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Effect of Sodium Selenite on Bone Repair in Tibiae of Irradiated Rats

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This study evaluated the radioprotective effect of sodium selenite on the bone repair process in tibiae of female rats. For such purpose, 100 female Wistar rats (*Rattus norvegicus, albinus*) were randomly assigned to 4 groups (n=25), according to the treatment received: administration of distilled water (control); administration of sodium selenite; gamma radiation; and administration of sodium selenite plus gamma radiation. A bone defect was prepared on both tibiae of all animals. Three days after surgery, the gamma radiation and selenium/gamma radiation groups received 8 Gy gamma rays on the lower limbs. Five animals *per* group were sacrificed 7, 14, 21, 28 days after surgery for evaluation of the repair process by bone volumetric density analysis. The 5 animals remaining in each group were sacrificed 45 days postoperatively for examination of the mature bone by scanning electron microscopy. Based on all analyzed parameters, the results of the present study suggest that sodium selenite exerted a radioprotective effect in the bone repair of tibia of irradiated rats.

Key Words: sodium selenite, ionizing radiation, radiation-protective-agents, bone, fracture healing.

INTRODUCTION

Bone is a metabolically active tissue, whose activity is essential to keep tissue integrity and body homeostasis. Maintenance of this process requires interaction of two types of cells, namely osteoblastic mesenchymal cells and hematopoietic osteoclastic cells (1).

Malignant lesions are frequently treated by the association between surgery and radiotherapy procedures. Since radical surgery is often mutilating, radiotherapy is also an aggressive treatment that could cause sequels to patients, including osteoradionecrosis (1). Thus, trauma healing in irradiated and fragile bones may be compromised. The effects of ionizing radiation are mediated by the formation of free radicals, which are highly reactive, removing hydrogen atoms from fatty acids, causing lipid peroxidation and consequently cell death (2).

Radioprotective agents have been developed to minimize these deleterious effects to normal tissues. These agents may eliminate the free radicals, acting as "sweepers" or in an indirect manner by the increase of antioxidant enzymes like peroxidase glutathione. Selenium has been indicated as component of this enzyme, which assigns it a radioprotective action (3). Thus, it is extremely important to evaluate the action of radioprotective agents on tissue repair, since the association between surgery and radiotherapy is still used for the treatment of some types of tumors. Based on this, the present study investigated, by quantitative and qualitative analyses, whether sodium selenite administration could reduce the radiation-induced damage to rat bone.

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MATERIAL AND METHODS

The research protocol was reviewed and approved by the Research Ethics Committee of Piracicaba Dental School, State University of Campinas, Brazil (Protocol #645-1) in compliance with the ethical guidelines for animal experimentation. One hundred adult female Wistar rats (*Rattus norvegicus, albinus*) weighing 200-250 g were used. The rats were housed in polycarbonate cages and maintained under climate-controlled conditions (12-h light/dark cycles thermostatically regulated room temperature). During the experimental period, the animals were fed solid rat chow and water *ad libitum*.

The rats were randomly assigned to 4 groups of 25 animals each, according to the treatment received: *control*, in which the rats received only distilled water; *selenium*, in which the rats received an intraperitoneal injection 1.0 mg/kg sodium selenite (Merck KgaA, Darmstadt, Germany) diluted in distilled water 15 h before sham irradiation; *gamma radiation*, in which the rats received distilled water and were irradiated with gamma rays; *selenium/gamma radiation*, in which the rats received an intraperitoneal injection of 1.0 mg/kg sodium selenite (Merck KgaA) diluted in distilled water 15 h before the irradiation with gamma rays.

For evaluation of bone tissue repair, a bore hole was drilled into both tibial cortical bones of all animals using a size 6 carbide bur (KG Sorensen, São Paulo, SP, Brazil) at low-speed under saline irrigation, in such a way to create a 6-mm-diameter defect on bone surface. Three days after preparation of the bone defect, the animals were anesthetized by an intramuscular injection of ketamine chlorhydrate (Dopalen; Agribrands do Brasil, Ltda., Paulínia, SP, Brasil; 0.1 mg/kg body weight), and the rats belonging to the gamma radiation and selenium/gamma radiation groups were stabilized on a table for irradiation of the lower limbs with Co^{60} by means of a single acute exposure of 8 Gy (Grays) of gamma radiation delivered by an Alcion CGR II device (Co^{60}, Siemens, São Paulo, SP, Brazil), source target distance of 80 cm and field of 31 x 16 cm. Control and selenium animals were anesthetized, but were not irradiated (sham-irradiated).

