Fracture in Irradiated Rat Femur: A Description of an Experimental Model and Evaluation of its Effectiveness

Fratura em fêmur irradiado de rato: Descrição de um modelo experimental e avaliação de sua eficácia

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Abstract

Objective We aim to describe an experimental model for studying femoral fractures in rats after exposure to ionizing radiation, demonstrating a way to apply a substance for analysis, the method for patterning fracture and irradiation, and how to evaluate its effectiveness based on radiographic studies.

Methods We used 24 rats divided into 2 groups of 12 animals each. The STUDY group was exposed to ionizing radiation and treated with saline solution, and the CONTROL group was not exposed to radiation and was treated with saline solution. All animals were subjected to standardized fracture of the right femur that was fixed with intramedullary wire. The efficiency of the bone union was assessed by radiographic exam.

Results Fracture healing was more efficient in bones not exposed to ionizing radiation ($p = 0.012$). All fractures met the criteria of being simple, diaphyseal, transverse or short oblique.

Conclusion The experimental model presented is an efficient alternative for the study of fractures in irradiated bones in rats.

Keywords
- fracture healing
- radiation effects
- radiotherapy
- fractures, spontaneous

Resumo

Objetivo Nosso objetivo é descrever um modelo experimental para estudo de fraturas de fêmur em ratos após exposição a radiação ionizante, demonstrando uma forma de aplicação de uma substância para análise, o método de padronização de fratura e irradiação e a forma de avaliação de sua eficácia com base em estudos radiográficos.

Métodos Utilizamos 24 ratos divididos em dois grupos de 12 animais cada. O grupo ESTUDO foi exposto à radiação ionizante e tratado com soro fisiológico, enquanto o grupo CONTROLE não foi exposto à radiação e foi tratado com soro fisiológico. Todos os animais foram submetidos à fratura padronizada do fêmur direito e sua fixação

Palavras-chave
- consolidação da fratura
- efeitos da radiação
- fraturas espontâneas
- radioterapia

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com fio intramedular. A eficácia da consolidação óssea foi determinada por exame radiográfico.

Resultados A cicatrização de fraturas foi mais eficiente em ossos não expostos à radiação ionizante ($p = 0{,}012$). Todas as fraturas atenderam aos critérios de serem simples, diafisárias, transversas ou oblíquas curtas.

Conclusão O modelo experimental apresentado é uma boa alternativa para o estudo de fraturas em ossos irradiados em ratos.

Introduction

Radiotherapy is an important therapeutic tool, especially for local control of tumors. It is used in the treatment of several oncological pathologies, contributing to their cure. However, its use is not free of complications. Healthy tissues exposed to its field of action undergo changes in their physiology, which can lead to complications that are of difficult resolution.[1]

Specifically in skeletal tissue, radiotherapy can lead to necrosis, actinic osteitis, osteomyelitis, and pathological fractures that can progress to nonunion. Although these complications are frequently described, little is known about their etiology and how to solve these problems.[1]

There are some studies that address fractures in irradiated bones.[2,3] However, they do not describe the method used to perform the fractures and irradiation. An experimental model must be reproducible and patternable as much as possible the variables to be studied.

We aim to describe an experimental model for studying femoral fractures in rats after exposure to ionizing radiation, demonstrating a way to apply a substance for analysis, the method for patterning fracture and irradiation, and how to evaluate its effectiveness based on radiographic studies.

Method

The present study was initiated after authorization from the Animal Use Ethics Committee (CEUA) under opinion number 40/2014.

In our study, we used 24 female Wistar rats, 3 months old, which were divided into 2 groups of 12 animals. The animals in the STUDY group underwent a radiotherapy session and received 0.3ml of saline solution at the fracture site. The CONTROL group was not submitted to radiotherapy, and also received 0.3ml of saline solution in the fracture focus.

Any procedure with potential physical or emotional suffering for the animals was performed under anesthesia. For this, they received an intraperitoneal dose of Ketamine Hydrochloride at a dose of 60 mg/kg and Xyliazine Hydrochloride at a dose of 15 mg/kg.

