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ULTRASTRUCTURAL EVALUATION OF THE RADIOPROTECTIVE EFFECT OF SODIUM SELENITE ON SUBMANDIBULAR GLANDS IN RATS

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ABSTRACT

The aim of this study was to evaluate the radioprotector effect of sodium selenite on the ultrastructure of submandibular glands in rats. Fifty-seven male albino Wistar rats were randomized to 4 groups: control, irradiated, sodium selenite and irradiated/sodium selenite. The animals in the sodium selenite and irradiated/sodium selenite groups received intraperitoneal injections of sodium selenite (0.5 mg/kg body weight) 24 h before irradiation. The animals belonging to the irradiated and irradiated/sodium selenite groups were submitted to 15 Gy of gamma radiation in the head and neck region. The submandibular glands were removed at 4, 8, 12, 24, 48 and 72 h after irradiation. The ionizing radiation induced damage to the secretory cells, especially the serous cells, right from the first period. Vacuolization, lysis of cytoplasmic inclusions and nuclear alterations occurred. The sodium selenite group also presented cellular alterations in the study periods, but with less damage compared to that caused by radiation. There was greater similarity between the irradiated/sodium selenite group and the control group than with the other groups treated in all study periods. Despite the alterations observed in the sodium selenite group, sodium selenite presented a radioprotective action on the secretory cells of submandibular glands.

Uniterms: Submandibular gland; Sodium selenite; Selenium; Ultrastructure; Radiotherapy; Ionizing radiation; Rat.

INTRODUCTION

The inevitable exposure of salivary glands to radiation occurs frequently during radiotherapy of the head and neck region, which results in decreased saliva secretion, called xerostomia, shortly after a few radiation fractions. This may persist for the rest of the patient's life, contributing to oral infections, caries and reduction in taste, and has been shown to be very prejudicial to the quality of life[4].

Of the cells that comprise the salivary glands, the secretory cells are the most radiosensitive, especially the serous secretors[4,7]. The submandibular gland has two types of secretory cells, serous and mucous cells, and it is frequently exposed during radiotherapy of the head and neck. In rodents, the submandibular serous cells are confined to the convoluted granular tubules, and the mucous cells are found in the acini[1].

Different methods have been used to estimate the impact of various ionizing radiation doses on secretory cells, such as qualitative descriptions of acute light and electron microscopic alterations[7,9,15,22,23]. In order to overcome the influence of fibrosis and different degrees of atrophy in different cells, morphometric determinations of different cell types have been applied at the level of light microscopy. However, such measurements do not reflect subtle alterations in the morphology of individual cell types[7].

Unfortunately, there is no adequate treatment for the deleterious effects of radiation on the salivary glands. Therefore, research has been undertaken on the administration of substances called radioprotectors that may inhibit or attenuate these effects. In a series of experiments demonstrating the radioprotecting effects of WR-2721, isoproterenol18 and cAMP17, the weight of the salivary glands was the only factor used to determine the relative radiation injuries under different experimental conditions.

Among the substances that promote radioprotection, sodium selenite, an inorganic selenium-based component, is outstanding. Selenium is a mineral essential to the organism

and has been shown to have a radioprotective action in rat intestines[10], to increase survival in animals[24] and cell cultures[14,20,25].

Therefore, the aim of this study was to perform an ultrastructural evaluation of the radioprotective effect of sodium selenite on the damage caused by gamma radiation on the submandibular gland secretory cells in rats.

MATERIAL AND METHODS

Fifty-seven 3-month-old male albino Wistar rats, weighing 250-300 g were used. The rats were housed in polycarbonate cages (5-6 rats per cage) under a light/dark (14/10 h) cycle. Food (a standard pellet diet) and water were given *ad libitum*. The entire experiment was performed in agreement with the Ethical Principles for Animal Research established by the Brazilian College for Animal Experimentation (COBEA). The project was approved by the institutional Committee for Ethics in Animal Research (State University of Campinas, UNICAMP) on March 11, 2004.

The rats were randomly assigned to 4 groups: control group, irradiated group, sodium selenite group and sodium selenite/irradiated group. The control group was composed of 3 animals. The other groups comprised 18 rats each. Except for the control group, the other groups were divided into 6 sub-groups in accordance with the time of removal of the submandibular gland after irradiation: 4, 8, 12, 24, 48 and 72 h.

The animals belonging to the sodium selenite and sodium selenite/irradiated groups received 0.5 mg/kg body weight of sodium selenite (Merck KgaA, Darmstadt, Germany) intraperitoneally and saline was administered to the others.

