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ORIGINAL ARTICLE

DIPYRONE HAS NO EFFECTS ON BONE HEALING OF TIBIAL FRACTURES IN RATS

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ABSTRACT

Objective: To evaluate the effect of dipyrone on healing of tibial fractures in rats. Methods: Fourty-two Wistar rats were used, with mean body weight of 280g. After being anesthetized, they were submitted to closed fracture of the tibia and fibula of the right posterior paw through manual force. The rats were randomly divided into three groups: the control group that received a daily intraperitoneal injection of saline solution; group D-40, that received saline injection containing 40mg/Kg dipyrone; and group D-80, that received saline injection containing 80mg/Kg dipyrone. After 28 days the rats were sacrificed and received a new label code that was known by only one researcher. The fractured limbs were then amputated and X-rayed. The tibias were disarticulated and subjected to mechanical, radiological and histological evaluation. For statistical analysis the Kruskal-Wallis test was used at a *s*ignificance level of 5%. Results: There wasn't any type of dipyrone effect on healing of rats tibial fractures in relation to the control group. Conclusion: Dipyrone may be used safely for pain control in the treatment of fractures, without any interference on bone healing. ***Level of Evidence II, Controlled Laboratory Study.***

Keywords: Tibial fractures. Fracture healing/drug effects. Rats.

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INTRODUÇÃO

Bone healing is a repair process that does not result in a scar, but rather in the reconstitution of damaged tissue, a lot like the original form. This process can be divided into three sequential phases: inflammation, repair and remodeling.[1]

In the inflammatory phase, there is bleeding from the bone and soft tissue ends with clot formation, vasodilatation and exudation of plasma and leucocytes.[2] In the repair phase, the organization of the hematoma occurs. This is invaded by fibrovascular tissue that replaces the clots and deposits collagen fibers and the matrix that is subsequently mineralized to form the primary callus.[1]

The process of bone remodeling occurs when the fracture line is filled by callus. At this stage, the mineralized cartilage is converted into bone tissue, which is modified into lamellar bone by organization of the haversian system. In this process, osteoclasts remove bone and osteoblasts deposit lamellar bone around the central capillary canal.[3] The mechanics of primary bone healing involves a complex inter-relationship of physical and biological factors.[4]

The control of postoperative pain is an essential part of the surgical procedure.[5] In many countries, pyrazolone derivatives, including dipyrone, are widely used as pain killers.[6] Baños *et al*.,[7] in a study performed in three Spanish hospitals, reported that dipyrone is the most frequently prescribed analgesic drug. Although dipyrone is widely used to promote analgesia, we did not find in the literature any work that assesses its effect on fracture healing.

MATERIALS AND METHODS

For this study, Federal Law number 6,638 of May 8, 1979, and the guidelines of *Colégio Brasileiro de Experimentação Animal* (Brazilian College of Animal Experimentation) have been followed. Forty two Wistar male rats (Rattus norvegicus *albinus*), with 280g average body weight and 101 days old.

The rats received a standard diet and water *ad libitum* and were housed four to five mice in cleaned cages. Animals were kept in a semi-controlled macro-environment, with dark/light 12 hours cycle, environmental noise intensity and natural moisture.

On the first day of the study, all rats were anesthetized with ketamine, weighed and submitted to close fracture in the middle third of the tibia and fibula of the right hind paw, by hand force by three-point support. All fractures were performed by the same researcher.

All the authors declare that there is no potential conflict of interest referring to this article.

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Only after the fracture the rats were divided by lot into three groups of 14 rats each. The control group rats received a daily intraperitoneal injection of 0.2 ml saline 0.9% NaCl; rats from group D-40 received a daily intraperitoneal injection of 0.2 ml saline containing 11.2 mg dipyrone, at a concentration of 40mg/Kg and rats from group D-80 received a daily intraperitoneal injection of 0.2 ml of saline containing 22.4 mg of dipyrone, at a concentration of 80mg/kg. The fractured limb was not immobilized and free movement was allowed.

Control of bias occurred at each step of the study, from the random distribution of mice in cages, the rotation of cages position on the shelves, the order of application of medicines and basic care performed by technicians at the facility.