Irradiation

Irradiation was performed at the Gamma Irradiation Laboratory of the Nuclear Technology Development Center / National Nuclear Energy Commission (LIG–CDTN/CNEN, in the Portuguese acronym). The laboratory uses a category II multipurpose panoramic radiator, manufactured by MDS Nordion (Canada), Model/serial number IR-214 and type GB-127, equipped with a Cobalt-60 (60Co) dry-storage source with maximum activity of 2,200 TeraBequerel (TBq) or 60,000 Curio (Ci) (►**Fig. 1**)

The focus of the radioactive beam was directed to the area of the right femur. The irradiation dose, with exposure of the area to be studied to the 60Co source in the dosage, was 18 Gy (2), in a single dose at a depth of 1.5 cm. The animals were placed 30 cm away from the radioactive source and exposed for 77 seconds.

As it is a panoramic radiator, the radiation was directed to the rat's right femur through lead shields developed specifically for this purpose. The lead shields have holes of five centimeters in diameter, which directed the radiation exclusively to the right femur of the animals (►**Fig. 1**).

Femoral Shaft Fracture

Two weeks after irradiation, the animals underwent a diaphyseal fracture of the right femur. To standardize the type of fracture, we used a guillotine developed specifically for this purpose, which produces a transverse or short oblique, diaphyseal fracture, similar in all animals (►**Fig. 2**).

The guillotine is composed of a stainless steel weight of 1.3 kg, which runs along a 50 cm rail, accelerated by the force of gravity. The impact is dampened by a 6.0-cm high spring, also in stainless steel. The impact is transmitted to the femur of the animals by the guillotine blade, which has a blunt tip. The femur is positioned on supports, also made of steel, with a distance between them of 2.5 cm.

Femoral Fracture Fixation

The animals were anesthetized and underwent surgical reduction of the femoral diaphyseal fracture. After anesthesia, surgical site trichotomy and skin asepsis were performed with an alcoholic solution of 5% Chlorhexidine Digluconate. Longitudinal surgical access was performed on the lateral aspect of the thigh, opening the skin and the musculature of the thigh of the animal. With the opening of the musculature, the fracture was exposed.

The fracture was fixed with a Kirschner wire measuring one millimeter in diameter and closed by suturing in layers. Initially, the muscular plane was closed and then the skin suture. After closure, infiltration was performed with 0.3 ml of 0.9% saline solution. The saline solution can be substituted

