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Polychlorinated dibenzo-*p*-dioxins and dibenzofurans via mother's milk may cause developmental defects in the child's teeth

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Abstract

Previous studies have shown developmental dental defects in rhesus macaques and rats experimentally exposed to dioxin. Now it was investigated if dioxin exposure from mother's milk in a normal breast-fed child population correlated with enamel hypomineralization of teeth that mineralize during the first 2 years of life.

We studied 102 6–7-year-old Finnish children breast-fed for an average of 10.5 months. Milk samples were collected when the child was 4 weeks old. The concentrations of 17 most toxic polychlorinated dibenzo-*p*-dioxin and furan congeners were determined. The total exposure to dioxins was calculated from the concentrations in milk and the duration of breast feeding.

Hypomineralization of the target teeth was found in 17 children. Both the frequency and severity of the lesions correlated with the total exposure. The results suggest that at the prevailing levels in human milk, dioxin may be an important cause of hypomineralization in the developing teeth of children.

Keywords: Dioxin; Human milk; Breast feeding; Dental enamel abnormality

1. Introduction

Once formed, dental hard tissues are not remodeled. Hence, disturbances in the function of odontoblasts (dentin forming cells) and/or ameloblasts (enamel forming cells) lead to definite morphological consequences which render it possible to determine the time of the damage. This makes the dental hard tissues unique as compared with other human tissues.

Etiologically the dental disturbances can be genetic, acquired (related to e.g. nutritional deficiencies, high fluoride intake, drugs such as cyclophosphamide and tetracyclines) or, most often, idiopathic. A Swedish study on the prevalence of idiopathic enamel hypomineralization reported that in the late seventies, an increasing number of children showed extensive and severe mineralization defects especially in the permanent first molars, and that children born in 1970 had more idiopathic enamel hypomineralization than those born before or after (Koch et al., 1987). The findings imply that the dental defects might be associated with environmental changes.

Polychlorinated dibenzo-*p*-dioxins (PCDD) and furans (PCDF), belonging to a class of environmental pollutants known as polyhalogenated aromatic hydrocarbons, are a focus of public concern because of their toxicity in animal studies, their ubiquity in our food and in the environment, and their involvement in some well-publicized environmental contamination incidents (Peterson et al., 1993).

After accidental exposures of human infants to polychlorinated biphenyls (PCB) and dioxin-like compounds a variety of dental changes such as mottled, chipping and carious teeth have been reported (Hara, 1985; Rogan et al., 1988). In rhesus macaques exposure via food has been shown to cause squamous metaplasia of specialized epithelial cells such as ameloblasts (McNulty, 1986) leading to defective amelogenesis. Also, studies on the continuously growing rat incisors have indicated impaired dentin and

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enamel formation after exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Alaluusua et al., 1993). Similar defects have been found in rat pups after their breast feeding dam was exposed to TCDD (Lukinmaa et al., unpublished observations). Since dioxin-like compounds are detected in human milk and thus infants may be exposed to high cumulative doses via breast feeding (Yrjänheikki, 1989) we studied if PCDD/F compounds could be among the causative agents in enamel hypomineralization, often found in newly erupted permanent first molars. The permanent first molars were chosen as the target teeth since they are mineralized during the first 2 years of life, the time when the child is exposed to PCDD/F via mother's milk.

2. Subjects and methods

2.1. Milk sample collection

Women were recruited between January and May, 1987, from one of the maternity hospitals in Helsinki, the capital, and the maternity clinic of Kuopio University Hospital in Eastern Finland. The study is part of a WHO/EURO coordinated follow-up study on the levels of PCDDs and PCDFs in breast milk. All women giving birth during that time period were invited, and about 150 mothers from both areas promised to collect a milk sample after 4 weeks, provided that they were still nursing. Altogether 168 milk samples were obtained, 77 samples from Helsinki and 91 samples from Kuopio.

2.2. Determination of PCDDs and PCDFs

About 40–80 ml of each sample, equivalent to 1.4 g fat, was spiked with 100 pg of ^{13}C-labelled PCDD/F standards. Milk fat was extracted with diethyl ether/hexane and the fat content determined. The extract was defatted in a silica gel column and cleaned up with activated carbon and alumina columns before analysis by high resolution gas chromatography mass spectrometry with resolution 10 000 (Vartiainen et al., 1995). Levels of the 17 most toxic PCDD/Fs were expressed in TCDD toxic equivalents calculated by using the international equivalency factors (I-TEQ, NATO/CCMS, 1988). A full description of the analytical methods used in this study and the detailed PCDD/F results will be published separately. The laboratory has participated successfully in international quality control studies for analysis of PCDD/Fs in cow milk samples organized by EU/BCR project in 1993 (Ryman, 1994; Schimmel et al., 1994).