Each group received the medication on a single dose once daily, uninterruptedly, starting from the day of the fracture until the day of sacrifice that occurred on the 28th day after the fracture, performed by inhalation of sulfuric ether overdose. Immediately before sacrifice, rats received a new code, known only to one researcher so that other researchers were unaware of the identification of the animals until the end of the period of analysis.

The fractured limbs were then amputated at the femur level, and the amputation parts were referred for obtaining digital radiographs for better visualization of soft tissue and callus. Then, tibia were disarticulated from the knee and ankle and carefully dissected, separating the bone from the soft parts, without interfering in bone formation.

Dissected tibias were subjected to three types of evaluation:

Mechanical: the proximal tibia were tied with cotton twine and hung on a metal base. Then, they underwent continuous pendulum traction distally, under gravity, starting with one pound and increasing one pound every 10 seconds, until fracture of the callus. The maximum weight immediately before the callus fracture was scored for each evaluated tibia.

Radiographic: seven evaluators graded the bone callus between zero and four through X-rays taken in anteroposterior and lateral views. The zero mark corresponded to the absence of bony bridges; graduation one corresponded to the callus in one cortical (anterior, posterior, medial or lateral); graduation two corresponded to the presence of callus in both cortices; graduation three, the callus was seen in three cortical; and grade four callus existed in all cortical. Histological: tibiae from each group were placed into a solution of 10% formalin and sent to the Anatomic Pathology Laboratory for slides preparation and evaluation of fracture foci. After decalcification, samples were embedded in paraffin for preparation of blocks, which were cut in the microtome in the fracture corresponding region, stained with hematoxylin-eosin, mounted on slides, and evaluated by a pathologist.

All researchers who participated in the mechanical, radiological and histological analysis were unaware to which group the analyzed mice tibiae belonged. Variance analysis by Kruskal-Wallis test was used for statistical analysis of specific magnitudes. It has been settled $p=0.05$ or 5.0% as rejection level of null hypothesis.

RESULTS

In mechanical evaluation, the average maximum weight supported by the callus just before break was 3,615 kg in the control group; 4,308 kg in D-40 group and 3,321 kg in D-80 group. Statistical analysis showed no significant difference between the results obtained in all groups.

In radiographic evaluation, the average graduation of the control group was 2,969; of D-40 group it was 2,684 and of D-80 group it was 2,541. (Figures 1 and 2) These results were also not statistically significant. (Table 1)

There was also no significant difference between control, D-40

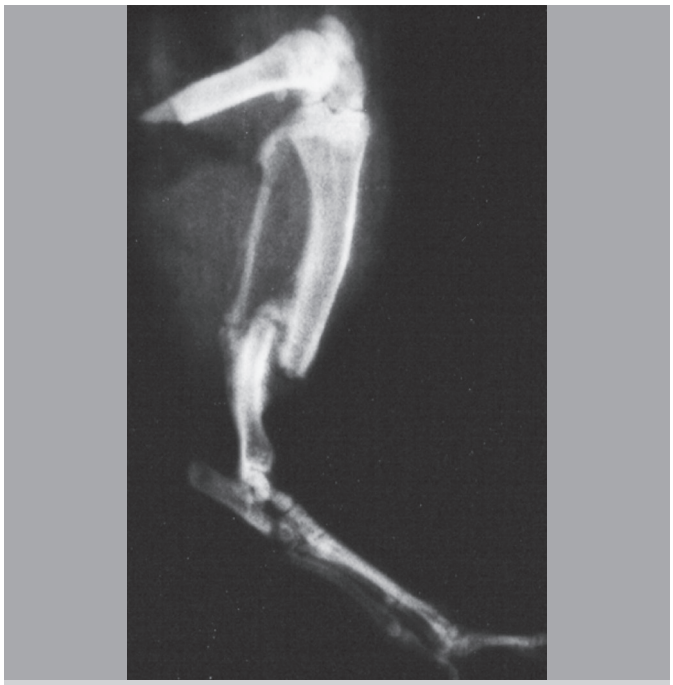

Figure 1. X-Ray of rat paw, in profile incidence, showing sparse tibia callus (average degree: 1.286).

