

Ultrastructural assessment of the radioprotective effects of sodium selenite on parotid glands in rats

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(Received 10 November 2009 and accepted 30 April 2010)

Abstract: The aim of this study was to assess the radioprotective effects of sodium selenite on parotid glands in rats by ultrastructural analysis of acinar cells. Four experimental groups were assessed; control, irradiated, selenium, and selenium/irradiated. The sodium selenite dose was 0.5 mg/kg, administered intraperitoneally 24 h before irradiation in the head and neck region with a single 15-Gy dose of gamma radiation. At 4, 8, 12, 48 and 72 h after irradiation, all animals were sacrificed and the parotid glands were removed. Radiation caused cellular changes from 4 h, and the organelles that presented the greatest alterations were the mitochondria and the secretion glands; nuclear alterations were also observed. Sodium selenite was found to have a radioprotective action, as the selenium/irradiated group presented with less damage when compared to the irradiated group. However, sodium selenite caused cellular alterations that were evident after 8 h, but with less damage when compared to those caused by radiation, which demonstrates a favorable risk-benefit for its use as a radioprotector. Thus, this research shows that sodium selenite has an effective radioprotective action in the parotid gland, which may contribute to the reduction of the adverse

effects brought by the radiotherapy. (*J Oral Sci* 52, 369-375, 2010)

Keywords: ionizing radiation; radiotherapy; free radicals; sodium selenite; ultrastructure.

Introduction

Among the organs injured by the action of ionizing radiation during radiotherapy of the head and neck are the salivary glands. Functional and structural changes have been observed, such as reductions in volume and changes in saliva composition, which may cause nocturnal oral discomfort, xerostomia and high susceptibility to dental infections, thus considerably diminishing patient quality of life (1).

Different methods have been used to estimate the impact of various doses of ionizing radiation on secretory cells, such as qualitative descriptions of acute light and electron microscopic alterations (2-4). However, such measurements do not reflect subtle alterations in the morphology of individual cell types (4). Ultrastructural studies of acinar cells have shown that radiation causes serious harm to the salivary glands (5-7). These alterations become more evident a short time after irradiation, demonstrating the acute effects of radiation on the acinar cells.

The parotid gland is the most sensitive, whereas the submandibular and sublingual glands are relatively radioresistant (8). This may be related to the larger quantity

of serous cells found in the parotid glands (1,8,9). Studies have demonstrated that the presence of heavy metals like zinc, iron and manganese in the serous secretion granules presents a cyclic redox reaction with the production of hydroxyl radicals, causing destruction of the granular membrane and releasing lytic enzymes into the cell (10,11).

The damage caused by radiation is well known, and research has been performed on substances that diminish its deleterious effects; these substances are known as radioprotectors. The action of radioprotectors, particularly selenium, is directly connected to the capture of free radicals formed by the interaction between ionizing radiation and live tissue (8,12,13).

Pontual et al. (8) observed that the sodium selenite protected the submandibular glands of rats subjected to irradiation at a dose of 15 Gy. Therefore, we investigated whether sodium selenite is also capable of protecting parotid glands.

Materials and Methods

Fifty-seven male albino Wistar rats, (150-300 g) were used. They were kept in polycarbonate cages under an alternating 12-h light/dark cycle, and fed laboratory chow and water *ad libitum*. The entire experiment was performed in accordance 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.

Rats were divided randomly into four groups: the control group, the irradiated group, the sodium selenite group and the sodium selenite/irradiated group. The control group was composed of three animals. The other groups comprised 18 rats each. With the exception of the control group, rats were divided into six sub-groups based on the time of removal of the parotid gland after irradiation; 4, 8, 12, 24, 48 and 72 h.

The animals in the sodium selenite and sodium selenite/irradiated groups received 0.5 mg/kg of body weight of sodium selenite (Merck KgaA, Darmstadt, Germany) intraperitoneally; saline solution 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). Animals in the irradiated and sodium selenite/irradiated groups had only the head and neck region irradiated with a single, fixed nominal dose (15 Gy) of gamma radiation ⁶⁰Co using an Alcion CGR II model with a yield of 1.07 Gy/min and an average of 1.25 MV. Limitation of the exposed area was ensured by collimating

the apparatus. The treatment distance to the focal point on the skin was 80 cm.

Subsequently, to obtain the glands, animals were anesthetized by intraperitoneal injection of sodium pentobarbital (Nembutal®, 30 mg/kg body weight) at 4, 8, 12, 24, 48 and 72 h after irradiation (3 animals at each time point). Rats were then sacrificed under general anesthetic with sodium pentobarbital (Nembutal®).

