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Effects of short-term treatment with systemic prednisone on bone healing: an experimental study in rats

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Abstract – Long-term systemic use of corticosteroids causes osteoporosis and increased risk of fracture. However, the effect of short-term use of corticosteroids on bone healing is not well defined. The aim of the present study was to test the influence of short-term systemic corticosteroid therapy on bone healing. Standardized bone defects (2 mm diameter) were formed in the middle of the femur in 40 male rats. Rats were divided into two groups; control group ($n = 20$) and prednisone-treated group ($n = 20$). Subcutaneous injection of either sterile normal saline (control) or 0.020 mg kg^{-1} dose of prednisone was administered just before surgery and thereafter daily for 3 days. Histopathological cross sections were taken 1, 2, 3 and 4 weeks after surgery. There was no statistically significant difference between the prednisone group and the control group. No inhibitory effects were seen following short-term corticosteroid treatment.

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Key words: corticosteroid; prednisone; bone healing; experimental study

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The body responds to a bone injury in much the same way it responds to a soft-tissue injury. Bone healing can be divided into three stages: inflammatory, reparative, and remodelling (1). During the inflammatory stage, haematoma formation occurs due to the disruption of the vascular structures (1, 2). Neutrophils and macrophages are the first to arrive at the injury site to clean up cellular debris and foreign substances (2). During the reparative stage, capillary proliferation occurs and fibroblasts lay down collagen at the fracture site. Fibroblasts and periostal cells work together to form a dense matrix of collagen and newly formed cartilage, which is called the temporary callus (2). Osteoblasts lay down new bone, gradually replacing the temporary callus with a bony callus during the maturation stage. This bony callus is composed of spongy bone that will eventually be replaced by compact bone (1).

Corticosteroids inhibit phospholipase A2 and alter the activity of peripheral leucocytes (2). By inhibiting phospholipase A2, corticosteroids prevent the release of arachidonic acid from cell membranes and therefore prevent the formation of luekotrienes, thromboxanes and prostaglandins (2). These products are thought to cause inflammation and oedema, which in turn cause pain due to increased pressure on tissues. Thus the blocking of phospholipase A2 by corticosteroids results in a blockage of important inflammatory and pain (2).

Leucocytes arriving at the site of injury can cause local tissue damage by the release of lysosomal enzymes. Corticosteroids stabilize the lysosomal membrane *in vitro*, thus preventing the release of these enzymes into the surrounding tissue (3).

Corticosteroids have become widely used for the alleviation of inflammation in almost every field of medicine (4).

Long-term treatments with corticosteroids are known to induce general osteoporosis and increase the tendency for spontaneous fractures (5–7). Furthermore, long-term (more than 1 week) treatment with corticosteroids inhibits bone healing. In some medical conditions only short-term medication with corticosteroids is needed. The outcome of this therapy on bone-healing metabolism is unclear and not well defined (5, 8). In some experimental studies analysis of

the fracture sites showed that all histological processes of repair are retarded in cortisone-treated groups (7). In contrast to these studies, some authors observed no delay in fracture healing and no change in the microscopic appearance of the process of fracture healing in corticosteroid-treated animals (7).

The aim of the present study was to establish a model in rats that could evaluate how short-term corticosteroid therapy may modulate the course of the healing process after standardized bone defects.

Material and methods

We used 40 male Wistar rats for the study, each of which weighed 200 g on average at the Physiology Laboratory of Faculty of Medicine of Atatürk University.

Rats were anaesthetised with the injection of 1 mg kg^{-1} dose of 50 mg ml^{-1} Ketalar (Parke-Davis, Ann Arbor, MI, USA). A cavity was carved by cooling serum physiologic on the right-back tibia of the rats in accordance with the principles of general surgery. The diameter of each cavity was 2 mm.

Derma and endoderm were sutured. One week following the operation, the wound was treated and dressed. Penicillin procaine (0.25 cc of 800.000 IU) was injected (i.m.).

Rats were divided into two groups: control group ($n = 20$) and prednisone-treated group ($n = 20$). Subcutaneous injection of either sterile normal saline (control) or prednisone (Prednisolon, Atlas-Fako, Turkey), (0.20 mg/100 g body weight) was administered just before surgery and thereafter daily for 3 days.

After the follow-up of 7, 14, 21 and 28 days, rats were grouped into equal numbers and killed by injecting high-dose Ketalar. Bone segments on which the experiment was performed were extracted. Sections were taken out by paraffin wax buried into blocks following fixation in formalin and formic acid decalcification. The specimens were dyed with haematoxylin–eosin (HE) and examined under a light microscope. The specimens were scored with a histopathological scale (Table 1) and the data were analysed statistically.

