

# Experimental Study of the Interference of Dissodium Phosphate of Dexamethasone in the Process of Rat Tibia Bone Repair

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## ABSTRACT

The present research assessed the interference of dexamethasone in the bone repair process in surgical wounds produced in rat tibia. The rats used as sample were divided in three groups. In all groups two monocortical perforations were produced in the tibia's diaphysis. The material was collected and processed for histological analysis, in period of 24 and 48 hours, 7 and 21 days, after surgical stage. The control group did not get intra muscular dexamethasone shots. One group received a dosage of 0.1mL solution of 8mg dexamethasone by 5mL of saline an hour prior to surgical procedures. The third group received one hour prior to the surgery and continued receiving the same dosage for three days after the surgery, every 8 hours. The comparative histological analysis of the repairing processes showed that, in the control group and in the group that received only one pre-surgical dosage, the repair of the perforations was determined on the twenty-first day. There was evident delay in the repairing process in the group that received one pre-surgical dosage and continued receiving it during the entire experimental period. On the twenty-first day after the surgery, a granulation tissue was still possible to be observed, and it was intermediated by an osseous blastema. Coagulum remnants were observed and the periosteum was not fully repaired. This remarks show a clear delay in the healing that will have to

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be taken into account at the moment of prescribing steroidal anti-inflammatory medicines when only the facial edema decrease is desired.

**KEYWORDS:** dexamethasone, bone repair, bone.

## INTRODUCTION

Inflammation is the natural response of tissue to injury, resulting in the release of histamine and other substances, which starts the process of protection of the organism. There is also release of fluids from capillaries in the injured area leading to its isolation followed by migration of leukocytes aiming to phagocyte the invading organism or its remains. As the process continues, normal healing is the ultimate result (MESSER; KELLER, 1975).

The importance of protein metabolism in wound healing is obvious. The tissue involved in the repair process is collagenous and has as characteristic the protein synthesis, which leads to complete maturation of the tissue in formation. The intricate relationship among carbohydrates metabolism is outlined when it is perceived that some systemic alteration affecting such metabolism modify the pattern of healing (GRANDINI, 1978).

The amount of endogenous cortisol, from the adrenal cortex, seems not to affect the process of inflammation. However high doses of exogenous cortisol, or synthetic steroids, blockes the stages of process of repair or healing. Steroids prevent diapedesis, the initial flow of fluid from capillaries, stabilizes the membrane of lysosomes, avoiding in this way the release of hydrolytic enzymes, as well as reducing the formation of bradycininine, which is a potent vasodilatator (MESSER; KELLER, 1975).

Steroids are the most important drugs in the preventive treatment of posttraumatic edema. Among the steroids, dexamethasone has the greatest antinflammatory action and less side effects. High doses of steroids, for a few days, seem to be innocuous. However, a long treatment carries some risks, which is not sufficient to justify the fearfulness to use the drug: the fear that the drug may interfere negatively in the healing process after extraction seems to be innocuous (SOUZA et al., 1988).

Authors that are against the use of steroid argue that these drugs have a negative effect in pituitarian hypothalamus resulting in suppression of the normal endogenous secretions, such as cortisol from the adrenal. However, the amount of suppression is time and dose dependent (WILLIAMSON et al., 1980).

The cortex to the suprarenal produces steroids, which are lipids showing in its nucleus ciclopentane in the pyridine fenantrene. During the biosynthesis process of hormones from cholesterol, chemical radicals are added or removed to this nucleus, which gives different activities to the many final products. Hormones are not stored in the cortical cells, but are synthesized and secreted according to the needs. Steroids secreted by the cortex can be grouped in three categories according to its main action:

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glucocorticoids, mineralocorticoids and androgen. Glucocorticoids mainly represented by cortisol (hydrocortisone) and corticosterone, acts particularly in the protein metabolism leading to increase in the production of glycogen (neoglycogenesis) and increase in glycemia. They also mobilize fat from its deposits of DNA, particularly in the lymphoid tissue attenuating the immune response. They also inhibit inflammation, i.e., they are anti-inflammatory (JUNQUEIRA; CARNEIRO, 1999).

