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Histopathological consequences of hyperzincemia on rat teeth. Experimental study

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Abstract

The aim of this study was to investigate the histopathological features of dental pulp in acute zinc (Zn) intoxication and to identify possible physiopathological mechanisms of the lesions. Twelve adult male Wistar rats were divided into two groups, the control one and the exposed group. Each animal from the experimental group received a single dose of zinc chloride ($ZnCl_2$) by intraperitoneal injection. Blood samples were collected from exposed animals at 2, 4, and 6 hours after the injection and plasma Zn concentrations were determined by spectrophotometry. After six hours of observation, the animals were sacrificed and two teeth from every rat were removed. Twelve teeth were processed by standard histological technique using Hematoxylin–Eosin (HE) and Szekely trichrome stainings, and the other twelve were subjected to Schliff cutting–grinding technique. The experimental group showed increased plasma zinc concentration (0.46±0.06 mg/L) after two hours and then slightly decreasing values in the next four hours. Undecalcified teeth did not showed any changing into the dentin or enamel structures, but decalcified teeth revealed numerous deposits into the dental pulp, which consisted of red acellular superposed sediments that could be made up of zinc with some plasma protein, or there could be an unknown compound which precipitated under the influence of zinc cation (Zn^{2+}). We can presume that the dental pulp may be an elective place for zinc accretion and so it must be considered a potential target for this metal.

Keywords: histopathological features, hyperzincemia, rat teeth, experimental study.

Introduction

Even though zinc is included in the periodic system of the chemical elements in the same group with two toxic metals, cadmium and mercury, it is a trace element relative harmless. Only at very high exposure, it has toxic effects, so acute zinc poisoning is a rare event. Moreover, unlike the other two metals, zinc has an essential role in cellular growth, cell proliferation, cell apoptosis, in the proper function of the immune system, as well as in the activity of numerous zinc-binding proteins, and also intervenes in the optimal metabolism of nucleic acids and proteins [1, 2].

In recent literature, there are articles presenting mostly the effects of dietary restrictions of zinc because this condition is widespread in the human population and causes immunodeficiency [3], low activity of various enzymes involved in beta-oxidation of unsaturated fatty acids [4], the compromise of the mammary glands function, altering the composition of milk from mothers who consume inadequate amounts of zinc [5]. Zinc deficiency may play a role in the pathogenesis of myocardial ischemia/reperfusion injury [6], growth retardation and abnormal bone formation, in regulation of dentin formation and periodontal tissues homeostasis, and in neurodegeneration [7].

Excessive intake of zinc is relatively rare, but many patients are chronically exposed to this metal on a regular basis through dentistry because of its use in certain restorative materials, mouthwashes, toothpastes, and denture adhesives. Of particular importance to dental professionals are various case reports concerning the neurological effects of excess zinc intake by patients who routinely use large quantities of zinc-containing denture adhesives [2].

Denture adhesives containing zinc salts in order to stabilize its composition are a possible source of hyperzincemia. This condition was first reported by Nations *et al.* [8], in 2008.

However, in recent years many articles were published about the effects of hyperzincemia on human and animal body. Researchers found microscopic lesions, even tissue destruction, in liver, pancreas, heart and stomach [9], kidney [10], spleen [11], lung [12], after oral or intraperitoneal administration of zinc oxide.

However, as far as we know, there are no data about zinc toxicity on teeth. Regarding zinc effects on the oral cavity structure, there is only one article that showed the effect of hyperzincemia on chemical characteristics and flow rate of saliva and the weight of salivary glands [13], but no histopathological effects were determined.

In this article, we investigated the histopathological features of dental pulp in acute zinc intoxication *via* a single intraperitoneal injection with zinc chloride ($ZnCl_2$) as the histological effects of plasmatic hyperzincemia on dentin–pulp complex were not studied until now even though Zn^{2+} cations effects were identified in these tissues related to zinc compound used in endodontic treatment.

Materials and Methods

Twelve adult male Wistar rats, 200±20 g body weight, were obtained from the Animal Facility of the „Grigore T. Popa" University of Medicine and Pharmacy, Iassy, Romania. They were divided into two groups, the control one and the exposed group, each consisting of six animals. They were placed in plastic cages located in the Animal Room of the Department of Human Anatomy, "Grigore T. Popa" University of Medicine and Pharmacy, Iassy.

They were maintained under standard conditions of temperature (22±2^{0}C), relative humidity (65±5%), and 12-h light/12-h dark cycle. The rats were fed appropriately using standard rat chow and water was provided *ad libitum* throughout the experiment.

