Effect of sodium fluoride on the morphological picture of the rat liver exposed to NaF in drinking water

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Abstract

Purpose: **Due to its efficacy in caries prophylaxis and easy application, sodium fluoride (NaF) is still used for caries prevention in the form of fluoridated drinking water, fluoride tablets, fluoridated salt or milk. Effect of fluorides on various metabolic levels in hard and soft tissues, namely respiration as well as carbohydrate, protein, enzymatic and vascular metabolism, can disturb detoxication of fluorine compounds administered orally. The study objective was morphological examination of the liver of young and mature rats exposed to NaF in drinking water from conception till maturity, as well as after its withdrawal.**

Material and methods: **In the initial stage of the experiment, 30 female Wistar rats, 180-200 g body weight, were divided into 3 groups: one control and two experimental groups (I, II). Female rats in the experimental groups received fluorine in aqueous solutions of sodium fluoride (NaF) at a concentration of 10.6 mg NaF/dm^3 (group I) and 32.0 mg NaF/dm^3 (group II).**

Results: **The pathomorphological changes observed in the liver, particularly of young rats exposed to fluorides at superoptimal doses can help determine to what degree oral fluoride caries prevention is safe and whether it should be implemented. The transitory nature of pathomorphological changes in hepatocytes indicates adaptive potentials or defence mechanisms against orally administered sodium fluoride.**

Key words: fluoride, liver, rat, morphological picture.

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Introduction

Due to its efficacy in caries prophylaxis and easy application, sodium fluoride (NaF) is still used for caries prevention in the form of fluoridated drinking water, fluoride tablets, fluoridated salt or milk [1]. Effect of fluorides on various metabolic levels in hard and soft tissues, namely respiration as well as carbohydrate, protein, enzymatic and vascular metabolism, can disturb detoxication of fluorine compounds administered orally [2-4].

Fluorine, considered to be one of the environmental toxins [5], does not occur free in nature but thanks to high affinity for the ions of calcium, sodium, magnesium and tin it forms chemical compounds with them, which are more or less soluble in water [6].

Because of good solubility in water, easy absorption from the alimentary tract as well as for economic reasons, sodium fluoride (NaF) is the most commonly used compound in collective endogenic oral caries prophylaxis. During oral exposure, it can positively affect the oral environment. However, when consumed with food via the alimentary tract it can change, depending on dose and exposure time, cell and tissue metabolism in the further stages [1]. The organ that reacts rapidly to xenobiotics reaching the body from the outside is the liver. It is there where detoxication processes take place and the resulting pathomorphological changes are the response to the orally administered preparation.

Objective

The study objective was morphological examination of the liver of young and mature rats exposed to NaF in drinking water from conception till maturity, as well as after its withdrawal.

Material and methods

In the initial stage of the experiment, 30 female Wistar rats, 180-200 g body weight, were divided into 3 groups: one control and two experimental groups (I, II). Female rats in the

Figure 1. **Picture of a 30-day-old rat hepatocyte, control group. H+E stained. Magn. x400**

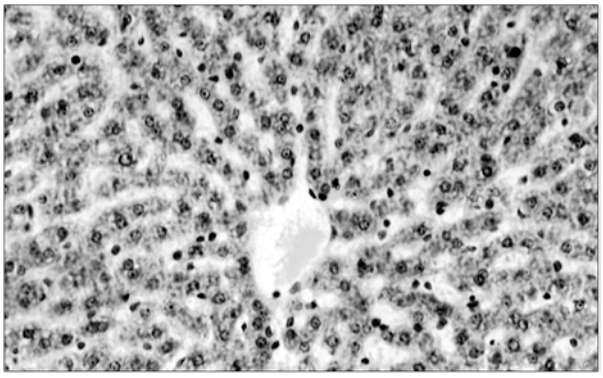
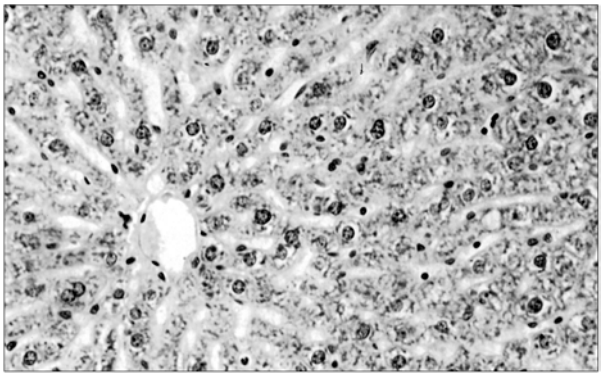


