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COMPARISION OF DOSIMETRIC MAPPING OF RADIATION INDUCED SKIN ULCER ANIMAL MODEL IN NUD MICE AND WISTAR RAT

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ABSTRACT

Skin ulcer (SU) is the damage caused to the skin by ionizing radiation, becoming evident at the end or after the conclusion of radiotherapeutic treatments. Technological advances have enabled dose increases in radiotherapy protocols, augmenting SU cases. In order to investigate potential therapies for the SU, an animal model (AM) was devised for Wistar rats, based upon the AM of the Nud mice. The AM dose rate (DR) was measured with silicium diode in the gamma irradiator and lead blocks. Three animals were positioned into immobilizers with their dorsal region skin pinched and held up by a suture point fixed in the immobilizer and exposed to 85 Gy. The DR variation in the immobilizer tangential point with the source median plane was non-significant, thus establishing an average DR. Such shielding reduced the DR in the rat in more than 93%. The difference in the immobilizers' dimensions impaired the comparison between the DRs; nevertheless, the DR comparison in the immobilizer tangential point with the source median plane became the reference point for AM comparison. The appearance of SU symptoms and their maximum extensions were similar, notwithstanding the difference regarding their healing periods. The specified dose induced the SU emerging. Mass variation exerted no influence onto the healing, despite having age affected it. The animals, throughout and after the experiment, showed normal health with just the SU symptoms. This work granted us the AM for the Wistar rats, which shall reinforce the investigation of new therapies for SU treatment.

Key words: Dosimetry, Wistar rat, Ionizing radiation, Dosimetric Mapping.

1. INTRODUCTION

So as to achieve the attenuation at acceptable levels or the discarding of objectionable damages arisen from the ionizing radiation (IR) through controlled proceedings (industry, research and health), it is mandatory to comply with the rules set forth by the National Committee of Nuclear Energy, more specifically those related to radio protection [1]. Amongst the undesirable damages, the skin ulcer (SU) when at highly serious levels [2, 3], is the main adverse radiotherapy side effect [4]. Radiotherapy is a treatment modality that basically makes use of RI for tumor treatment, the efficiency of which depends on the balance between the dose maximization in the tumor and the dose minimization in the adjacent tissues [3, 5]. According to the National Cancer Institute's estimate for the biennial 2012/2013, an

increase of more than half million new cases of cancer is expected in Brazil, out of which, more than 60% of such patients will eventually undergo radiotherapy treatment [6], thus foreseeing an increase in the number of oncological patients who will show some kind of skin deterioration, consequently leading to the increase in the number of SU cases. SU appears predominantly in two situations: at the end of the radiotherapy treatment sessions, which will interfere in the continuation of such therapy, or after its conclusion, which will compel the patients to be subjected to other therapies. In both situations, the used treatment will bring about results that will compromise the patient's quality of life [6], allied to a higher level of social costs. SU worsening is also associated to factors which are not related to ionizing radiation, such as: genetic, environmental, infectious diseases, radiosensitizations, albeit being the dose and the dose rate used in the radiotherapeutic treatments, the predominant factors for the appearance of this type of ulcerations [7]. The technological advances encouraged alterations in radiotherapy protocols, increasing the therapeutic doses for tumor control, allied to the concurrent use of other drugs, both of which could intensify the appearance of SU as a side effect [7-9]. Such effects can be clinically treated depending on their consequences, however, at present, there exist few techniques to inhibit or eliminate the skin ulcer process induced by RI, thus an animal model (AM) for the Wistar rats was devised, based upon the AM of the Nud mice [10-12] and verified through a dosimetric mapping, aiming at investigating potential therapies for the SU, arisen from radiotherapeutic treatment.

2. MATERIALS AND METHODS

2.1. Animal model dosimetry

The dosimetric mapping was performed in the Irradiator of ^{60}Co Panoramic Type FIS 60-04 series 226 (YOSHIZAWA KIKO CO. LTDA - JAPAN) with cylindrical geometry source. The animal immobilizer (I) measuring 7.2 cm of $Ø_{external}$ and 19.1 cm of height, comprised a PVC plastic tube and two rubber lids. The immobilizer's side surface and the lead block A's face are tangent to the source's median plane (figure 1). The reading of the dose rate in the I, were obtained in the axial plane in positions **1** to **4** in the respective heights 10.0, 13.5, 14.0, 14.5 and 15.0 cm (figures 1 and 2). Such readings were achieved from a distance of 18.7 cm from the source up to the irradiation point, tangent to the immobilizer's surface. The DRs were measured by the dosimetric kit, which comprised the electrometer Keithley model 617, Silicium diode, model SFH00206, computer program LabVIEWTM developed in the Institute of Energetic and Nuclear Research (IPEN/CNEN-SP) and lead blocks A and B, respectively, with respectively (9 x 10 x 20) e (5 x 10 x 20) cm^3.

