INFLUENCE OF CIPROFLOXACIN ON FEMORAL FRACTURES HEALING IN RATS

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ABSTRACT

Objective: To present an experimental study about the effects of ciprofloxacin on the bone callus strength on femoral fractures in rats. *Methods:* The animals were divided in two groups of 8 rats each: the group study (cipro) and the control group. The animals were fixed with an intramedullary nail and submitted to a standardized femoral fracture. The group study was treated with ciprofloxacin and the control group with saline solution for six weeks. After that period, the femurs were analyzed using X-ray images and biomechanical three-point test, which measured the flexion strength in Newtons. *Results:* The X-ray images and the macroscopic analysis showed that all fractures healed. The results found in the biomechanical study between the two groups were compared, and the cipro group presented a mean resistance to flexion force of 71.11 and the contol group, mean flexion force of 74,78. There was no statistically significant difference (p = 0.601, t-test).*Conclusion:* The results from the biomechanical tests performed on femoral fractures in rats receiving ciprofloxacin revealed no statistical significance from measures of callus flexion strength when compared to the control group.

Keywords: *Ciprofloxacin. Fracture healing. Fracture healing/drug effects. Rats.*

Citation: *Kupczik F, Vialle LRG, Nobre LO, Vieira LA, Fernandes AEO. Influence of ciprofloxacin on femoral fractures healing in rats. Acta Ortop Bras. [online]. 2009; 17(4):228-31. Available from URL: http://www.scielo.br/aob.*

INTRODUCTION

Lower limbs' long bones fractures are very common in orthopaedic trauma, with femoral shaft fractures presenting an annual incidence of 1 to 4:10.000 individuals.[1] They most frequently occur in patients younger than 40 years as a result of high-energy trauma.[2]

Femoral fractures can be either closed or open, and treatment is predominantly surgical with internal or external fixation providing the required stability for bone union.[2,3] Complications of these fractures can be systemic and/ or local, with infection being one of the most feared.[2,4,5]

Ciprofloxacin is one of the antibiotic agents employed both for prophylaxis and therapy in these cases. In addition, it is used simultaneously in the treatment of infection at external fixation screws sites and remote infections. Its use is increasingly frequent in medical practice.[6-13]

Adverse side effects have been described for this drug on the musculoskeletal system. On tendons, degenerative changes occur, which may evolve to spontaneous ruptures, particularly in the elderly population. On joint and growth cartilages, chondrocyte changes, such as cell death and matrix degeneration with resultant changed collagen production have been demonstrated both *in vitro* and *in vivo* by several studies.[14-20]

The clinical relevance of this study is due to the fact of being able to change the routine use of ciprofloxacin in patients with fractures associated to bone and remote infections. Should the hypothesis of negative influence on bone callus strength is confirmed, the use of this antibiotic agent for prolonged periods of time would delay union process, extend the hospitalization time, and imply in new surgical procedures, increasing therapy costs and postponing patient's return to daily life activities.

OBJECTIVE

To assess the influence of ciprofloxacin on bone callus strength of standardized femoral fractures on rats by means of X-ray studies and biomechanical three-point flexion force test.

METHODS

Sixteen male Wistar rats, with ages ranging from 100 to 120 days and with mean weight of 324g (range: 296g - 352g) supplied by the Central Animal Lab at Pontifícia Universidade Católica do Paraná - PUCPR.

The procedures followed the rules of the *Guide for the Care and Use of Laboratory Animals of International Association for Assessment and Accreditation of Laboratory Animal Care* (1996) and the Federal Law nr. 6,638 of May 8th, 1979. By the approval and release of the Committee of Ethics in Animal Experiments (CEUA - PUCPR) under registration at CEUA/PUCPR nr. 197, the rats were divided into two groups: study group (cipro) and control group (saline solution). All animals were anesthetized with an intramuscular injection of ketamine hydrochloride at a dosage

All the authors state no potential conflict of interest concerning this article.