Immediately after removal, glands were sectioned into fragments of approximately 10 mm × 10 mm and fixed by immersion in 2.5% glutaraldehyde at pH 7.3, 0.1 M Na-cacodylate buffer and 0.1 M sucrose for 24 h at 4°C. 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, and 100%), and embedded in Araldite resin (14). For light microscopy, 1- μ m sections were cut on an MT2B Sorvall Porter Blum ultramicrotome, followed by staining 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, operated at 60 kV. The alterations in the serous and mucous cells were evaluated by qualitative descriptions. Only changes that were observed in all three rats in each sub-group were considered. The figures depicting the results are representative of three rats from each sub-group.

Results

Control group

The ultrastructures of the parotid gland in the control group showed acinar cells, with a large quantity of electron-dense serous secretion granules, throughout the cellular cytoplasm. Several cytoplasmic organelles, such as mitochondria, Golgi complex, and granular endoplasmic reticula were present. The nuclei (N) presented with normal chromatin, surrounded by nuclear membranes of normal thickness (Fig. 1).

Irradiated group

Four hours after irradiation, the irradiated group presented with evident ultrastructural alterations, with acinar tissue disorganization, represented by accentuated cytoplasmic degeneration and formation of amorphous structures, known as clear and cytolitic bodies. Atrophic nuclei with condensed chromatin were present, indicating apoptosis. Greater damage was observed in the secretion granules followed by the mitochondria (Fig. 2A).

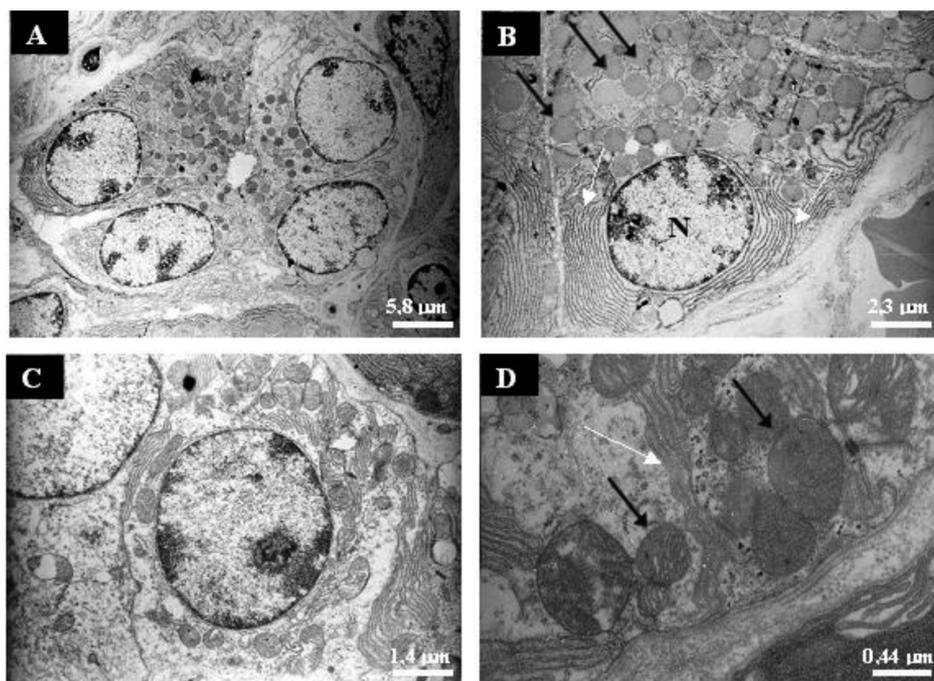


Fig. 1 Transmission electron microscopy of the control group. A: Acini with serous cells showing secretion granules. B: Serous cell with secretion granules (black arrow), nuclei (N) and rough endoplasmic reticulum (white arrow). C: Nuclei surrounded by organelles. D: Mitochondria (black arrow) and rough endoplasmic reticulum (white arrow).

