Experimental model of cutaneous radiation injury in rabbits[1]

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ABSTRACT

PURPOSE: To describe an experimental model of cutaneous radiation injury in rabbits.

METHODS: On this study eight six-month-old New Zealand male rabbits, with an average weight of 2.5kg were used. They were distributed in four groups (n=2 per group). The control group did not receive radiotherapy and the others received one radiotherapy session of 2000, 3000 and 4500 cGy, respectively. Photographic analysis and histopathological evaluation of the irradiated areas were carried out.

RESULTS: After 30 days, the animals from the control group had all their hair grown. In spite of that, the animals from group 2000 cGy had a 60-day alopecia and from group 3000 cGy, a 90-day alopecia. After the 30th day, the 3000cGy group demonstrated 90-day cutaneous radiation injuries, graded 3 and 4. One of the animals from group 4500 cGy died on the 7th day with visceral necrosis. The other from the same group had total skin necrosis. A progressive reduction of glands and blood vessels count and an increase on collagen deposition was observed.

CONCLUSION: The proposed experimental model is reproductable. This study suggests that the dosage 4500cGy is excessive and the 3000 cGy is the most effective for this experimental model of cutaneous radiation injury in rabbits.

Key words: Radiation Injuries. Radiotherapy Dosage. Models, Animal. Rabbits.

Introduction

Around 60% of cancer patients receive radiotherapy during their treatment[1,2]. The most common complication of radiotherapy is the cutaneous radiation injury. The lack of experimental models of cutaneous radiation injury is one of the research challenges[3]. There are some experimental models described for small animals (murine, rats and mice)[3]. In spite of that, there are no reproductable models in literature for medium size animals.

The rabbits, medium size animals, because of their immunological similarity to humans are the most frequently used for cutaneous tests such as the Draize test[4]. Their antibody development is considered resistant to radiation, differently than rats, mice and murines that aren't capable of presenting a similar immune response[5].

A experimental model is a materialization of a reality and it is as similar as possible to the desired study[6-8]. Therefore, the development of an efficacy treatment for radiodermitis requires a reproductable experimental model with animals immunologically similar to humans, such as rabbits.

Methods

All the procedures followed the protocol approved at the Ethical Committee: UNIFESP/EPM-0264/12.

Eight six-month-old New Zealand male rabbits, with an average weight of 2.5kg, were used. They were distributed in four groups (n=2 per group). The control group did not receive radiotherapy and the others received one radiotherapy session of 2000, 3000 and 4500 cGy.

The animals were anesthetized with Ketamine intramuscular (gluteal region) and the dose was 0.5 ml/kg.

The dorsal skin was epilated (10x10 cm) with an OSTER A5 hair removal –blade size 40-10mm. The borders of epilation were standardized (from the occipital region, a 10x10cm square was drawn, having the vertebral column as the mid line).

The dorsal skin was elongated and fixed between two wood sticks. The sticks were fixed with an elastic string, giving five laps on each side (Figure 1).

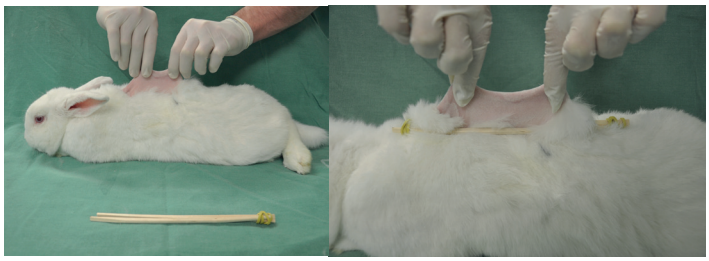

FIGURE 1 - Standardized epilation and fixation. Use of two wood sticks and elastic string with five laps on each side to elongate the dorsal skin.

The radiotherapy was performed in pairs of animals. After anesthesia, with the elongated dorsal skin, they were positioned on lateral decubitus and fixed together with three nylon 4.0 sutures, with a 10cm distance between them. They were then fixed to the table with adhesive tape (Figure 2).

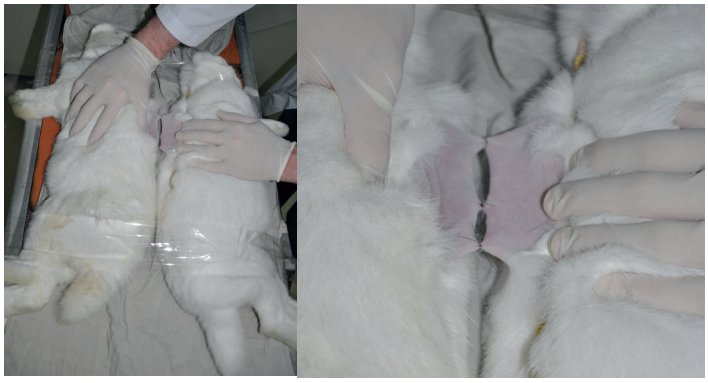

FIGURE 2 - Position: lateral decubitus of the rabbits fixed to the table with adhesive tape: 10 cm distance between them, fixed with nylon sutures.