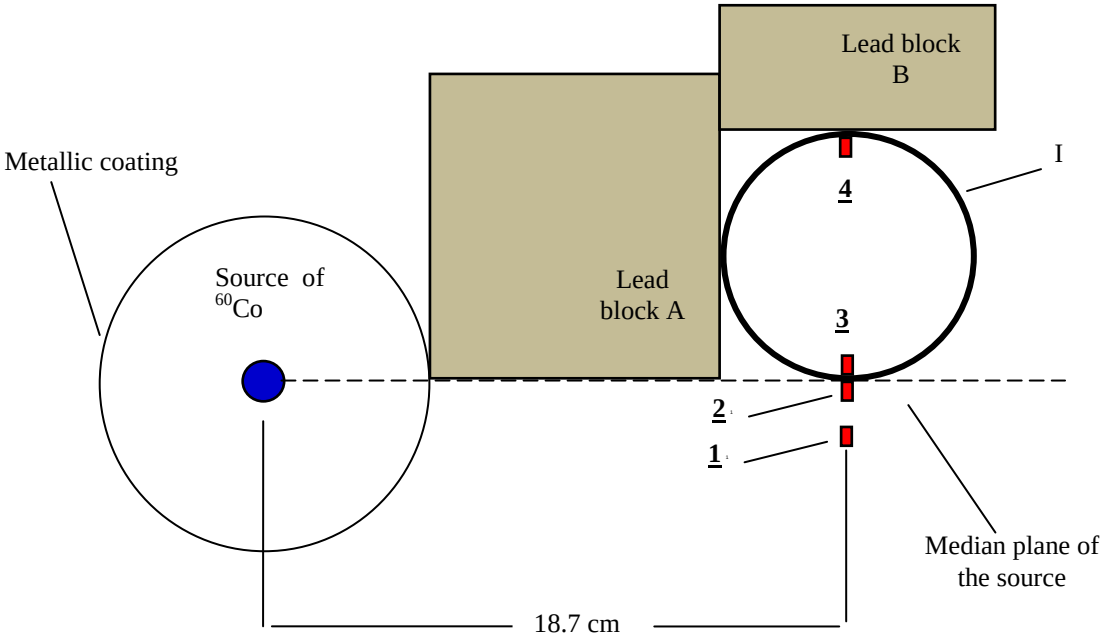

Figure 1: Upper view of irradiation configuration. The number 1 to 4 indicate the positions held by the diode (red rectangle) during the verification of the dose rate in the immobilizer's axial plane.

