A preliminary study of the submandibular gland of the rat after one-year cadmium intoxication. Part II. Pathomorphology and ultrastructure

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Abstract

The aim of the present study was to establish to what degree a one-year exposure of rat females to 5, 50 and 100 mg of Cd/l affects cell morphology of the submandibular glands. After one-year cadmium exposure of female rats, at doses of 5, 50 and 100 mg Cd/l, a pathomorphological examination revealed periductal fibrosis in the submandibular glands of rats in all the three experimental groups, which increased with cadmium dose. We also found foamish cytoplasm in the cells of the submandibular glands in all the experimental groups, the intensity of that phenomenon also increasing with Cd dose. The ultrastructural examination revealed no abnormalities in Group I. However, in Groups II and III, we observed numerous granules with a secretion, varying in shape and size that filled up the cytoplasm both in the mucous and serous cells.

Key words: submandibular gland, rat, cadmium, pathomorphology, ultrastructure.

Introduction

Cadmium (Cd) is a trace metal found in high concentrations in the polluted air of large urban agglomerations and highly industrialized areas. It penetrates all body organs, including the submandibular glands [1, 2]. The presence of cadmium was detected in the saliva of workers who were professionally exposed to cadmium and in young subjects, inhabiting large urban agglomerations [1, 3]. An increase in salivary cadmium level in humans was also observed after cadmium isotope administration [2]. The aim of the present study was to establish to what degree a one-year exposure of rat females to 5, 50 and 100 mg Cd/l affects cell morphology of the submandibular glands.

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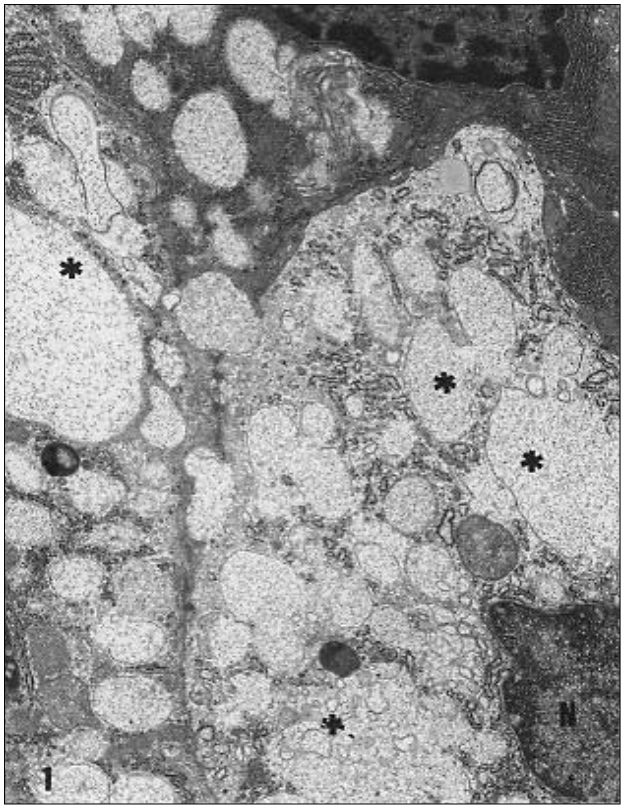
Material and methods

For the study, twenty-two young female Wistar rats were used, allocated to 4 groups. Six control rats received only redistilled water to drink. Eighteen experimental rats were given aqueous solution of cadmium chloride ($CdCl_2$) to drink: six rats from Group I received a dose of 5mg Cd/l, six animals from Group II received a dose of 50 mgCd/l and the remaining four from Group III - 100 mg Cd/l. After one-year of the experiment, the animals were sacrificed in pentobarbital narcosis. A fragment of one submandibular gland was fixed in Bouin's fluid. 5 µm sections were stained with H+E and, according to Azan's method. Sections for ultrastructural examinations were fixed in 3.6% glutaraldehyde at temp. of 4^0C for 2 hours, postfixed in 2% osmium tetroxide, dehydrated in alcoholic series, propylene oxide and embedded in Epon 812. Semithin preparations were stained with toluidine blue, while ultra thin sections were contrasted with uranyl acetate and lead citrate, and evaluated in an OPTON 900 PC transmission electron microscope.

Results

After one-year of the experiment, a histopathological examination of the submandibular glands in Group I revealed slight periductal fibrosis and, in some cells, an increased translucence of the cytoplasm. In Group II, periductal fibrosis was enhanced, compared to that in Group I and in the control, and single cells with foamish cytoplasm and single atypical cells could still be observed. In Group III, the submandibular glands showed increased periductal fibrosis, in comparison to that in Groups II, I and in the control; some cells exhibited more pronounced nuclear atypia and more foamish cytoplasm. In some fields of vision, cellular membranes and basement membranes of the

Figure 1. Group II. 50 mg Cd/l. Numerous secretory granules, irregular in shape and binding together, with disrupted limiting membranes. (*) N-cell nucleus with irregular contours. (original magnification X 4400).



gland tube were blurred. No abnormalities were observed in the ultrastructural examination of the submandibular glands in either Group I or the control. Changes, which could be seen in Groups II and III, were similar, being more pronounced in the latter. We found: 1) irregular contours of cell nuclei with clumping of nuclear chromatin on the periphery (Fig. 1); 2) activation of Golgi apparatus in the form of a higher number and dilation of cisterns (Fig. 1); 3) blurring of the internal structure in some of the mitochondria with segmental destruction of the limiting membranes. In mucous cells, secretory granules combined to form large vacuoles which, sometimes, filled up the cytoplasm and pressed the cell nucleus (Figs. 1, 2). The limiting membranes of the secretory vacuoles were frequently disrupted and their contents were flowing out to the cytoplasm (Fig. 1). Serous cells contained numerous secretory granules, which varied in shape and size (Fig. 3), among which single large granules were frequently encountered. The lumina of serous vesicles and mucous tubes had a smoothed surface and were, sometimes, completely devoid of microvilli (Fig. 3). In the stroma, around the vesicles, rare collagen fibres were observed.

