EFFECT OF FLUORIDE IN DRINKING WATER ON THE MINERAL COMPOSITION OF VARIOUS AREAS OF RAT MOLAR DENTIN

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SUMMARY: The amounts of calcium, phosphorus, fluorine, sodium, magnesium and zinc in various dentin zones in rats receiving 0, 1 or 19 ppm fluoride in their drinking water were determined. Wistar rats of both sexes were weaned at 22 days, weighed, marked and allotted to one or other of three groups of 10. All of the animals were fed ad libitum on a modified Stephan-Harris diet containing 43% sucrose plus 22% wheat flour, 32% skim milk powder, 2% whole liver powder and 1% corn oil, and distilled water, to which 0, 1 or 19 ppm of fluoride had been added. Elements of dentin samples were determined in a JEOL JXA-733 Super Probe electron-probe microanalyser (EPMA). The high level of fluoride in the drinking water (19 ppm) resulted in a significant increase in F, Na and Zn levels in the dentin close to the pulp. Ca, P and Mg were evenly distributed throughout the dentin, although slight accumulation of Mg to the dentino-enamel junction could be seen.

Key words: Dentin; Mineral composition; Rat; Spectrum analyses; Tooth calcification; Water fluoride.

Introduction

Fluoride has been shown to reduce dose-dependently the area of dentin formed in molar crowns as well as to reduce the incidence of caries in young rats fed a cariogenic diet. The anti-caries effect of fluoride in drinking water was more pronounced with 19 ppm of fluoride than with 0, 1 or 7 ppm of fluoride in reducing occlusal caries in rats. Combination of fluoride in drinking water (19 ppm) and a high-cariogenic diet diminished dentin apposition in rat molars.

A negative correlation between areas of caries in dentin and dentin formed under the lesions has been observed in young rats. Apposition of dentin and progression of dentinal caries have been found to be ten times greater in young rats than in adult rats. These findings suggest that fluoride, especially when concentrations of sucrose in the diet are high, could affect progression of dentinal caries and quantity and/or quality of dentin formed during carious challenge.

The aim of the study reported here was to discover whether fluoride in drinking water, in addition to its effect on dentin apposition during carious challenge, affects the mineral composition of dentin. Concentrations of Ca, P, F, Na, Mg and Zn in the dentin of rats fed a cariogenic diet and 0, 1 and 19 ppm fluoride in their drinking water were determined.

Materials and Methods

In the present study the same experimental design, which was known to result in quantitative differences in morphometric features of dentin apposition and significant differences in progression of dental caries was followed. These experiments resulted in mean total occlusal caries incidence of 12.0, 10.2 and 9.8 in the animals receiving 0, 1 or 19 ppm fluoride in their drinking water, respectively, and the mean incidences of advanced occlusal dentin caries were 9.7, 4.6 and 0.1 in those animals.
Wistar rats of both sexes were weaned at 22 days, weighed, marked and randomly distributed into three groups of 10. All animals were fed ad libitum a modified Stephan-Harris diet containing 43% sucrose plus 22% wheat flour, 32% skim milk powder, 2% whole liver powder and 1% corn oil and distilled water to which 0, 1 or 19 ppm fluorine in the form of its sodium salt had been added (Merck 6449). The calcium content of the diet was 0.59, and the content of phosphorus was 0.39 percentages by weight. To cause the cariogenic infection all animals were inoculated with a fresh suspension of *S. sobrinus* (ATCC 27351 K1 Fitzgerald) on days 23 and 24.

The fluoride content of the rat food was measured by a modified microdiffusion method after perchloric acid digestion. An Orion fluoride-sensitive electrode 94-09 was used, with a pH-mV meter. The free fluoride content of the sucrose-supplemented food was zero, and the total fluoride concentration of it was 5 ppm.

The animals were housed two per cage under normal atmospheric conditions at 21°C. Each animal was subjected to the same regimen of lightning (12 hours light, 12 hours dark), and times of feeding, human handling and noise remained the same.

On day 49 the animals were killed under slight ether anaesthesia, and their mandibles were sectioned sagittally, following the method of Keyes. (Figure 1).

Figure 1. Sagittal sectioning of rat mandible according to the method of Keyes.

For scanning electron microscopy analyses, bulk sample specimens were embedded in epoxy resin, leaving the sectioning plane free. The polished specimens were then coated with carbon for detailed mineral analyses in a JEOL JAXA-733 Super Probe electron probe microanalyzer (EPMA) with a ZAF correction program and nature hydroxyapatite as a standard at the Institute of Electron Optics, University of Oulu. Calcium (Ca), phosphorus (P), fluorine (F), sodium (Na), magnesium (Mg) and zinc (Zn) were determined. The magnification used in determination of mineral composition was 1000 X, the examining spot diameter was 10 μm. Three measurements were performed on each tooth: 1) near the dentino-enamel junction (DEJ), 2) in the middle of the dentin layer (MID) and 3) at the dentin close to the pulp (PULP) (Figure 2). If there was a carious lesion in dentin, the sample was taken from the sound dentin beside the lesion. Samples were taken from intertubular dentin.