Animal handling was ethically done according to the agreed guidelines for Animal Care and Use Committee of the "Grigore T. Popa" University of Medicine and Pharmacy, Iassy. All the animals were kept for five days for acclimatization to the laboratory conditions before the experiment started.

Blood samples (1 mL) from the tail veins were collected from all animals at the beginning of the experiment. Then, each animal from the experimental group received a single dose of $ZnCl_2$ by intraperitoneal injection (0.05 mEq/kg). Blood samples (1 mL) from the tail veins were collected again from the exposed animals at 2, 4, and 6 hours after the injection. The plasma zinc concentrations were determined for all blood samples by spectrophotometry.

At the end of six hours of observation, all the animals were given narcotics and they were sacrificed by sectioning the carotid arteries.

Two teeth for each animal, from the control group and the experimental one, were harvested. Firstly, the teeth were grossly viewed. Then, one tooth for each sacrificed animal were placed in 10% buffered neutral formalin as fixative solution and then in 10% ethylenediaminetetraacetic acid (EDTA), pH 7.3, as demineralizing solution.

After standard tissue processing, paraffin-embedded samples were sectioned into 5 μm thickness and mounted onto glass slides. The Hematoxylin-Eosin (HE) and Szekely trichrome techniques were used to stain the slides, and they were viewed using an optical microscope (Leica, Germany).

The other twelve teeth from sacrificed animals were subjected to Schliff cutting–grinding technique and viewed using the same optical microscope.

A one-way ANOVA (analysis of variance) for significance was performed to evaluate the toxicological data. All statistical analyses were performed by using freely available SPSS software, version 19. 0.

Results

Toxicokinetic study

After $ZnCl_2$ intraperitoneal injection of each animal from the experimental group, their plasma zinc concentration increased to 0.46±0.06 mg/L after two hours and then slightly decreased in the next hours until their sacrifice (Table 1).

Table 1 – ***Plasma zinc concentration: summary of toxicokinetic parameters***

Plasma levels	Zn [mg/L]	*P*
Initially	0.05±0.01	
2 hours	0.46±0.06	<0.01
4 hours	0.42±0.06	<0.01
6 hours	0.35±0.05	<0.01

P is calculated towards the initial values.

Gross pathology

At the macroscopic examination, no differences in terms of color or external features could be traced between the teeth of the experimental animals and the teeth of the animals from the control group.

Histopathological study

Control group

The histology of the control animal teeth was perfectly normal. Those treated by cutting–grinding technique revealed the dentin with its normal metabolic dentinal units and the enamel showed a layer with irregular thickness that covered the coronar dentin (Figure 1).

Figure 1 – ***Undecalcified tooth harvested from an unexposed rat revealed the normal structure of dentin and enamel (cutting–grinding technique, ×100).***

On HE and Szekely stainings, the dental pulp presented its two characteristic regions: the peripheral area made of odontoblasts, fibroblasts, Weil–Höhl cells, capillary blood vessels and nerves which formed a vascular-neural network beneath the layer of odontoblasts, and the central region, containing loose connective tissue, capillary blood vessels and nerves.

Experimental group

All the harvested teeth treated by Schliff technique did not showed any apparent changing into the dentin or enamel structures (Figure 2), but decalcified teeth showed similar histopathological features.

Figure 2 – ***Undecalcified tooth harvested from an exposed rat did not show any histological changes into the dentin or enamel structures (cutting–grinding technique, ×200).***

On HE-stained sections, it could be seen a mild inflammatory response with only few polymorphonucleated inflammatory cells, and numerous dilated and anastomosing capillaries filled with red deposits, mainly situated into the central part of the dental pulp. Despite the presence of these accretions, a relative normal histological structure of the dental pulp was preserved and no evidence of necrotic pulp tissue was seen. Histological sections prepared with Szekely trichrome staining also showed numerous dilated and branching capillaries filled with red amorphous deposits, mainly situated into the central part of the dental pulp (Figure 3, a–c).

With a high-power objective, we could established that these deposits were made by the superposition of amorphous, acellular sediments with various dimensions, but always filling the capillary lumina (Figure 3d).