Discussion

The present study has revealed that a one-year exposure of female rats to cadmium at doses of 5, 50 and 100 mg Cd/l leads to irreversible changes in the submandibular glands, being manifested in periductal fibrosis, observed in histopathological examination. In the ultrastructural examination, collagen fibres were visible in the stroma. Fibrosis was found to increase with

Figure 2. Group III. 100 mg Cd/l. Secretory granules in the cytoplasm, blending to form the, so-called, "pools" (*) (original magnification X 3000).

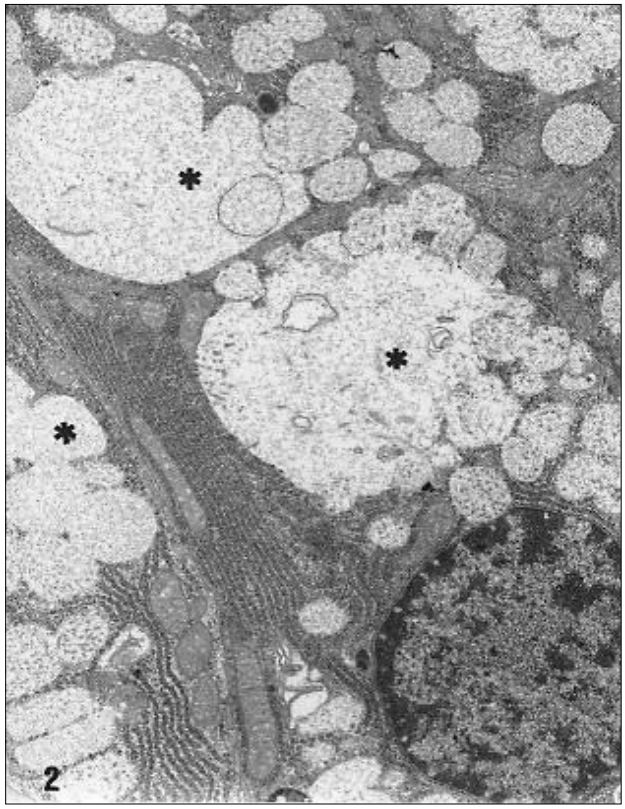
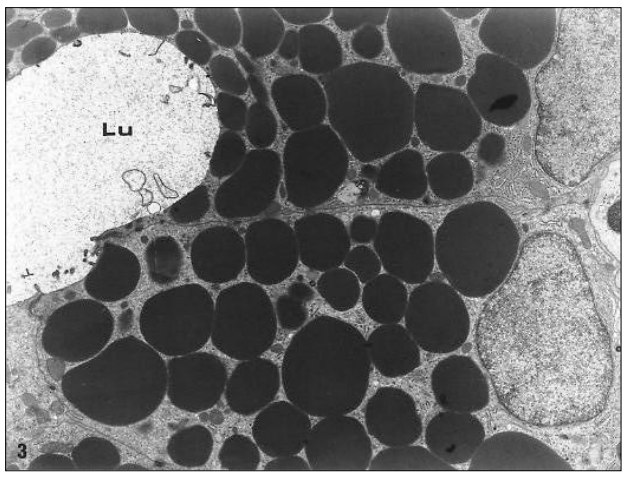


Figure 3. Group III. 100 mg Cd/l. Numerous serous granules, varying in size and shape, accumulated around the vesicular lumen (Lu) with smoothed surface (original magnification X 3000).



the increase in cadmium dose, administered to the rats. The remaining changes, detected in the submandibular glands of female rats, both in histopathological and ultrastructural examination, were reversible. Nevertheless, they caused secretion disorders in mucous and serous cells of the submandibular glands, expressed by the presence of foamish cytoplasm in histopathological examination and seen as secretory granules, filling up the cytoplasm in the ultrastructural examination. Other authors have observed similar changes in the form of either single or multiple vacuoles in serous cells of the rat parotid glands [4]. According

to Maier [5], cadmium is not only accumulated in the rat parotid gland but it is also "excreted via the parotid gland" to the saliva. We think that the same phenomenon may occur in the rat submandibular gland. It is likely that an active excretion of cadmium to the rat saliva inhibits massive secretion of the secrete, accumulated in cytoplasm of the submandibular gland. The pathomorphological changes, observed in the present study in the submandibular glands of female rats after one-year exposure to cadmium, were not only cadmium-specific, but they may also occur, following intoxication with other toxic compounds, such as herbicides [6]. According to other authors, pathomorphological changes, observed in the salivary glands of rats, exposed to cadmium, may occur, both due to its accumulation in the gland and be caused by general intoxication with this metal [4].

Conclusions

The present experiment indicates that one-year exposure to cadmium at doses of 5, 50 and 100 mg Cd/l induces dose-dependent pathomorphological and ultrastructural changes both of reversible and irreversible nature.

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