Figure 2. COMPO image of the enamel (white area on top) and the dentin (porous structure) under the main central transverse fissure of a mandibular molar of a rat supplied with drinking water containing no fluoride. Measurement sites are shown as white spots at the dentino-enamel junction (DEJ), in the middle of the dentin (MID) and at the dentin close to the pulp (PULP).
The specimens were also analyzed by means of back-scattered electron images (COMPO) (JSM-6400 Scanning electron microscopy, JEOL Ltd, Tokyo, Japan; Link TETRA back-scattered detector and Link eXL energy-dispersive image-processing analyzer with GIPFOUR color pallet, Link Analytical, High Wycomb, England), so that the change of minerals in the dentin could be visualized. The accelerating voltage was 15 kV, the beam current exactly 1.5 nA, and the working distance 25 mm. In the COMPO images, the grey levels are determined by the mineralization degree, with white resembling the best mineralization (sound enamel) and black resembling the areas with no minerals.

Statistical analysis of results was undertaken by the Mann-Whitney test.

Results

The highest concentration of fluoride (19 ppm) in drinking water resulted in a doubling of F content in dentin close to the pulp (P < 0.001) as compared to the reference samples (Figure 3). In groups drinking water containing fluoride, dentin

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Figure 3. Levels of F and Mg in teeth (percentages by weight, ordinate) measured from three sites of dentin: DEJ = dentino-enamel junction, MID = middle of the dentin and PULP = dentin close to the pulp (abscissa), in rats supplied with drinking water containing no fluoride, or 1 or 19 ppm of fluoride.
F levels decreased from the dentin near to the pulp chamber towards the dentino-enamel junction. F levels were uniform at all sample sites in the group with no fluoride in their drinking water.

The Mg level was nearly constant throughout the dentin, except at the dentino-enamel junction, where the Mg level was slightly increased in the high-fluoride samples ($P < 0.01$) as compared to the controls (Figure 3). High fluoridation of drinking water increased Na levels at all sample sites ($P < 0.001$) (Figure 4). The highest concentration of fluoride (19 ppm) in drinking water resulted in a considerable increase in Zn levels in dentin close to the pulp ($P < 0.05$) as compared to the control samples (Figure 4).

Ca content varied from 27.6 (± 5.1) to 31.1 (± 1.4) percentages by weight, and P content from 14.2 (± 3.2) to 16.6 (± 0.7). The contents of Ca and P in the dentin close to the pulp were similar to those at other measurement sites, regardless of the amount of fluoride in the drinking water. Slight increases in Ca and P levels were found at the dentino-enamel junction of animals supplied with drinking water containing fluoride.

Figure 4. Levels of Na and Zn in teeth (percentages by weight) expressed in the same way as in Figure 3.
In the COMPO images, the highly mineralized, white bands of dentin were clearly seen in the dentin formed during the experiment in the specimens of the high-fluoride group (Figure 5b and 5c), but were absent in the samples of the non-fluoride animals (Figure 5a).

Figure 5a (below). COMPO image of the tooth structure adjacent to main central transverse fissure of a second mandibular molar of a rat receiving no fluoride in the drinking water. The density of dentin is slightly decreased under the enamel due to initial dentin caries, but is uniform otherwise throughout the dentin. No hypermineralized areas.

Figure 5b (upper right). COMPO image of the tooth structure adjacent to main central transverse fissure of a second mandibular molar of a rat receiving 19 ppm fluoride in the drinking water. Hypermineralized areas are seen as white bands in the dentin (arrows).

Figure 5c (lower right). COMPO image of the tooth structure adjacent to main central transverse fissure of a second mandibular molar of a rat receiving 19 ppm fluoride in the drinking water. A large area of decreased density due to dentinal carious lesion is situated under the dentino-enamel junction. Hypermineralized bands in dentin are seen even more clearly than in Figure 5b.
Discussion

The animals fed a cariogenic diet and treated with fluoride at concentrations 1 or 19 ppm in their drinking water have been reported to have fewer carious lesions than those which did not receive fluoride, and the progression of dentinal lesions is efficiently inhibited with 19 ppm fluoride in drinking water. The apposition of dentin was decreased in rats receiving 1 or 19 ppm fluoride in their drinking water as compared to rats receiving no fluoride. The experiment here reported was designed to find out if there were, in addition to those quantitative differences, any alterations in amounts of some elements of dentin mineral, which are considered to be of importance in dentin apposition and mineralization.

In the present study fluoride levels were greatest in the dentin next to the pulp chamber and decreased peripherally in animals drinking fluoridated water, but were evenly distributed in reference animals (Figure 3.). In human permanent and deciduous dentition, highest concentrations of fluoride have been found at the pulpal surface. In the deciduous molars of 7-year-old children, fluoride concentrations at the pulpal surface were 250 ppm in a low-fluoride area (< 0.1 ppm), whereas in a high-fluoride area (2 ppm) pulpal concentrations were almost 1000 ppm. From the results of the secondary ion mass spectrometry (SIMS) studies it was concluded that the fluorine contents of water directly influence the fluorine contents of both deciduous and permanent teeth.

