The effect of methylprednisolone on the morphology of rabbit knee joint after partial synovectomy

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Abstract

The research was done on 33 rabbits, which underwent partial synovectomy of the right knee. 24 of them were treated with Depo-Medrol twice- immediately after and 7 days after surgery. The knee joints were taken 21 days after surgery, from sacrificed animals, and fixed in formaline. The histological examination of joint cartilage, synovium, fibrous membrane of the joint capsule and muscles of methylprednisolone- treated animals showed the absence of cellular reactions (giant cells and mononuclear cells) in synovium and fibrous membrane. In similar samples from the control group a lot of granulomas were present with foreign-body type giant cells and less or more intense infiltrations of mononuclear cells.

This finding can explain previously reported good functional results of animals injected by Depo-Medrol after knee surgery.

Key words: *rabbit's knee surgery, methylprednisolone, joint morphology.*

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Introduction

Daily surgical practice shows many postoperative problems connected with gaining good function of operated joint. Restriction of mobility after surgery may be connected with post-operative inflammation and fibrosis in the joint area. In the previous paper, we reported beneficial effect of methylprednisolone on the post-operative range of the knee joint movement in rabbits after partial synovectomy [1]. In this paper we present the results of histological examination of knee joints collected from these rabbits 3 weeks after surgery.

We performed this study in order to evaluate if applied treatment can influence the morphological structure of articular cartilage, synovial membrane, fibrous capsule, and muscles.

Material and Methods

The research was done on 33 crossbreed rabbits of both sexes. The control group was made up of 10 rabbits, not treated with methylprednisol /Depo Medrol -/. The research group was made up of 23 rabbits treated with methylprednisol /Depo Medrol +/.

At the beginning of the experiment the weight of animals ranged from 2.7 kg to 3.7 kg.

Glucocorticoid as suspension of Methylprednisolone acetate (Depo-Medrol, Upjohn) was administered generally in the early postoperative period. The medicine was being injected intramuscularly into surroundings of triceps muscle after an operation and 7 days later, in 1 mg/kg doses.

Surgical technique was described previously [1]. Briefly, all rabbits underwent partial synovectomy of the right knee under local anaesthesia with 0.5% of xylocaine, followed by immobilization of the operated joint in sitting position. The plaster cast was left for 2 weeks, then removed and unrestricted rabbit's activity within the cage was permitted.

On the 21-st day the animals were put to death by intravenous injection of Morbital in 10 ml dose. The knee joints were taken from dead animals. From each joint, fol-

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lowing samples were taken for microscopic examination: joint cartilage from patella, joint capsule with post-surgery scar (medially to patella) and femoral quadriceps muscle. Samples were fixed in 10% formalin, embedded in paraffin and slices were stained with:

- cartilage – Haematoxylin and Eosin (H&E), Alcian Blue,
- joint capsule – H&E, Van Gieson method,
- muscle – H&E, Van Gieson method.

For all experiments animals were handled according to the Polish law on the protection of animals and NIH standards. All experiments were accepted by the local Ethical Committee.

Results

Joint cartilage

In the examinated group (DepoMedrol +), like in the control group (DepoMedrol -) similar chondrocytes distribution was observed with smaller and flattened cells on the surface comparing to deeper layers. In both groups significant amount of necrotic and non-active cells ("cell shadows") was observed with various intense chondrocytes proliferation (should be interpreted as a result of 2-week immobilization). Positive stains for glycosaminoglycans were present in both groups.

Joint capsule

A. Synovium

In synovium samples from the control group (DepoMedrol -), a lot of resorptive granulomas around surgical sutures were present with foreign-body type giant cells and less or more intense infiltrations of mononuclear cells.

In examined group, despite the presence of surgical sutures, cellular reactions were absent (giant cells nor mononuclear cells).

B. Fibrous membrane

There were sparse inflammatory infiltrations of mononuclear cells in the control group, which were absent in the examined group.

Muscles

In both groups only insignificant hyalinization of single muscular fibers was seen.

Discussion

Described in the earlier paper analysis of knee joint mobility of rabbits which were subject to general methylprednisolone treatment /Depo-Medrol +/ showed bigger range of movement by average 34.7° after 2 weeks (37.1% of mobility of the control group) and 33.7° 3 weeks after the surgery (30.5% of mobility of the control group), with statistical significance level of $p<0.001$ [1]. Perhaps, our present findings of histological examination of operated joints can explain better functional results of animals injected by DepoMedrol postoperatively.

