Ultrastructural study of the submandibular gland of the rat after 6-month exposure to cadmium and zinc in drinking water

Dąbrowska E[1], Szynaka B[2], Kulikowska-Karpińska E[3]*

[1] Department of Social and Preventive Dentistry, Medical University of Białystok, Poland
[2] Department of Medical Pathomorphology, Medical University of Białystok, Poland
[3] Department of Toxicology, Medical University of Białystok, Poland

Abstract

Purpose: Cadmium toxicity in the exposure of the general, professional and cigarette smoking populations has been well known. From the dental point of view, it is important to find out whether and how separate and joint exposures to cadmium and zinc affect the structure and function of the submandibular gland, which is the major saliva-releasing gland. Cadmium, a particularly active xenobiotic, damages cellular metabolism at the level of various enzymatic systems of the cell, which may disturb functioning of the salivary glands. Mutual interactions of cadmium and zinc suggest a protective role of zinc through the induction of metallothionein which inactivates cadmium effect.

Material and methods: The aim of the study was to assess the ultrastructural picture of chosen cell organelles of the submandibular salivary gland of the rat exposed to cadmium and zinc. The study used 90 male Wistar rats, of the initial b.w. 150-180 g. The animals were exposed to cadmium and/or zinc for 6 months. Cadmium was received in aqueous solutions of cadmium chloride with drinking water at a concentration of 5 mg Cd/dm^3 or 50 mg Cd/dm^3. Zinc was also given in aqueous solutions of zinc chloride ad libitum at concentrations of 30 mg Zn/dm^3 and 60 mg Zn/dm^3.

Results: The ultrastructural changes in the mucous and serous cells of the submandibular salivary gland were most pronounced at cadmium concentration of 50 mg Cd/dm^3 and were mainly observed in the cell nucleus, Golgi Apparatus and secretory granules of the salivary gland cells.

Conclusions:

1. Exposure to cadmium induces ultrastructural changes in the submandibular gland, which are dose and time of exposure-dependent.
2. Exposure to zinc did not affect significantly the ultrastructural picture of cells of the submandibular gland.
3. Zinc administered together with cadmium reduces the intensity of ultrastructural changes in the submandibular gland.

Key words: ultrastructure, submandibular gland, cadmium, zinc.

Introduction

The civilization advances and growing environmental pollution bring the effect of various xenobiotics, including heavy metals, on the functioning of the living organism [1-3]. Cadmium toxicity in the exposure of the general, professional and cigarette smoking populations has been well known [4]. Its effect on long bones, kidneys and liver has been investigated in experimental rat models [5-10]. From the dental point of view, it is important to determine whether and how separate and joint exposures to cadmium and zinc affect the structure and function of the submandibular gland, the major saliva-releasing gland. The oral cavity can be the first site through which the xenobiotic gets via the respiratory and alimentary tract to the body, and the saliva is a "protective coat" for the oral structures. Cadmium, a particularly active xenobiotic, interferes with cellular metabolism at the level of various enzymatic systems of the cell and may thus disturb functioning of the salivary gland. Biological consequences of the effects of the respective metals and their mutual interactions on the structure of submandibular gland cells can be assessed only using animal experimental models. Mutual interactions of cadmium and zinc suggest a protective role of zinc through the induction of metallothionein which inactivates the cadmium effect [7,11].

The aim of the study was to assess the ultrastructural picture of chosen cell organelles of the submandibular gland of the rat exposed to cadmium and zinc. There is no data on an interaction influence of cadmium and zinc together administrated in a chronic poisoning of cadmium.