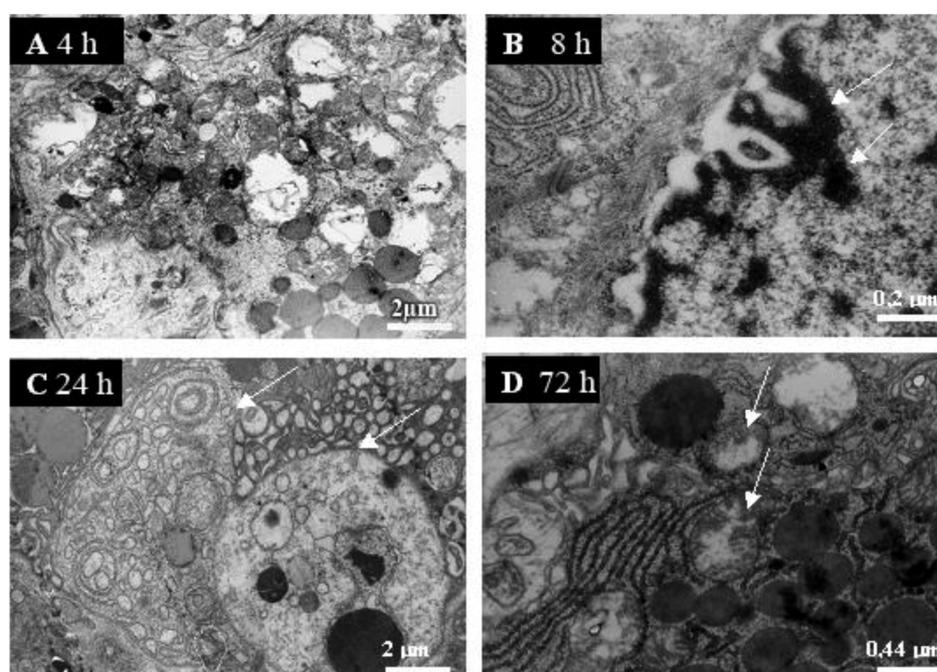


Fig. 2 Transmission electron microscopy of the irradiated group. A: Cytoplasmic degeneration at 4 h after gamma irradiation. B: Pleomorphic nucleus with thickened membrane (white arrow) and chromatin condensation, surrounded by free polyribosomes 8 h after gamma irradiation. C: Vacuoles with varying contents of nucleus and cytoplasm (white arrow) at 24 h after gamma irradiation. D: Degenerated organelles (white arrow) and free polyribosomes at 72 h after gamma irradiation.

At 8 h after irradiation, the tissue showed accentuated cytoplasmic compromise, and destruction of organelles was observed. There was a greater concentration of nuclei with condensed chromatin, various chromatin fragments were present. Alterations in the nuclear membrane were also observed (Fig. 2B).

Glandular tissue repair began 24 h after irradiation, but the presence of some nuclei with condensed chromatin and cytoplasmic fragments and the presence of cytolitic bodies were still noted in some regions (Fig. 2C). A larger number of whole organelles, such as mitochondria, secretion granules, Golgi complex and endoplasmic reticula, were observed at 24 h when compared to 4, 8 and 12 h.

At 48 and 72 h, a progression was seen in the glandular tissue repair process (Fig. 2D).

Irradiated selenium group

Four hours after irradiation, cytoplasmic degeneration was observed in some regions, but to a lesser degree than in the irradiated group. Cellular organization and tissue preservation were clear when compared with the irradiated group. The greatest changes were noted in the mitochondria, in contrast to the irradiated group, in which the most compromised organelles were the secretion granules. Nuclei were normal (Fig. 3A). Eight hours after irradiation, the tissue continued to be more organized than in the

irradiated group, but more compromised when compared to the previous time point. At the other times assessed, there was progressive glandular tissue repair, with better organization when compared to the irradiated group and a smaller amount of cytoplasmic degeneration.

At 24 and 48 h, cytolitic bodies were observed (Figs. 3B, C), which suggests that the intense alterations in the first periods are characterized by disseminated degeneration, but the tissue repair process at more advanced stages shows more localized alterations, forming the cytolitic bodies. At 72 h, the general aspect was much more preserved, with the presence of whole organelles (Fig. 3D). However, some regions with cytoplasmic degeneration and a small number of vacuoles remained present, thus demonstrating that although the tissue was more organized when compared with earlier times, completely regenerated tissue was not present.

Selenium group

At 4 h after irradiation, tissue was generally preserved, and was comparable to that of the control group, showing normal nucleus and cytoplasm (Fig. 4A).