The radiation machine used was ALCYON II for Telecobaltotheraphy. The distance applied was 70cm and the Bolus used was 0.5cm. The radiation field was 5x10cm (width x length) and the dosages were 2000cGY, 3000cGY and 4500cGy.

The procedures were performed by the same researcher, assisted by a veterinarian and a radiotherapist at Arnaldo's Institute at Faculty of Medical Sciences of Santa Casa of Sao Paulo.

Serial photography of the dorsal skin surface was performed to evaluate for macroscopic evidence of radiation injury on days 15, 30, 60 and 90 post radiotherapy. The images were analyzed and classified by the same blinded radiotherapist.

The radiodermitis is classified based on skin appearance[9-12]. Cox *et al*.[13] published the Acute Radiation Morbidity Scoring Criteria to classify the effects of radiotherapy, grading 0 (no reaction), 1 (light eritema, dry desquamation, epilation), 2 (moderate eritema, exudative dermatitis and moderate edema), 3 (exudative dermatitis, intense edema) and 4 (ulceration, necrosis).

The camera used was a NIKON D90 on macro and

automatic feature, with a 30cm distance from the animal.

The dorsal skin was divided in four quadrants and skin biopsy specimens were taken from the zone of irradiation on day 15, 30, 60 and 90 after external beam radiation therapy and fixed in 10% formalin (n=2 per group). Specimens were paraffin-embedded and sectioned for hematoxylin and eosin staining. The histopathological analysis was performed by the same blinded pathologist. Fibrosis was assessed by picrosirius red stain (collagen content). The 'point-counting' technique proposed by Gundersen *et al*.[14] was used to quantify the number of blood vessels, sweat glands, sebaceous glands, hair follicles and collagen using a reticulum of 100 points and 50 lines. Five random and non-coinciding fields were examined, totalling 500 points per slide at a magnification of x200. Points that were not incident upon solid areas were not considered. The values were expressed in percentage of positive points, in the total of points in solid areas, according to the following formula: P points= (Pi x100)/P parenchyma; where P points is the corrected percentage of marked points, Pi is the number of points that are incident upon the positivity of the items measured and P parenchyma is the total number of points that are incident upon the solid area.

Results

After 30 days of radiotherapy, the control group animals had all their hair grown. In spite of that, the animals from group 2000 cGy had a 60-day alopecia and from group 3000 cGy, a 90-day alopecia. The irradiated area from group 3000cGy demonstrated radiodermitis graded 3 (exudative dermatitis, edema) and 4 (ulceration – Figure 3) at 60 day and at 30 day, respectively. One of the animals from group 4500 cGy died on the 7th day after radiotherapy, because of visceral necrosis. The other animal from the same group had total skin necrosis after 30 days of radiotherapy and was sacrificed.

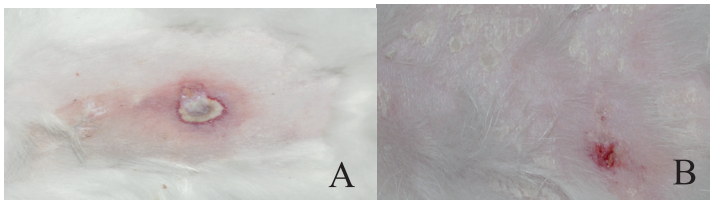

FIGURE 3 - Group 3000 cGy 30 days (**A**) and 60 days (**B**) of radiotherapy. Observe lesion graded 4 with ulceration and graded 3 with exudative dermatitis, intense edema.

The epilated area was classified based on the Oncology Radiotherapy Group Scale (grade 0-4: Figure 4)[15].

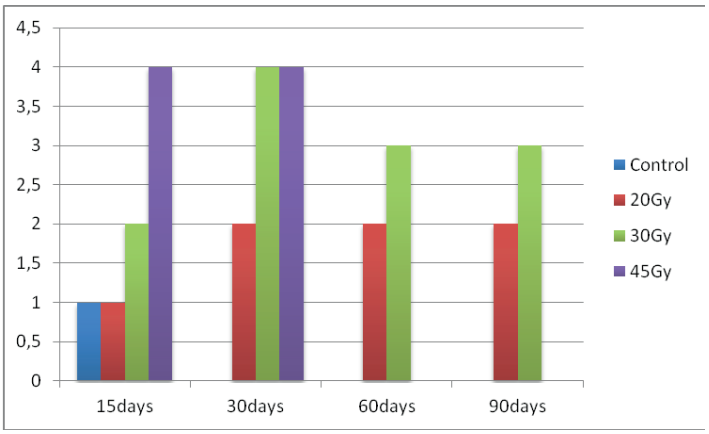

FIGURE 4 - Classification of radiodermitis[15]. Graded 0-4. Group 3000cGy demonstrated stable classification graded 3-4.