* CORRESPONDING AUTHOR:
Department of Social and Preventive Dentistry
Medical University of Białystok, Poland
ul. Akademicka 3, 15-089 Białystok
(Ewa Dąbrowska)

Received 27.03.2006 Accepted 11.07.2006

Material and methods

The study used 90 male Wistar rats (initial b.w. 150-180 g). The animals were exposed to cadmium and/or zinc for 6 months. Rats received cadmium in the form of aqueous solutions of cadmium chloride with drinking water at a concentration of 5 mg Cd/dm^3 or 50 mg Cd/dm^3. Zinc was also given in aqueous solutions of zinc chloride ad libitum at concentrations of 30 mg Zn/dm^3 and 60 mg Zn/dm^3. Control animals received water to drink without cadmium and zinc. The rats had unlimited access to LSM diet. Throughout the experiment the intake of drinking water and body mass increase were monitored. The rats were divided into 9 groups according to metal concentration: group I – control, group II 30 mg Zn/dm^3, group III – 60 mg Zn/dm^3, group IV – 5 mg Cd/dm^3, group V – 5 mg Cd/dm^3 + 30 mg Zn/dm^3, group VI – 5 mg Cd/dm^3 + 60 mg Zn/dm^3, group VII – 50 mg Cd/dm^3, group VIII – 50 mg Cd/dm^3 + 30 mg Zn/dm^3 and group IX – 50 mg Cd/dm^3 + 60 mg Zn/dm^3. After the exposure termination, the animals were anaesthetised (with Vetbutal) and then, immediately, 4 sections of 1-2 mm^3 vol. each were cut off from the submandibular gland in 3 animals from each group. The sections were obtained from the same part of the organ examined.

The tissue material was collected and prepared for ultrastructural analysis following generally accepted principles. Sections for ultrastructural examinations were fixed in 3.6% glutaraldehyde at a temp. of 4°C 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 ultrathin sections were contrasted with uranyl acetate and lead citrate, and evaluated in a transmission electron microscope OPTON 900 PC [12]. The experiment was approved by the Bioethical Committee (2004/03).

Results

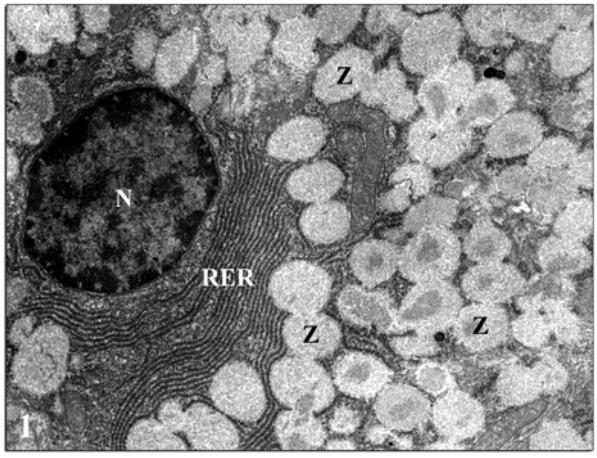
Group I (control)

The ultrastructure of the submandibular gland was normal. Mucous acinar cells had oval nuclei located in the parabasal part. In the vicinity of the nucleus, there were parallel channels of the rough endoplasmic reticulum, Golgi Apparatus, as well as oval or elongated mitochondria with numerous crests and matrix of moderate electron density. In the central and apical part of the cell, bright mucous granules containing microgranular material were seen in great numbers (*Fig. 1*). Cellular membranes of the adjacent cells were indented and linked together. Moreover, the cells were joined together by desmosal junctions.

Serous acinar cells had round or oval nuclei, which were located in the central or parabasal parts. The rough endoplasmic reticulum channels were shorter and less numerous than in mucous cells. In the vicinity of cell nuclei, Golgi Apparatus and many slightly elongated mitochondria could be observed. A large part of the cell was filled with relatively numerous secretory granules of high electron density.

In the stroma, a large number of tiny blood vessels were seen between the vesicles.

Figure 1. **Group I (control). Fragment of normal mucous cells of the submandibular salivary gland. N-cell nucleus, RER – rough endoplasmic reticulum, Z – secretory granules. Mag. x 4400**



Group II (30 mg Zn/dm^3)

The ultrastructural picture of the cells of the submandibular gland in this group did not differ from the control one.

Group III (60 mg Zn/dm^3)

The ultrastructural picture of the cells of the submandibular salivary gland in this group also did not differ significantly from the control one. However, the picture showed numerous secretory granules, both in mucous and serous acinar cells, which accumulated around the smoothed-surface lumen. Mitochondria with slightly increased matrix translucence were seen.