Glucocorticoids are steroids secreted by the suprarenal cortex and are responsible for the most used therapeutic actions in the clinic including their anti-inflammatory, antialergic and immunosupresor activities (LOPES et al., 1998).

Head and neck surgery may induce severe pain, dysfunctions and edema. The surgical removal of third molars is a common procedure in oral surgery, which may be completed by such occurrences. Factors that contributes to post-operative edema, trismus and pain are complex and a good technique associate to careful handling of tissues minimizes these sequels although do not prevent them (WILLIAMSON et al., 1980).

Ware et al. (1963) stated that, regarding the operative technique in oral surgery, the excess of facial edema, followed by trismus and discomfort, might lead to a late healing. Notwithstanding, the same authors based on their studies, could not recommend dexamethasone as a routine procedure for the prevention of edema and trismus after oral surgery. In 1973, Amier reported that, despite disturbs to normal healing of wounds after dental extraction are interrelated, each disturb is a result of a very specific characteristic for each stage, but modifying the alveolar healing as a whole.

According to Hooley and Hohl (1974), since 1974 the systemic and topic therapies with steroids have been stimulated to the prevention of edema resulting from oral surgery procedures leading to a reduction in the period of admission of patients in the hospital after major oral operations.

For Bahn (1982), despite the controversies, the prophylactic use of steroids in traumatic oral surgeries has not demonstrated consistent advantages to patients, taking into consideration the cost/effectiveness of side effects and risks.

By evaluating the effect of dexamethasone in the control of traumatic facial edema in patients undergoing surgery of retained inferior third molar, Souza et al. (1988) concluded that a single IM doses of 20 mg of dexamethasone does not show any negative effect in the repair process of tissues although the evaluation of the normality it the process has been done by clinical exams in the 5<sup>th</sup> and 10<sup>th</sup> post operative day.

Pedersen (1985) while investigating the role of decadronphosphate in the relief of post operative symptoms of surgery of the third molar, concluded that 4 mg of dexamethasone reduces edema and trismus after removal of impacted mandibular third molars. Despite the reduction in 1/3 of post operative pain, the effect had not statistical significance.

According to Butler et al. (1983) the transoperative and post-operative use of steroid is a protocol procedure in oral surgery to modulate the

inflammatory response and to reduce edema. The effects offer comfort to patients and reduce the course of postoperative symptoms.

The use of synthetic steroids, such as dexamethasone, is well documented. Synthetic steroids have a suppressive effect on the hipotalamus-hipophysis-adrenal mechanism, resulting in suspension of endocrine secretion of normal cortisol. Prolonged adrenal suppression, due to long term therapy with steroids, is a well-known phenomenon. The clinical experience derived from patients treated with steroids, due to allergic problems, does not allow an accurate estimation of the duration of treatment needed for adrenal suppression. Laboratorial data suggest that suppression occurs within one or two weeks (WILLIAMSON et al., 1980).

Physiological functions of steroids, besides their pharmacological actions, are manifold. They influence in the carbohydrates metabolism, proteins and fat; hydroelectrolitical balance and in the function of the cardiovascular system, kidneys, nervous system, among others. It provides to the organism its capability of resistance to stress and environmental modification, being considered as the basis of the organic homeostasis. Its anti-inflammatory effects are particularly notable (WILLIAMSON et al., 1980).

Devlin and McCord (1995) mention that the healing dental pocket is an excellent model of bone healing due to absence of cartilaginous formation, to the rapid bone reshaping and to its obvious clinical relevance.

Healing of post-exodontic wounds has been reported in many studies in animal models with various techniques: histological studies, radiological analysis and histochemical studies. These have established many steps of the process of alveolar repair (GRANDINI,1978).

Systemic alteration, experimentally produced in rats, demonstrated various grades of disturbance in the healing process of dental extraction wounds. In the rat, the end of the wound repair occurs in the 21<sup>st</sup> day after the operation (BARRETO et al.,1982).