Rabbits exposed to radiation doses greater than 2000 cGy developed progressive skin thickening. The skin was thickened and inelastic with areas of non-healing ulceration (Figure 3). The histopathological analysis demonstrated thickening of the dermis, with exuberant and progressive collagen deposition over time (Figure 5).

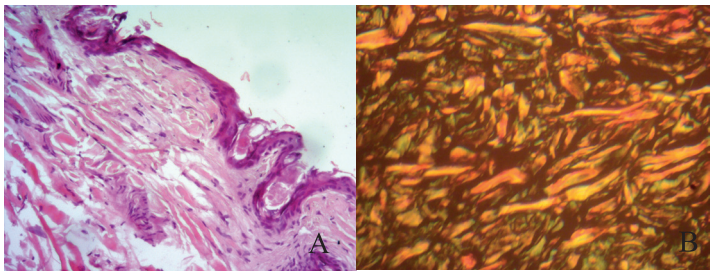

FIGURE 5 - Group 3000 cGy after 60 days of radiotherapy (Hematoxilin-eosin-HE-**A**)/(Picrosirius Red-**B**). A decrease on number of blood vessels, sweat glands, sebaceous glands, hair follicles is observed (**A**) and an increased picrosirius red staining, a marker of fibrosis (**B**).

A progressive reduction of glands and blood vessels count and an increase on collagen deposition was observed (Table 1).

TABLE 1 - Analysis of blood vessels, glands (sebaceous, sweat glands and hair follicles) and collagen. P POINT= Pi x100/ P parenchyma (average results).

	15 days			30 days			60 days			90 days		
	Blood vessels	Glands	Collagen	Blood vessels	Glands	Collagen	Blood vessels	Glands	Collagen	Blood vessels	Glands	Collagen
Control	2.1	12.4	10.6	2.4	14.9	11.2	2.3	21.7	11	2.4	19.2	11.2
20 Gy	1.9	11.2	16.2	1.6	7.4	16.4	1.8	9.1	18.2	1.6	8.4	18.4
30Gy	1.9	10.8	16.2	1.4	6.4	18.2	1.2	5.3	18.4	1.2	5.1	18.6
45Gy	1.8	10.7	16.4	-	-	-	-	-	-	-	-	-

Discussion

Although new techniques and sources of radiotherapy have been developed, the cutaneous tolerance is frequently a limiting factor during the radiotherapy treatment[3]. The skin exposition to ionized radiation causes acute and chronic effects. During the acute phase, the inflammatory cells, such as macrophages, are triggered and eritema, exudative dermatitis and ulceration may appear. The chronic phase is known as a proliferative phase, with an increase of myofibroblasts. The combination of perivascular fibrosis is due to myofibroblasts activation and its effects to the endotelial cells may cause hypovascularization and chronic fibrosis[16-20]. This fibrosis is characterized by excessive collagen deposition, with stromal encasement of blood vessels, loss of hair follicles, and dermal thickening[21,22]. The progressive collagen deposition, skin thickening and alopecia were features also found in our study and mimic a human radiation injury.

The dosages used on this study were similar to the murine model described by Thanik *et al*.[3] On their study, from the 25 treated animals, three in the highest dose groups (≥ 4500 cGy) had inadequate lead shielding, resulting in tail exposure with subsequent necrosis and infection, and died within the first week. On our study, both animals from group 4500 cGy did not have a desired outcome.

The alopecia was noticed on groups 2000 cGy and 3000 cGy. The alopecia noticed after radiotherapy is caused by the damage to follicular cells associated with fibrosis[23]. After radiotherapy, the tissue may become thick, inelastic, hyperchromic and hairless. Effects are thought to be dose, technique, and location dependent[24]. Our results demonstrate this dose dependency since the alopecia was more persistent on group 3000cGy.

This model accurately reproduces the changes seen in humans subjects from radiotherapy-induced injury. It was possible to observe and classify the irradiated area by photographic analysis. This classification was not previously described for an experimental study. In spite of that, it could be used, since the dosages were comparable to dose rates to human treatment protocols and the macroscopic changes were similar[25].

The establishment of a reliable rabbit model to mimic the radiation-injury and study its pathogenesis and potential treatment is essential. Although, studies on radiation injury in rabbits have been described previously, there is no established rabbit model for creating a reproducible cutaneous injury pattern. Using a rabbit model offers many advantages in the study of radiation–induced cutaneous injury. It is ease handling, low cost and the rabbit, has an adequate donor site of fatty tissue, the dorsum, differently than mice[26].

Conclusion

The proposed experimental model is reproductable. This study suggests that the dosage 4500cGy is excessive and the 3000 cGy is the most effective for this experimental model of cutaneous radiation injury in rabbits. Therefore, a study with a greater sample is required to determine the most satisfactory dosage for this model.

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