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Microstructure of Temporomandibular Joint Cartilage after Intra-Articular Betamethasone Sodium Phosphate/Betamethasone Dipropionate Injection during the Early Stage of Experimental Osteoarthrosis

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Abstract

Objective: to study the morphological changes in cartilage after a single intra-articular betamethasone sodium phosphate (BSP)/betamethasone dipropionate (BDP) injection during the early stage of experimental osteoarthrosis (OA) of the temporomandibular joint (TMJ).

Material and Methods: The experiment was performed on 18 male rabbits aged 6 months .The first group consisted of 9 healthy rabbits. The second group included 9 rabbits with mechanically induced TMJ OA. For 5 days, 3 hours daily, a load (with a force of 200N) on the TMJ was imposed. In the left TMJ of the second group of rabbits, betamethasone was injected intra-articularly in different doses: 0.1 ml (n=3), 0.3 ml (n=3), and 0.5 ml (n=3). The right TMJ was used for comparison. A combined anesthesia was applied each experimental day. Rabbits of both groups were sacrificed on days 7, 14, and 30 with introductory combined anesthesia and intravenous injection of Zoletil 100®20 mg/kg to stop their breathing.

Results: Betamethasone caused destruction of the chondrocytes, fragmentation of collagen fibers, deficit of proteoglycans (PGs) and glycosaminoglycans (GAGs), thinning of the cartilage, and contributed to the progression of TMJ OA.

Conclusion: The optimal dose of BSP/BDP for intra-articular injection in the early stages of TMJ OA must be within the range of 0.1-0.3 ml|0.7-1.5 mg.

Keywords: *temporomandibular joint cartilage; experimental osteoarthrosis; betamethasone sodium phosphate/betamethasone dipropionate.*

Introduction

Intra-articular glucocorticoid (GC) injections in synovitis and rheumatoid and juvenile arthritis are used for quick relief of inflammation symptoms—pain, swelling, and impaired joint function [1-3]. Rheumatologists have extensive experience treating gonarthrosis with local GC therapy [4-9].

The action mechanism of GC is mediated through its direct anti-inflammatory effect on the synovium [10]. Histological study of the GC effect has revealed reduction of inflammation and hypertrophy of the synovium in rheumatoid arthritis [11-13]. However, histological changes in the joint tissues after GC injection in OA have not been sufficiently studied.

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Traditionally, for intra-articular injections, triamcinolone, methylprednisolone, or betamethasone has been used. GCs pharmacological properties differ in their duration of solubility and anti-inflammatory activity, which should be considered during treatment. The GC dosage also depends on the size of the affected joint. The highest doses of betamethasone (7-14 mg) and triamcinolone (40-80 mg) were administered in large joints (hip, knee), a two-fold lower dose (3.5-7 mg and 20-40 mg, respectively) in the middle joints (shoulder, elbow, ankle), and the lowest dose (0.7-1.5 mg and 5-10 mg, respectively) in small joints (metacarpophalangeal, interphalangeal) [14]. Triamcinolone hexacetonide is recommended for administration in large joints and methylprednisolone acetate and BSP/BDP for medium, small, and hard-to-get-at joints [4].

Many rheumatologists use intra-articular GC injection during the early stages of OA to quickly eliminate synovitis and reduce pain [5]. Currently, for the treatment of synovitis in

TMJ OA, the optimal GC dose has not been defined, and there are yet no results from controlled studies [15].

Objective of this study was to investigate the morphological changes in cartilage after a single intra-articular BSP/BDP injection during the early stage of experimental OA of the TMJ.

Material and Methods

The experiment was performed on 18 male rabbits aged 6 months in a Central Research Laboratory vivarium of Ural State Medical University. Experiment was performed in accordance with the Guide for the Care and Use of Laboratory Animals (the institute of LAR, 1996) and with approval of local Ethics Committee (Protocol #8 of 12/16/08).

Experimental animals were divided into two groups. The first group consisted of 9 healthy rabbits. The second group included 9 rabbits with mechanically induced TMJ OA. For 5 days, 3 hours daily, a load (with a force of 200N) on the TMJ was imposed (Patent of the Russian Federation of 03/20/12, the application number 2445711). After that, in the left TMJ of the second group of rabbits, betamethasone was injected intra-articularly in different doses: 0.1 ml (n=3), 0.3 ml (n=2), and 0.5 ml (n=3). For intra-articular administration, Diprospan® Suspension (Schering-Plough, Belgium), containing 5 mg of betamethasone as dipropionate and 2 mg of betamethasone as sodium phosphate in 1ml, was used. The right TMJ was used for comparison. A combined anesthesia was applied each experimental day: XylaVet® 2% (Interchemie, Holland) + Zoletil 100® (Virbac Sante Animal, France) at a dose of 0.1mg/kg was administrated intramuscularly. Rabbits of both groups were sacrificed on days 7, 14, and 30 with introductory combined anesthesia and intravenous injection of Zoletil 100® 20 mg/kg to stop their breathing.