Figure 2. **Picture of a 30-day-old rat hepatocyte, examined group II (10.6 mg NaF/dm^3). Vacuolar degeneration. H+E stained. Magn. x400**



experimental groups received fluorine in aqueous solutions of sodium fluoride (NaF) at a concentration of 10.6 mg NaF/dm^3 (group I) and 32.0 mg NaF/dm^3 (group II), corresponding to a dose of 1.2 mg F/kg b.w. (group I) and 3.6 mg F/kg b.w. (group II). Sodium fluoride (NaF), (crystalline powder Natrium fluoride, Sigma, Germany) was dissolved in tap water. After a two-week adaptation period, during which the rats drank an average amount of 50 ml of water, the females were covered. They received NaF with drinking water 3 days before covering, during pregnancy and lactation. On day 30 of life, young rats were separated from mothers but were still given sodium fluoride with drinking water in the concentrations as above, ad libitum. Control animals received tap water ad libitum, in which the level of fluorine did not exceed 0.2 mgF/dm^3. On day 90 of life, NaF was withdrawn in groups I and II, and tap water alone was administered to all the animals. The experiment was terminated on day 120 of life.

Of various experimental models of intoxication with fluorine compounds, the one with fluoride administration via the alimentary tract in rat is the closest to oral fluoride prophylaxis in man. The concentration of 10.6 mg NaF/dm^3 drinking water, applied in the current study, results from a 10-fold lower sensitivity of rat to fluorine [7] and is considered to be the 'optimum' fluorine level for rat as compared to the acceptable level for man being 0.8-1 mg F/dm^3 of drinking water. The choice of NaF can be justified by its easy solubility in water, easy absorption in the alimentary tract and simple application in oral, both collective and individual, caries prophylaxis. Deionised water was not used to dilute NaF, as we believe that all micro- and macroelements contained in tap water are necessary for metabolic processes in tissues.

The animals were kept in standard environmental conditions [8]. The young were subjected to the action of fluoride from conception, through the foetal period, nest period (till day 30 of life) and maturity (day 90). After 90 days of exposure, NaF was withdrawn. The experiment was terminated on day 120 of life. Rats were fed on standard granulated LSM diet.

90 young rats were included in the experiment, 5 in each study subgroup. The animals were weighed and subjected to autopsy at the following age intervals: day 4 (newborn), day 14 (young rat fed on mother's milk); day 30 (young rat, receiving mother's milk, drinking water and standard diet from day 14 to day 30 of life – on that day, the young were separated from mothers but the experimental model was continued), day 60 (mature rat), day 90 (adult rat) and day 120 (NaF was not applied for 30 days). In all experimental animals, sections were collected from the anterior lobe of the liver. The material for light microscopic examination was fixed in 10% formalin, and embedded in paraffin cubes, cut on a rotary microtome into 7 μ sections, and stained with haematoxylin and eosin (H+E) [9].

The weight of the selected animals was monitored. The experiment was approved by the Bioethics Committee, Medical University of Białystok.

Results

Histological findings H+E

Control group (animals drinking tap water)

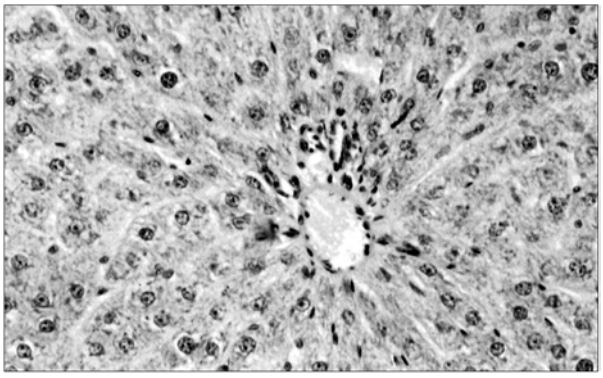
In the animals dissected on day 4 of the experiment, hepatocytes in all fields of vision formed chaotic spatial arrangements. Pictures of the liver showed the predominance of blood vessels and very numerous haemopoietic system cells that formed islets. Hepatocytes had a foamy, acidophilic cytoplasm. Cell nuclei were well stained, with distinct chromatin lumps and nucleoli. In 14-day-old rats, hepatocytes showed a distinct trabecular arrangement and contained acidophilic cytoplasm with markedly stained nuclei and nucleoli. In most fields of vision, lobules were distinct. The haemapoietic cells sporadically formed islets. In 30-day-old animals, the liver structure was already mature (*Fig. 1*). In 60-, 90- and 120-day rats, the structure of the organ was typical for adult animals. Slight vacuolar degeneration could be sporadically seen in the peripheral parts of the lobules.