Twenty-four hours after administration of sodium selenite, all rats were anesthetized by intraperitoneal injection of sodium pentobarbital (Nembutal®, 30 mg/kg body weight). The animals in the irradiated and sodium selenite/irradiated groups had only the head and neck region irradiated with a single, fixed nominal dose of 15 Gy of gamma radiation Co^{60}. Limitation of the exposed area was obtained by collimating the apparatus. The treatment distance to the focal point on the skin was 80 cm, and the apparatus used was an Alcion CGR II model with a yield of 1.07 Gy/min, with an average of 1.25 MV.

At the previously established times, the animals were anesthetized by intraperitoneal injection of sodium pentobarbital (Nembutal®, 40 mg/kg body weight), for surgical removal of the right submandibular gland. Subsequently, the rats were sacrificed under general anesthetic with sodium pentobarbital (Nembutal®).

The glands were sectioned into fragments of approximately by 1 mm and fixed by immersion in 2.5% glutaraldehyde at pH 7.3, 0.1 M sodium cacodylate buffer and 0.1 M sucrose for 24 h at 4°C. The specimens were post-fixed by immersion for 1 h in 1% osmium tetroxide, 0.1 M buffered in a 0.1 M phosphate buffer (pH 7.3) at 25°C. They were then dehydrated in a graded acetone series (50%, 70%, 80%, 90%, 100%) and embedded in Araldite resin[8]. For light microscopy, 1-µm–thick sections were cut on an MT2B Sorvall Porter Blum ultramicrotome and stained with toluidine blue. After light microscopy field selection (area with terminal secretory portions and convoluted ducts), ultrathin sections (60 nm) were cut with an MT2C ultramicrotome for transmission electron microscopy. These sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM-10 transmission electron microscope (Zeiss,. Oberkochen, Germany), operated at 60 kV. The alterations in the serous and mucous cells were evaluated by qualitative descriptions. Only alterations that were observed in 3 selected rats of each sub-group were considered. The figures presented in the Results section are representative of these 3 rats *per* sub-group.

RESULTS

Four hours after irradiation, vascularization, cytoplasmic and nuclear alterations were observed in both types of secretory cells in the irradiated group. Diminished concentration, union, undefined limits and alterations in the electrodensity of serous granule components (Figure 1B) and the presence of electrodense content inside some mucous granules (Figure 1D) were found in the secretion granules. With regard to the nuclei, pleomorphism, chromatin condensation, thickening and rupture of the membrane were observed. Around these, altered and destroyed organelles, such as endoplasmic reticulum and mitochondria, were found (Figure 1G). Due to the rupture of the rough endoplasmic reticulum, there was an increase in free ribosomes. In addition to the rupture, the spaces between the mitochondrial crests were larger. The vacuoles showed amorphous material, organelles and/or nuclear debris (Figure 1H).

In all the studied groups, the mucous cells showed greater integrity than the serous cells in the first time period studied (Figure 2A, B). However, the behavior among the groups and times was similar for the two types of cells. In the mucous secretory cells, the sodium selenite and sodium selenite/irradiated groups presented the same alterations, but with less intensity than that found in the irradiated group, mainly in the sodium selenite/irradiated group. In the serous secretory cells, the intensity of the alterations was similar to that found in the irradiated group, except for the greater integrity of the secretion granules in the sodium selenite/ irradiated group (Figure 2C, D).

At 8 and 12 h after irradiation, the intensity of the alterations in the two types of cells in the irradiated group, was greater, with more tissue destruction and disorganization (Figure 3A). Both types of cells in the sodium selenite and sodium selenite/irradiated groups presented with diminished alterations at 8 h after irradiation (Figure 3B, C). Regression of the alterations only occurred in the irradiated group from 24 h (Figure 3D), while the sodium selenite and sodium selenite/irradiated groups presented greater integrity (Figure 3E, F).