Samples for histological examination were collected in three subgroups: subgroup 1 (n=9) - TMJ of healthy rabbits; subgroup 2 (n=9) –induced OA of the TMJ; and subgroup 3 (n=9) - induced OA of TMJ and intra-articular BSP/BDP injection. Tissue samples of the TMJ were fixed in 10% neutral formalin, and then were decalcified and processed with an increasing alcohol concentration and embedded in paraffin; next, microtome sectioning was carried out. Histological sections were stained with Hematoxylin and Eosin, PAS-reaction was performed.

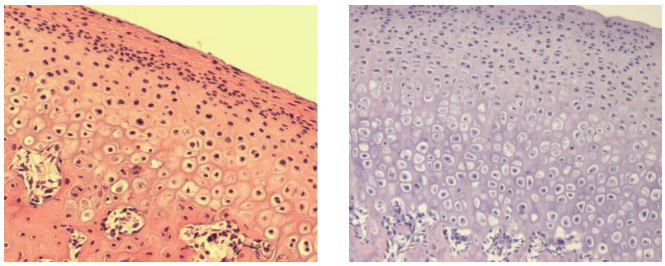
The histological study was carried out in CRL's pathology department. Visual assessment of stained tissue sections was performed under a light microscope Axiosnar (Zeiss, Germany) using x40, x200, and x400 lenses. The total thickness of the cartilage and the thickness of the cellular and fibrous portion of the cartilage were measured using an ocular micrometer with a power of M06-x increasing to 10x15. Micrometers were converted to millimeters.

Results were statistically processed using the software package «Statistica 7, Statsoft, INC». The mean (M) and standard error of the mean (SEM) were deduced. For data with normal distribution, inter-group comparisons were performed using Student's t-test A value of $P<0.05$ was considered statistically significant.

Results

Cartilage microstructure of healthy rabbits (subgroup 1)

For articular cartilage, three zones are defined: superficial, intermediate, and basal (Fig.1a). The superficial area has a fibrous layer consisting of bundles of collagen fibers oriented parallel to the surface of the cartilage and tightly adjacent to each other. The chondrocytes of this area are small, flattened, and individually located among the fibers. The cartilage matrix is not expressed and is pale colored (Fig.1b).



A *B*

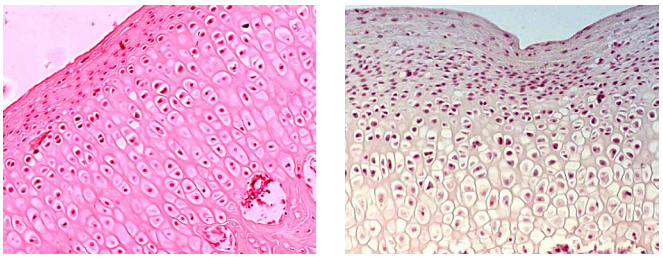
Fig. 1 .

The microstructure of TMJ cartilage of healthy rabbits (subgroup 1). A – H&E staining; B- PAS-reaction x200

The intermediate zone is represented with typical chondrocytes. The cartilage matrix is well defined and brighter colored than in the superficial zone. Among the inter-territorial matrix, the primary lacunae containing isogenic groups of 2-3 chondrocytes are arranged. The intermediate zone goes into the basal area without clear boundaries adjacent to the subchondral bone. In the basal zone, chondrocytes are large, hypertrophied, and form a column of 5-10 cells (Fig.1a). Single chondrocytes penetrate the subchondral bone. The cartilage matrix is brightly colored (Fig.1b) and the basal line is contoured. The total thickness of the cartilage in the middle is 1.02±0.02 mm, with the thickness of the fiber portion (0.14±0.001 mm) being 6.3-fold thinner than the cell portion (0.88±0.02 mm).

Cartilage microstructure of the subgroup 2 rabbits after modeling the early stages of OA

The general structure of cartilage during the early stages of OA modeling remained, but zoning was violated—the fibrous layer was extended, collagen fibers were swollen with areas of fiber degeneration and mammock decay (Fig.2a). Intermediate zone chondrocytes were flattened. Lacunae were pinched. In the basal zone, chondrocytes were randomly arranged in columns of 15-20 cells, and the cell nuclei were pycnotic (Fig.2b).