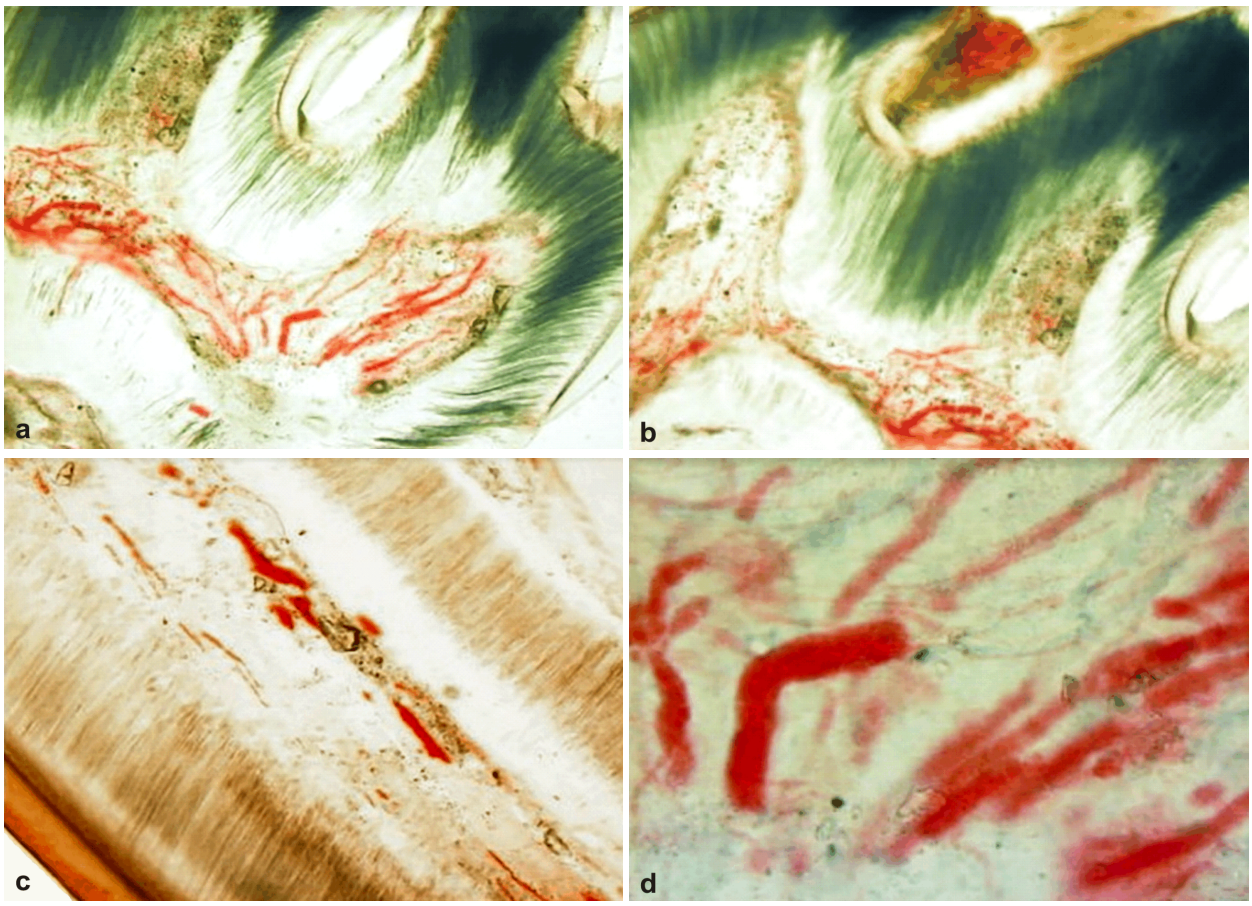

Figure 3 – ***Decalcified teeth from exposed rats: (a–c) In the central part of the dental pulp, there was a rich network of dilated capillaries containing red deposits in their lumina and mild inflammatory infiltrate around them; (d) High-power objective picture of dental pulp showed that the deposits were made by the superposition of some red amorphous acellular sediments. Szekely trichrome staining: (a and b) ×100; (c) ×200; (d) ×400.***

Discussion

In recent years, many published articles showed the high interest of the Romanian researchers to understand the pathological effects of endodontic and orthodontic treatment on dental pulp or dentin in order to identify the best way of treatment for their patients [14, 15].

For the same purpose, we investigated the effects of zinc chloride ($ZnCl_2$) on dental pulp, as we did not identify any other research in this field. This chemical substance, which is a white crystalline solid obtained by the action of hydrochloric acid on granulated zinc, is extensively used in endodontic treatment as part of the dental cements, or as component of the dental adhesives. It represents an element in the chemical formula of mouthwashes, which treat plaque, gingivitis, stomatitis and halitosis, as it has germicidal, fungicidal, anesthetic and healing properties [16], and it is contained in toothpastes used for polishing the teeth. Also, zinc chloride is used frequently as a hemostatic agent to control bleeding

gums or local bleeding in dental surgeries [16, 17]. This substance acts as an astringent and may be administered by retraction cords already impregnated with the agent or by applying it to cotton pellets. Zinc chloride affects only the superficial layer of gingival mucosa and precipitates the proteins, but do not penetrate cells. Therefore, this substance determines superficial and local coagulation [16].

