

# Beneficial effect of methylprednisolone on the postoperative function of rabbit knee

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## Abstract

The research was done on 33 crossbreed rabbits of both sexes. The control group was made up of 10 rabbits, not treated with methylprednisolone. The research group was made up of 23 rabbits treated with methylprednisolone /1 mg/kg/day/. Depot type /Depo-Medrol of Upjohn/ made the experiment easier and decreased the risk of local infection.

All rabbits underwent partial synovectomy of the right knee under local anesthesia 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 a cage was permitted.

Examination of mobility range in the knee-joint was done by a goniometer on operated and unoperated knees in experimental and control group immediately after the removal of the plaster cast i.e. 2 weeks after surgery and 1 week after free in cage activity.

The functional results 2 weeks after surgery revealed difference of 48.5 degrees of movement between operated comparing to unoperated knee in control group, and only 7.5 degrees in the group treated with Depo-Medrol.

The 3 weeks after surgery the difference of range of movement in control group was 36 degrees and only 2.6 in group treated with steroids.

There was no difference in cells counts of blood, level of alkaline phosphates and calcaemia between control and steroids group of rabbits. The rabbits treated with Depo-Medrol showed small and transitory hyperglycaemia and hyperglyceridemia.

Further investigations made in orthopedic practice have to be done to introduce steroids as a safe and proof treatment of some difficult cases in human knee surgery.

**Key words:** rabbits, knee joint surgery, methylprednisolone.

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## Introduction

“One of the most common complication due to joint injuries or surgeries is joint mobility restriction” [1]. Notwithstanding the fact of the great development of orthopaedic surgery and lapse of time, this opening address by Bross and Hilarowicz at 28 Polish Society Surgery Congress in 1935 is still valid.

Daily surgical practice shows many postoperative problems connected with gaining regular range of movement in operated joint.

Many patients suffer from big mobility restriction both in flexion and in joint extension, as a result of the presence of inflammatory and fibrous tissue in a joint area.

Senior author (L.J.) found this problem in rheumatoid patients with fibrous ankylosis of the knee, after surgery done in patients with psoriatic arthritis, and in some cases of unexplained limited motion after total knee replacement. On the basis of theoretical deduction steroids (prednisone) was used immediately post-op in human with the risk of restricted movement after surgery. The results

were more than satisfying, so experimental studies on rabbits were undertaken.

Experimental work on rabbits was conducted in order to evaluate:

- a. If and to what degree applied glucocorticoids influence mobility of a rabbit knee joint after partial synovectomy operation with 2 week immobilization afterwards?
- b. To what degree methylprednisolone short-term treatment changes basic blood morphological and biochemical parameters?

## **Material and Methods**

### **Experimental animals**

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

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

Regarding accessibility of these animals, rabbits were chosen for the experiments.

The sizes of lower limbs permitted the range of joint mobility measurement exact to 5°.

In the case of smaller animals with shorter limbs, the higher margin of measuring error should be taken into account.

### **Medicine used in the experiment**

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. Based on data in professional literature referring to the steroid use in experiments on rabbits the medicine dose was predetermined on the level of 1mg/kg/day [2-4]. Depot type made the experiment easier and decreased the risk of local infection after the multiple intramuscular steroid injections of short duration.

According to Upjohn Co, the effect of administration of Depo-Medrol lasts from 1 to 3 weeks.

However as the clinical observations and pilot research before the experiment had shown, the effect of administration approximates 1 week, and wherefore the injections were repeated 7 day after the operation.

### **Surgical technique**

The surgery was done upon the rabbit's right knee, and the left limb was used as the comparative one for the individual specimen. After knee and a part of thigh and shank depilation the rabbits were put on their backs and a laboratory assistant kept this position by having a grasp of lower and upper limbs. After cleaning the skin thrice with 1%

solution of hibitan in 70% spirit, a knee was wrapped with sterilized dressings. The anaesthetic was done by intracutaneous, subcutaneous, periarticular, and intra-articular injections with 5 ml of 0.5% solution of Xylocaine. No general or pain responsiveness to a surgery done in local anaesthetic were observed. In strict aseptic conditions, the skin was cut above the joint by an anterior, straight incision, and a joint was opened at a medial parapatellar approach. After lateral luxation of a patella, with Luer's forceps infrapatellar fat pad was partly removed together with synovial membrane within reach. Granted that a haematoma after intra-articular oozing of blood would be a good basis to create adhesions intra-articular haemostasis was not done. After washout a joint and a wound with 1% solution of neomycin an articular capsule was closed by solitary dexon sutures and a skin with Mersilk by Ethicon Co.

After protecting a wound by the sterilized dressings and wrapping with a paper tape, a plaster cast with a strip around pelvis was put in a knee and a hip flexion, while an ankle joint was left free. This type of immobilization let an animal stay in a rest position. This joint position was chosen because the trials to immobilize a knee in extension made an animal stay in a grotesque position and caused high discomfort and nervous animal activity.