A *B*

Fig.2.

The microstructure of TMJ cartilage of the subgroup 2 rabbits after modeling the early stages of OA. A – H&E staining; B- PAS-reaction x200

Many lacunae looked empty as a result of cytolysis. Basophilia and the content of Schick-positive substances were reduced (Fig.2b). A structureless mass at the boundary of the cartilage appeared on the 30th day of the experiment. Deeper implementation of proliferating chondrocytes in the subchondral bone was noted. Basal line contours were blurred.

After OA modeling, the total thickness of the cartilage was changed. It has decreased on the 7th day and increased on the 30th day of the experiment (Table 1). On 7th day of the experiment, the ratio of the fibrous portion thickness to the cellular portion thickness was 1:2.2; on the 14th day - 1:2.8; and on the 30th day - 1:3.2. Changing of the total cartilage thickness was due to an increase in the thickness of the fibrous portion by 1.2-fold, and in the cellular portion by 1.8-fold.

Table 1.

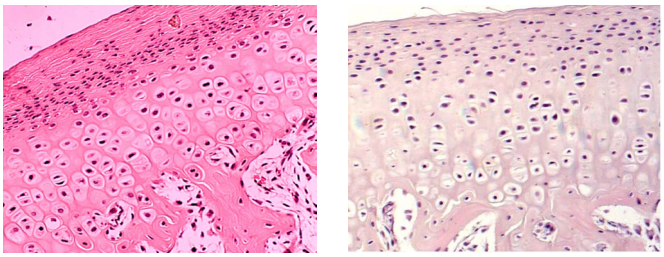
Thickness (mm) of TMJ cartilage of the subgroup 2 rabbits after modeling the early stages of OA

Morphometry of TMJ cartilage	Modeling the early stages of TMJ OA			Average value
	7th day	14th day	30th day	
Total thickness	0.67±0.03	0.79±0.04	1.09±0.06	0.87±0.03
Thickness of the FP	0.21±0.009	0.21±0.006	0.26±0.007	0.23±0.005
Thickness of the CP	0.46±0.03	0.59±0.03	0.83±0.06	0.65±0.03

Note: FP - the fibrous portion CP - the cellular portion

Cartilage microstructure of the subgroup 3 rabbits after modeling the early stages of OA and intra-articular BSP/BDP injection

Histological examination of cartilage revealed changes in all areas. In the superficial area, a small degeneration of collagen fibers was found. Chondrocytes were small, flattened, and not orderly located (Fig.3a). In the intermediate zone, small chondrocytes of conventional shape were located in lacunae. Only a few empty lacunae were found (Fig.3a,b). The cartilage matrix was pale-colored; the content of the Schick-positive substances was reduced. In the basal zone, chondrocytes formed columns of 10-12 cells. A few empty lacunae were noted (Fig 3b). The territorial matrix was more basophilic and Schick-positive than the inter-territorial matrix (Fig.3b). The basal line was partially formed.



A ***B***

Fig.3.

The microstructure of TMJ cartilage of the subgroup 3 rabbits after modeling the early stages of OA and intra-articular BSP/BDP injection. A – H&E staining; B- PAS-reaction x200

The total thickness of OA-modified cartilage was the same after administration of 0.1 ml and 0.3 ml BSP/BDP. After administration of 0.5 ml BSP/BDP, cartilage thickness was the least (Table 2). After administration of 0.1 ml BSP/BDP, the ratio of the fibrous portion thickness to the cellular portion thickness was 1:2.2; at 0.3 ml, it was 1:2.5; and at 0.5ml, it was 1:1***.9.***

Table 2.

Thickness (mm) of TMJ cartilage of the subgroup 3 rabbits after modeling the early stages of OA and intra-articular BSP/BDP injection

Morphometry of TMJ cartilage	*Intra-articular BSP/BDP injection*			Average value
	0.1 ml	0.3 ml	0.5 ml	
Total thickness	0.85±0.04	0.85±0.05	0.72±0.03	0.80±0.02
Thickness of the FP	0.26±0.007	0.24±0.006	0.25±0.005	0.25±0.003
Thickness of the CP	0.58±0.04	0.61±0.05	0.47±0.03	0.55±0.02

Note: FP - the fibrous portion CP - the cellular portion

*Comparative evaluation of morphological changes in cartilage of different subgr*oups

Normal TMJ cartilage is a species of hyaline cartilage with a fibrous layer in the superficial area; the perichondrium is absent. Collagen fibers create a 'skeleton' of cartilage. Their greatest accumulation in the superficial zone forms an elastic articular surface of the mandible capitulum, which is resistant to deterioration.

