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Effect of methylprednisolone, hyaluronic acid and pioglitazone on histological remodeling of temporomandibular joint cartilage in rabbits affected by drug-induced osteoarthritis

Wpływ metyloprednizolonu, kwasu hialuronowego oraz pioglitazonu na histologiczną przebudowę chrząstki stawowej stawu skroniowo-żuchwowego u królików prezentujących polekowe zapaleniem chrząstki stawowej

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

Krzysztof Kałużynski^{1, A, B, C, D, E, F}, Grzegorz Trybek^{1, A, B, C, D, E, F},
Tomasz Smełka^{3, D, E, F}, Marek Masiuk^{2, B, D}, Leszek Myśliwiec^{1, B, D},
Katarzyna Sporniak-Tutak^{3, D, E, F}

¹Department of Dental Surgery, Pomeranian Medical University in Szczecin, Poland

²Department of General Pathology, Pomeranian Medical University in Szczecin, Poland

³Department of Maxillofacial Surgery, Pomeranian Medical University in Szczecin, Poland

Summary

Background:

The aims of this study were to assess the anti-degenerative effects of pioglitazone and to compare these effects with those of methylprednisolone and hyaluronic acid on drug-induced osteoarthritis in rabbits' temporomandibular joint cartilage.

Material and Methods:

The experiment was conducted on 40 Californian white rabbits. Degenerative changes were induced by intra-articular injections of papain. Subsequently, all of the animals were randomly assigned to one of four groups:

- 1) a control group that received no medications;
- 2) a group treated with 4 intra-articular injections of 2 mg (0.2 ml) of hyaluronic acid at weekly intervals;
- 3) a group treated with 4 intra-articular injections of 2 mg (0.1 ml) of methylprednisolone at weekly intervals;
- 4) a group administered pioglitazone orally in daily doses of 2 mg/kg of body weight. Four weeks after the beginning of drug administration, the rabbits were sacrificed. Sagittal sections of the intra-articular cartilage (discs) and mandibular condyles were stained with hematoxylin and eosin by the PAS technique and with van Gieson's solution. Histologic examinations, as well as cartilage thickness and number of cell layers measurements, were performed.

Results:

Histologic assessment in cases of arthritis-associated pathologies revealed that changes occurred most frequently in the control group and least frequently in the pioglitazone group. There were no differences in the histological structures of the intra-articular discs. Cartilage thickness measurements demonstrated the thinnest cartilage in group 2 and the thickest in

Conclusion:	group 3. Analysis of cell layer numbers showed the most numerous layers in the pioglitazone group and the least in the control group.
Key words:	Pioglitazone and hyaluronic acid showed anti-degenerative properties compared to methylprednisolone in an animal model.
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Author's address: dr n. med. Tomasz Smełka, Maxillofacial Surgery Department, 70-111 Szczecin, al. Powstańców Wlkp. 72, tel. 91 4661736, e-mail: smektala.t@gmail.com

INTRODUCTION

The degenerative process is the outcome of loss of balance between the degradation and regeneration of tissue. General risk factors for the occurrence of degenerative changes in the area of the temporomandibular joint are psychogenic stress, neurological and endocrinological disorders, osteoporosis, infectious diseases, genetic factors and developmental disabilities. Local factors include occlusion disorders, habitual displacement of the condylar head, occlusal and non-occlusal parafunctions, trauma, infections and neoplastic lesions [23,25].

Studies of pharmacological treatment for osteoarthritis have resulted in three groups of drugs: non-steroidal anti-inflammatory and analgesic drugs, glucocorticosteroids and medicines supplementing synovial membrane components.

Recently, increased attention has been paid to the role of cytokine regulation in the processes of degradation and regeneration of the articular structures and to the secondary inflammation accompanying the development of degenerative changes [1,6,7,8,19,23,25]. One of the groups of pharmaceuticals modulating cytokine-based mechanisms related to joint inflammation is the thiazolidinedione derivatives, i.e., hypoglycemics used in the treatment of type 2 diabetes. Pioglitazone is one of the latest medicines belonging to this group. Therefore, the aims of this study were to assess the anti-degenerative effects of pioglitazone and to compare these effects with those of methylprednisolone and hyaluronic acid in an animal model of drug-induced osteoarthritis.