Findings

Postoperative day 7

Fibrous bone union was seen in all specimens in the first week. New bone formation with a thin stratum on the sides and base of all defects in prednisone group were observed. The defects were full of fibrose connective tissue. In control group it was observed that the defects were full of fibrous connective tissue and with an inflammatory area on it (Table 2).

Table 1. Histopathological criteria of bone defect healing from Heiple et al. (11)

A-Union
(0) No sign of union
(1) Fibrous union
(2) Osteocondrial union
(3) Bone union
(4) Completed reorganisation of bone

B-Formation of Spongiosa
(0) No osseous cellular activity
(1) Early apposition of new bone
(2) Active apposition of new bone
(3) Reorganised spongiosa formation
(4) Completely reorganised spongiosa

C-Formation of Cortex
(0) No sign of cortex
(1) Beginning to appear
(2) Beginning of cortex formation
(3) Reorganised cortex
(4) Completed reformed cortex

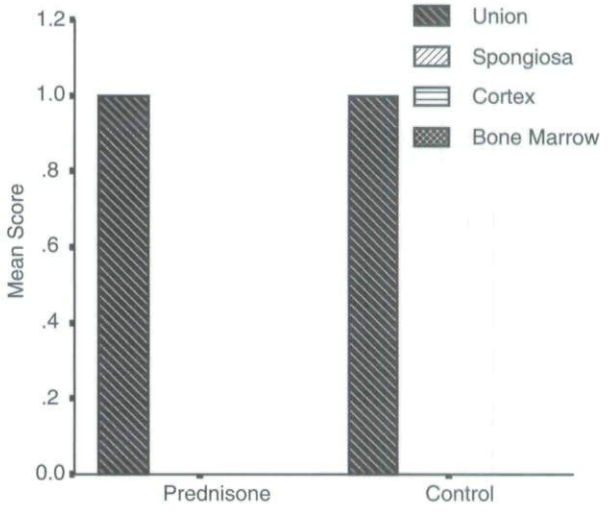
D-Bone marrow
(0) No sign of marrow
(1) Beginning of bone marrow formation
(2) More than half of defect
(3) Completed red bone marrow
(4) Adult-type fatty marrow

Postoperative day 14

In one case in the prednisone group there was osteocondrial healing and in other cases fibrose bone union was in progress. New spongiosal bone formation and cellular activity was observed in one case (Fig. 1).

In three cases of the control group, fibrose bone formation continued. In two other cases, only indirect spongiosa bone formation was observed. Active bone formation existed on the base in two cases. No bone marrow formation was recorded in all specimens (Fig. 2) (Table 3).

Table 2. Graphic of the histopathologic scores of 7th day



Postoperative day 21

In two cases of the prednisone group, osteocondrial bone formation was observed. In three other cases fibrose bone union was determined. In two control cases fibrous bone union was seen, and in three other cases osteocondrial bone union and active new bone formations were determined (Table 4).

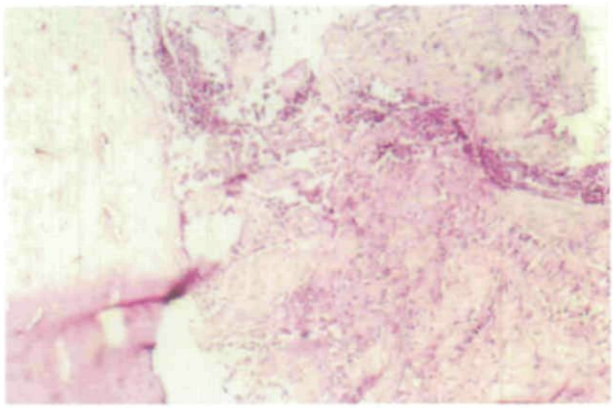


Fig. 1. Prednisone group 14th day post-procedure – new spongiosal activity and cellular activity is observed.

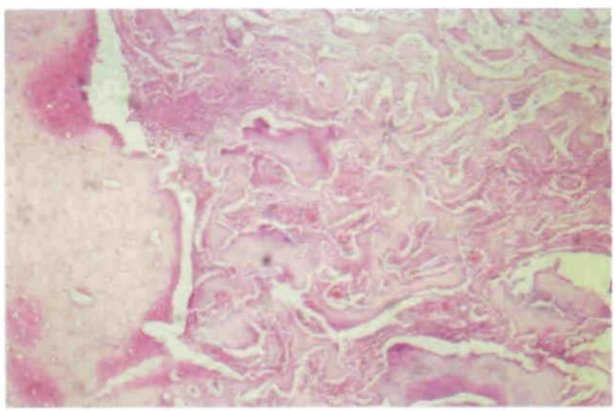


Fig. 2. Control group 14th day postprocedure – new bone formation is seen.