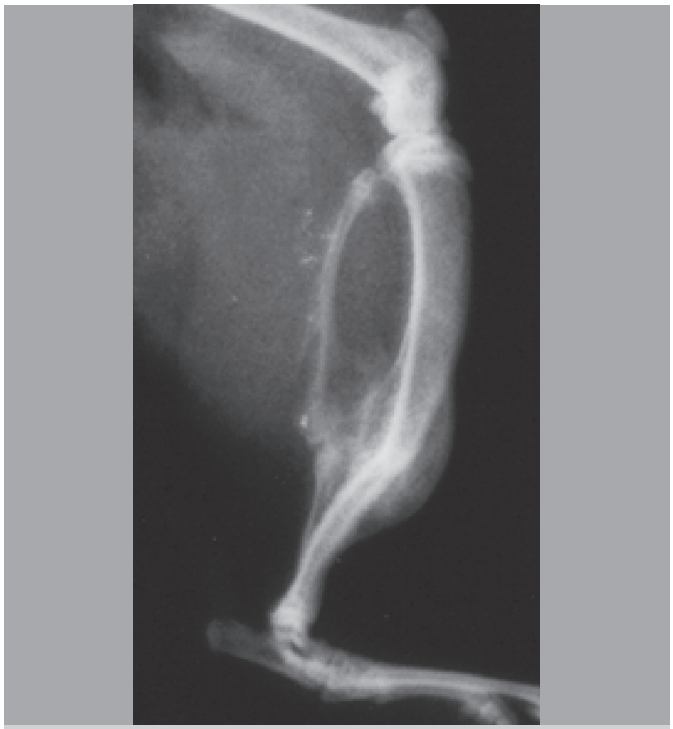

Figure 2. X-Ray of rat paw, in profile incidence, showing exuberant tibia callus (average degree: 3.714).

and D-80 groups regarding histologic evaluation of the consolidation. The examination of the parts showed very similar aspects: cortical was always thicker, unlike the medullary trabeculae, which were thinner and populated by numerous osteoblasts. (Figures 3 and 4)

Therefore, it was not observed any effect of dipyrone in the consolidation of mice tibia fractures compared to the control group on mechanical, radiographic and histological assessment.

Table 1. Average of radiographic assessment in each group.

	D-40 Group	D-80 Group	Control Group
Evaluator 1	2.714	2.857	2.929
Evaluator 2	2.786	2.286	2.214
Evaluator 3	2.643	2.571	3.071
Evaluator 4	2.929	3.000	3.286
Evaluator 5	2.286	2.286	3.000
Evaluator 6	2.571	2.143	3.071
Evaluator 7	2.857	2.643	3.214
Mean	2.684	2.541	2.969

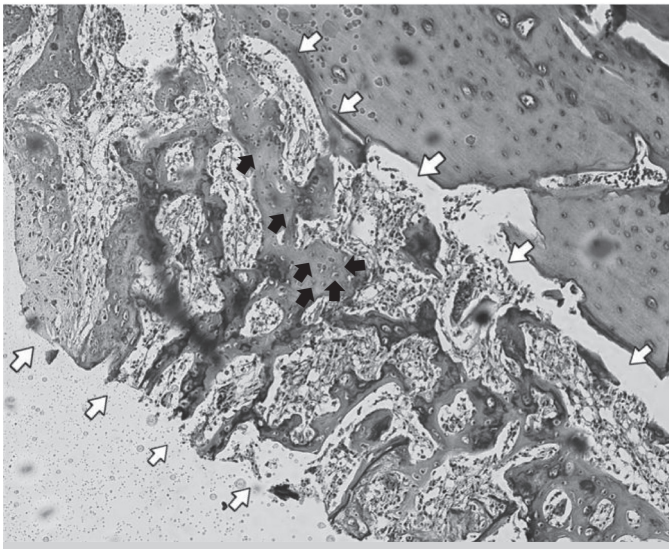

Figure 3. 10x magnification photomicrograph showing area of abundant new bone formation around mature bone (white arrows). Numerous osteoblasts (black arrows) synthesizing a new bone matrix can be seen.