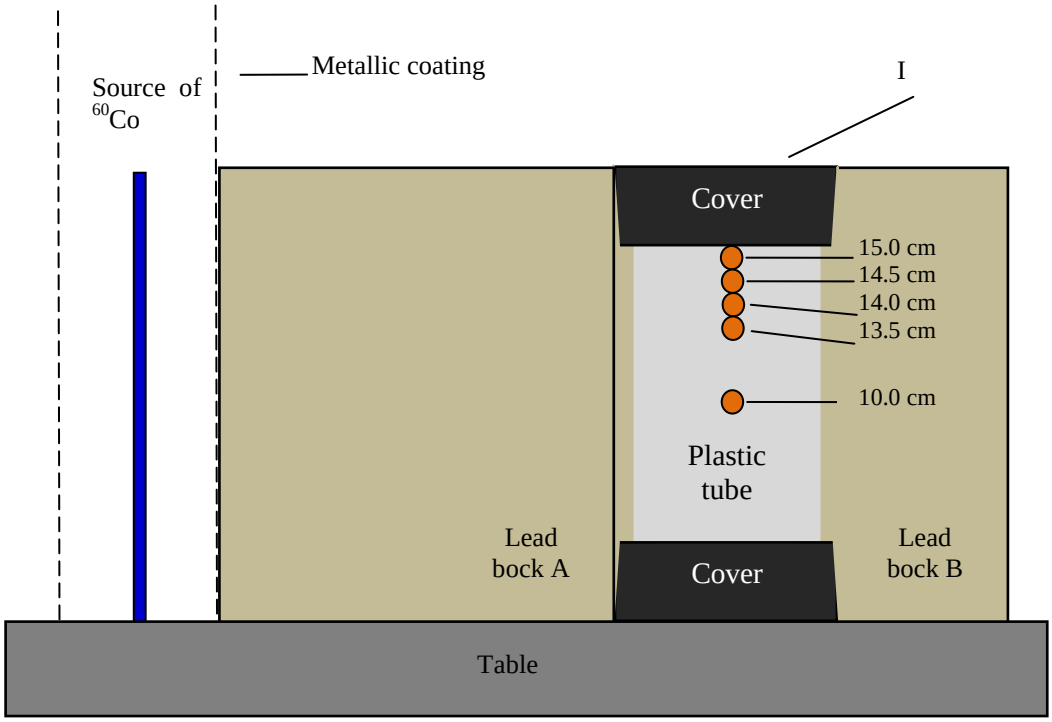

Figure 2: Profile view of the irradiation configuration. The points in orange indicate the different heights of the quartz tube with openings for the exposure of the animal skin.

2.2. Irradiation of the Wistar rat

Three male rats (Rat 1, aged 4 weeks and weighing 294 g, Rat 2, aged 20 weeks and weighing 385 g, Rat 3, aged 9 weeks and weighing 372 g) were held in individualized cages in acclimatized environment (22^{o}C), controlled luminosity with 12 hour-periods of clear/dark, being fed without water and ration restriction, in the facilities of IPEN Bioterium. The adopted procedures for the use of such rats were approved by the IPEN's Ethics Committee for the Use of Animals (CEUA, in Portuguese), registered under number 89/11/CEUA-IPEN/SP. Prior to irradiation, the rats were weighed and subsequently anesthetized (0.3 mL of xylazine and 0.4 mL ketanine) in the intramuscular region of the thigh and, subsequently trichotomized in the upper dorsal region by means of an electric equipment. Each animal was positioned inside the immobilizer, having their dorsal region skins pinched through the quartz tube in heights 14.5 and 15.0 cm (figure 2). The pinched skin was pierced by a suture point and fixed on to the immobilizer, which held it exposed and irradiated with a single 85 Gy dose. When the irradiation was over, the rats returned to the bioterium in individualized cages, being checked every two days through photographic register of the irradiated place and through the measuring of the SU area until its healing.

3. RESULTS AND DISCUSSION

3.1. AM dosimetry of the Wistar rat

The values of the dose rate indicated in table 1 in the positions **1**, **2**, **3** and **4** have not showed significant variations related to their average dose rate in heights 13.5, 14.0, 14.5, 15.0 cm (figure 2), thus allowing the use of a fixed value of the median dose rate for the animals' irradiation in the immobilizer. The existence of a gradient of dose rate between 6.0 and 7.4% in positions **1** and **2** led to a value of the median dose rate (1.276 Gy**.**min^{-1}) for point **2**, as the experiment's reference value. The gradient of the dose rate in the axial plane in position **2** for position **3** (figure 1) displays a reduction bigger than to 93%, which grants physical integrity to the rat exposed to radiation throughout the experiment. Lead block A gives protection to the rat regarding the direct radiation and block B attenuates the spread radiation and has a secondary function of mechanical support to the immobilizer.

Table 1: Dose rate for animal model of the Wistar rat

Height (cm)	Dose rate			
	1	**2**	**3**	**4**
15.0	1.367	1.275	0.087	0.000
14.5	1.355	1.278	0.089	0.000
14.0	1.353	1.264	0.083	0.000
13.5	1.386	1.291	0.078	0.000
Average	1.365	1.277	0.084	-
Standard Deviation	0.009	0.006	0.003	-

The dosimetric verification of the animal model of the Wistar rat and the verification of the animal model for the Nud mice, with theoretical correction for the same date, are reported in table 2. Due to the difference in shape and size of the immobilizers for the Nud mice and the Wistar rats (figure 3), there occurred limitations in the comparison between the dose rate in the axial plane; nonetheless, such comparison was feasible by using positions **1** (Nud) and **2** (Wistar), which coincided with the median plane of the source (figure 1), being, thenceforth, the reference positions for the comparison of the animal models.

Table 2: Comparison between the AM's dose rates

Animal	Height (cm)	Dose rate ($Gy.min^{-1}$)			
Wistar	10.0	**1**	**2**	**3**	**4**
		1.399	1.276	0.079	0.000
Nud		1	2	3	4
		1.355*	0.514*	0.019*	0.014*