Barreto et al. (1982), evaluating the influence of vitamin D3 in the repair process of dental extraction wounds, in a clinical and histological study in rats, concluded that animals receiving vitamin D3 have an earlier bone neoformation.

Barroso et al. (1979/1980) state that the repair process after exodonty has been studied under many variables, among which those related to hormonal problems. In a histological study on repair of dental extraction wounds in rats treated by tyrocalcitonine, they concluded that there was no modification on the repair process.

The influence of external factor in the process of alveolar repair, after exodonty, seems to be well documented. Saad Neto et al. (1985) analyzed the influence of local anesthetics in the repair process of alveolar bone in rats following terminal infiltration and alveolar irrigation. They concluded that alveolar irrigation with anesthetic solution, with or without epinephrine, plus terminal infiltrative anesthesia, retards the chronology of alveolar repair. The delay is more marked when the anesthetic solution has epinephrine and is also more mischievous to the repair than the simple and abundant irrigation of the alveolus.

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## MATERIAL AND METHODS

In this study were used 48 male and female Wistar rats (*Rattus albinus*) from the animal farm of the University of the Sacred Heart.

Animals were allotted in three groups with 16 rats each, being eight animals kept in each cage:

Group 01 – Control: animals in this group underwent surgery for tibial perforation.

Group 02 – Experimental: animals in this group underwent surgery for tibial perforation with previous IM injection of dexamethasone one hour before the procedure. Each animal received 0.1 mL of a solution of 8 mg of dexamethasone.

Group 03 – Experimental: animals in this group underwent surgery for tibial perforation plus an IM injection of the same solution of dexamethasone. In addition, these animals continued to receive the same drug, every 8 hours, for three days.

The above groups were subdivided in four groups with four animals each. For each of these groups, four animals were sacrificed at 24 hours, 48 hours, 7 days and 21 days after the surgical procedure.

During the procedures animals were anesthetized with sulphuric ether. The animals were placed on a sterilized towel on tables cleaned with a solution of iodine alcohol.

After tricotomy on the leg, the area was cleaned with the iodine alcohol solution. The surgeon used dischargeable gown, mask and sterile gloves.

After antisepsis a skin incision was made in the central aspect of the leg with a #15 blade leading to the deep tissues and the periosteum of the tibia.

Another incision was made in the periosteum for undermining and visualization of the bone, which received two monocortical perforations with a number 4 spherical drill under continuous irrigation with saline.

The suture was made with 4/0 nylon after cleaning of the wound with saline. Animals were kept in their cages with water and ration *ad libitum*. Sacrifice was done by inhalation of abundant sulphuric ether. After that, the legs were removed and fixed in 10 % formalin, stained by H&E and analyzed on optical microcopy.

## RESULTS OF HISTOLOGICAL SLIDES

The analysis of the histological slides of the three groups of rats led to the following results:

In the control group (sacrifice at 24h), the bone cavity was filled with blood clot and hyperemia circumjacent to the clot was found in the medullar vessels (FIGURE 1).

In the rats sacrificed at 48 h the clot in the surgical wound was edematous and there was still hyperemia of vessels, although more marked.

In the rats sacrificed at the 7<sup>th</sup> day there was steady invasion of fibroblasts and bone progenic cells in the clot (healing organization). Besides that, there was also capillary proliferation and clear macrophagic activity. In this period it was already possible to observe bone neoformation, with blastema and mild bone trabecula (FIGURE 2).

In the animals sacrificed at the 21<sup>st</sup> day, the blood clot in the cavity was almost replaced by bone trabecula. The intramembranous ossification fills the whole surgical wound and it is possible to visualize restructuring of the periosteum (FIGURE 3).

In the group of animals that received a dose of dexamethasone one hour before the operation it was possible to observe that in the group of rats sacrificed at 24h the bone cavity was filled with blood clot and there was hyperemia close to the clot.

In the rats sacrificed at 48h there was a swelling clot with clear disorganization.