Five animals *per* group were sacrificed by halothane inhalation 7, 14, 21, 28 and 45 days after the bone defect surgery. The tibias removed at days 7, 14, 21 and 28 were fixed in 10% buffered formalin for 72 h, dehydrated in 5% EDTA (Titriplex III, ACS, ISO, Merck, Darmstadt, Germany), embedded in paraffin, sectioned longitudinally to obtained 6-µm-thick sections that were stained with hematoxylin and eosin for volumetric density analysis. The tibias removed at 45 days were immersed in buffered 2.5% glutaraldehyde and analyzed with a scanning electron microscope (SEM) (Zeiss DSM 940A; Zeiss, Axiolab, Jena, Germany).

In order to evaluate the volumetric density (in mm^3/mm^3) of bone surface, two measurements were made in different areas and observed with a light microscope at ×10 magnification using a 100-point grid, in which each point was equal to 0.065 mm^3. Next, bone volumetric density was calculated using the following equation: $Vv = P \times (0.065)^3/100 \times (0.065)^3\ mm^3/mm^3$, where Vv is the volumetric density and P is the number of impact points.

Data were analyzed statistically by ANOVA and Tukey's test at 5% significance level.

RESULTS

Quantitative Evaluation of Volumetric Density

At 7 days, control and gamma radiation groups presented the highest and the lowest bone volumetric density values, respectively. No statistically significant differences ($p>0.05$) were found between the gamma radiation and selenium/gamma radiation groups. The selenium and control groups showed statistically similar ($p>0.05$) bone volumetric density values. However, significant differences ($p<0.05$) were found between the selenium/gamma radiation and selenium groups as well as the gamma radiation and control groups (Table 1), emphasizing the deleterious effect of irradiation.

At 14 days, the selenium group presented the highest bone volumetric density and differed significantly ($p<0.05$) from the control group. The gamma radiation group presented significantly higher ($p<0.05$) bone volumetric density than the selenium/gamma radiation group. No significant differences ($p>0.05$) were found between the control and the gamma radiation groups.

At 21 days, the control group showed the highest volumetric density among all groups. No significant differences ($p>0.05$) were observed among the experimental groups.

At 28 days of repair process the selenium and control groups presented the highest bone volumetric density without statistically significant difference ($p>0.05$) between them. The gamma radiation group presented the lowest bone volumetric density and no statistically difference was observed (p>0.05) from the selenium/gamma radiation group. Significant differences (p<0.05) were observed between the selenium/gamma radiation and selenium groups and between the gamma radiation and control groups.

Qualitative Evaluation

SEM (Fig. 1) analysis was performed to evaluate the mature bone tissue after 45 days of tissue repair. In the control, selenium and selenium/gamma radiation groups, it was possible to see thick cortical bone plates with spongious bone between them. On the other hand, in the gamma radiation group, it was evident the total absence of spongious trabecular bone between the two cortical plates in the tibia, being different from the control, selenium and selenium/gamma radiation groups.

DISCUSSION

Bone fracture and some surgical procedures cause a modification in tissue integrity. Therefore, good healing is necessary for this tissue to exert its normal activities (4). Remodeling events occur during bone healing, which can also suffer influence from ionizing radiation in the form of modification or delaying of the repair process (5). Carvalho et al. (6) observed thin trabecular bone with presence of osteoblasts at least in the apical and middle thirds of tooth sockets of Wistar rats, after 7 days of repair. Thus, the outcomes of the present study at 7 days occurred slightly earlier compared to those of Carvalho et al. (6). However, it should be mentioned that, in that study, the repair process was evaluated after tooth extraction. In such situation, some factors can even minimally interfere with the normal bone repair process, especially at its onset, when there may be rupture of the blood clot. In the present study, bone defects were created in rat tibiae, thereby constituting a different situation. Chicarelli et al. (7) studying the effect of gamma rays on bone repair, observed the formation of bone trabeculae 7 days after preparation of bone defects in control groups. Similar to our results, their data showed higher bone volumetric density than that observed in the gamma radiation group at day 7, with significant difference between them.

Table 1. Volumetric density (mm^3/mm^3) of the bone tissue at days 7, 14, 21 and 28 of the repair process.