Group I

(NaF concentration in drinking water – 10.6 mg/dm^3)

In the animals dissected on day 4, pictures of the liver did not differ markedly from those seen in the control group. In some fields of vision, hepatocytes contained vacuoles. In the livers of 14-day-old rats, in some hepatocytes the cell membrane was blurred. Blood vessels were slightly dilated. In 30-day-old

***Figure 3*. Picture of a 120-day-old rat hepatocyte, examined group II (10.6 mg NaF/dm^3), a few cells with features of vacuolar degeneration. H+E stained. Magn. x400**



rats, vacuolar degeneration-type changes occurred. Necrotic lesions were sometimes seen in hepatocytes (microfocal lesion). In 60- and 90-day-old rats the liver had a normal structure. In the peripheral parts of rare lobules slight vacuolar degenerations were found. In 120-day-old rats in this group, i.e. 30 days after NaF withdrawal, the vacuolar degenerations persisted, particularly in hepatocytes of the peripheral lobules.

Group II

(NaF concentration in drinking water – 32.0 mg/dm^3)

In this group, morphological changes in the liver were more pronounced in all the animals regardless of the time of dissection. Already in the livers of 4-day-old rats a large number of hepatocytes with vacuoles in the cytoplasm were seen. Blood vessels were dilated and filled with clotted acidophilic fluid. On day 14, hepatocytes showed features of damage, visible in the structure of cell and nuclear membranes. In the vicinity of blood vessels, inflammatory infiltration of neutrophilic granulocytes was observed. In the livers of 30-day-old rats, the changes were further intensified, and hepatocytes showed distinct vacuolar degeneration and micronecrotic foci (*Fig. 2*). In 60-day-old rats, the picture of intoxication in the liver was still present. In the livers of 90-day-old rats, the changes were becoming weaker. However, vacuolar degeneration-type changes still persisted in the livers of 120-day-old rats despite NaF withdrawal (*Fig. 3*).

Discussion

According to research surveys conducted by the International Research Agency for Fluorination, the toxicity of fluorine compounds is frequently ignored by medical doctors, dentists and paramedical staff involved in healthcare. Therefore, measures should be taken to obtain undoubted benefits resulting from the application of fluorine compounds in caries prevention and to minimize any side-effects [6,10-14].

Assuming that the liver is involved in the metabolism of toxic compounds produced during systemic transformations and exogenous toxins getting to the organism from the environment, we could expect to find both pathomorphological and metabolic changes as reactions to NaF. Therapeutics or toxins, to which NaF, depending on its dose, can be included, are likely to impair liver function and induce morphological changes in the liver [15,16-23]. Hepatotoxic action is manifested by cell respiration disorders that interfere with oxidation and reduction mechanisms, by impairment in protein, carbohydrate and lipid metabolism and by disturbances in intra- and extracellular transport. In consequence, whole cell or its cytoplasmic organelles can be damaged. Most frequently the damage is expressed as parenchymal vacuolar degeneration, necrosis of hepatocytes or disorders in the activity of metabolic enzymes [15,24-29]. In the livers of newborn rats in group II, slight changes were found in Browicz-Kupfer cells at higher fluoride concentration. Distinct blood vessel dilation could suggest metabolic disturbances in the liver, thus indicating a potential toxic effect of fluoride on the organ as early as in the foetal period. The livers of 14-day-old rats exposed to NaF in group I and II, receiving fluorine only with mother's milk, showed vacuolar degeneration-type changes, damage or blurring of cell or nuclear membrane and vessel dilation. The changes were more pronounced in group II, i.e. at higher fluoride concentration.