After modeling the early stages of OA, TMJ cartilage is characterized by impaired zoning, proliferation, chaotic arrangement of chondrocytes, decreased cell density, and lack of PGs and GAGs. Reduction of PGs and GAGs is uniform in the territorial and inter-territorial cartilage matrix.

After intra-articular BSP/BDP injection in OA-modulated TMJ, cartilage was characterized by moderate destruction, reduction in size of chondrocytes, emergence of cell-free lacunae, and a moderate deficiency of PGs and GAGs. In the inter-territorial matrix, the content of PGs and GAG was significant decreased.

Comparison of the characteristics of TMJ cartilage thickness showed cartilage thinning in subgroups 2 and 3 (Table 3). In the subgroup 2, cartilage total thickness decreased by 13.9%, the thickness of the fibrous portion increased by 64.2%, and the thickness of the cell portion decreased by 26.1%. These differences were statistically significant when compared with the subgroup 1 characteristics (Table 3).

Table 3.

Comparative characteristics of the TMJ cartilage thickness (mm) in rabbits of the various subgroups

Groups	Total thickness	Thickness of the fibrous portion	Thickness of the cellular portion
Subgroup 1	1.02±0.02	0.14±0.001	0.88±0.02
Subgroup 1	0.87±0.03	0.23±0.005	0.65±0.03
P_{1-2}	P=0.0006	P<0.0001	P<0.0001
Subgroup 1	0.80±0.02	0.25±0.003	0.55±0.02
P_{1-3}	P<0.0001	P<0.0001	P<0.0001

In the subgroup 3, cartilage total thickness decreased by 20.8%, the thickness of the fibrous portion increased by 78.6%, and the thickness of the cell portion decreased by

37.5%. These differences were statistically significant when compared with the subgroup 1 characteristics (Table 3).

Discussion

Questions about the relationship between the administered dose of GCs in the joint and the treatment effect and side effects are still to be discussed. After intra-articular hydrocortisone injections, arthralgia was amplified in 2-3% of the subjects, destruction of the articular surfaces increased in 3%, and hypopigmentation and local tissue atrophy appeared in 2% [4].

Betamethasone relates to fluorinated GCs of long-term effect and consists of two esters: solubilized BSP, which is rapidly absorbed and provides fast effect (during 30 min), and slow release of BD micro-crystals, which are slowly absorbed and provide prolonged action (4 weeks and more). Intra-articular betamethasone injections are well tolerated and give long clinical effect [16]. Earlier studies have shown that intra-articular GC injections inhibit cartilage proteoglycan synthesis, degrade cartilage structure, and can cause degenerative lesions in normal cartilage [17].

In an experiment on dogs, intra-articular injections of small doses of triamcinolone acetate (5 mg) reduced osteophyte formation and cartilage damage (reduction of erosions and cracking, preservation of matrix proteoglycans) on later stages of OA [18]. In experimental OA on dogs, methylprednisolone acetate intra-articular injections reduced the frequency and size of osteophytes and the severity of cartilage damage due to inhibition in synthesis of stromelysin by chondrocytes [19].

In the present study, in subgroup 2, the general cartilage structure was preserved after modeling the early stages of OA, but zoning was broken, the fibrous layer became thicker, and the cellular layer thinner. The ratio of the fibrous portion thickness to the cellular portion thickness was 1:2.8. The thickness of the cartilage in the whole was decreased by 1.2-fold. In subgroup 3, after intra-articular BSP/BDP injection in OA-modulated TMJ on the early stages of the process, somewhat more cartilage zoning was broken than in subgroup 2. The ratio of the fibrous portion thickness to the cellular portion thickness was 1:2.2. The thickness of the cartilage in the whole was decreased by 1.3-fold.

Thus, BSP/BDP causes the destruction of the same chondrocytes, fragmentation of collagen fibers, a deficit of PGs and GAG, and cartilage thinning, which contributes to the progression of TMJ OA. The study has shown that administering into the TMJ cavity of 0.5 ml BSP/BDP causes the greatest degenerative changes to the cartilage.

Conclusion

Betamethasone causes changes in the cartilage matrix; namely, reduction in content of PGs/GAGs and their distribution. Betamethasone inhibits the synthetic function of chondrocytes and exacerbates damage in the joint, which has also been described in other studies [12,20]. BSP/BDP administration in the early stages of OA moderately deepens the degenerative changes in articular tissues. The optimal dose of BSP/BDP for intra-articular injection in the early stages of TMJ OA must be within the range of 0.1-0.3 ml|0.7-1.5 mg.

Competing interests

The authors declare that they have no competing interests.