*corrected values

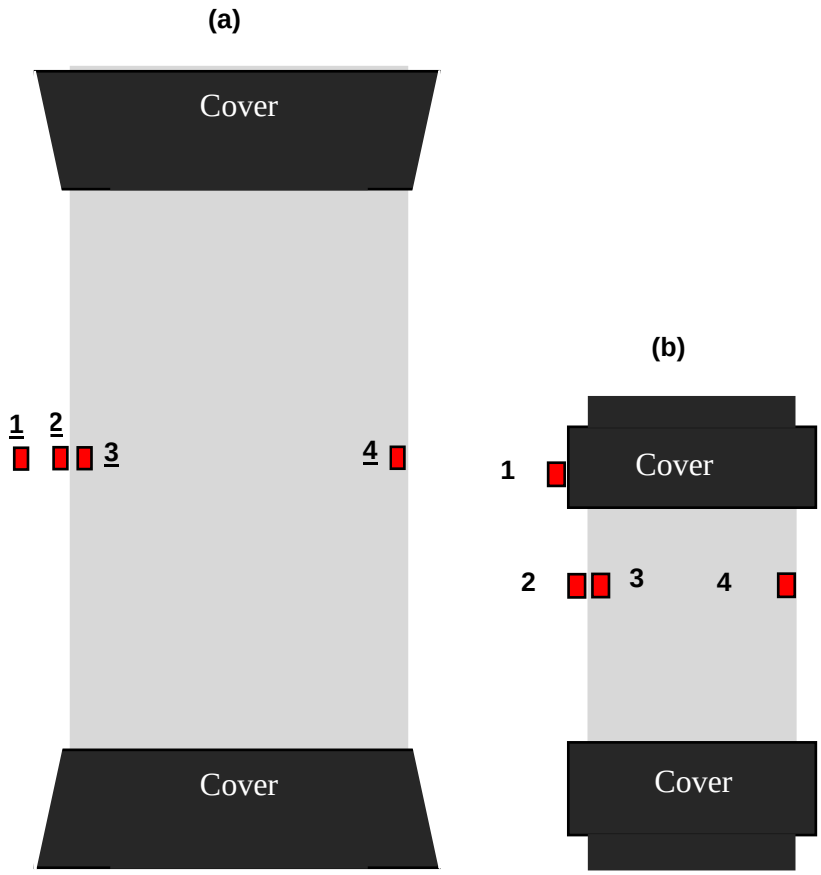

Figure 3: Immobilizers of the Wistar rats (a) and Nud mice (b). The markers in red (1 to 4) indicate the diode positions for the dosimetry.

3.2. Wistar rat's behavior towards irradiation

Throughout and after the experiment's period, the animals showed normal health conditions, when the sole alteration presented was the appearance of the skin ulcer. Three months later, the animals were still in good health, having developed no sequels in the irradiated area.

Registers were made related to the evidence of the exposure effects onto the skin on the 7^{th} day and the first vestiges of skin ulcer arose between the 9^{th} and the 11^{th} day, peaking it a maximum SU extension around the 16^{th} and the 18^{th} days (figure 4). Such behavior is similar for the three rats (table 3); nevertheless, the healing period of rat 2 needed about a 20-day additional period, when compared to rat 1 and 3 healing periods (table 3).

Table 3: SU evolution, estimated in days, subsequent to irradiation

Animal	Signs of the Exposure (day)	Necrosis Evidences (day)	Skin ulcer maximum size (day)	Healing period (days)
Rat 1	7º	9º	16º	28
Rat 2	7º	9º	18º	49
Rat 3	7º	11º	18º	30

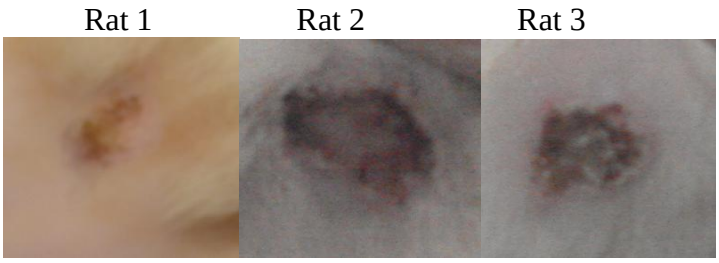

Figure 4: Skin ulcer maximum extension in rats 1, 2 and 3 in the respective 16^{th}, 18^{th} and 18^{th} days.

The results point out that with the 85 Gy established dose it was possible to impel the appearance of a skin ulcer, keeping the animal healthy, and that the mass variation of the rats (294 g, 385 g and 372 g) was not relevant for the SU healing process, notwithstanding age having seemingly played greater influence in this process, since Rat 2 (49 days for healing) was 20 weeks old and Rats 1 and 3 (28 and 30 days for their healing) were respectively 4 and 9 weeks old.

4. CONCLUSION

The results stemming from this work lead us to the conclusion that we have obtained the animal model with the Wistar rats, indicated for the generation of induced skin ulcer through ionizing radiation. This model opens up a channel for the investigations of new therapies for the treatment or the prevention of side effects caused by radiotherapy.

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