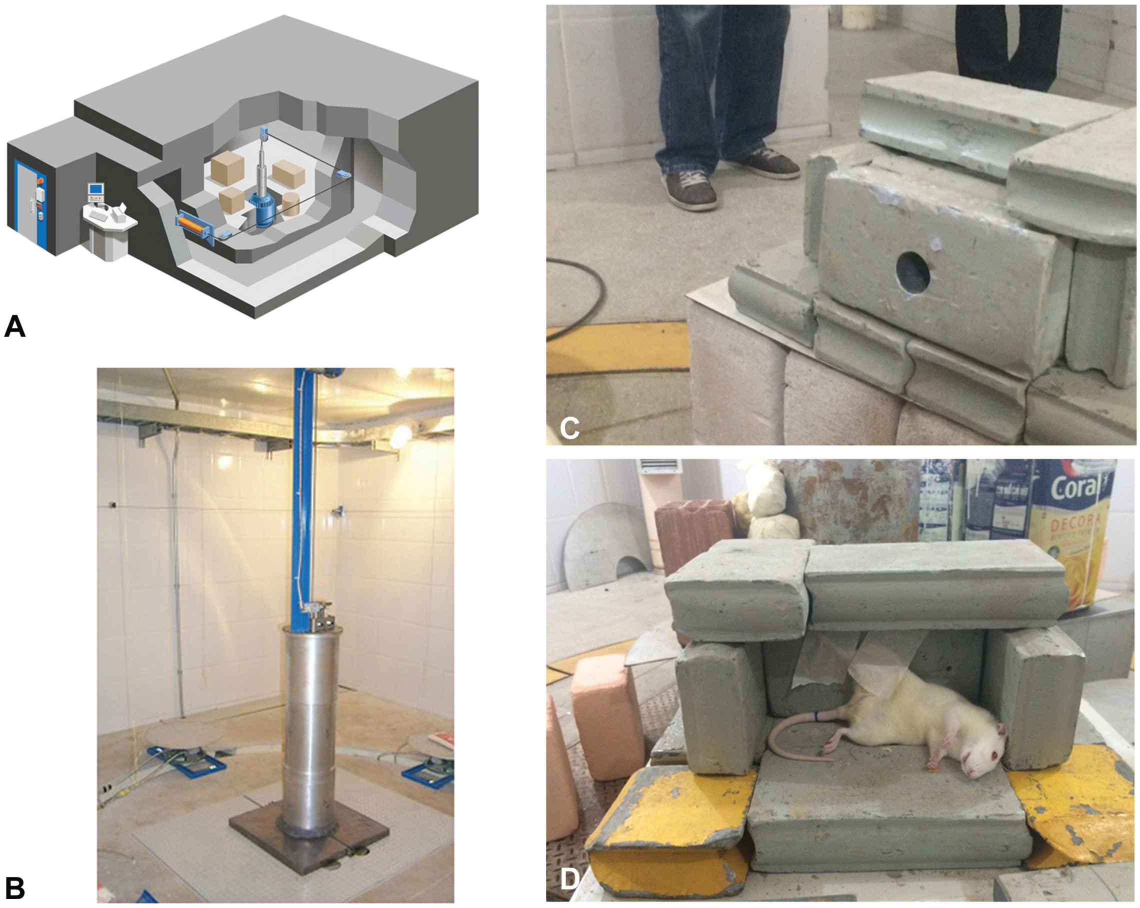


Fig. 1 Method for irradiation. (A) 3D plan of the collimator room; (B) Cobalt 60 Source; (C) Lead blocks for directing the radioactive beam; (D) Anesthetized animal, positioned for procedure.

Fig. 2 Guillotine developed for femoral diaphyseal fractures. On the left, the entire structure can be seen. In the center, a detail of the weight at the end of the track. On the right, the tip of the guillotine with a simulation of fracture production.