Lack of distinct morphological and enzymatic changes in the livers of 14-day-old animals of group I (lower NaF concentration) can be explained by the protective role of mother's milk [30]. Since morphological and ultrastructural changes were most pronounced in the livers of 30-day-old rats, we would like to discuss them more widely and compare with the findings of other authors. Up to day 30 of life, the rats stayed in nests, receiving both mother's milk, standard diet and NaF-enriched water to drink, which resulted in combination of drinking water fluorides with mother's milk fluorides. Already in group I, at the lower NaF concentration, apart from vacuolar degeneration also micronecrotic foci was observed. In group II, at the higher NaF concentration, vacuolar degeneration and numerous micronecrotic foci were seen to multiply as compared to group I.

After NaF withdrawal the changes in the liver in both groups were subsiding. The amount of glycogen increased in hepatocytes, and cell nuclei and endoplasmic reticulum were normal in appearance. Only mitochondrial polymorphism was maintained and damaged endothelial blood cells were sporadically seen.

Endoplasmic reticulum reacts rapidly to the action of toxic compounds [31]. Its rough component undergoes vacuolization (vacuolar degeneration) and loses ribosomes, which leads to a decrease in RNA. These changes are associated with the impairment in protein synthesis within the cell [32]. The smooth part of the endoplasmic reticulum can also be subject to vacuolisation or proliferation, which causes a considerable decrease in the count of glycogen granules in the affected sites [27,33]. This has been confirmed by our previous ultrustructural findings [34]. The electron microscope examinations revealed dilation of channels of the rough endoplasmic reticulum in 30-day-old group II animals, i.e. those exposed to the higher concentration of fluoride ions. Similar changes in the liver found in the rough endoplasmic reticulum have been described by Lavrushenko [34]. Detachment of ribosomes from the reticular membranes may indicate disorders in protein production within the cell, which may be caused, as Pasternak suggests, by a drop in tRNA

aminoacylation in the presence of fluoride ions [35]. A decrease in proteins produced by the rat liver at the time of exposure to fluoride has been also described by Wędzisz [36].

Additionally, in group II rats older than 30 days, the sinusal lumen of the liver sometimes showed collagen bundles accompanied by micronecrosis observed in the morphological examination, which could suggest the beginning of liver fibrosis.

From day 30 till day 90 of NaF administration, the pathomorphological changes showed a gradual decrease in intensity and only considerable dilation of blood vessels with endothelial swelling was observed. Taking into account a continuous exposure to NaF, the decrease could be the result of adaptive mechanisms of the organism to fluoride, which have been discussed by Machoy-Mokrzyńska [37]. Hepatic hyperaemia after administration of acetate acid to rats has been observed by Luty [38]. In some systemic diseases, hyperaemia seems to be beneficial, e.g. in myocarditis. The findings of laboratory and epidemiologic studies conducted during water fluoridation period suggest that the mortality rate due to heart infarct may have decreased due to fluorine compounds present in drinking water [39,40].

Some literature reports as well as our own findings (unpublished) seem to prove that fluorine accumulates in the liver [41]. Its blood level depends on fluoride supply, which refers to all fluorine forms in blood serum [30]. As revealed by Chlebna-Sokół, due to high homeostasis of the serum, blood fluoride levels remain constant even in the case of overdosage [42].

The NaF-induced morphological changes in rat hepatocytes create a picture similar to those observed after intoxications with other toxic compounds administered to experimental animals. It can be assumed that the liver is involved in detoxication of excessive fluorine doses. After NaF withdrawal, the changes in the liver in both groups were subsiding. The glycogen count increased, but in a considerable number of cells vacuolar degeneration persisted.

Remission of most pathomorphological changes after NaF withdrawal may suggest their transitory nature. However, at the time of exposure sodium fluoride affects the development of the organism both in the prenatal and postnatal period of experimental animals.

Conclusions

Although the findings obtained for rats cannot be directly referred to the human body, the pathomorphological changes observed in the liver, particularly of young rats exposed to fluorides at superoptimal doses can help determine to what degree oral fluoride caries prevention is safe and whether it should be implemented. The transitory nature of pathomorphological changes in hepatocytes indicates adaptive potentials or defence mechanisms against orally administered sodium fluoride.

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