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Received in: 07/21/08; approved in: 09/08/08

of 20 mg/kg and xylazine hydrochloride 50 mg/kg, followed by trichotomy and asepsis of the surgical wound site with 1% iodine solution.
The surgical procedure was performed on a surgical table at the Laboratory of the Discipline of Surgical Technique of this institution, and consisted of a median incision at the level of the joint between femur and the left tibia for exposing quadriceps fibers. By performing a longitudinal dissection of quadriceps fibers and lateral patellar dislocation, femoral condyles were exposed for producing an entrance point with a 40 x 1.2 mm needle at the intercondylar region. Then, with the aid of a driller, a sterile 1-mm wide steel wire (Kirshner) was introduced into spinal cord canal, passing through shaft and major trochanter in a retrograde fashion up to its emergence beneath the skin. At this site another incision was made through which the wire was cranially pulled into femoral shaft, removing it from animal's knee joint. The cranial end was cut off and folded in order to avoid migration into the joint and buried beneath animal's skin. The wounds were closed with mononylon 3-0 suture.
While anesthetized, the rats were submitted to fracture of left femur shaft at its mid third with a device mimicking a blunt guillotine to produce standardized fractures, according to the methodology described on studies by Vialle *et al*.[21], Pedroni[22] and Mussi Filho *et al.*[23] also conducted in trauma research approach of the Surgical Practice Post-Graduation Program at PUCPR.
The wire position and the confirmation of fracture by X-ray imaging were checked. (Figure 1)

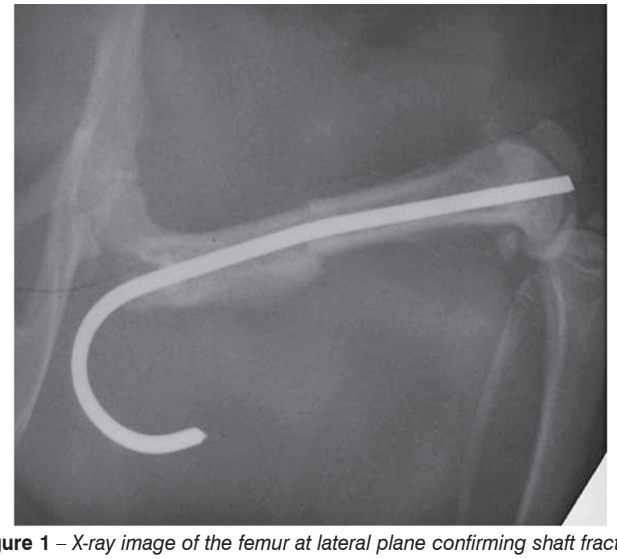

Figure 1 – *X-ray image of the femur at lateral plane confirming shaft fracture fixated with intramedullary metal wire.*

The rats, in groups of 2 animals each, were lodged in polypropylene cages with specie-standardized dimensions at the PUCPR's Central Animal Lab. The light cycle was monitored (12-hour bright-dark cycle) at room temperature 20±2ºC. Humidity and noise levels were kept stable. All animals received water and ration ad libitum.
Postoperative pain was controlled with the use of analgesic and anti-thermal medication, Flunixine meglumine BANAMINE® brand (0.1 ml intramuscularly once a day), for two days (according to the provisions of Resolution nr. 196/96, by the Ministry of Health and International Guides for Animal Protection).
On the cipro group, twelve hours after fracture, the administration of ciprofloxacin (Halexistar®) 50mg/kg was initiated at 12-hour intervals for six weeks. On control group, 0.9% 2 ml saline solution was administered similarly to the study group.
After the six-week period, the animals were sacrificed according to the Resolution 714/2002, using a lethal dose of ketamine hydrochloride (148 mg/kg) intraperitoneally.
Fractured and intact bones were disconnected, assessed, submitted to X-ray imaging tests and stored into 5% formalin solution. After one week, the prepared femurs were taken to PUCPR's Laboratory of Destructive Assays, where the biomechanical study was carried out on a three-point flexion strength assay device (tension/compression) EMIC® brand, model DL-500, connected to a computer with built-in *software* M-test for mechanical assays. A load cell SV100 was employed with correction value equal to 11.05769. Fractured femurs (bone callus) and non-fractured femurs (normal femurs) were tested for both groups.

Statistical Methods

The calculation of the sample size was statistically made for a significance level of 5%, test power of 90% and minimum relevant difference to be detected equal to 15N of the strength observed on control group.
Initially, cipro and control groups were assessed for bone callus and normal femur strength. Then, bone callus was compared to normal femur in each group.
The study results were expressed as mean, standard deviation, median, minimum and maximum values.
For comparing groups for strength, the Student's t-test was employed for independent samples. For comparing bone callus to normal femurs in each group, the Student's t-test was employed for paired samples. The assessment of normality status of the variable was made with the Shapiro-Wilks' test. Values of $p<0.05$ indicated statistical significance.