Tissue alterations became evident at 8 h, with the presence of alterations in the cytoplasmic organelles, such as the mitochondria, secretion granules and granular endoplasmic reticula. The presence of cytolitic bodies was also observed, but in fewer numbers than in the

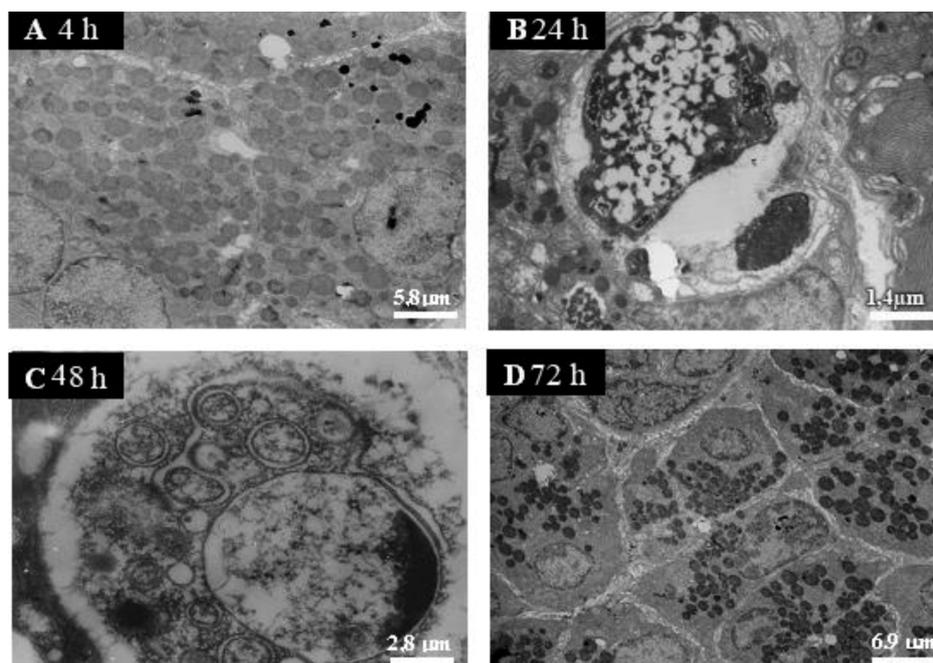


Fig. 3 Transmission electron microscopy of the irradiated/selenium group. A: Serous cells at 4 h after gamma irradiation. B, C: Cytolitic bodies observed at 24 and 48 h after gamma irradiation. D: Serous cells 72 h after gamma irradiation.

irradiated and selenium/irradiated groups (Fig. 4B).

At 24 h, greater cytoplasmic and nuclear alterations were observed, with the presence of nuclei with condensed chromatin and a larger number of mitochondria and degenerated secretion granules (Fig. 4C).

At 48 and 72 h, the tissue was generally more organized and cytoplasmic degeneration and vacuolization were observed only in some regions. The presence of a large number of whole secretion granules and extensive granular endoplasmic reticulum (Fig. 4D) denoted a greater power of synthesis, and suggested that the tissue was undergoing repair.

Discussion

The results showed that 15-Gy gamma irradiation caused degenerative processes in both types of secretory cells, but with greater destruction of serous cells, in agreement with the findings of Stern et al. (7) and Vissink et al. (3). Secretion granules, followed by mitochondria and granular endoplasmic reticulum, were also shown to be altered in other studies (2,5-8,10). There was a greater concentration of nuclei with condensed chromatin, various chromatin fragments as well as alterations in the nuclear membrane. These findings were similar to those of Vissink et al. (3) who reported distension of the nuclear membrane.

These findings may correspond to a reduction in the acute effects on the cytoplasm, but still in the active phase in the nuclei, which may be verified by the characteristics of nuclear apoptosis. The glandular tissue repair process started at 24 h, by which time the cytoplasmic degeneration was localized and not sparse in the cellular tissue, as observed at 4 h. At the following times, progressive repair of the irradiated tissue was observed, but areas with small alterations were still present.

In all the groups assessed, the alterations were less pronounced when sodium selenite was used, demonstrating its efficacy as a radioprotector. The alterations in cytoplasmic organelles were less substantial when compared to the irradiated group, particularly with regard to the nuclei and secretion granules. Thus, the secretion granules having been compromised to a lesser extent in this period may represent greater tissue protection. Therefore, sodium selenite appears to have prevented the rupture of secretion granule membranes, as glutathione peroxidase prevents cellular destruction caused by hydrogen peroxide.

The protective effects of various substances have been studied with regard to irradiation of the salivary glands (8,10,11,15-18). Similarly to the findings of Pontual et al. (8), in the present study, sodium selenite was an effective radioprotector for the selenium/irradiated group.

