasia. After euthanasia, femur and fibula together were dissected for gross morphological examination.

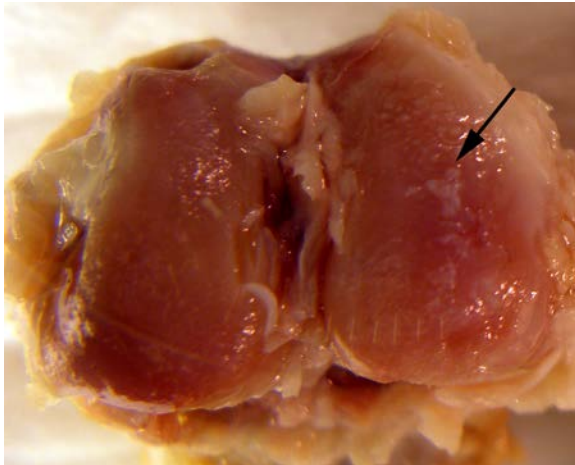
### 3. Results and discussion

Morphology of the lateral and medial femoral condyle in the healthy control group shows normal appearance and smooth surface of the cartilage (Figure 1). Morphological changes in osteoarthritic control group without treatment were visible in the medial and lateral area of the condyles of the femur, but more pronounced in the medial condyle. The surface of the articular cartilage had been roughened and severe erosions were found (Figure 2).

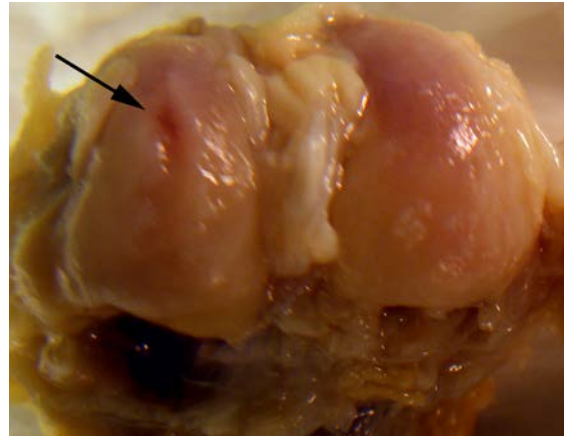


**Figure 2.** The macroscopic morphology of the femoral condyles-untreated control

investigated the bioavailability and pharmacokinetics of glucosamine sulfate in an open, randomized, crossover study, which concluded that increased doses of glucosamine sulfate, similar doses to those used in this experiment, no longer increases the effect of glucosamine. The macroscopic morphology of the femoral condyles in the group treated with glucosamine sulphate (GS1) had a significantly roughened surface with mild to severe erosions (Figure 3).



**Figure 3.** The macroscopic morphology of the femoral condyles-group GS1



**Figure 4.** The macroscopic morphology of the femoral condyles-group GS2

The mutual comparison of groups treated with glucosamine sulfate alone at different doses (GS1, GS2) show statistical significant differences. Similar results have been documented by Kim et al. [6] in the experiment with the enzyme-induced osteoarthritis in the rabbit.

Results of animal studies, pilot clinical studies and multicenter clinical studies argue in favor of the favorable action of sodium hyaluronate and glucosamine to structural changes in the joint [7, 8, 9]. Symptomatic slow-acting drugs for osteoarthritis are defined as drugs that improve pain and joint function with some delay, but the beneficial effect persists even after treatment. Their effect occurs after two to four weeks of treatment and persists for more than two months after its completion [10]. Action profile is therefore strictly distinguished from fast acting analgesics and nonsteroidal antirheumatic drugs [11].

Glucosamine sulfate is a derivative of the naturally occurring amino-monosaccharide glucosamine. Original crystalline glucosamine sulphate (also used in the model of osteoarthritis) is chemically well defined neat substance with low molecular weight (456.42), which is easily distinguished from the more non-defined macromolecular cartilage extracts of animals [5, 12]. Glucosamine sulphate dissociates into a sulfate ion and D-glucosamine, which is then absorbed in the small intestine. Serum reaches a maximum after 60 minutes and absolute bioavailability after oral administration is about 72%. Following absorption of glucosamine

accumulates in joint cartilage [13]. Glucosamine has an important role in the metabolism of cartilage as it forms the polysaccharide chains of proteoglycans joint synovial fluid and cartilage matrix [14]. Glucosamine sulfate is an essential substrate for the biosynthesis of cartilage proteoglycans [15]. Glucosamine is synthesized by chondrocytes from glucose in the presence of the glutamine [16]. Subsequent biochemical reactions glucosamine is used for the synthesis of glycosaminoglycans and proteoglycans [17]. In experimental animal models, the glucosamine sulfate is able to slow the development of osteoarthritis [18].

#### 4. Conclusions

Osteoarthritis is a disease that although the first place affects the articular cartilage and subchondral bone, but at the same time also affects other joint structures and the surrounding tissue. Osteoarthritis is perceived as a process which proceeds from pathobiochemical changes in articular cartilage to morphologic changes.

Morphological changes in osteoarthritic control group without treatment were visible in the medial and lateral area of condyles of femur, but more pronounced in the medial condyle. Differences between the control groups and experimental groups were statistically significant. The severity of articular cartilage damage during development of osteoarthritis in the knee joints of rabbits proportionally decreased with increasing doses of glucosamine sulfate.

This study showed that oral administration of glucosamine sulphate slowing the degenerative process and play important role in the protection of cartilage in the early stages of the development of osteoarthritis.

### **Acknowledgements**

This work has been supported by VEGA project No. 2/0037/16 (Ministry of Education, Slovak Republic).

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