Zinc enters into the human body by three major routes: inhalation, skin, or ingestion. In each case, there are specific effects affecting specific parts of the body [1].

The effects of Zn^{2+} cations on different organs were assessed by experiments made with various zinc compounds (zinc oxide, zinc gluconate, zinc chloride) administrated in various ways in experimental animals.

Some researchers showed that dental pulp filled with zinc oxide–eugenol (which is used temporary in dentistry works) stimulated an inflammatory reaction in the exposed pulpal tissue and this fact can be consider the proof of pulp response to the metal presence [18, 19]. Zinc oxide based root canal sealers injected subcutaneously also showed acute inflammatory response in the tissue with polymorphonuclear neutrophils even from the first day of injection [20]. Also, the injection of zinc gluconate into the cat testis firstly determined inflammatory cells response and after three months atrophic and dilated seminiferous tubules, a decrease in the number of germ cells, and incomplete spermatogenesis appeared [21].

Some researchers consider that zinc chloride is not toxic *per se*. Someya *et al.* [22] found that if this substance is used in endodontic medication, it failed to induce chromosome aberrations as some other dental medication did. In 2011, Lin *et al.* [23] showed that zinc chloride could promote odontoblastic differentiation of dental pulp stem cells through the up-regulation of gene metallothionein. However, it was demonstrated recently that drinking zinc chloride in over-doses causes corrosive gastroenteritis with vomiting, abdominal pain, diarrhea, and finally death, as it is a powerful irritant poison [24].

Also, Salvaggio *et al.* [25] demonstrated that zinc chloride had toxic effects on calcification process and can produce skeletal malformations. Banerjee & Chandra demonstrated by histopathological analysis of the respiratory organs of some fish that zinc chloride intoxication induced fine blood capillaries from the sub-epithelial connective tissues to anastomose extensively and to form an intensive network, which show congestion. The authors also found that the red blood cells acquired a globular shape and Zn^{2+} cations bounded with various S-containing proteins from the mucus [26].

In our work, we used the intraperitoneal injection of $ZnCl_2$ in order to obtain an acute toxicity with zinc and systemic effects. We found that the dental pulp from the teeth of our experimental animals exhibited mild inflammatory response with only few polymorphonucleated inflammatory cells, capillary hyperemia, edema, probably due to the presence of Zn^{2+} cations into the plasma of pulpal capillaries.

Also, we identified red acellular deposits in the dilated capillaries situated mostly in the central part of the dental pulp of all the teeth harvested from the experimental animals, but not from the control animals. We do not know the nature of these deposits, but we presumed that they could be made up of some compound of zinc with some plasma protein, or there could be an unknown compound, which precipitated under the influence of Zn^{2+} cations. We can only hypothesize the mechanism, as it is known that any chemical substance found in the blood can reach the dental pulp through the capillary vessels [27]. The presence of Zn^{2+} cations in the dental pulp capillaries could determine the pulpal vascular beds to become more permeable because the bivalent ion probably reacts as an irritant. The increased permeability of pulpal blood vessels promotes the release of blood plasma proteins into the pulp, and so pulpal fluid becomes more protein-rich than the normal one. These plasma proteins (mostly albumin and globulin) may bind with Zn^{2+} cations and could contribute to the development of those red acellular accretions.

In our experiment, the plasma zinc concentration increased in the first two hours and slightly decreased in the four coming hours after the injection. Choi *et al.* [12] showed that the Zn^{2+} cations disappear in 48 hours as they are eliminated from the body through urine and feces. These findings and our own results let us to presume that high levels of plasma zinc concentrations for a short period of time are not toxic *per se*, but could determine some changes in time as they can deposit in the dental pulp and could act later like an irritative substance.

Conclusions

Regarding our research, we must emphasize the fact that we found red accretions only in the dental pulp and not in dentin or enamel; such deposits could be probably correlated with loose connective tissue that can be found at this level. We can presume that the dental pulp may be an elective place for zinc accretion and so it must be considered a potential target for this metal, especially in case of hyperzincemia. However, the tissues of the oral cavity are, in general, the first, which make contact with zinc compounds in endodontic treatment, and a direct absorption through dentin into the dental pulp can appear. Therefore, these facts should aware the dental doctors and they should counsel their patients about the possible effects on dental pulp of the dental products containing zinc.

Conflict of interests

The authors declare that they have no conflict of interests.

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