MATERIALS AND METHODS

This study protocol was approved by the Local Bioethics Committee (20-05 W-1). Forty healthy 12-month-old

Californian white rabbits weighing between 3.5 and 4 kg were used as experimental animals for the study. Before the study, the general health of the rabbits was monitored for 10 days. The rabbits were kept in standard individual cages and were fed a standard laboratory diet and water ad libitum. The animals were maintained in a room with controlled temperature and humidity (approximately 22°C and 60%) under a 12 h light/dark cycle.

Degenerative changes in the animals' temporomandibular joints were induced by three intra-articular injections of 0.2 mg papain solution at three-day intervals^{2,17}. Twenty-four hours after being administered the last dose of the medication, the rabbits were randomly, by rolling dice, assigned to 4 equal subgroups:

- 1) a control group that received no medication;
- 2) a group treated with 4 intra-articular injections of 2 mg (0.2 ml) of hyaluronic acid at weekly intervals;
- 3) a group treated with 4 intra-articular injections of 2 mg (0.1 ml) of methylprednisolone at weekly intervals; and
- 4) a group administered pioglitazone orally in a daily dose of 2 mg/kg of body weight, for a period of 4 weeks.

Owing to the loss of 2 rabbits, one from the control group and one from the methylprednisolone group, over the course of the experiment, the final number of animals that underwent analysis was 38.

Four weeks after the beginning of drug administration, the rabbits were sacrificed. The intra-articular cartilage (discs) and mandibular condyles were prepared in the form of sagittal sections stained with hematoxylin and eosin by PAS technique and with van Gieson's solution. The samples were histologically assessed, measured and photographed using a Leica Q600 QWin (Leica Microsystems Cambridge, Cambridge, United Kingdom) semiau-

tomatic image analyzing system and a Zeiss Axiophot (Carl Zeiss Microscopy, Thornwood, United States) fluorescence microscope with 4×, 10× and 25× magnification.

Measurement of tissue thickness was performed relative to the whole condylar cartilage sample, in each of 3 zones (superficial zone, transitional zone and deep zone) and in the intra-articular discs. Additionally, the number of cell layers constituting each of the 3 cartilage zones was assessed. The thicknesses of the cartilage were measured at 5 points of the cartilage zone, all of which were distributed at equal distances from one another. Then, the arithmetic mean of the values obtained was calculated. To calculate the number of cell layers in specific zones of the cartilage, the shortest line connecting the boundaries of a given zone was drawn at each of the 5 measurement points distributed at equal distances on the surface of the tissue section, and the number of cells located along each such line was determined. The arithmetic mean was calculated based on the values obtained at each of the 5 measurement points.

The statistical data obtained were evaluated using the Kruskal-Wallis test; the outcomes were then additionally analyzed and verified using Scheffé's and Tukey's tests. Results yielding $p < 0.05$ were considered to be statistically significant.

RESULTS

The histological analysis of the condylar cartilage revealed differences among all of the groups. The reaction of the intercellular substance of the control group to hematoxylin and eosin staining showed the lowest response (Fig. 1).

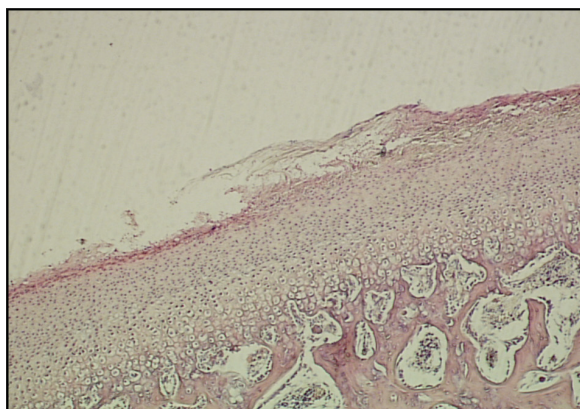


Fig. 1. The cartilage of the mandibular condyle of animals in group 1. Visible poor staining of the inter-cellular substance, thickened and fibrous superficial zone and wide transitional layer. Hematoxylin and eosin staining. Image magnified 4×

The condylar cartilage of the rabbits treated with hyaluronic acid (group 2) reacted strongly to basophilic staining, and the presence of evenly spread subcartilaginous bone cells was observed in the condylar cartilage. Histological investigation also revealed numerous osseocartilaginous trabeculae in the subcartilaginous layer of the bone.