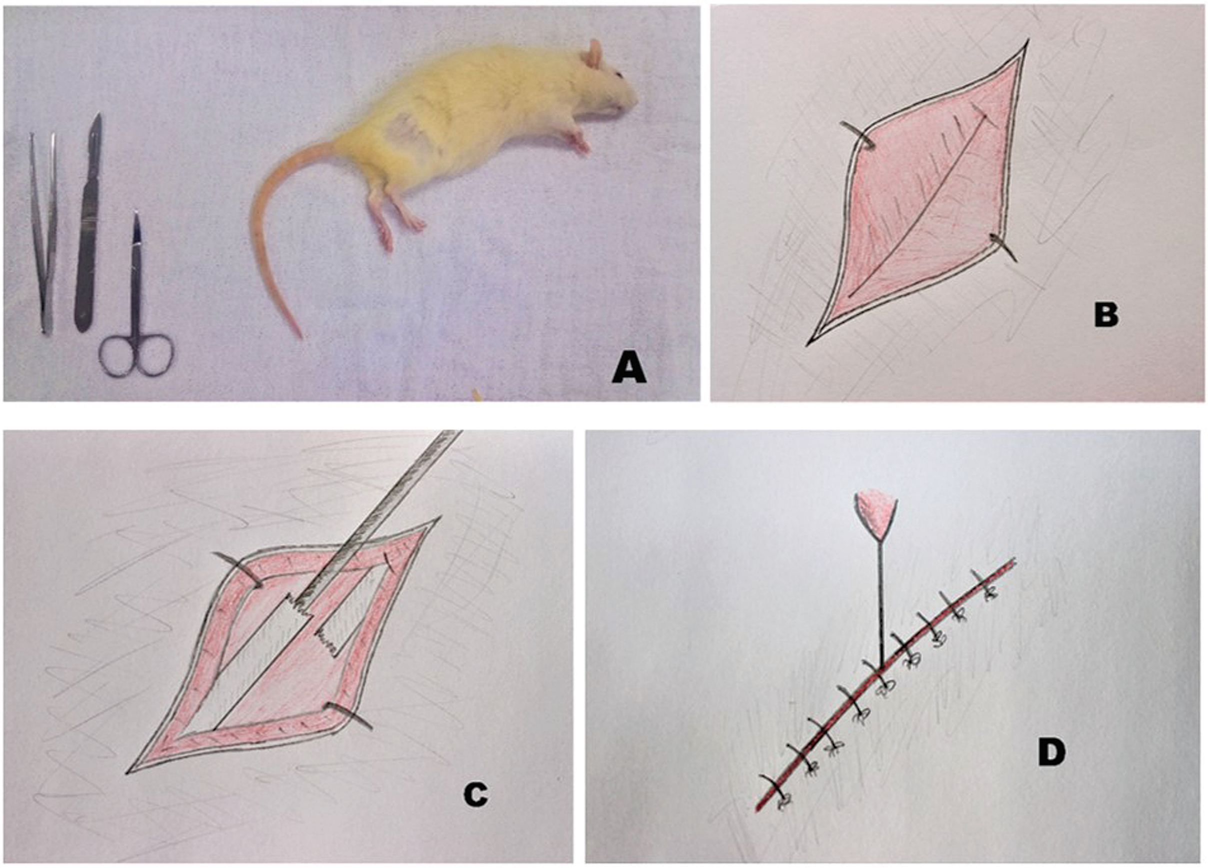


Fig. 3 Example of a surgical procedure performed on animals. (A) Anesthetized animal, after trichotomy, prepared for surgical procedure. (B) Schematic representation of surgical access in cutaneous and muscular planes. (C) Schematic representation with an open fracture focus, being fixed with a smooth intramedullary wire. (D) Schematic representation of wound after closure receiving saline treatment.

for any substance to be studied. ►**Fig. 3** demonstrates the surgical procedure schematically.

Radiographic Study

Three weeks after the fracture, the animals were euthanized and the right femurs were removed. Imaging exams were performed using digital radiographs. The removed right femur was placed in a 10% buffered formalin solution for 5 days, being radiographed after this time.

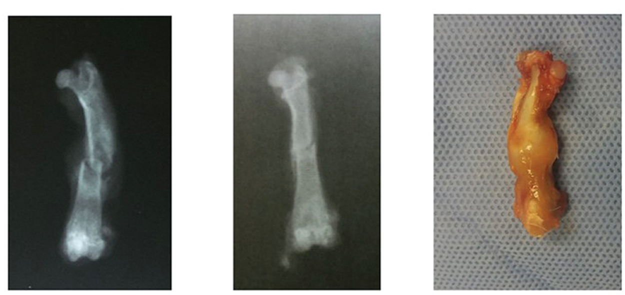
Digital radiographs were taken using a radiographic device (Raiotécnica, model 30 × 60, series 2577) coupled to an AGFA processor (model CR30X). Images were printed by an OKI printer (model C911 MDI). The radiographs were taken at a dose of 40 kilovolts (kV), 2mAs at 1 m away from the X-ray source, in an air-conditioned environment at 20°C.

The radiographs were evaluated for the presence of the fracture, the degree of fracture comminution, the location of the fracture, and its evolution to bone union. Comminuted or segmental fractures would be excluded, and only simple, transverse, or short oblique fractures would be included in the study.

To measure the degree of consolidation of fractures on radiography, we used the Lane–Sandhu classification[4,5]: (0) complete absence of consolidation; (1) initial callus formation; (2) initial ossification; (3) initial disappearance of the fracture line; (4) complete consolidation of the fracture. ►**Fig. 4** discriminates the classification used.

Lane–Sandhu Scoring System		
Type	**Description**	**Consolidation**
0	Complete absence of healing	Insufficient
1	Initial callus formation	Insufficient
2	Initial ossification	Sufficient
3	Initial disappearance of the fracture line	Sufficient
4	Completer fracture healing	Sufficient

A



B

Fig. 4 Classificação radiográfica para consolidação de fraturas em ratos (A) Exemplo radiográfico da consolidação (B) À esquerda, a radiografia do animal número 20, classificado como tipo "1," ou seja, consolidação Insuficiente. Ao centro, a radiografia do animal número 4, classificado como tipo "3," ou seja, consolidação Suficiente. A direita a peça anatômica no momento da radiografia.

For statistical purposes, types 0 and 1 were grouped under "Insufficient Consolidation" and types 2, 3 and 4 under "Sufficient Consolidation."

Statistical Analysis

Data comparison was performed using IBM SPSS Statistics for Windows version 22 (IBM Corp., Armonk, NY, USA). Data were compared using the chi-squared test with Fisher correction. We established a test power of 80%, with a 5% confidence interval (CI). We consider differences for $p < 0.05$.

To assess the effectiveness of the method for studying fractures in irradiated bone, we compared the union between the groups. A group with a higher concentration of samples with inefficient consolidation indicates that the method is effective. To evaluate the fracture standardization method, we will evaluate the frequency with which we obtained comminuted fractures, as we want short transverse or oblique fractures.

Results

Fracture healing, assessed by radiographic examinations, was more efficient in bones not exposed to ionizing radiation ($p = 0.012$). In the STUDY group, of the 12 animals, 10 had insufficient consolidation. In the CONTROL group, 3 of the 12 animals had insufficient consolidation (►**Table 1**, ►**Fig. 5**).