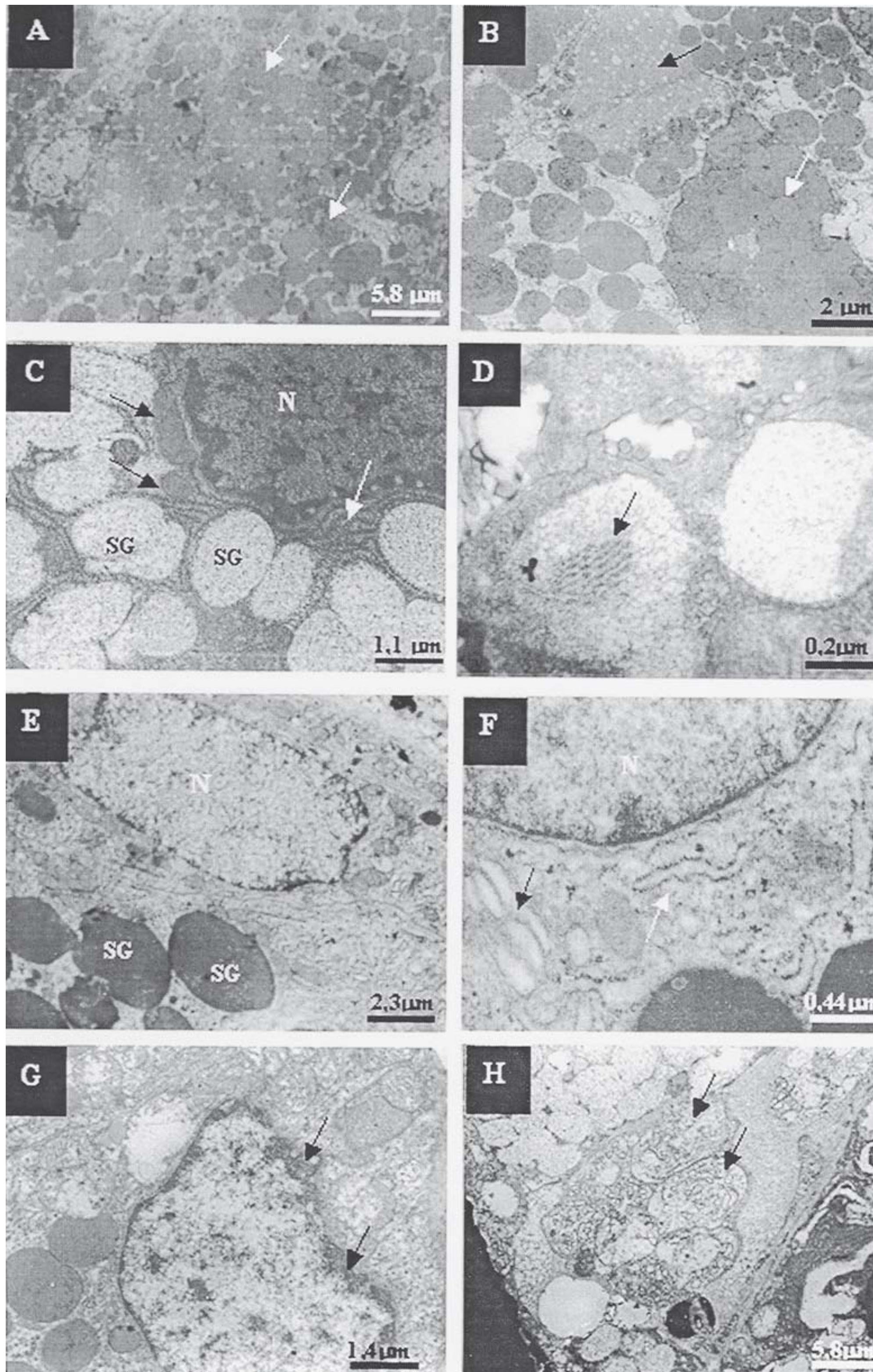

FIGURE 1- Transmission electron microscopy of the control group and irradiated submandibular salivary gland tissue 4 h after gamma ray irradiation. (A) Control group: convoluted granular tubules with serous cells presented secretion granules (white arrow). (B) Irradiated group: convoluted granular tubules with serous cells presented serous secretion granules with bonds (white arrow) and decreased electrodensity of contents (black arrow). (C) Control group: mucous cell presented mitochondria (black arrow), secretion granules (SG), nuclei (N) and rough endoplasmatic reticulum (white arrow). (D) Irradiated group: acini with mucous cells presented fibril-like condensations (arrow) in mucous granules. (E) Control group: serous cell presented nuclei (N) and secretion granules (SG). (F) Control group: serous cell presented nuclei (N), rough endoplasmatic reticulum (white arrow) and Golgi complex (black arrow). (G) Irradiated group: pleomorphic nucleus with thickened membrane (black arrow) and chromatin condensation, surrounded by degenerated organelle (white arrow) and free polyribosomes. (H) Irradiated group: vacuoles with varying content of nucleus and cytoplasm (arrow).