Day	Sodium selenite	Non-irradiated	Irradiated
7	Yes	0.22 (0.07) Aa	0.027 (0.00) Ab
	No	0.23 (0.06) Aa	0.015 (0.03) Ab
14	Yes	0.48 (0.03) Aa	0.22 (0.18) Bb
	No	0.20 (0.03) Ba	0.32 (0.09) Aa
21	Yes	0.18 (0.10) Aa	0.21 (0.06) Aa
	No	0.22 (0.19) Aa	0.18 (0.09) Aa
28	Yes	0.22 (0.04) Aa	0.07 (0.02) Ab
	No	0.21 (0.04) Aa	0.06 (0.04) Ab

Values are expressed as mean (standard deviation). Means followed by different uppercase letters in the same column and lowercase letters in the same row, in each time, differ significantly (Tukey's test; p<0.05).

After the surgical procedure, the normal bone architecture is lost, and so a large number of free radicals are released. Thus, a faster bone tissue repair, demonstrated by higher bone volumetric density values, was expected in animals receiving sodium selenite as compared to the control group, which was clearly demonstrated at days 14 and 28. The selenium group presented features and values of volumetric bone density closer to or higher than those of the control group in all experimental periods, emphasizing its antioxidant mechanisms.

At day 7, the gamma radiation group showed the greater delay in bone repair, caused by ionizing radiation damage. Other authors (1,8) have also reported radiation damage in bone tissue in animal experiments and in individuals undergoing radiotherapy (9). According to Gal et al. (10), cells subjected to ionizing radiation present lower growth rate, with decrease in collagen production and inihibition of osteoblast proliferation. High radiation doses, such as 4 Gy, used by Dare et al. (11), could exert a different effect on cell proliferation and osteoblast differentiation. This could explain our results of delay in the repair process at 7, 21 and 28 days in the gamma radiation group, since a dose of 8 Gy was used.

Although no significant differences in bone volumetric density was observed, the amount of bone trabeculae was greater in the selenium/gamma radiation group when compared to the gamma radiation group at 7, 21 and 28 days. The process of bone repair in the selenium/gamma radiation group presented more bone trabeculae associated with red bone marrow. Thus, a radioprotective effect of sodium selenite could be assumed.

The antioxidant property of most selenium derivatives, including sodium selenite, is widely known (12) and the radioprotective action of sodium selenite has been demonstrated (13,14). According to Tappel (12), the antioxidant action of selenium is related to its ability to inhibit peroxidation, catch free radicals and repair molecular damages. All these functions seem to be related to a known antioxidant enzyme: peroxidase glutathione.

Sieber et al. (15) showed that dietary supplementation with selenium at high doses immediately after 10 Gy total body irradiation exposure decreased significantly radiation damage to kidneys. Those authors administered a dose of 10.95 μg/mL *per* day of sodium selenite dissolved in distilled water, and the total dose during the whole experimental period was 0.2 mg. In the present study, each animal received, on average, 0.25 mg of sodium selenite, which was well tolerated. Ellenhorn and Barceloux (3), however, reported toxicity with doses ranging from 1 to 5 mg/kg, 24 h after administration. Thus, despite the radioprotective action, the possibility of sodium selenite causing adverse effects should not be discarded.

SEM analysis was used to examine the mature bone, since Vizioli et al. (16) observed that maturation of bone trabeculae initiates at 23 days. The lack of bone trabeculae in the gamma radiation group may be related to a resorption process caused by radiation, altering the normal physiology of bone remodeling. This lack of bone trabeculae may also be explained by the fact that the mineral content of newly formed bone is different in irradiated animals. Therefore, this bone is more susceptible to force application, altering the bone remodeling. Another factor that could be related to lack of bone trabeculae in the gamma radiation group is the mineral deficiency in that bone trabeculae may have been formed and destroyed