RESULTS

Gross and X-ray evaluations of rats' fractured femurs after six weeks postoperatively showed fracture union in all animals.
The results of the biomechanical tests on each group are shown on Table 1.
Initially, for bone callus and normal femur, the null hypothesis of the mean strength is equal for both groups was tested versus the alternative hypothesis of different averages. Then, for each group, the null hypothesis of the mean value for a bone callus is equal to the mean value for normal femur was tested versus the alternative hypothesis of different averages (Table 2 and Figure 2).
For bone callus, in the comparison of cipro and control groups, no significant difference was found ($p=0.601$).
For normal femur, in the comparison of cipro and control groups, no significant difference was found ($p=0.054$).
For the difference between normal femur and bone callus, in the comparison of cipro and control groups, a significant difference was found ($p=0.007$). On table 2, for control group, the difference between normal femur and bone callus is smaller than the difference for cipro group.
For control group, in the comparison of bone callus to normal femur, a significant difference was found ($p<0.001$).
For cipro group, in the comparison of bone callus to normal femur, a significant difference was found ($p=0.002$).

Table 1 – *Results of the assessment of bone callus and normal femur strength to flexion forces, as Newtons, for cipro and control groups*

Cipro Group			
Rat	**Bone Callus**	**Rat**	**Normal Femur**
1	61.17	**1**	97.87
2	91.76	**2**	103.99
3	61.17	**3**	128.46
4	42.82	**4**	91.76
5	79.52	**5**	122.34
6	79.52	**6**	91.76
7	91.76	**7**	110.11
8	61.17	**8**	116.22
Control Group			
Rat	**Bone Callus**	**Rat**	**Normal Femur**
9	65.39	**9**	79.40
10	68.69	**10**	83.37
11	63.25	**11**	76.16
12	76.71	**12**	93.47
13	82.09	**13**	115.31
14	77.50	**14**	99.52
15	76.71	**15**	92.98
16	87.90	**16**	106.93

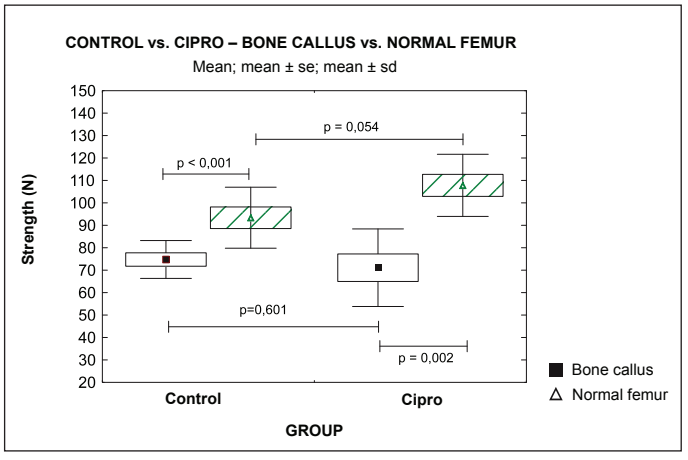

Figure 2 – *Comparison between mean and standard deviation values for bone callus strength and normal femurs on cipro and control groups.*

Table 2 – *Results (as Newtons) of mean, median, minimum, maximum, and standard deviation values for bone callus strength in mechanical assays carried out on cipro and control groups.*

Variable	Group	n	Mean	Median	Minimum	Maximum	Standard Deviation
Bone callus	Control	8	74.78	76.71	63.25	87.90	8.44
	Cipro	8	71.11	70.35	42.82	91.76	17.28
Normal femur	Control	8	93.39	93.23	76.16	115.31	13.60
	Cipro	8	107.81	107.05	91.76	128.46	13.85

DISCUSSION

Antibiotics are frequently used in the treatment of fracture complications, either for prophylaxis or as infection control therapy. One example is ciprofloxacin, which is being indicated for several cases.[6-13] This is a bactericide antibiotics belonging to 2nd generation quinolones class, of which mechanism of action is to inhibit bacterial DNA synthesis by its cleavage. Its action is more effective when its serum concentration is thirty times higher than the minimum inhibitory concentration. It is properly absorbed, both orally and endovenously, shows stronger tissue and cell penetration than plasma concentration, being mainly eliminated through the kidneys. It has an antimicrobial activity especially on Gram-negative, atypical pathogens and *Pseudomonas aeruginosa*.[5,17]

The side effects caused by quinolones occur mostly on gastrointestinal system and on the central nervous system, affecting 2-20% of the patients.[24] However, the musculoskeletal system has been frequently pointed out by literature as a target site for this antibiotics' side effects.[15-20] On tendons, degenerative changes occur, which can evolve to spontaneous ruptures. On cartilaginous tissues, especially on joint surfaces and growth plaques, chondrocyte changes are seen, leading to cell death and matrix degeneration, as well as changes on collagen production.[25] Therefore, the use of quinolones is contra-indicated for children and pregnant women.