After 48 and 72 h, the alterations diminished in all groups; the sodium selenite group, and especially the sodium selenite/irradiated group presented greater similarity to the control group than the irradiated group (Figure 4). These latter times were characterized by an increase in the size of the nuclei and the number of organelles, mainly the rough endoplasmic reticulum.

DISCUSSION

Gamma radiation (15 Gy) caused degenerative processes in both types of secretory cells, but with greater destruction of the serous cells, in agreement with the findings of Stern, et al.[19] (1976) and Vissink, et al.[22] (1991). The greater radiosensitivity of serous cells is explained by the hypothesis put forward by Abok, et al.[1] (1984) according to which, the serous secretion granules have proteolytic and metallic transmission enzymes, while the mucous secretion granules mainly contain glycoproteins. The transmission materials are known to promote the induction of oxidative stress, potentiating the damage to the serous granule membranes. As a result, the proteolytic enzymes may infiltrate and damage the cytoplasm, causing autolysis and cellular death. Later research has confirmed the involvement of the serous secretion granules in the increased damage caused by ionizing radiation[4,2,11].

The alterations found in the present study are consistent with those observed in similarly investigations[15,21]. The rupture of the rough endoplasmic reticulum increased the amount of free polyribosomes in the cytoplasm, as previously reported elsewhere[15,19,22]. The radiosensitivity of the mitochondria has also been observed in other studies involving submandibular glands[9,15,19,22,23].

The destruction and decrease in the number of secretion granules observed right from the first assessment after irradiation have been previously observed[2,15,19,22]. In the mucous granules, in addition to these alterations, electrodense fibrils were also found, corroborating the findings of Vissink, et al.[22] (1991), who suggested they were a sign of repair and regeneration.

Nuclear alterations were noticed in the present study and in other ultrastructural studies[15,19,21] on the secretory cells of submandibular glands. Among these alterations, chromatin condensation is outstanding, indicating apoptosis[20]. Nuclear rupture and less electrodensity of the euchromatin were found as well, characterizing cellular necrosis according to Rafferty, et al.[13] (2003).

In both types of secretory cells, alterations were observed right from the earliest assessment. The alterations found in the present research after 4 h were similar to those observed in other investigations[15,22,23] but were in disagreement with the evaluation by Stern, et al.[19] (1976), who observed the first alterations as from the fourth day after exposure to 2 Gy of neutron radiation.

In relation to the time of greatest destruction, there was greater intensity after 8 and 12 h for both cells, but Stern, et al.[19] (1976), found that the first great alterations occurred on the fourth day. Reade and Steidler[15] (1985), using an 8 Gy dose of X radiation, reported greater alterations after 48 h,