Group IV (5 mg Cd/dm^3)

In the ultrastructural picture of the submandibular salivary gland of rats exposed to low doses of cadmium, cell nuclei had a plicate nuclear membrane with large distinct nucleoli. The Golgi Apparatus was activated and with dilated cisterns. Mitochondria were slightly translucent and only sporadically myelinic structures could be seen within them.

Group V (5 mg Cd/dm^3 + 30 mg Zn/dm^3)

The ultrastructural picture of the acinar cells showed very subtle changes and thus it was not significantly different from the control one. Only in mucous cells, large secretory granules were seen.

Group VI (5 mg Cd/dm^3 + 60 mg Zn/dm^3)

In mucous cells, the nuclei frequently had irregular contours. Serous cells contained plenty of large secretory granules. Condensations of mucous cell granules were rare.

Group VII (50 mg Cd/dm^3)

The nuclei of mucous cells of animals exposed to this high concentration of cadmium had distinctly irregular contours and very large nucleoli (*Fig. 2*). The rough endoplasmic reticulum showed local degranulation and had an irregular course. Golgi

Figure 2. **Group VII (50 mg Cd/dm³). Fragment of mucous cells with numerous secretory granules accumulated around the acinar lumen /L/ with smoothed surface. The nuclear cell /N/ shows plicate margins and a large nucleolus /n/. M – mitochondria exhibited damaged crests. Mag. x 3000**

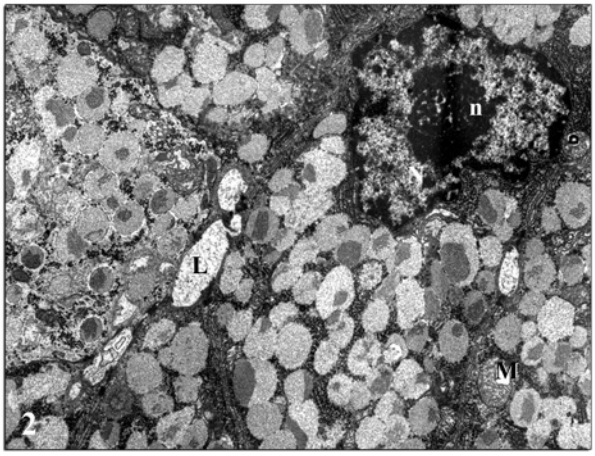
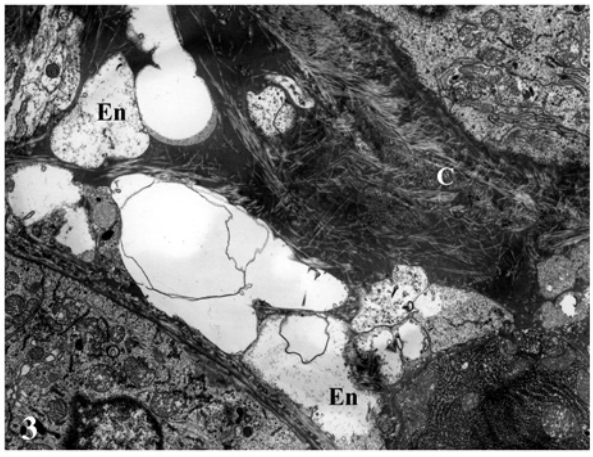


Figure 3. **Group VII (50 mg Cd/dm³). Fragment of blood vessel with damaged endothelial cells /En/. In the vicinity, a number of collagen fibres /C/. Mag. x 3000**



Apparatus was evidently well developed and had dilated cisterns. Numerous mitochondria exhibited damaged crests and matrix of increased translucence. Secretory granules in mucous cells contained numerous irregularly arranged "paracrystalline" condensations, some of which merged. The acinar lumen had a smoothed surface (*Fig. 2*).