In the rats sacrificed at the 7<sup>th</sup> day the slides showed fibroblastic, capillary and progenic cells proliferation. It was possible to identify regions with clear intramembranous ossification in which fine trabecula invades the space formerly occupied by the blood clot.

In the animals sacrificed at the 21<sup>st</sup> day, the surgical wound is filled with neoformed bone tissue and the periosteum is restored.

The group that showed greatest interference of the dexamethasone was the one that received a doses of anti-inflammatory, IM, one hour before surgery and continued to receive the same drug for three days every 8 hours.

In the histological slides of animals sacrificed at 24h the bone cavity is filled by the blood clot. There us a clear hyperemia of the surrounding bone marrow.

In the rats sacrificed at 48h the blood clot still persists. Hyperemia is mild and there is less swelling.

After 7 days, it is possible to observe mild capillary and fibroblastic proliferation. Remaining of the clot can be still seen, being substituted for loose connective tissue with discrete macrophagic activity.

The bone neoformation is delayed if compared to the control group and to the group that received only one dose. It is also possible to observe a mild invasion of bone trabecula replacing the clot in the surgical wound.

In the rats sacrificed at the 21<sup>st</sup> day, the bone cavity is not fully filled with bone trabecula revealing a marked delay in the healing process. It is still possible to observe remnants of the blood clot and there is not a complete repair of the periosteum (FIGURE 4).

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FIGURE 1: Control group (sacrificed at 24h). Note the blood clot in the region of the perforation.

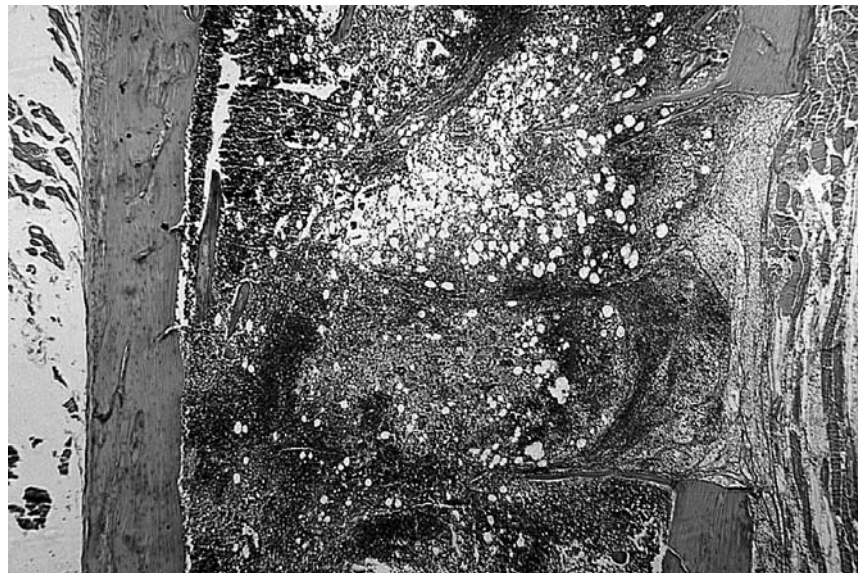


FIGURE 2: Control group (Sacrificed at the 7<sup>th</sup> day). Note the evolution of the healing process with bone neoformation.



FIGURE 3: Control group (Sacrificed at the 21<sup>st</sup> day. There is ongoing normal bone healing.

