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Effect of cefazolin and cefuroxime on fracture healing in rats

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KEYWORDS

Fracture healing
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ABSTRACT

Objective: To determine the effect of cefazolin and cefuroxime (the two most commonly used beta lactam antibiotics) at therapeutic doses in fracture healing in an animal model.
Material and Methods: 75 adult male wistar rats (3 months old) were selected. They were divided into three groups of 25 animals each (placebo, cefazolin and cefuroxime). A closed fracture was made in the middle third of the right femur of each rat and fixed with a Kirschner wire. Each group was treated with either saline (placebo), cefazolin or cefuroxime at therapeutic doses during fracture healing. Four weeks after fracture rats were killed and femora analysed through mechanical and histological testing.
Results: The group treated with cefuroxime showed a lower mechanical resistance of the healing callus and a lower histological grade than placebo. The group treated with cefazoline showed a similar mechanical resistance and histological grade of callus to placebo.
Conclusion: Cefuroxime appear to disturb fracture healing more than cefazolin or placebo in Wistar rats. If those results are similar to human, the use of cefuroxime during fracture healing should be avoided in the clinical setting, if possible.

Introduction

Surgical wound infection is one of the most common complications for the orthopedic surgeon. It is estimated that in America there are between 500,000 and 780,000 per year [1,2]. Beta lactam antibiotics are currently the most widely used antibiotics for treatment of infection and prophylaxis of infection in trauma and orthopedic surgery. The Beta lactam antibiotics have a low incidence of adverse effects and are generally considered very safe. Nevertheless, there are no studies "in vivo" that confirm this security. To date, there are only studies "in vitro" [3] which seem to confirm that cefazolin is secure, but there are no studies for other beta-lactams antibiotics.

The objective of this study was to determine the effect of cefazolin and cefuroxime (the two most commonly used beta lactam antibiotics) at therapeutic doses in fracture healing in an animal model.

Materials and Methods

The study followed the ethical requirements of the American Physiology Academy [4] and the standards of Spanish laws (law 1201/2005 of 10 October, on protection of animals used for experimental and other scientific purposes). The study was accepted and supervised by the Committee on Research and Animal Care of our centre (University of Jaen).

75 male adults wistar rats (3 months old) were used. The rats were housed at 22°C with light/ dark cycles of 12 hours. They were given unrestricted access to water and a standard diet. The animals were divided randomly into three groups of 25 rats each. The control group received 1 ml of saline 0.9% in a daily subcutaneous injection. The cefazolin group received 50 mg/Kg in a daily subcutaneous injection. The cefuroxime group received 100 mg/Kg in a daily subcutaneous injection. All injections were given till sacrifice (four weeks).

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An experimental unilateral fracture was made in the middle third of right femur of all rats. An apparatus constructed using the principles established by Bonnarens and Einhorn [5] and previously employed by our group was used [6,7]. The rats were anaesthetised with an intraperitoneal injection of a solution composed of 25 mg/ml of ketamine chlorhydrate (Ketolar®, Parke Davis Laboratories, Barcelona, Spain), 0.1 mg/ml atropine (Atropina Braun®, Braun Laboratories, Barcelona, Spain), and 0.4 mg/ml diazepam (Valium®, Roche Farma Laboratories, Madrid, Spain), administered at 0.25 ml/100 mg of the animal's weight intraperitoneally. To minimise post-operative pain after the fracture, all the rats received 0.10 mg/100 mg weight of buprenorphine 0.3 mg/ml (Buprex, Shering-Plough Laboratories, Madrid, Spain) by subcutaneous injection. Before the fracture was made an intramedullary Kirschner wire 1 mm in diameter was inserted into the femur (pre-nailing). For this prenailing, an anterior approach was made to the knee, lateralising the patella and exposing the two femoral condyles. The wire was inserted through the intercondylar line till the major trochanter without drilling (manually) and cut under the cartilaginous surface. To prevent invasion of the wire into the articulation, a small bent was made in the wire in the major trochanter. The incision was sutured with 3/0 silk and 3/0 absorbable sutures. The closed fracture was made immediately afterwards (by dropping a weight into the prenailed femur) and confirmed by a dental X-ray machine (Figure 1) (dental Trophy 70 CCX, Iris Paris, France: exposure 0.10 s, 70 Kv, 80 mA). When no fracture resulted, the process was repeated, and when the fracture was not transverse the animal was discarded from the experiment and a new rat was added to the group. After the procedure, the rats were placed in their cages, and the affected limbs were allowed to bear weight freely. Four weeks after the fracture, the rats were killed [5], the fractured femur was removed, cleaned of soft tissue, and the intramedullary wire was taken out. None of the wires presented an angulation of more than 5° nor were broken during careful wire extraction. The bones were wrapped in gauze soaked in saline 0.9% and preserved at −80°C until tested.