The distribution of chondrocytes in the deep zone of the condylar cartilage was regular; the cells formed characteristic rows and were visibly hypertrophic (Fig. 2).

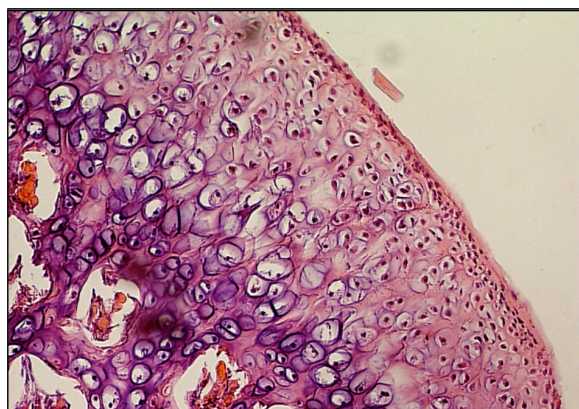


Fig. 2. The cartilage of the mandibular condyle of animals in group 2. Visible strong staining of the inter-cellular substance, in particular, in the deep zone and calcified cartilage layer (arrows). Hematoxylin and eosin staining. Image magnified 10×

Specimens from the third group presented thickening of all of the cartilage zones, particularly the transitional zone formed by undifferentiated mesenchymal (chondrogenic) cells. The deep zone cells were evidently hypertrophic. Moreover, osseocartilaginous trabeculae were unevenly spread along the boundary between the deep zone of the condylar cartilage and the subcartilaginous layer of the bone (Fig. 3).

Analysis of the group receiving pioglitazone showed a proper cartilage structure (Fig. 4).

There were no differences in the histological structures of intra-articular discs among the groups of animals.

Additional evaluation under 4x magnification demonstrated injuries characteristic of degenerative processes,

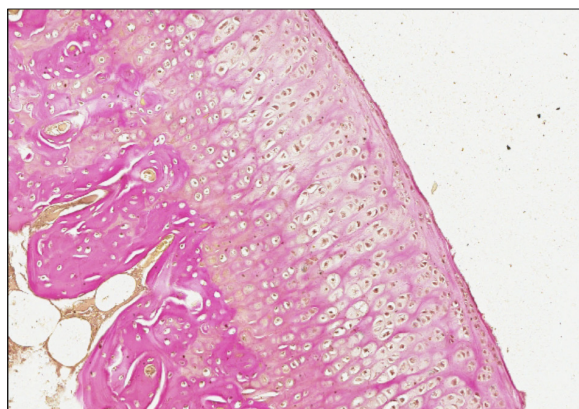


Fig. 3. The cartilage of the mandibular condyle of animals in group 3. Visible cartilage of a considerable thickness. Staining with van Gieson's solution. Image magnified 4×

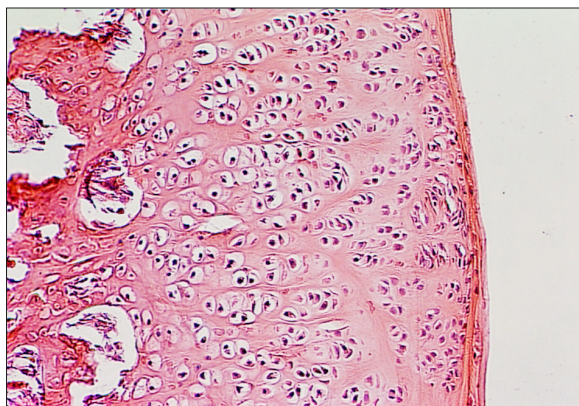


Fig. 4. The cartilage of the mandibular condyle of animals in group 4. Image of a normal cartilaginous tissue structure magnified 10×. Hematoxylin and eosin staining

i.e., roughness of the surface of the cartilage, erosion, fissures or delaminations, tears, fragmentation and deformation of the cartilage. These changes occurred most frequently in group 1 and least frequently in group 4.