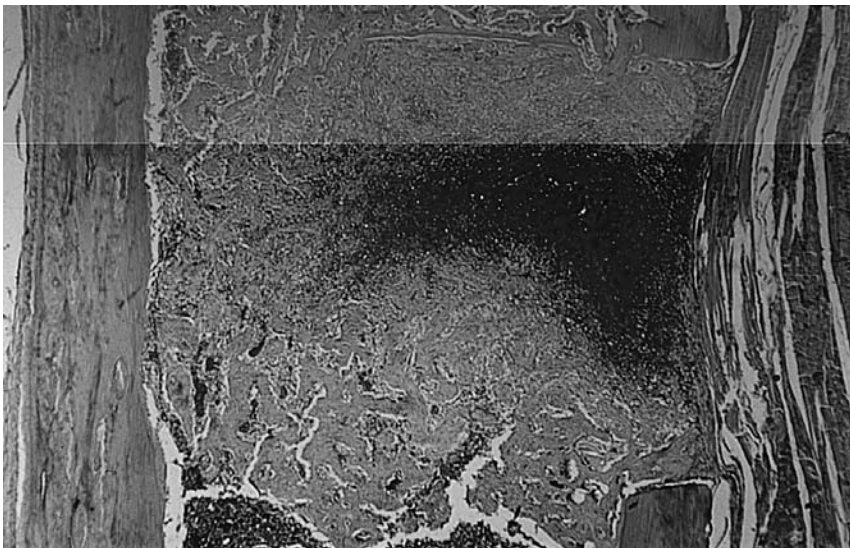


FIGURE 4: Group of animal that received dexamethasone for 3 days. Sacrificed at the 21<sup>st</sup> day. It is possible to note the delay in the bone healing due to the interference of the anti-inflammatory as compared to FIGURE 3.

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## DISCUSSION

According to Messer and Keller (1975) the amount of endogenous cortisol, from the adrenal cortex, seems not to influence the inflammation process. However, a great dose of exogenous cortisol or synthetic steroids tends to block steps in the process of repair or healing. Steroids prevent diapedesis, the leakage of fluid from capillaries stabilizes the membrane of lysosomes, preventing the liberation of a great amount of hydrolytic enzymes, besides reducing the formation of bradycinin, which is a potent vasodilator.

It is well established in the literature that steroid and its derivatives diminish the hyperemic reaction as they inhibit the release of bradycinin. As a result of the stabilization of the diameter and the permeability of vessels, swelling is discrete.

Regarding the persistence of swelling and surrounding hyperemia to the clot in the present study, a possible explanation is the fact that capillaries in the bone marrow are of the discontinuous and sinusoidal type. In places where these types of capillaries occur, it is not possible to control the edema since the vessels are extremely permeable once there is a wide space between the endothelial cells.

Souza et al.(1988) report that steroids are the most important drugs in the preventive and curative treatment of traumatic edema. Among steroids, dexamethasone has more antiedema action and fewer side effects. High doses of steroids, during the period of a few days, seem to be innocuous. However, long treatments bear risks, which is not sufficient to avoid the use of this drug: the fear that steroids may interfere negatively in the process of healing of extraction wound seems unsustainable.

Regarding the importance of the preventive and curative treatment of traumatic edema by steroid, we have the same philosophy as Souza et al. (1988), since we demonstrated that in the group of animals that received one doses of dexamethasone one hour before the surgical procedure and continued to receive the same drug for three day at every 8 hours interval, there was a reduction in the edema as observed in the histological slides of animals sacrificed after 48 hours.

However, we do not agree with these authors that maintain that long use of steroids do not have negative interference in the healing process. Indeed, its is possible to see, in the slides of animals sacrificed at 21 days and that received steroids for three days, a clear delay in the healing process. It was also possible to detect remaining of the blood clot and the partial repair of the periosteum.

For Barreto et al. (1982) the systemic alteration experimentally induced in rats demonstrated varied grades of perturbation in the process of wound repair of dental extraction in rat. The end of the repair process occurs in the 21<sup>st</sup> postoperative day.

According to the analysis of the histological slides, we observed that bone healing of the monocortical perforations finished, in the control group, at the 21<sup>st</sup> day, which agrees with the study by Barreto et al.

(1982). However, the group of rats that received dexamethasone for three days showed in the histological slides a delay in the bone healing in those animals sacrificed at the 21<sup>st</sup> day.

## CONCLUSION

The data obtained in this histological study it was possible to observe that there was interference of dexamethasone in the healing process of bone repair.

This finding should be taken into consideration by the clinics while evaluating the advantages or disadvantages in prescribing steroids just to prevent edema but potentially risking the healing process after the surgery.

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