Bone union is a continuous process that can be divided into three phases: inflammation, repair and remodeling. The inflammatory phase is characterized by fracture hematoma, providing a medium to which inflammatory cells migrate and release inflammatory mediators and growth factors. In the repair phase, which is marked by neovascularization, mesenchymal cells and fibroblasts migration, the soft callus is originated, formed by cartilaginous tissue[5]; it is suggested that at this moment ciprofloxacin would cause a negative influence. Bone tissue gradually replaces the cartilaginous tissue by endochondral ossification, generating a hard callus and stability of fracture fragments.[5,26] A rat's bone consists of a primary bone with a non-Harversian lamellar structure, distinguishing it from a human bone in this sense. However, the physiological basis of remodeling is similar to the one of humans, which indicates it for carrying out studies.[27]

Biomechanical studies on animals and human beings addressing strengths acting both on femur and on formed bone callus have been carried out.[28] The flexion strength, one of the destabilizing forces of fractures, was assessed on bone callus after the use of ciprofloxacin, with a three-point mechanical test.[29] This assay was chosen due to the reliability of the method, which obtains numeric values in Newtons and allows for comparisons and statistical studies with a safe specificity.[28]

The selection of rats as experimental model for biomechanical analysis is due to the fact that these are animals that are easy to handle and care during the research, and for having bone biomechanical characteristics that are similar to those of humans.[30]

The studies by Huddeleston and Tuncay demonstrated by histological, X-ray and biomechanical analysis that bone union was delayed with the use of therapeutic concentrations of ciprofloxacin.[31,32]

In the study by Huddeleston, the authors conducted histological, X-ray, biomechanical and ultra-structural analyses. They concluded that bone union was delayed with the use of therapeutic concentrations of ciprofloxacin.[31] However, fractures were produced on both femurs; they did not report if they were fixated or not; the

control group was administered with no substance; the antibiotics administration occurred only one week after the fracture; the rats were sacrificed at week 4; the biomechanical test was carried out by assessing strength and stiffness with torsion failure, and; gross evaluation of bones was not reported after bone disconnection.

Concerning the study by Tuncay, the authors have also conducted an experimental study on rats and histologically assessed bone union after the administration of four kinds of quinolones (ofloxacin, norfloxacin, pefloxacin and ciprofloxacin). They concluded that all those quinolones delayed bone callus formation in rats. Consistently to the first study, fractures were produced on both sides, quinolones were only administered after seven days of fracture, for three weeks, and the animals were sacrificed at week four.[32]

Differently from previous reports showing a negative influence of ciprofloxacin on bone union of rats[31,32], this experimental study did not confirm the hypothesis that ciprofloxacin does change bone callus strength. Concerning to those studies, some differences exist to our method. First, the time for ciprofloxacin introduction was established as the first day of study, because, as previously reported, as early as in the second week, cartilaginous cells can already be found. The contralateral femur was also assessed, which allowed for using the non-fractured bone as control on the same animal, this being important to rule out bone changes. We chose to fixate the fracture in order to avoid major deviations, differently from those studies where fractures were not fixated, potentially leading to inaccurate results. The intramedullary fixation technique employed in this study was shown to cause no change on bone union process.[22,23,28,33,34]

The biomechanical study was conducted with a three-point flexion test, which is safer and more reliable than the torsion test, because the latter is much influenced by fracture deviations. Also, it must be outlined that biomechanical tests were performed after 6 weeks postoperatively, because, in this phase, bone union is completed and the bone callus is shown to be stronger than after 4 weeks, when tests were conducted in the first study.[33]

Despite of the histological changes reports on cartilaginous cells during bone union process with the use of cirpofloxacin[31,32], the biomechanical analysis carried out in this study has shown that there was no statistical difference for bone callus strength compared to control group after six weeks of fracture. Further studies are warranted, including histopathological and immuno-histochemical analysis in order to prove a negative influence exerted by this drug on fractures. By doing so, we could possibly conclude if ciprofloxacin does or does not change bone union process.

CONCLUSION

There was no statistically significant difference on bone callus strength on standardized rats' femoral fractures with the use of ciprofloxacin when compared to control group.

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