Table 3. Graphic of the histopathologic scores of 14th day

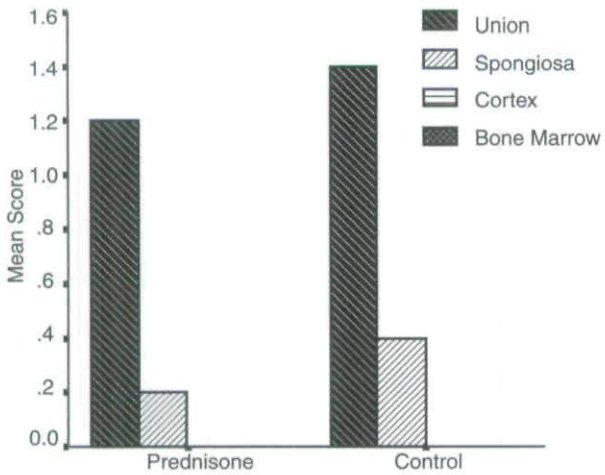
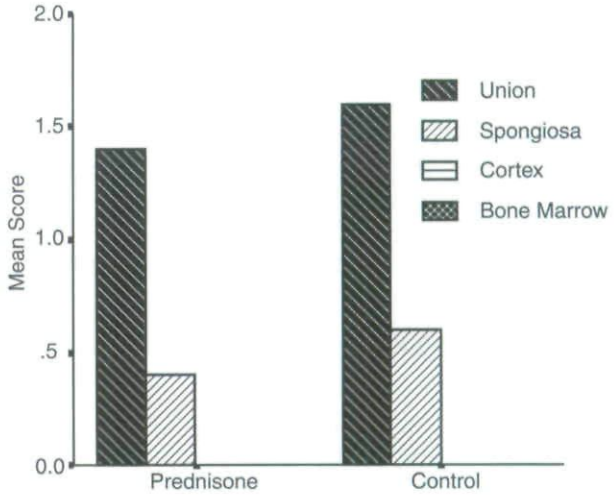


Table 4. Graphic of the histopathologic scores of 21st day



Postoperative day 28

In two cases of the prednisone group osteocondrial bone union were observed. In addition, in one case active bone marrow formation was observed. No cortex formation was seen in all cases (Fig. 3).

In two cases of the control group there was active new bone formation, in three other cases early bone formation was seen. Only in two cases active bone marrow formation was observed but no cortex formation occurred (Fig. 4) (Table 5).

Kruskal–Wallis and Mann–Whitney *U* statistical analyses were performed on histopathological scores of the periods. No statistical difference was found between all parameters and periods.

Discussion

The ability of corticosteroids to suppress the inflammatory reaction is well documented (4).

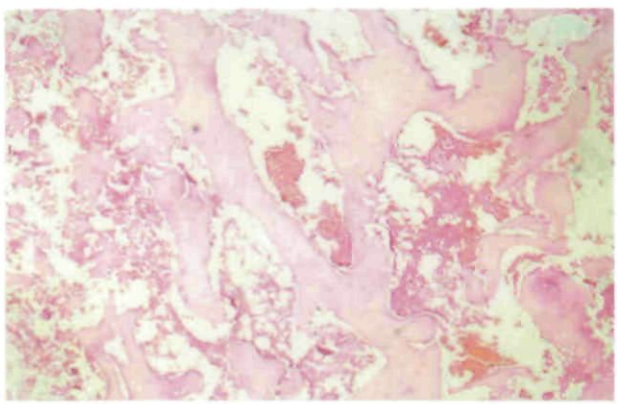


Fig. 3. Prednisone group 28th day postprocedure – no cortex formation can be seen.

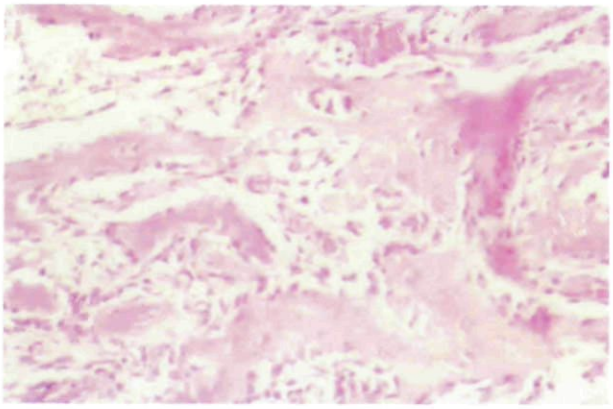
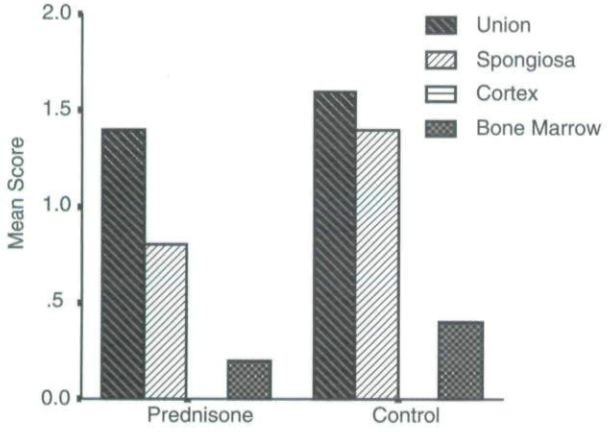


Fig. 4. Control group 28th day postprocedure – active bone marrow formation is seen, but no cortex formation occurred.