References

1. Ravelli A, Martini A. Juvenile idiopathic arthritis. Lancet 2007; 369:767-78.
2. Hashkes PJ, Laxer RM. Medical treatment of juvenile idiopathic arthritis. JAMA 2005; 294:1671-84.
3. Wallace CA. Current management of juvenile idiopathic arthritis. Best Pract Res Clin Rheumatol 2006; 20:279-300.
4. Lanni S, Bertamino M, Consolaro A, Pistorio A, Magni-Manzoni S, Galasso R, et al. Outcome and predicting factors of single and multiple intra-articular corticosteroid injections in children with juvenile idiopathic arthritis. Rheumatology (Oxford) 2011; 50:1627-34.
5. Cleary AG, Murphy HD, Davidson JE. Intra-articular corticosteroid injections in juvenile idiopathic arthritis. Arch Dis Child 2003; 88:192-6.
6. Keen HI, Wakefield RJ, Hensor EM, Emery P, Conaghan PG. Response of symptoms and synovitis to intra-muscular methylprednisolone in osteoarthritis of the hand: an ultrasonographic study. Rheumatology (Oxford) 2010; 49:1093-100.
7. Arroll B, Goodyear-Smith F. Corticosteroid injections for osteoarthritis of the knee: meta-analysis. Br Med J 2004; 328:10.
8. Bellamy N, Campbell J, Robinson V, Gee T, Bourne R, Wells G. Intraarticular corticosteroid for treatment of osteoarthritis of the knee. Cochrane Database Syst Rev 2006; 19:CD005328.
9. Uthman I, Raynauld J-P, Haraoui B. Intra-articular therapy in osteoarthritis. Postgrad Med J 2003; 79:449-53.
10. Creamer P. Intra-articular corticosteroid injections in osteoarthritis: do they work and if so, how? Ann Rheum Dis 1997; 56:634-6.
11. Filippucci E, Farina A, Carotti M, Salaffi F, Grassi W. Grey scale and power Doppler sonographic changes induced by intra-articular steroid injection treatment. Ann Rheum Dis 2004; 63:740-3.
12. Schueller-Weidekamm C, Krestan C, Schueller G, Kapral T, Aletaha D, Kainberger F. Power Doppler sonography and pulse-inversion harmonic imaging in evaluation of rheumatoid arthritis synovitis. Am J Roentgenol 2007;188:504-8.
13. Terslev L, Torp-Pedersen S, Qvistgaard E, Danneskiold-Samsoe B, Bliddal H. Estimation of inflammation by Doppler ultrasound: quantitative changes after intra-articular treatment in rheumatoid arthritis. Ann Rheum Dis 2003; 62:1049-53.
14. Caldwell JR. Intra-articular corticosteroids. Guide to selections and indications for use. Drugs 1996; 52:507-14.
15. Scott C, Meiorin S, Filocamo G, Lanni S, Valle M, Martinoli A. et al. A reappraisal of intra-articular corticosteroid therapy in juvenile idiopathic arthritis. Clin Exp Rheumatol 2010; 28:774-81.
16. Hetland ML, Stengaard-Pedersen K, Junker P, Lottenburger T, Hansen I, Andersen LS, et al. Aggressive combination therapy with intra-articular glucocorticoid injections and conventional disease-modifying anti-rheumatic drugs in early rheumatoid arthritis: second-year clinical and

radiographic results from the CIMESTRA study. Ann Rheum Dis 2008; 67:815-22.
17. Pelletier JP, Haraoui B, Martel-Pelletier J. Modulation of cartilage degradation in arthritic diseases by therapeutic agents. In: Woessner JF., Howell DS. eds. Joint cartilage degradation. New York: Marcel Dekker. 1993: 503-28.
18. Pelletier JP, Martel-Pelletier J. Protective effects of corticosteroids on cartilage lesions and osteophyte formation in the Pond-Nuke dog model of osteoarthritis. Arthritis Rheum 1989; 32:181-93.
19. Pelletier JP, Mineau F, Raynauld JP, Gunia-Smitt Z, Martel-Pelletir J. Intraarticular injections with methylprednisolone acetate reduce osteoarthritic lesions in parallel with chondrocyte stromelysin synthesis in experimental osteoarthritis. Arthritis Rheum 1994; 37:414-23.
20. Karim Z, Quinn MA, Wakefield RJ, Bromn AK, Green MJ, Hensor EM. et al. Response to intramuscular methyl prednisolone in inflammatory hand pain: evidence for a targeted clinical, ultrasonographic and therapeutic approach. Ann Rheum Dis 2007; 66:690-2.