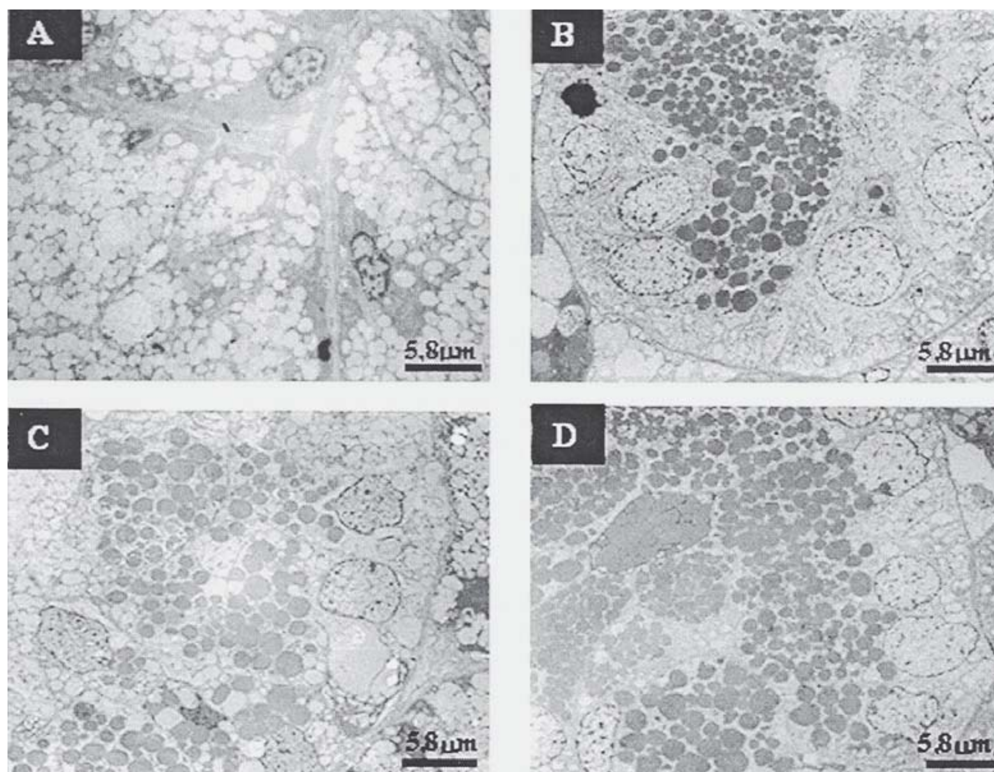

FIGURE 2- Transmission electron microscopy of submandibular gland tissues for each groups at 4 h. (A) Mucous cells from the irradiated group. (B) Serous cells from the irradiated group presenting greater degranulation than the mucous cells. (C) Serous cells from the sodium selenite group. (D) Serous cells from the sodium selenite/irradiated group.

while Vissink, et al.[22] (1991) observed the greatest changes by light and electron microscopy at 72 h. With respect to the period at which destruction diminished, indications of recovery were observed from 24 h, with decrease in damage at the last time assessed. This result differed from those of other studies, in which the indications of recovery appeared from the sixth day[15,22].

With the aim of avoiding or attenuating the effects of ionizing radiation, radioprotective substances are used, which act by means of antioxidant action[18,11]. According to Aruoma[3] (1996) animals have lines of defense like antioxidant enzymes that are able to inhibit the production of free radicals. Manganese superoxide dismutase, and copper and zinc superoxide dismutase enzymes are considered to be agents in the first line of defense against free radicals, removing the hydrogen superoxide and peroxide[3]. But the most important hydrogen peroxide removal is done by the peroxidase glutathione enzyme, which acts in the presence of selenium[3,21].

Selenium is a mineral essential to the organism and has antioxidant properties[6,5,16]. Its probable mechanism of action occurs by means of its covalent binding to proteins, forming selenoproteins, with emphasis on peroxidase glutathione[3,5,13,14,20,21] and thioredoxin reductase, which have similar antioxidant properties[5,19,21]. Among the selenium based components, inorganic selenium components, such as sodium selenite, are the most effective antioxidants[14].

In the present study, sodium selenite diminished the effects of radiation at all the times studied in both secretory cells, but did not prevent the sodium selenite/irradiated group from undergoing alterations similar to those of the irradiated group. Four hours after irradiation, the sodium selenite/