20 randomly elected healed femora in each group were submitted to a low-speed torsion test (10°/min) using a SERVOSIS machine, model MT-10 + PCD-2k; Servosis Enterprise Pinto, Madrid, Spain), with a sensor capable of detecting torque pressures of less than 10 miliNm. Adhesive cement (Osteopak®, Biomet-Merck, Sjöbo, Sweden) was applied to the ends of the bones to fit them into the heads of the machine and avoid slippage. Each test provided the following information: XY curve (torque on the bone), maximum torque applied before breakage (maximum torque), angle deformation by maximum torque, sample rigidity calculated by the slope of the XY curve (between 10% and 50%), and energy absorbed by the sample, calculated as the area below the XY curve.

Fig. 1. Radiograph showing a typical transverse fracture of the femur with a Kirschner wire inserted.

The histological test was performed in 5 specimens for each group, chosen randomly. After thawing at room temperature, the bone was fixed with buffered formol (pH 7.4) for 24 hours and decalcified with nitric acid-formalin for 24 hours. The tissue was then embedded in paraffin and sliced using a high-precision rotation Minot microtome (Leica Microsistemas SA, Barcelona, Spain), providing a series of single, thin sections (3 μm; in the sagittal plane). The sections were placed over the microscope slides, removing the paraffin and hydrating them for staining with haematoxylin and eosin. The central section of each piece was used. The samples were photographed with a digital camera (Nikon Coolpix 4500, Barcelona, Spain) equipped with an optical microscope and a specific visor to more clearly delineate the areas of the different zones in each section. The different areas of the tissues that formed the fracture callus (bone, cartilage, fibre) were manually measured using the University of Texas Health Science Center at San Antonio image tool program. An area of every tissue (bone, cartilage, fibrous tissue) was in the section. Next, a single blinded observer (MN) examined the data and the stained slides. The fracture calluses were graded according to the amount of fibrous tissue, cartilage, woven bone and mature bone [8,9]. Grade 1 indicated fibrous tissue, grade 2 predominantly fibrous tissue with some cartilage, grade 3 equal amounts of fibrous tissue and cartilage, grade 4 all cartilage, grade 5 predominantly cartilage with some woven bone, grade 6 equal amounts of cartilage and woven bone; grade 7 predominantly woven bone with some cartilage, grade 8 entirely woven bone, grade 9 woven bone and some mature bone, and grade 10 lamellar (mature) bone.

Statistical analysis was performed using R and R-commander software (http://www.R-project.org). Normality of data was confirmed by the Kolmogorov-Smirnov test. So, the information provided by mechanical and histological tests (cited before) were compared between groups with non-parametric tests (Kruskal Wallis). The significance level was set at $p = 0.05$ with the 95% confidence interval (CI).

Results

In five rats the fracture obtained was not transverse, so they were replaced by another five. No rat died during fracture healing, so 60 rats were available for mechanical testing and 15 for histological testing at sacrifice.

Results of the mechanical testing are shown in Table 1, Figure 2. The mechanical strength (maximum torque) of the group treated with Cefazolin (0.26) is very similar to the placebo group (0.25) (Figure 2), N. S. The group treated with cefuroxime had a clear lower mechanical strength (0.18), (Figure 2), being the differences statistically significant (Figure 2) ($p = 0.011$ to placebo).

Results of the histological testing is shown in Table 2, Figure 3. The histologic grade for the group treated with cefuroxime was significantly lower ($p = 0.003$) than placebo.

Discussion

The results of our study indicate that the mechanical resistance of the fracture callus and the histological grade were significantly lower in the wistar rats treated with cefuroxime during fracture healing.