The plaster cast was left for fortnight, and after its removal, an unconstrained rabbit's activity within a cage was permitted.

### **Knee-joint' range of movement examination**

The examination of mobility range in a knee-joint was done by a goniometer in the most obtainable free side position of the animal. The range of movement in a knee joint was tested immediately after the removal of the plaster cast *i.e.* 2 weeks after the surgery and 1 week after free in-cage activity. The examination was done upon both the operated /right/ limb and the unoperated one.

**Blood samples** for tests were taken from a marginal vein of the rabbit's vein to a thrombus test-tube containing disodium edetate. Blood was taken four times in the morning hours before feeding *i.e.* on the surgery day, and on the 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> day following the surgery. After the last blood test on the 21<sup>st</sup> day the animal was put to death by intravenous injection of Morbital /pentobarbital sodium/ in 10 cm<sup>3</sup> dose.

Serum was used to group:

- the level of glucose (orthotoluid method),
- the level of triglycerides (Eggstein's method),
- the level of alkaline phosphates (King-Jegatheesan's method),
- the level of calcaemia (photometric 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.

### Statistical analysis

Statistical evaluation of the data from experiments was done by Statsoft, Inc. (1997) Statistica PL (Software system data analysis), version 5. ANOVA was used to test whether there were statistically significant differences in knee joint range of movement between all groups of means. To confirm these differences, the post hoc Tukey test for unbalanced designs was used for multiple comparisons ( $p < 0.05$ ).

### Results and Discussion

Observations of the range of joint movement performed after 2 weeks following the surgery revealed that in the control group /Depo-Medrol -/ there were huge mobility limitations observed in a right knee joint. Average range of joint movement was  $93.5^\circ /SD \pm 13.5^\circ$ , while for unoperated knee  $142^\circ /SD \pm 15.7^\circ$ . The difference of average range of joint movement was  $48.5^\circ$ .

In the test group /Depo-Medrol +/- average range of joint movement of the operated knee was  $128^\circ /SD \pm 21.3^\circ$ , while for unoperated knee  $135.7^\circ /SD \pm 15.2^\circ$ . Difference of average range of joint movement for both knees was only  $7.5^\circ$ . The results are presented on Figure 1.

In the summary of the research which was done 2 weeks after the surgery it should be emphasized that the average range of the joint movement / $128.2^\circ$ / in the operated knees at the rabbits treated with methylprednisolon was bigger by  $34.7^\circ$  than the range of joint movement in the control group / $93.5^\circ$ /. The received result demonstrates statistical significance level of  $p < 0.001$ .

Observations 3 weeks after the surgery revealed that in the control group /Depo-Medrol-/ average range of joint

movement of the operated right knee was  $110.5 /SD \pm 17.8^\circ$ , and left knee  $146.5^\circ /SD > 3.9^\circ$ . The difference was  $36.0^\circ$ .

In the test group /Depo-Medrol +/- average range of joint movement of the operated knee was  $144.2^\circ /SD \pm 5.7^\circ$ , while for the unoperated one  $146.8^\circ /SD \pm 4.4^\circ$ : the difference was only  $2.6^\circ$ . The results are presented on Figure 2.

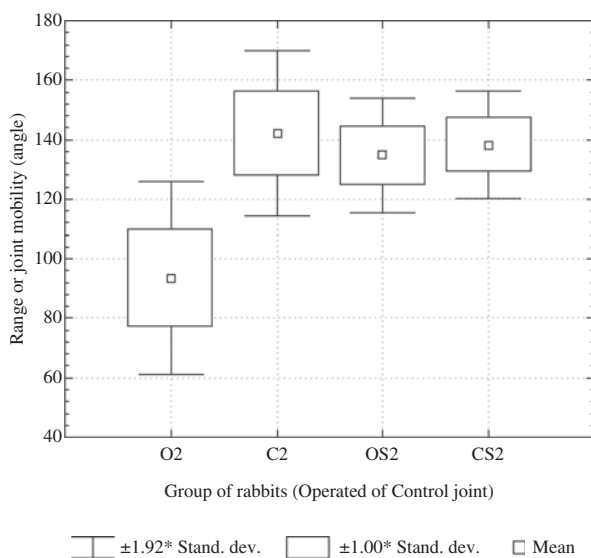
It should be emphasized that 3 weeks after the surgery the difference in average range of movement is  $33.7^\circ$  in favour of the animals treated with methylprednisolone / $144.2^\circ$ / in comparison with the control group / $110.5^\circ$ /. The indices differ relevantly  $p < 0.001$ .

There was no difference in cells counts of blood, level of alkaline phosphates and calcaemia between control and steroids group of rabbits. The rabbits treated with Depo-Medrol showed small and transitory hyperglycaemia and hyperglyceridemia.