All fractures met the criteria of being simple, diaphyseal, transverse or short oblique.

Discussion

Fractures are frequent complications of radiotherapy treatments. However, this is an understudied subject. The creation of a reproducible experimental model has great potential to contribute to filling this gap in the literature. The development of an accessible method, standardizing variables such as the type of fracture, dose and direction of the radiation beam, sample processing, and its availability for imaging or histology studies are important to study this problem.

The ideal animal for experimental studies on long bone fractures is the rat (Rattus norvegicus).[6] It is an animal capable of reproducing the healing process of human bone tissue, with long bones of sufficient size to carry out studies based on a standardized experimental model. In our study, we used female Wistar animals, 3 months old. These have long bones of sufficient size to allow proper manipulation in the laboratory.

Table 1 Results and statistical analysis of data regarding radiographic classification

Radiographic Evaluation	Study	Control	*p-value*
Insufficient	10 (83.3%)	03 (25.0%)	0.012
Sufficient	02 (16.7%)	09 (75.0%)	

Fig. 5 Examples of radiographs of animals in each group. On the left, we have a typical radiograph of animals in the STUDY group. It is classified as type "0" – Insufficient. On the right, is an example of a typical image from the CONTROL group, classified as type "3" – Sufficient.

In our study, we observed differences in fracture healing when comparing the groups. Animals in the STUDY group had less efficient consolidation than those in the CONTROL group. As the only difference between them is the femoral exposure to ionizing radiation, this result suggests that the method is efficient for the experimental reproduction of the fracture that occurs in irradiated bone.

There are some alternatives to tissue exposure to ionizing radiation. Most publications use a specific device for hospital radiotherapy to perform it. In our model, we used a panoramic radiator with a Co60 source, with the radioactive beam directing through lead shields. In this way, we use the same radioactive isotope that most radiotherapy devices use. The effectiveness of this method was proven by the relevant difference in the healing of fractures in the STUDY and CONTROL groups, assessed in radiographic examinations.

Our results also support the effectiveness of the parameters used in the study. We highlight the exposure in a single dose and the radiation dose used. We used a dose of 18 Gy, in a single dose, at a depth of 1.5 cm. This was the same used by Nicholls et al.,[2] also with proven efficiency.

An important limitation of this experimental model is its difference in relation to the pathology that occurs in humans. Here, we present a way to study the healing of a fracture that occurs in a previously irradiated bone, which is different from a pathological fracture after radiotherapy. In humans, the fracture occurs due to mechanical failure, probably related to factors such as tissue necrosis and local inflammatory response, occurring after a long period of time.[1,7,8]

The methodology applied for fracture simulation is an important point of the present study. Other published models do not describe how the standardization of fractures was performed.[2,9,10] It seems relevant to us that the fractures have the same pattern. We achieved this standardization using a guillotine designed specifically for this purpose. All fractures obtained were transverse diaphyseal or short oblique, a fact that demonstrates the efficient standardization of the fracture type. Therefore, we recommend using this method for standardizing the femoral fracture.

We performed surgical treatment of fractures by open reduction and intramedullary fixation with a 1.0mm thick Kirschner wire. In our experience, we observed that retrograde intramedullary fixation is efficient for fracture stabilization, promoting its consolidation. We used a time interval of 3 weeks between the fracture and the death of the animals. This time is enough for an initial consolidation of the fracture to occur. We believe that this is the optimal period to evaluate the results in rats.

After closing the surgical wound, we infiltrated 0.3 ml of saline solution into the fracture site. In studies designed to assess the effectiveness of any compound, they can be inserted into the fracture focus in the same way.

In the present study, we chose to assess the outcome based on digital radiographic exams. Other ways of evaluating the obtained samples are described.[3,11,12] The sample obtained can be decalcified and placed in formalin blocks for histological study, if this is the chosen option.

There are some options for the experimental study of fractures in irradiated bones. The use of a cobalt-60 source directed by lead shields to the point to be studied, with standardization of radiation dose, the method of producing the fracture, its fixation, and the way to administer the material to be studied are central points for the reduction of confounding factors. Here, we present an efficient and reproducible method for this purpose.

Conclusions

1. The irradiation method presented is an efficient alternative for the study of fractures in irradiated bones in rats.
2. The fracture standardization method proved to be efficient, producing diaphyseal, transverse or oblique fractures.

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Conflict of Interests
The authors have no conflict of interests to declare.

Home Institution / Laboratory
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