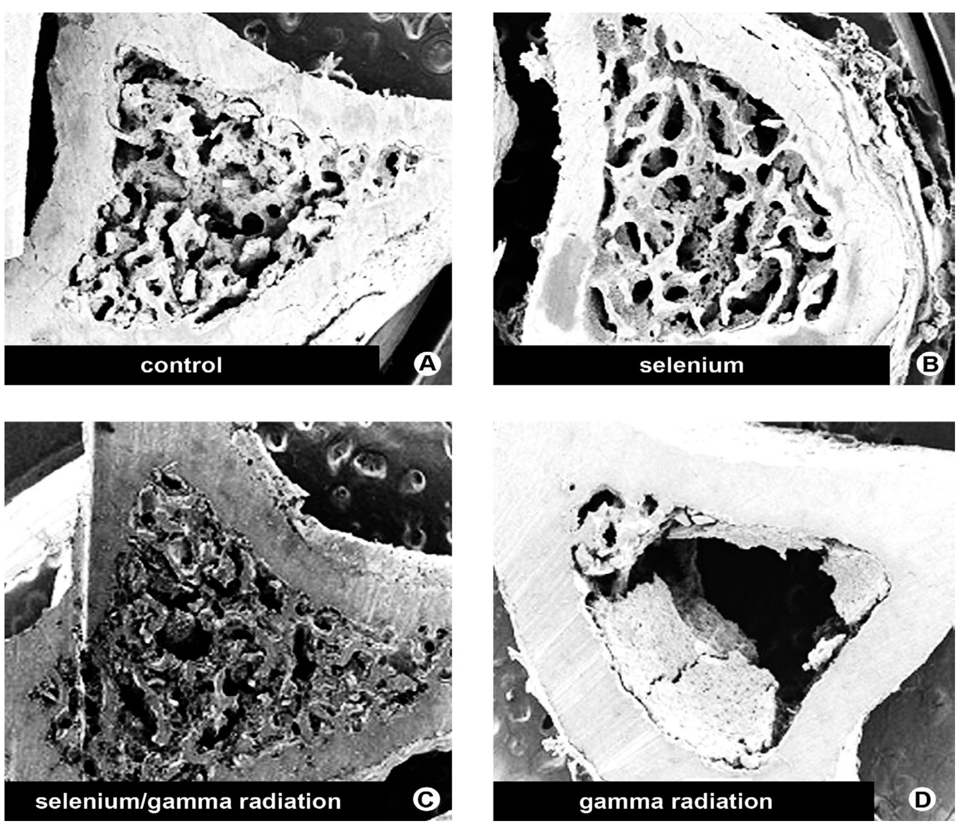

Figure 1. Panel of SEM micrograph on the 45th day of bone repair. Note the presence of thick cortical bone plates with spongious bone between them in the control (A), selenium (B) and selenium/gamma radiation (C) groups. In the gamma radiation group (D), it can be observed an empty space with total absence of spongious trabecular bone between the cortical plates.

during preparation for the SEM analysis. However, this does not exclude the assumption of tissue differences between the studied groups. The difference between the gamma radiation and selenium/gamma radiation groups may be associated to the action of selenium on the bone metabolism. According to Turan et al. (17), the lack of selenium and vitamin E is associated with the Kashin-Beck syndrome, leading to chronic degenerative osteoarthrosis. In their study, rabbits with deficiency of these substances presented evidence of osteomalacia. Sun and Liu (18) observed accelerated osseointegration in animals receiving titanium implants and 10 micrograms of selenium postoperatively. A selenium-poor diet led to growth delay in the second generation of rats, which according to Moreno-Reys et al. (19) is associated with bone metabolism and osteopenia. According to Jacob et al. (20), the expression of reductase thioredoxin and other selenoproteins in bone cells is an important means of regulation of bone resorption and remodeling.

It is important to highlight the risk/benefit of sodium selenite in the process of bone repair in individuals undergoing radiotherapy. It is believed that the possible sequels of radiotherapy entirely justify the use of this radioprotective agent, thus contributing to improve the quality of life of patients. Based on the results of this study, it may be concluded that sodium selenite exerted a radioprotective effect in the bone repair of tibia of irradiated rats.

RESUMO

Este estudo avaliou o efeito radioprotetor de selenito de sódio no processo de reparação óssea em tíbias de ratas. Para isto, 100 ratas Wistar (*Rattus norvegicus, albinus*) foram aleatoriamente divididas em 4 grupos (n=25), de acordo com o tratamento recebido: administração de água destilada (controle); administração de selenito de sódio; irradiação gama; e administração de selenito de sódio mais irradiação gama. Um defeito ósseo foi realizado em ambas as tíbias de todos os animais. Três dias após a cirurgia, apenas os animais dos grupos irradiado e selênio/irradiado receberam 8 Gy de radiação gama na região dos membros inferiores. Cinco animais por grupo foram sacrificados 7, 14, 21 e 28 dias após a cirurgia para avaliação do processo de reparo ósseo pela análise da densidade óssea volumétrica. Os cinco animais remanescentes em cada grupo foram sacrificados aos 45 dias do pós-operatório para avaliação da maturação óssea por meio da microscopia eletrônica de varredura. Baseado em todos os parâmetros analisados, os resultados do presente estudo sugerem que o selenito de sódio exerceu efeito radioprotetor no reparo ósseo de tíbias de ratas irradiados.

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