Thickness measurements revealed that the thinnest cartilage occurred in group 2 (mean 105.4 μm) and the thickest in group 3 (mean 162.3 μm) (Fig. 5). The mean thickness values in groups 1 and 4 were 130.6 and 114.5 μm , respectively. The differences were statistically significant. The thickness of the deep zones of cartilage also varied from the highest value in group 3 (mean 109.78 μm) to the lowest value in group 2 (mean 62.89 μm) (Fig. 6). Discrepancies between the thicknesses of the superficial and tran-

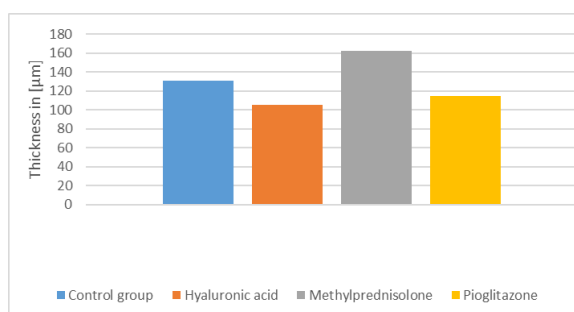


Fig. 5. Mean thickness of the condylar cartilage

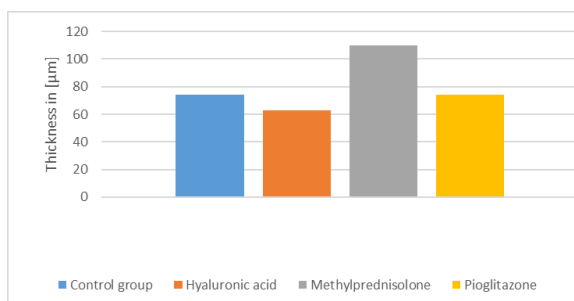


Fig. 6. Mean thickness of the deep zone of the condylar cartilage

sitional zones of the cartilage and average thicknesses of the intra-articular discs were not significant.

The number of cell layers within each of the 3 condylar cartilage zones varied according to the group. In the cartilage samples from group 4, the cell layers were the most numerous, whereas the least numerous were found in groups 1 and 2 (Figs. 7 and 8). Statistically significant differences were found in the transitional zones and deep zones of tissue.

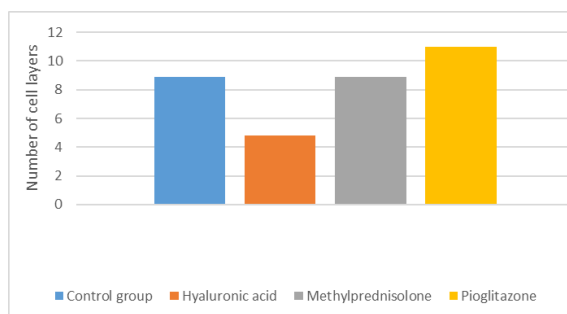


Fig. 7. Mean number of cell layers within the transitional zone of the condylar cartilage

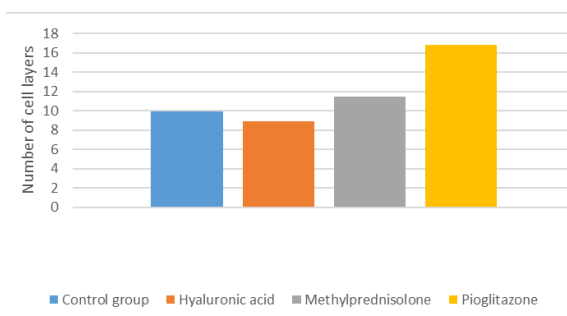


Fig. 8. Mean number of cell layers within the deep zone of the condylar cartilage

DISCUSSION

The structure of temporomandibular joint cartilage in rabbits is similar to humans and consists of four zones: 1) a superficial zone, formed by collagen fibers and sparse cells; 2) a transitional zone, including undifferentiated mesenchymal (chondrogenic) cells; 3) a deep zone, in which the enlarged chondrocytes form rows; and 4) calcified cartilage, adjoining the bone tissue [6,7,10]. This similarity and numerous reports of experimental models for the degenerative changes in cartilage resulted in the selection of Californian rabbits for this study [3,17,18,22].