Serous acinar cells contained a large number of secretory granules that varied in size. In tissue stroma, blood vessels with badly swollen endothelial cells and an increased number of collagen fibres were seen (*Fig. 3*).

Group VIII (50 mg Cd/dm³ + 30 mg Zn/dm³)

In this group, the nuclei of mucous acinar cells had irregular contours, large nuclei and chromatin condensed on the periphery. Some of the mitochondria showed increased matrix translucence, but with no distinct features of damage. Secretory granules of these cells merged to form "little lakes" of mucosa (*Fig. 4*).

In serous acinar cells, the granules were large and numerous. Increased cytoplasm translucence was sometimes observed. In tissue stroma, blood vessels with swollen endothelial cells and numerous collagen fibres were seen.

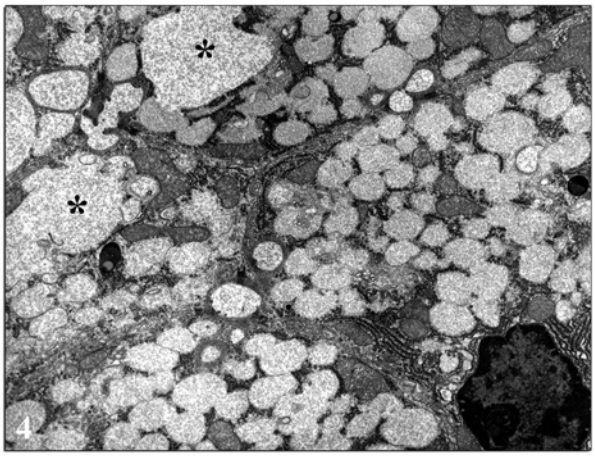
Group IX (50 mg Cd/dm³ + 60 mg Zn/dm³)

The ultrastructural picture of acinar cells was similar to the one observed in group VIII. There were, however, numerous regularly arranged condensations of mucous granules.

Discussion

Cadmium, lead, iron and mercury, known as particularly active xenobiotics, are the focus of many research studies. They manifest toxicity by damaging cell metabolism and disturbing membranous transport, cellular respiration, lipid metabolism and protein synthesis [7,13].

Figure 4. **Group VIII (50 mg Cd/dm³ + 30 mg Zn/dm³). Fragment of mucous cells with secretory granules merging to form the so called "little lakes" /*/. Mag. x 3000**



In the current study, the ultrastructural changes in the secretory cells of the submandibular salivary gland of rats were cadmium concentration-dependent. In the group with lower cadmium intoxication (5 mg Cd/dm³) no significant deviations were found as compared to control. In the rats receiving higher cadmium concentration (50 mg Cd/dm³), the ultrastructural picture showed very distinct damage to cell organelles, with the cell nucleus, mitochondria, Golgi Apparatus and rough endoplasmic reticulum being affected. The nuclear membrane was plicate and the number and size of nucleoli were increased, which may indicate activation and intensification of metabolic functions of the salivary gland cells. Degranulation of the rough endoplasmic reticulum and various degrees of mitochondrial damage could be seen, usually with swelling and increased translucence of the matrix. Czykier et al. [14], studying the submandibular

salivary gland in acute cadmium intoxication (50 mg Cd/dm^3) also observed nuclear membrane plication and Golgi Apparatus activation. However, at a concentration of 100 mg Cd/dm^3 these authors described secretory granules merging to form "little lakes" and with no damage to organelles, which may suggest the development of adaptive mechanisms or increased saliva secretion. Also in the current study, we observed an increase in the quantity of secretory granules varying in shape and accumulating around the acinar lumen. Smoothing of the acinar lumen and accumulation of numerous secretory granules may indicate a cadmium-induced disturbance in calcium ion homeostasis and membranous transport [15-17]. Damage to mitochondria can cause disturbances in cellular respiration, and degranulation of the rough reticulum may suggest disorders in the synthesis of the cell-specific proteins. Cadmium, competing with other metals in metalloenzymes affects protein synthesis, energetic processes, membranous transport, metabolism of lipids and nucleinic acids [17]. This metal reacting with the SH groups of proteins inactivates cytosolic and mitochondrial enzymes, thus reducing the activity of main mitochondrial enzymes – succinic dehydrogenase and cytochrome oxidase, which interferes with the processes of oxidative phosphorylation [18]. Changes in the biochemical processes of the Krebs cycle, due to cadmium binding to SH-groups, lead to a decrease in ATP and to metabolic deficiency of the cell. This may occur even at a low cadmium exposure [15]. In the cell, cadmium binds to proteins of the cytoplasm, mitochondrial and lysosomal membranes, and cell nuclei, which may affect the membranous transport of the cell [18]. Literature evidence seems to suggest that the toxic effect of cadmium can be accompanied by the formation of reactive oxygen forms which may exert a negative effect on cell metabolism [7].