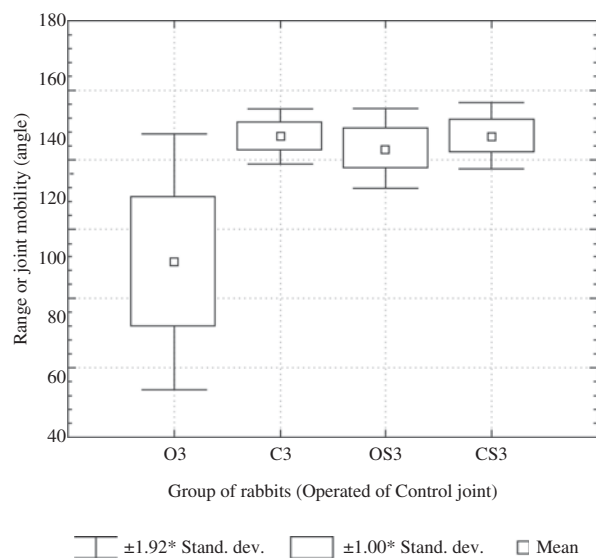
However, we observed some postoperative complications. Among 10 animals of the control group /Depo-Medrol -/ 2 cases of empyema in operated knee joint were observed.

In the test group of 23 rabbits there were found after-mentioned complications:

1. One case of paralysis probably as a result of improper removal of plaster cast
  - slew 14 days after the surgery.
2. 3 septic complications:
  - empyema of operated knee,
  - diffuse back phlegmon (died 17 days after the surgery),
  - extensive foot phlegmonous abscess with cachexia (died 15 days after the surgery),
3. Diarrhoea with dehydration, and impoverishment (died 18 days after the surgery).



**Fig. 1.** Range of joint motility 2 weeks after surgery. O – operated joint; C – non-operated joint; S – steroid-treated rabbits ( $\pm SD$ )



**Fig. 2.** Range of joint motility 3 weeks after surgery. O – operated joint; C – non-operated joint; S – steroid-treated rabbits ( $\pm SD$ )

Side effects of corticosteroids therapy were described [5-8]. These untoward effects divided into high-dose phenomena (myopathy and septic necrosis) and low-dose problems (growth suppression and osteoporosis). For the clinician, the former group may be an uncommon experience whereas the later group is highly predictable.

Undertaken research was supposed to deliver replies to a question if the experimental results carried out on the animal model correspond around with clinical observations concerning advantageous influence of steroid therapy on mobility of operated knee joints in chosen diseases of musculoskeletal system (psoriatic arthropathy, rheumatoid arthritis, fibrous ankylosis of knee joint).

The presented study done on the rabbits proved beneficial influence of the general steroid therapy on the postoperative knee joint mobility, what corresponds to the results obtained by Weckesser [9], who administered triamcinolone into the knee joints of rats and had found it effective in preventive joint stiffness in joints immobilized for three weeks.

The results obtained from the animal model in correlation with the clinical observations justify the purposefulness of general treatment with steroids in the chosen morbid conditions of the human knee joint.

More information about clinical use of glucocorticoids in the treatment of joint surgery was recently presented by us in review paper [10].

## References

1. Bross W, Hilarowicz H (1935): Kwasica salmiakowa w leczeniu skostnień okołostawowych i zeszywnień pooperacyjnych stawów. *Pol Przegl Chirur* 14: 713-717.
2. Goldlust MB, Rich LC (1981): Chronic immune synovitis in rabbits. Modulation by anti-inflammatory and anti-rheumatic agents. *Agents Actions* 11: 729-735.
3. Goldlust MB, Rich LC, Harrity TW (1977): Effects of anti-inflammatory agents on the response of immune synovitis in rabbits. *Arthritis Rheum* 20: 937-946.
4. Hunneyball IM (1992): Use of experimental arthritis on the rabbit for the development of antiarthritic drugs. *Adv Inflamm Res* 7: 249-261.
5. Hollister JR (1992): The untoward effects of steroid treatment on the musculoskeletal system and what to do about them. *J Asthma* 29: 359-61.
6. Charalambous CP, Tryfonidis M, Sadiq S et al. (2003): Septic arthritis following intra-articular steroid injection of the knee – a survey of current practice regarding antiseptic technique used during intra-articular steroid injection of the knee. *Clin Rheumatol* 22: 386-390.
7. Kumar N, Newman RJ (1999): Complications of intra- and peri-articular steroid injections. *Br J Gen Pract* 49: 465-466.
8. Raynauld JP, Buckland-Wright C, Ward R et al. (2003): Safety and efficacy of long-term intraarticular steroid injections in osteoarthritis of the knee: a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 48: 370-377.
9. Weckesser EC (1978): Some results using triamcinolone on immobilized joints. *Hand* 10: 267-275.
10. Jung L, Skorupski M, Mazurkiewicz M, Skopińska-Różewska E (2008): Glucocorticoids in the treatment of joint surgery. *Centr Eur J Immunol* 33: 153-157.