Table 5. Graphic of the histopathologic scores of 28th day



Short-term corticosteroid treatment is commonly used in inflammatory diseases and in acute bone injury. In orthognathic and orthopaedic surgery short-term therapy with corticosteroids may be routinely administered to reduce postoperative pain and swelling and to modify the clinical course after elective surgery or accidental trauma (4, 5, 8, 9). The medical effects of this therapy on bone healing are unclear. No adverse effects of short-term corticosteroid treatment have been detected in clinical practice (7). Long-term treatment with corticosteroids clearly induces general osteoporosis, spontaneous fractures and, development of pseudo-arthrosis, in fracture healing in animals as well as suppressed immunological response to infections (5–7). Corticosteroids also diminish the production and activity of growth factors that are important for bone healing (7). However, short-term therapy with corticosteroids may not induce such undesired effects (5, 8).

In their *in vitro* study Lukert et al. used corticosteroids in the rat calvarias. They showed that corticosteroids inhibit collagen production and osteoprogenitor and osteoblast cell activation (10).

Waters et al. found that chronic, systemic administration of a corticosteroid (prednisone) impaired bone healing in rabbits in a non-critical sized ulnar defect model. Their data indicated a smaller callus, less density at the osteotomy site, and a very low rate of union in the ulnae of prednisone-treated rabbits when compared with controls (7).

Mbugua et al. used glucocorticosteroids in soft tissue and bone injuries in dogs. They found no adverse effect of glucocorticosteroids on wound and bone healing (8).

Hogevold et al. found experimental bone defects in short-term prednisone-treated rats. According to their study, short-term therapy with prednisone did not suppress the healing when evaluated 6 weeks after surgery. They found that short-term prednisone therapy did not influence bone healing (5).

In conclusion, bone union and spongiosa formation were remarkable in the control group than the prednisone group on day 14, 21 and 28, but were not statistically significant (Tables 2–5). The prednisone-treated group showed better histopathological appearance but it was not statistically significant.

References

1. Liu SH, Yang RS, al-Shaikh R, Lane JM. Collagen in tendon, ligament, and bone healing. A current review. Clin Orthop 1995,(Sep);318:265–78.
2. Fleischli JW, Adams WR. Use of postoperative steroids to reduce pain and inflammation. J Foot Ankle Surg 1999;38:232–7.
3. Fauci AS, Dale DC, Balow JE. Glucocorticosteroid therapy: mechanisms of action and clinical considerations. Ann Intern Med 1976;84:304–15.
4. Olstad OA, Skjelbred P. Comparison of the analgesic effect of a corticosteroid and paracetamol in patients with pain after oral surgery. Br J Clin Pharmacol 1986;22: 437–42.
5. Hogevold HE, Grogaard B, Reikeras O. Effects of short-term treatment with corticosteroids and indomethacin on bone healing: a mechanical study of osteotomies in rats. Acta Orthop Scand 1992;63:607–11.
6. Aaron JE, Francis RM, Peacock M, Makins NB. Contrasting microanatomy of idiopathic and corticosteroid-induced osteoporosis. Clin Orthop 1989;243:294–305.
7. Waters RV, Gamradt SC, Asnis P, Vickery BH, Avnur Z, Hill E et al. Systemic corticosteroids inhibit bone healing in a rabbit ulnar osteotomy model. Acta Orthop Scand 2000;71:316–21.
8. Mbugua SW, Skoglund LA, Skjelbred P, Lokken P. Effect of glucocorticoid on the post-operative course following experimental orthopaedic surgery in dogs. Acta Vet Scand 1988;29:43–9.
9. Skjelbred P. The effect of corticosteroids on postoperative edema. Plast Reconstr Surg 1988;81:816–7.
10. Lukert B, Mador A, Raisz LG, Kream BE. The role of DNA synthesis in the responses of fetal rat calvariae to cortisol. J Bone Miner Res 1991;6:453–60.
11. Heiple KG, Goldberg VM, Powell AE, Bos GD, Zika JM. Biology of cancellous bone grafts. Orthop Clin North Am 1987;18:179–85.