Morphological changes of various degrees have been also observed in the salivary gland cells exposed to lead and were dose and exposure time-dependent [19]. In the cells of the parotid salivary gland of the rat, the authors described plication of the nuclear membrane, chromatin clumping, dilation of RER channels, Golgi Apparatus activation and features of considerable mitochondrial damage. They also found large lipid deposits and a smaller numbers of secretory granules in comparison to control.

Morphological changes have been noted in the liver and kidney due to cadmium exposure [20]. Among the early lesions in the kidney, swollen mitochondria with condensed matrix predominated and proliferation of smooth endoplasmic reticulum could be seen [8]. The ultrastructural picture of hepatocytes chronically exposed to cadmium showed an increase in perichromatin granules in the nuclei and nucleolar condensation [9]. This may suggest various mechanisms of metal actions and different cell "sensitivity" to a xenobiotic [21].

The combined administration of cadmium and zinc in the current study showed a protective role of zinc during exposure to cadmium. In the ultrastructural examination of the submandibular salivary gland exposed to cadmium + zinc, the latter reduced the intensity of morphological changes in the cells. With an increase in zinc concentration and no change in the level of cadmium, lesions in cell organelles were decreased. Numerous condensations in regularly arranged mucous granules indicated normalization of secretory functions of the submandibular salivary gland.

According to some authors, normalization in the picture of the salivary gland exposed to the simultaneous action of both metals may result from different mechanisms of the effect of zinc or can be due to interactions between the metals [22]. The interactions between zinc and cadmium can be explained by their affinity to metalloenzymes. Zinc is involved in many metabolic processes in the body and is necessary for their normal course. Adequate zinc concentration conditions the activity of over 200 enzymes. Due to its presence in many enzymes, zinc controls the pro- and antioxidative balance [23,24]. At high concentrations, zinc can also show antioxidative effects, regardless of the enzymes it builds. In the extracellular spaces, zinc protects sulphydryl groups against oxidation and prevents generation of reactive oxygen forms in the presence of such transitory metals as cadmium [25].

Conclusions

1. Exposure to cadmium induces ultrastructural changes in the submandibular gland, which are dose and time of exposure-dependent.
2. Exposure to zinc did not affect significantly the ultrastructural picture of the submandibular gland cells.
3. Zinc administered together with cadmium reduces the intensity of ultrastructural changes in the submandibular gland.

References:

1. Moniuszko-Jakoniuk J, Brzóska MM. Environment pollution and Health. Lek Wojsk, 1999; 75, 7-8: 419-26.
2. Kulikowska E, Moniuszko-Jakoniuk J, Miniuk K. Rola cynku w procesach fizjologicznych i patologicznych organizmu. Pol Tyg Lek, 1991; XLVI, 24-26: 470-3.
3. Waszkiel D, Dąbrowska E, Stokowska W. Influence of the work environment of the dentition glassblowers. Czas Stomat, 1998; 51, 1: 13-7.
4. Wielgus E, Kamiński M, Włoch S, Pawlicki K. Morphometric analysis of placenta terminal villi in women cigarette smokers. Acta Pol Toxicol, 2001; 9, 2: 153-63.
5. Brzóska MM, Moniuszko-Jakoniuk J. Disorders in bone metabolism of female rats chronically exposed to cadmium. Toxicol Appl Pharmacol, 2005; 207, 3: 195-211.
6. Plewka A, Plewka D, Nowaczyk G, Brzóska MM, Kamiński M, Moniuszko-Jakoniuk J. Effect of chronic exposure to cadmium on renal cytochrome P450-dependent monooxygenase system in rats. Arch Toxicol, 2004; 78, 4: 194-200.
7. Floriańczyk B. Toksyczne i kancerogenne właściwości kadmu. Now Lek, 1995; 64, 6: 737-45.
8. Nishizumi M. Elektron microscopic study of cadmium nephrotoxicity in the rat. Arch Environ Health, 1972; 24: 215-25.
9. Stoll RE, White JF, Miya TS, Bousquet WF. Effects of cadmium on nucleic acid and protein synthesis in rat liver. Toxicol Appl Pharmacol, 1976; 37: 61-74.
10. Czykier E, Szynaka B, Moniuszko-Jakoniuk J, Sawicki B. Ultrastructural and enzymatic studies of rat liver after acute cadmium exposure. Ann Acad Med Bial, 2002; 47: 203-12.
11. Żak I, Steibert E. Biochemiczne aspekty toksykologii kadmu. Post Hig Med Dośw, 1980; 34: 249-72.
12. Sjonstrand FS. Electron microscopy of cells and tissues, Vol I Instrumentation and techniques. Academic Press New York and London; 1967.
13. Stohs J, Bagachi D. Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med, 1995; 18: 321-36.
14. Czykier E, Szynaka B, Dzięcioł J. A preliminary study of the submandibular gland of the rat after one-year cadmium intoxication. Part

II. Pathomorphology and ultrastructure. Ann Acad Med Bial, 2004; 49 (Suppl. 1): 175-7.

15. Martel J, Marion M, Denizeau F. Effect of cadmium on membrane potential in isolated rat hepatocytes. Toxicology, 1990; 60: 161-72.

16. Brzóska MM, Jurczuk M, Moniuszko-Jakoniuk J, Roszczenko A, Bartosiewicz E. Effect of cadmium on calcium metabolism in rat. Bromat Chem Toksykol, 1996; 29, 1: 35-9.

17. Friberg L, Elinder CG, Kjellstrom T. Cadmium environmental health criteria. 1992; 134 World Health Organization, Geneva.

18. Norberg GF, Kjellstrom T, Norbert M. Kinetics and metabolism. Cadmium and Health: a toxicological and epidemiological appraisal. Eds: Friberg L, Elinder CG, Kjellstrom T, Norberger G, CRC inc., Boca Raton, Florida, 1985; vol. 1: 103-78.

19. Szynaka B, Andrzejewska A, Stokowska W. Ultrastructural evaluation of the parotid gland in the course of intoxication with lead acetate. Ann Acad Bial, 1994; 39: 121-9.

20. Brzóska MM, Kamiński M, Supernak-Bobko D, Zwierz K, Moniuszko-Jakoniuk J. Changes in the structure and function of the kidney of rats chronically exposed to cadmium. I. Biochemical and histopathological studies. Arch Toxicol, 2003; 77: 344-52.

21. Kamiński M, Wiaderkiewicz R. Czy i jak komórki uczestniczą w szacowaniu ryzyka stworzonego przez leki lub inne oddziaływania środowiskowe. Roczn Państw Zakł Hig, 2003; 54: 11-2.

22. Brzóska MM, Moniuszko-Jakoniuk J. Interactions between cadmium and zinc in the organizm. Food Chem Toxicol, 2001; 39: 967-80.

23. Ji LL. Exercise at old age: Does it increase or alleviate oxidative stress? Ann NY Acad Sci, 2001; 928: 236-47.

24. Kulikowska-Karpińska E, Moniuszko-Jakoniuk J. Influence of zinc on the activity of antioxidant enzymes in the liver of lead-exposed rats. Pol J Environ Stud, 1999; 8: 222-5.

25. Powell SR. The antioxidant properties of zinc. J Nutr, 2000; 130: 1447-54.