Histologic examinations of specimens from our study presented degenerative changes that have also been reported by other authors[11,18]. One of these findings was the presence of areas with irregular cartilage thickness. This finding was related, on the one hand, to the loss of volume in the

layer including differentiated cartilaginous cells and the replacement of healthy cartilaginous tissue by tissue affected by fibrosis and, on the other hand, to excessive proliferation of chondrocytes and focal sclerotization of the deep zone, accompanied by the formation of osteocytes [1,6,7,14,16,19,21,22,23,24,25]. Other histological features described in papain-induced degeneration models were: reduction in chondrocyte numbers, empty areas in the extracellular matrix, poor staining of the inter-cellular substance resulting from the lack of safranin staining, and the presence of ossification and sclerotization areas within the deep layers of cartilage^{12,17,18,22}. Studies of osteoarthritis induced by factors other than papain have shown the same findings of cell apoptosis, distorted cell distributions within all cartilage zones and areas of increased ossification in the deep zone [4,6,10,20].

Inter-group analysis showed numerous differences. In group 1, the result of staining of the intercellular substance with hematoxylin and eosin was poorer than in the other groups. One of the causes could be decreased numbers of basophilic proteoglycans in the matrix.

The examination of group 3 revealed hypertrophic cells covering the condylar processes and enlarged isogenic clusters of chondrocytes in the deep zone, which might be evidence of the proanabolic influence of glucocorticosteroids on the metabolism of cartilage and the stimulation of cell proliferation. Nonetheless, typical pathological changes, i.e., erosion, fissures and delaminations, areas of tears, fragmentation and deformation of cartilage, were observed more frequently in both of the above-mentioned groups than in groups 2 and 4. These symptoms could be evidence of the tissue's decreased fracture resistance and its susceptibility to mechanically induced injury. The similarity between the results of the examinations of groups 1 and 3 might confirm the low efficacy of intra-articularly administered glucocorticosteroids in the treatment of degenerative changes [5,9,12,15].

In our experiment, the most numerous normal or nearly normal histological images were found in group 4. There were no symptoms of tissue remodeling either, which might have been the result of inhibition by pioglitazone of cartilage structure disorders, as has also been reported by other authors. These results corresponded to those obtained by Kobayashi et al. [13] and Boileau et al. [5] in dogs and guinea pigs, respectively.

The characteristic features of the samples collected from animals treated with hyaluronic acid were symptoms of ossification, accompanied by the penetration of the deep zone of the cartilage by the subcartilaginous bone and the presence of evenly spread osseocartilaginous trabeculae. This finding

might be related to an increased rate of ossification and the activity of reparative processes in the condyles. These observations do not prove the existence of any irregularities, either in the structure or in the arrangement of cartilage, in group 2; rather, they might imply the efficacy of hyaluronic acid therapy.

The results of thickness measurements of condylar cartilage revealed the highest values in the group treated with methylprednisolone. Unfortunately, we did not find information in the literature about the thickness changes in cartilage submitted to treatment with glucocorticosteroids. Nevertheless, these results might confirm the influence of proanabolics on cartilage cell proliferation, as well as increased water storage in intercellular spaces.

Discrepancies in the deep-zone thicknesses corresponded to the differences in measurements of whole tissue sections. This finding might be related to the deep zone of the condylar cartilage being the thickest zone, thus determining its total thickness. Although no significant differences were found in the thicknesses of the intra-articular discs, the average value obtained in group 4 was the highest.

Assessment of the number of cell layers showed the most visible discrepancies in the deep zone of cartilage. The values obtained in groups 1 and 2 were the lowest, while in group 4, they were the highest. Apoptosis of the articular cartilage cells, particularly of the chondrocytes, is a characteristic feature of degenerative changes in tissues. Most studies devoted to the histological description of osteoarthritis have referred to the disappearance of cells and the resulting presence of smaller or larger empty spaces within the cartilaginous tissue [4,6,10,12,20,22].

CONCLUSION

Histologic assessment in the case of arthritis-associated pathologies showed that degenerative changes occurred most frequently in the control group and least frequently in the pioglitazone group. The results obtained in the group treated with hyaluronic acid also revealed a positive anti-degenerative effect. No obvious influence of methylprednisolone on the improvement of the condition of cartilage was found, but the anabolic processes and cell proliferation were clearly visible.

If the anti-degenerative property of the thiazolidinedione derivative drugs is confirmed by further research, they may be used for treatment of diabetic patients suffering from osteoarthritis of the temporomandibular joints, as an additional element of their therapy.

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The authors have no potential conflicts of interest to declare.