Noteworthy, the authors of majority of the reports seem to consider postoperative joint contracture as related to the development of intra-articular adhesions, created on the basis of a haematoma, and related to an excessive fibrosis within the articular capsule [2].

The articular capsule consists of 2 layers. In the synovial membrane 2 areas can be distinguished: ependymal – made of synovial cells, and subsynovial with differentiated histopathological structure /reticular type, fibrous type, adipose type/. Synovial membrane composes the ependyma of a joint and participates in nutrition of its elements.

External layer called the fibrous membrane functions above all as a mechanical structure, and not only limits the joint, but also presses joint surfaces. Thickness of the fibrous membrane is differentiated depending on the place of its location, it is thicker in the places of stronger tension. Collagenous fibres dominate in histopathological structure, the cell elements /mainly fibrocytes and fibroblasts/, and basic substance are scarceness.

Fibroplasias prevention is correlated with all possible methods of inhibiting the development of fibrous tissue as the final phase of inflammation process [3]. Chemical surrounding of the damaged tissue may activate fibroblasts to increased proliferation and synthesis of structural macromolecules, glucosaminoglicans, and collagen. Glucocorticoids inhibit the migration, reproduction or growing up of fibroblasts. Judging from periarticular and intra-articular adhesions, excessive fibroplasia of the cicatrix, steroid treatment should be linked with influence of the glucocorticoids on the fibroblasts, and the production or degradation of collagen [2].

Research on wound healing proved presence of fibroblasts in 2 days after an injury, while the highest level of collagen synthesis on the whole wound surface in 5-7 days after the injury.

Collagen is the main protein that builds the collagen fibres. In earlier research there could be found 5 types of collagen differentiating physicochemical properties and the places of occurrence. The newest research show 12, and even 13 types of collagen.

It is common knowledge that glucocorticoids inhibit the collagen synthesis [2-6]. Recent research shows that the fundamental control phase of the collagen production is the early phase of biosynthesis- transcription. These observations however were done during the keloid fibroblasts growth, but in the opinion of the scientists keloid provides an easy and acceptable model in research of the mechanism which leads to tissue fibrosis [7]. Glucocorticoids hamper more the synthesis of collagen than the synthesis of proteins. Steroid hormones given to the lab animals de-

crease the level of collagen in skin and change the qualitative reactions between collagen types III and I in favour of collagen III. In morphology the change is indicated by the increase of the reticulin fibrils content. Literature describes also collagenolitic activity of glucocorticoids [2, 6, 7]. It is also common knowledge that glucocorticoids influence immune and inflammatory reactions [2, 8-10].

In the presence of above-mentioned facts concerning the results of the carried experiment a very important problem should be solved. It was puzzling that during pathomorphological examination there had not been any inflammatory infiltration found only at the group treated with methylprednisolone. There were no however other features differentiating the articular capsule of the control group from the test one. Concerning the different sizes of the tested animals, the test of different thickness of the articular capsule of individual specimen was not done.

Per analogiam to keloid it can be presumed that the amount of collagen in a capacity unit of the tested tissue does not change, however summarily increased or decreased the amount of collagen is connected with thickness of pathologically changed tissue.

In the assessment of the tested method, the influence of glucocorticoids on articular cartilage cannot be omitted. Most of reports emphasize disadvantageous influence of the treatment with multiple intra-articular glucocorticoid injections which cause the degradation of the cartilage. There were no differences in the microscope cartilage material noted among the animals treated with methylprednisolone and the animals of the control group. The observed changes in the form of big proportion of dead cells in the different levels of cartlilage may be the result of joint immobilization and secondary malnutrition of cartilages [11-14].

Troyer in the research on the rabbits observed all pyknotic cells in the superficial cartilage area just 4 days after the immobilization of the joint in extension [15].

More information about anti-inflammatory and immunotropic activity of glucocorticoids and their clinical use in the treatment of joint surgery was recently presented by us in review paper [2].

Conclusions

1. Short-term general treatment with methylprednisolone does not influence the changes in the articular cartilage of the rabbit's knee.
2. Short-term administration of methylprednisolone inhibit the development of inflammatory infiltration in fibrous membrane of joint capsule in the rabbit's knee.
3. Microscopic tests on synovial membrane of rabbit's knee confirm inhibitory influence of glucocorticoids on inflammatory reaction.
4. We feel that the further clinical studies in this field are necessary.

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