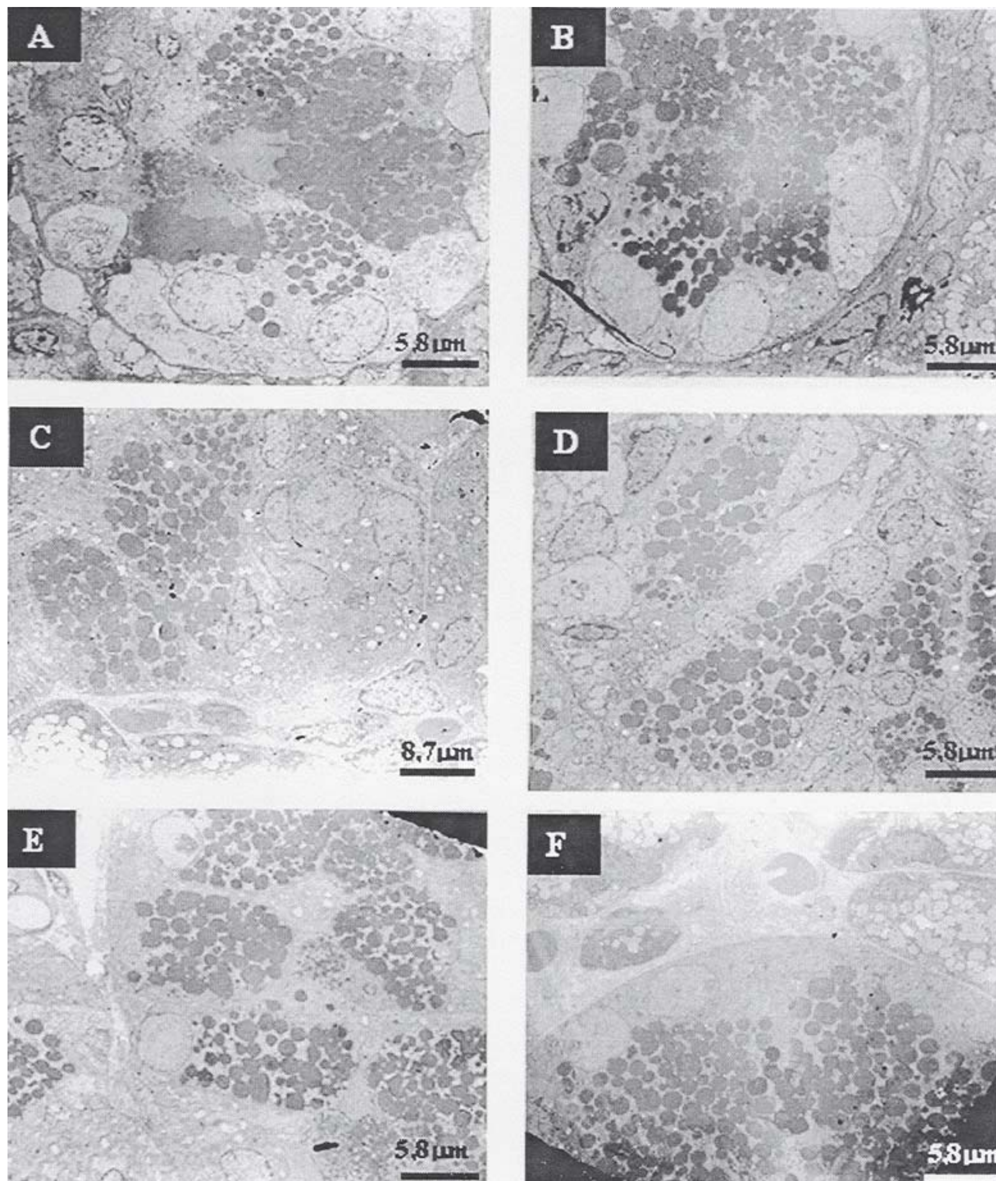

FIGURE 3- (A) Serous cells from the irradiated group 12 h (A,B) and 24 h (C,D) after irradiation. (B) Serous cells from the sodium selenite group at 12 h. (C) Serous cells from the sodium selenite/irradiated group at 12 h. (D) Serous cells from the irradiated group at 24 h. (E) Serous cells from the sodium selenite group at 24 h. (F) Serous cells from the sodium selenite/ irradiated group at 24 h.

irradiated group differed from the irradiated group only by the larger number of whole secretory granules. At the following times, the sodium selenite/irradiated group presented significant and progressive tissue integrity and organization in relation to the irradiated group. In view of these observations, sodium selenite probably helped to maintain the integrity of the secretion granules initially, preventing leakage of their contents into the cellular cytoplasm, with the consequent rupture of the organelles and cell destruction found with greater frequency in the irradiated group. A similar effect of sodium selenite was found by Tuji, et al.[21] (2005) in wound healing in irradiated rats.

In spite of having antioxidant action, selenium in excess has toxic activity, as demonstrated by studies on cell cultures[14,20,25] and *in vivo* studies[5,6]. Its toxicity is characterized by promotion of oxidative lesions in DNA[14], cell death[14], inhibition of cellular proliferation[6] and morphologic alterations[5]. The cytotoxicity of selenium is associated with the oxidation of glutathione and other thiols, giving rise to selenodiglutathione and selenopersulfide glutathione which promote the formation of hydrogen superoxide and peroxide radicals[14,24,25].

The sodium selenite dose used in the present study (0.5 mg/kg body weight) was chosen because it promoted a radioprotector effect on wound healing in rats in a previous study[21], without causing histomorphologic damage in the rats treated only with sodium selenite. Although this dose promoted radioprotection in the present study, this same dose in the sodium selenite group caused degenerative processes similar to those caused by the radiation in the irradiated group. This difference in the results can be explained by the fact that the work of Tuji, et al.[21] (2005) was a histomorphologic analysis and the present study is an ultrastructural analysis. However, it is worth pointing out that during the course of the present study, no animal treated with sodium selenite died.