A possible explanation of peripulpal accumulation of fluoride in dentin is ready access to ionic exchange between dentin and the vascular system. Fluoride uptake is probably related to the tubular structure of dentin, amount of dentinal fluid and metabolic activity of the tissue. Accumulation of fluoride in dentin may result partly from passive diffusion from the vascular system but may also be partly in result of active transport by the odontoblasts. Transportation of Ca²⁺ ions through the odontoblasts into the dentin close to the pulp has been described recently.

In the study reported here, except for the higher levels of Mg at the dentino-enamel junction if fluoride was present than if it was absent, the Mg level is nearly constant within the dentin (Figure 3). High tissue Mg levels in the presence of fluoride have been recorded before. In precipitation studies, Mg²⁺ ions have been found to promote the formation and stability of dentin apatite crystal-lites. Magnesium, although chemically related to Ca, is for size reasons relatively unwilling to substitute into apatite, and such incorporation probably entails qualitatively adverse effects.

In the earlier SIMS studies the level of Na has been found to be constant throughout the human dentin, also in teeth from high-fluoride areas. This is in disagreement with the study presented here, in which the Na levels in all sample sites were higher in the high-fluoride group as compared to the non-fluoride group. However, an accumulation of Na at the interface between the pulp and predentin has been found in a recent SIMS study. The differences in results may be due to different ways of sample preparation.

In the study reported here fluoridation of water (19 ppm F) significantly raised levels of Zn in the dentin close to the pulp. Results of animal studies have shown a clear relationship between Zn deficiency and high levels of caries, with greater penetration of the lesion into dentin. Zinc has important metabolic functions and
is found in many enzymes. Zinc induces increases in levels of the alkaline phosphatase (AFOS) which is related to DNA synthesis and, as a result, stimulates bone growth. AFOS has been demonstrated in excised, homogenized tissue of odontoblasts and adjacent dentin in rat maxillary incisor teeth. In a series of papers by Engström and coworkers, the enzyme activity was reported in teeth of normal rats and of rats fed a diet deficient in vitamin D and calcium. In human teeth, alkaline phosphatase is found mainly in the predentin layer and subodontoblastic layer of pulp. Higher Zn levels (234 - 325 µg/g) have been found in the circum-pulpal area of human coronal dentin than in other layers of dentin. So, the amounts of fluoride and zinc are high in the same areas of dentin, and also in those areas the formation of alkaline phosphatase is increased, which might result to alterations in DNA synthesis and in quality and quantity of dentin apposition.

Levels of Ca and P in dentin were the same in animals drinking fluoridated water and non-fluoridated water in the study reported here. After fluoride therapy of patients with osteoporosis, the content of Ca and P in bone samples remained unchanged, although there were crystallographic and chemical changes which were similar to those caused by prolonged use of drinking water containing two to four ppm fluoride. Levels of Ca and P, and levels of F, Na, Mg and Zn measured in the study reported here are similar to those measured in human deciduous teeth.

COMPO images have been used to visualize the changes in tooth mineral density. Hietala and coworkers compared rat dentin caries recording with COMPO images, dentin fluorescence and Schiff's reagent. They found that the COMPO images correlated well with the expression of dentin caries by Schiff's reagent and the change in the dentin fluorescence. So, in the study reported here the black areas just below the enamel in Figures 5a and 5c are considered to be due to the caries process. As shown in Figures 5b and 5c there are great differences in the mineral density of the dentin beneath the enamel in rats receiving 19 ppm F in drinking water. However, advanced dentin lesions occur also in high-fluoride groups although the incidence of those lesions has been found to be 0.1 compared to 9.7 in rats receiving no fluoride in their drinking water. Krook and Ferretti have found similar alterations of mineral density in COMPO images of rat molar dentin as in this study. In their opinion the phenomenon was an expression of enhanced dentin resorption, which they believe to be the primary event in experimental caries in rat.

In the study reported here the visual difference between the COMPO images from the high-fluoride samples and non-fluoride samples could clearly be seen (Figures 5a, b and c). If the carious process in dentin was advanced, the lamination phenomenon in dentin was even more evident (Figure 5c). In a recent in vitro study it has been shown that as a result of a demineralization-remineralization cycling of a fluoride-enriched dentin specimen, there was a tendency for mineral distribution deeper in the dentin lesion leading to a lamination phenomenon. The authors concluded that the stability of the incorporated fluoride and the presence of a remineralized surface layer makes the dentin more resistant to cariogenic challenges.

Fluoride in drinking water at concentrations of 1, 7 and 19 ppm reduced dentin apposition in the animals fed a highly cariogenic diet. In the study reported here a high concentration of fluoride in drinking water led to high levels of F, Na, and Zn in rat dentin close to the pulp, but Ca and P were evenly distributed throughout dentin.
This finding supports the possibility that fluoride ions may have an odontoblast-mediated effect on dentin formation because the changes were observed in the area of dentin where the nuclear parts of the odontoblasts are located. The presence of fluoride ions in tissue fluid is likely to have an influence on interactions between minerals but could also affect nearby odontoblasts involved in tissue growth and development via collagen synthesis and dentin mineralization.

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References