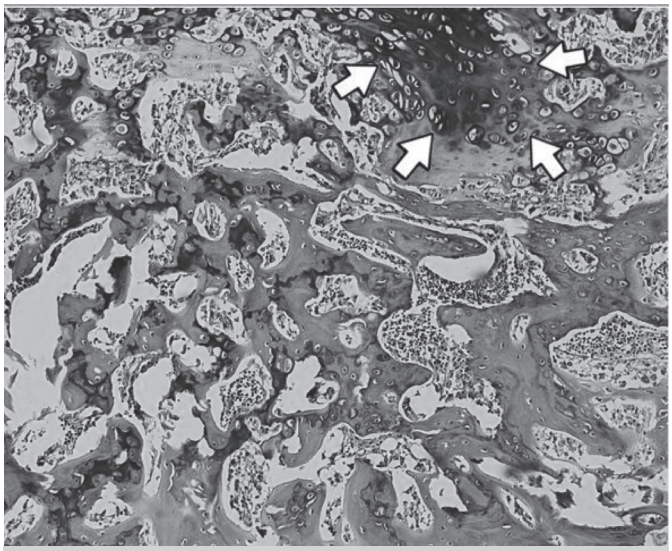

Figure 4. 10x magnification photomicrograph showing area of abundant new bone formation with early surface hyaline cartilage (white arrows).

DISCUSSION

Dipyrone is a cyclooxygenase inhibitor, with rapidly reversible effect. Its administration orally has been shown to be more effective than the same dose of aspirin or paracetamol for postoperative pain relief.4

There are controversies regarding the possibility of dipyrone may cause agranulocytosis. A study performed in Sweden reported the incidence of agranulocytosis in at least one in 1439 prescriptions.[8] Moreover, in a study performed in São Paulo, the incidence of agranulocytosis induced by dipyrone was 0.44 to 0.82 cases/million inhabitants/year.[9] In a recent study in the Netherlands, showed that dipyrone is a safe and effective drug in controlling acute pain, especially when compared to anti-inflammatory steroids.[5]

Many studies have evaluated the effect of pain relieve drugs which were administered to mice during the fracture healing process. Ibuprofen and indomethacin can slow the process of bone healing,[10] while celecoxib[11,12] and acetominofen[12,13] did not influence this process. Simon and O'Connor,[14] on the other hand, reported that celecoxib significantly reduces mechanical properties of the callus in the early stages of repair of fractures and increases the proportion of pseudoarthrosis in later stages.

Müller *et al*.[15] concluded that sodium diclofenac has changed the consolidation process and bone metabolism, leading to delay in the maturation of the callus and lower stiffness of the intact bone. Giordano Neto *et al*.[16] reported that Tenoxicam slows the consolidation process of tibial fracture in rats.

We are not aware of any research that has been done to evaluate the effects of dipyrone on bone consolidation. The option for concentrations of 40 and 80 mg/kg dipyrone was based on studies that used that dose in rats[17] and in humans. The dosage of 80mg/kg is above the recommended daily limit in humans and was used to verify whether, at this concentration, there could be any effect on bone healing.

We opted for closed fracture through manual angulation of the tibia; we did not use osteosynthesis materials in order not to interfere in the fracture focus.

We decided by the sacrifice of mice in the fourth week after the fracture for being a period of time which already provides for the presence of callus, according to the natural biology of bone healing. Udupa and Prasad[18] identified four distinct phases of bone healing in rats: fibroblastic phase in the first week; collagen phase in the second week; osteogenic phase in the third to fourth weeks, and remodeling phase from the fifth to sixth weeks.

As well as Castro *et al*.,[13] in our methodology the dissection of tibia was done only after radiographic examination, in order to cancel the possibility of damage to bone callus formation and because soft parts are also integrant of the fracture healing phenomenon.

In the mechanical evaluation, rats tibia were subjected to gravitational pull with progressively heavier weights until the bone

callus fracture. Despite the difference in the average weight to the occurrence of fracture in each rats group, there was no significant difference among the groups in the statistical analysis. Similarly, although there are differences in the subjective scaling of radiographic union among the seven assessors who evaluated the radiographs, there was no statistically significant difference between the ranks of the groups. It has not also been observed any significant difference between the groups regarding the growth of bone tissue in the histological analysis. In this assay the tissues studied were extremely similar.

CONCLUSION

There was no difference in either mechanical, radiographic and histological assessments in the consolidation of tibial fractures in rats between the control group and those in which dipyrone were administered.

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