Ionizing radiation results in a decrease in the accumulation of selenium by breaking its bond to proteins, involving the union of sulfur groups[24]. In addition, this may also explain the decreased concentration of selenium in the cell, used to diminish the concentration of hydrogen peroxide as a result of the ionizing radiation, as opposed to its greater accumulation in the sodium selenite group and the resultant oxidation of thiols. Thus, it is suggested that the difference in the results between the sodium selenite and the sodium selenite/irradiated groups is due to the changes in protein metabolism after irradiation, leading to the reduced accumulation of selenium in the cells.

Nordman, et al.[12] (1976) stated that selenite uptake is around 5-7 times greater in malignant cells than in normal tissues. Therefore, in view of the dichotomy of sodium selenite acting as a radioprotective substance, and as a toxic substance at greater concentrations, *in vivo* studies about the toxicity of sodium selenite in various organs are suggested, in order to find doses that may simultaneously cause toxicity in neoplastic cells, accentuate the deleterious effects of ionizing radiation, and lastly, provide radioprotection to normal cells.

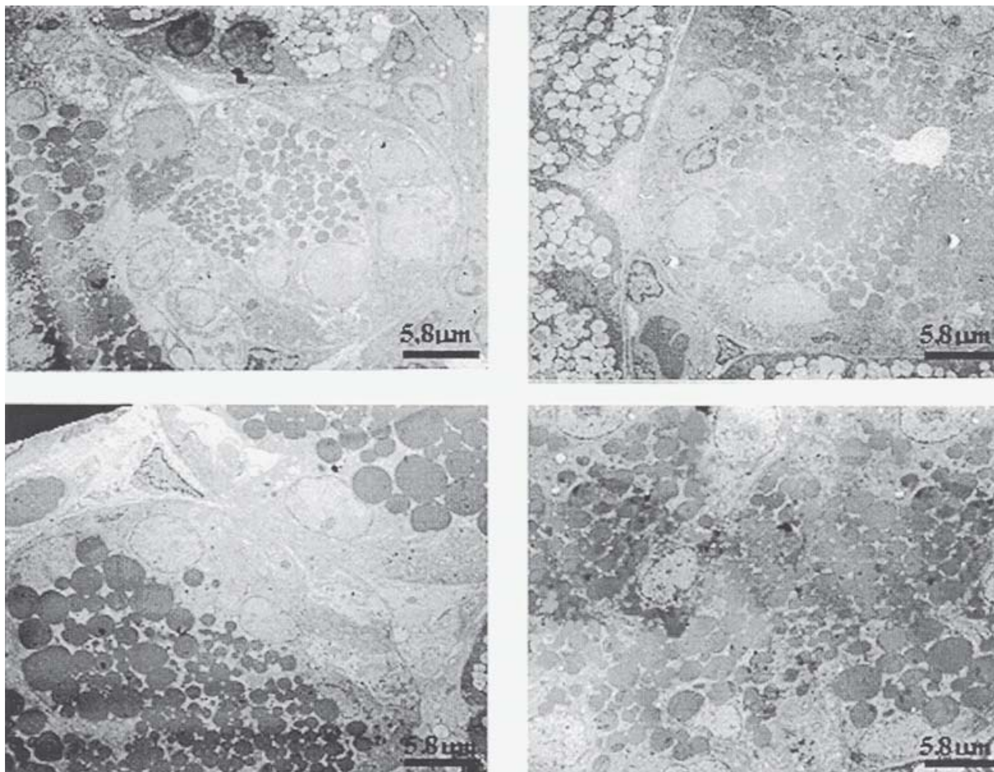

FIGURE 4- (A) Serous cells from the irradiated group at 72 h after irradiation. (B) Serous cells from the sodium selenite group at 72 h. (C) Serous cells from the sodium selenite/irradiated group at 72 h. (D) Serous cells from the control group.

CONCLUSIONS

Under this experimental conditions it was concluded that despite the alterations observed in the sodium selenite group, sodium selenite has a radioprotective action on the secretory cells of submandibular glands.

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REFERENCES

1- Abok K, Brunk U, Jung B, Ericsson J. Morphologic and histochemical studies on the differing radiosensitivity of ductular and acinar cells of the rat submandibular gland. Virchows Arch B Cell Pathol Incl Mol Pathol. 1984;45:443-60.

2- Ahlner BH, Hagelqvist E, Lind MG. Influence on rabbit submandibular gland injury by stimulation or inhibition of gland function during irradiation- histology and morphometry after 15 gray. Ann Otol Rhinol Laryngol. 1994;103:125-34.

3- Aruoma OI. Characterization of drugs as antioxidant prophylactics. Free Radic Biol Med. 1996;20:675-705.