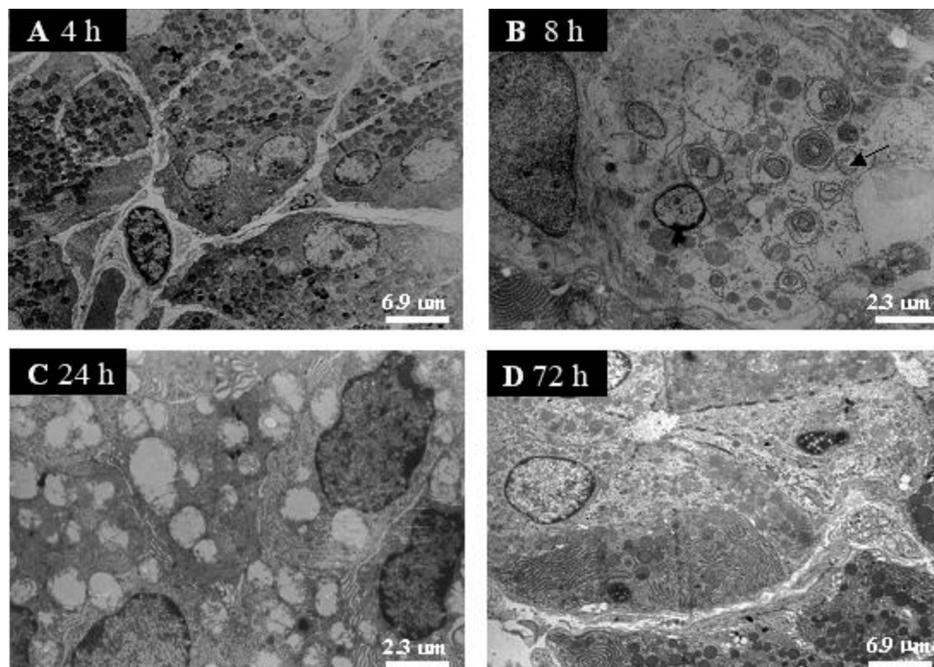


Fig. 4 Transmission electron microscopy of the selenium group. A: Serous cells at 4 h after gamma irradiation. B: Vacuoles with varying nuclear and cytoplasmic contents at 8 h after gamma irradiation. C: Serous cells at 24 h after gamma irradiation showing pleomorphic nuclei with chromatin condensation, surrounded by degenerated organelles. D: Serous cells at 72 h after gamma irradiation.

The radioprotective effects seen in this study may be due to increases in glutathione peroxidase, which acts directly on the capture of free radicals formed when ionizing radiation interacts with irradiated tissue (12,13,19-21). However, glutathione peroxidase may also be involved in another radioprotection mechanism, specifically for salivary glands. Several authors (10,11) have demonstrated that the cell death caused by radiation in the salivary glands occurs immediately after the secretion granule membranes rupture, due to heavy metals. Thus, it may be inferred that the increases in glutathione peroxidase, caused by administration of sodium selenite, may not only neutralize the free radicals produced by the direct effect of the radiation, but also prevent the secretion granule membranes from rupturing, thus reducing the number of these radicals.

Despite its antioxidant action, excess selenium is toxic, as demonstrated by studies on cell cultures (22,23) and *in vivo* (24,25). 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 (22,23,26).

Although the 0.5 mg/kg of body weight dose of sodium selenite promoted radioprotection in studies by Tuji et al. (27) and Pontual et al. (8), as well as in the present study, the sodium selenite dose caused degenerative processes similar to those caused by the radiation in both the present study and the study by Pontual et al. (8). This difference in the results can be explained by the fact that the study by Tuji et al. (27) was a histomorphologic analysis. However, it is worth noting that during the course of the present study, no animals 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 (26). This may also explain the reduced concentrations of selenium in the cell, and the reduced concentrations of hydrogen peroxide after ionizing radiation, as opposed to its greater accumulation in the sodium selenite group and the subsequent oxidation of thiols. Thus, the difference in the results for the sodium selenite and the sodium selenite/irradiated groups is probably due to changes in protein metabolism after irradiation, leading to the reduced accumulation of selenium in the cells.

Nordman et al. (28) stated that selenite uptake is around 5-7 times greater in malignant cells than in normal tissues. Therefore, due to the dichotomy of sodium selenite acting as a radioprotective substance, and as a toxic substance at greater concentrations, *in vivo* studies on the toxicity of sodium selenite in various organs are necessary, in order

to find doses that may simultaneously cause toxicity in neoplastic cells, accentuate the deleterious effects of ionizing radiation, and provide radioprotection to normal cells.

This study demonstrates that sodium selenite presents evident toxicity, and its radioprotective effects were confirmed at all time points assessed.

Acknowledgments

The authors wish to thank Dr. Eliene Novaes for her help and advice with this experiment. This study was supported financially by grants from the National Council for Scientific and Technological Development-CNPq, Project Number 301185/95-6.

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