4- Coppes RP, Vissink A, Konings AWT. Comparison of radiosensitivity of rat parotid and submandibular glands after different radiation schedules. Radiother Oncol. 2002;63:321-8.

5- Delilbasi C, Demiralp S, Turan B. Effects of selenium on the structure of the mandible in experimental diabetics. J Oral Sci. 2002;44:85-90.

6- Gøenback H, Frystyk J, Ørskov H, Flyvbjerg A. Effect of sodium selenite on growth, insulin-like growth factor-binding proteins and insulin-like growth factor-I in rats. 1995;145:105-12.

7- Grehn AL, Gustafsson H, Franzén L, Thornell LE, Henriksson R. Ultrastructural morphometry of parotid acinar cells following fractionated irradiation. Oral Oncol. 1997;33(1):23-8.

8- Luft JH. Improvements in epoxy resin embedding methods. J Biophys Biochem Cytol. 1961;9:409-14.

9- Messelt EB, Dahl E. Influence of X-ray irradiation on the ultrastructure of rat submandibular gland striated-duct cells. Acta Odontol Scand. 1983;41:277-82.

10- Mutlu-Turkoglu U, Erbil Y, Öztezcan S, Olgaç V, Toker G, Uysal M. The effect of selenium and/ or vitamin E treatments on radiation-induced intestinal injury in rats. Life Sci. 2000;66:1905-13.

11- Nagler RM, Mammary Y, Fox PC, Baum BJ, Har-El R, Chevion M. Irradiation- induced damage to the salivary glands: the role of redox-ative iron a copper. Radiat Res. 1997;147:468-75.

12- Nordman E, Jászsági-Nagy E, Rekonen A. Changes in tumour cell selenite (75 Se) affinity due to irradiation. Ann Clin Res. 1976;8:43-7.

13- Rafferty TS, Beckett GJ, Walker C, Bisset YC, McKenzie RC. Selenium protects primary human keratinocytes from apoptosis induced by exposure to ultraviolet radiation. Clin Exp Dermatol. 2003;28:294-300.

14- Rafferty TS, McKenzie RC, Hunter JAA, Howie F, Arthur J R, Nicol F, et al. Differential expression of selenoproteins by human skin and protection by selenium from UVB-radiation-induced cell death. Biochem J. 1998;332:231-6.

15- Reade PC, Steidler NE. X- irradiation induced degranulation of cells of convoluted granular tubules of murine submandibular glands. J Biol Buccale. 1985;13:307-15.

16- Saito Y, Yoshida Y, Akazawa T, Takahashi K, Niki E. Cell death caused by selenium deficiency and protective effect. J Biol Chem. 2003;278:39428-34.

17- Sodicoff M, Conger AD. Radioprotection of the rat parotid gland by cAMP. Radiat Res. 1983;96:90-4.

18- Sodicoff M, Conger AD. Radioprotection of the rat parotid gland by WR-2721 and isoproterenol and its modification by propanolol. Radiat Res. 1983;94:97-104.

19- Stern MH, Turner JE, Jett LS, Mincer H, McGinnis JP. Electron microscopic changes in rat parotid and submandibular glands subsequent to total body irradiation with fast nêutrons. Oral Surg Oral Med Oral Pathol. 1976;42:620-30.

20- Stewart MS, Spaallholz JE, Neldner KH, Pence BC. Selenium compounds have disparate abilities to impose oxidative stress and induce apoptosis. Free Radic Biol Med. 1999;26:42-8.

21- Tuji FM, Almeida SM, Bóscolo FN, Manzi FR. Avaliação do efeito radioprotetor do selenito de sódio no processo de reparação tecidual em ratos. Radiol Bras. 2005;38(5):359-64.

22- Vissink A, Kalicharan D, S-Gravenmade EJ, Jongebloed WL, Ligeon EE, Nieuwenhuis P, et al. Acute irradiation effects on morphology and function of rat submandibular glands. J Oral Pathol Med. 1991;20:449-56.

23- Vissink A, S-Gravenmade EJ, Ligeonne E, Konings AWT. Effects of split-dose X irradiation on rat salivary gland function. Radiat Res. 1991;127:52-7.

24- Weiss JF, Srinivasan V, Kumar KS, Landauer MR. Radioprotection by metals: selenium. Adv Space Res. 1992;12:223-31.

25- Yan L, Spallholz JE. Generation of reactive oxygen species from the reaction of selenium compounds with thiols and mammary tumor cells. Biochem Pharmacol. 1993;45:429-37.