The total PCDD/F exposure of a child was calculated from the concentrations in milk (after having taken into account a yearly 25% first-order decline during lactation) and the duration of breast feeding (Table 1). The rate of decline was based on previous results (Lindström, 1988), and the present finding indicating approximately 25% decrease in PCDD/F from primiparae to those who nursed the second baby (data not shown). This decline corresponds to a reasonable elimination coefficient of about $k_e = 0.001/\text{day}$.

When the child was 4 weeks old the mother filled a questionnaire on smoking habits during the pregnancy (cigarettes/day), social status and work. The mothers were also asked about an exposure to harmful compounds in their work. In association with the dental examination of the child the mother was interviewed on the duration of nursing.

2.3. Dental examination

6–7 years later we studied the dentitions of 102 children of mothers whose milk had been analyzed. Mineralization defects were classified as severe (loss of enamel in association with affected dentin), moderate (loss of enamel only) and mild (color change: white, yellow or brown).

Table 1
Mineralization defects of the permanent first molars in relation to the total exposure to polychlorinated dibenzo-*p*-dioxins/furans [a] via mother's milk

Mineralization of the permanent first molars	Number of children with		
	Low exposure (< 8.0)	Moderate exposure (8.0–16.0)	High exposure (> 16.0)
Normal	22	41	22
Mild defect in only one tooth	1	5	2
Moderate defect or mild defect in more than one tooth	0	3	4
Severe defect	0	0	2
All	23	49	30

[a] The total exposure value (pg × year/g milk fat) was calculated from the AUC formula:

$$\text{AUC} = \frac{C \times (1 - e^{-k_e t})}{k_e}$$

where AUC is area under the curve C and the duration of breast feeding, C is the measured concentration of polychlorinated dibenzo-*p*-dioxins/furans as international toxic equivalents, k_e is elimination constant of polychlorinated dibenzodioxins/furans to milk ($k_e = -0.2877/\text{year}$) and t is time of nursing (in years).

The size of the defect was classified as large (about 4.5 mm or larger), moderate (about 3.5 mm) or small (diameter about 2 mm). To facilitate the assessment of the size, the size of the defect on the tooth surface was compared with drawings of three example sizes on a study model. In the assessment, tooth surface was treated as two units: occlusal surface together with the cusps formed one unit and the proximal surfaces formed another unit. If both units had a defect the larger one was chosen to represent the status of the tooth. The small defects were not included in the final analysis. Hypoplastic defects were recorded (FDI Commission on Oral Health, 1992) but not included in the final analysis. In the records all changes diagnosed as dental fluorosis or major disturbances in general health were excluded. Heritable defects in tooth structure were not seen. In all cases neither the examiner nor the mother had knowledge of the results of the TCDD/F analyses before the clinical examination.

2.4. Treatment of data

Mineralization changes in the permanent first molars were scored into four levels (Table 1): normal mineralization of enamel in all teeth examined (level 0); mild defect in mineralization in only one tooth (level 1); moderate defect or mild defect in more than one tooth (level 2); and severe defect (level 3). The changes were compared with PCDD/F TEQ exposure values using Mann-Whitney U test (level 0/level 1–3), Mantel Hänszel χ^2 test and regression analysis (the level as dependent variable and the total exposure value as independent). The duration of breast feeding in children who had mineralization changes and in those who had normal appearing teeth were compared by Mann-Whitney U test. Mann-Whitney U test was also used to study the association of mineralization changes and I-TEQ of the mother's milk at the time when the child was 4 weeks old.

3. Results

A total of 17 children (17%) showed mineralization changes in the permanent first molars (Fig. 1). One out of the four permanent first molars was affected in eight children, two molars were affected in two, three in five, and all four molars in two children. Of the lesions, 20 were in maxillary teeth and 15 in mandibular teeth. Of the lesions 29 were mild and six moderate or severe. When moderate or severe, the contralateral tooth was always also affected. Ten children had lesions of moderate size and seven children of large size.

I-TEQs ranged from 3.8 to 99.4 pg/g milk fat (mean $\pm$ S.D., 19.8 $\pm$ 10.9 pg/g). The duration of breast feeding ranged from 1 to 36 months (10.5 $\pm$ 5.5 months). In Table 1, the distribution of the total exposure (classified in three groups) and the levels of mineralization changes is presented. The changes in the permanent first molars occurred more often (Mann-Whitney U test, $P = 0.017$) and were more severe (regression analysis, $R = 0.3$, $P = 0.003$, Mantel Hänszel χ^2, $P = 0.010$, Table 1) in children who were exposed to a higher amount of PCDD/F compounds via mother's milk than in those who were less exposed. The duration of breast feeding alone, was not associated with mineralization changes (Mann-Whitney U test, $P = 0.44$). I-TEQs of the milk when the child was 4 weeks old were also not alone significantly associated with the occurrence of mineralization changes (Mann-Whitney U test, $P = 0.17$).

None of the mothers reported an exposure to putative harmful compounds in their work environment. 12 mothers

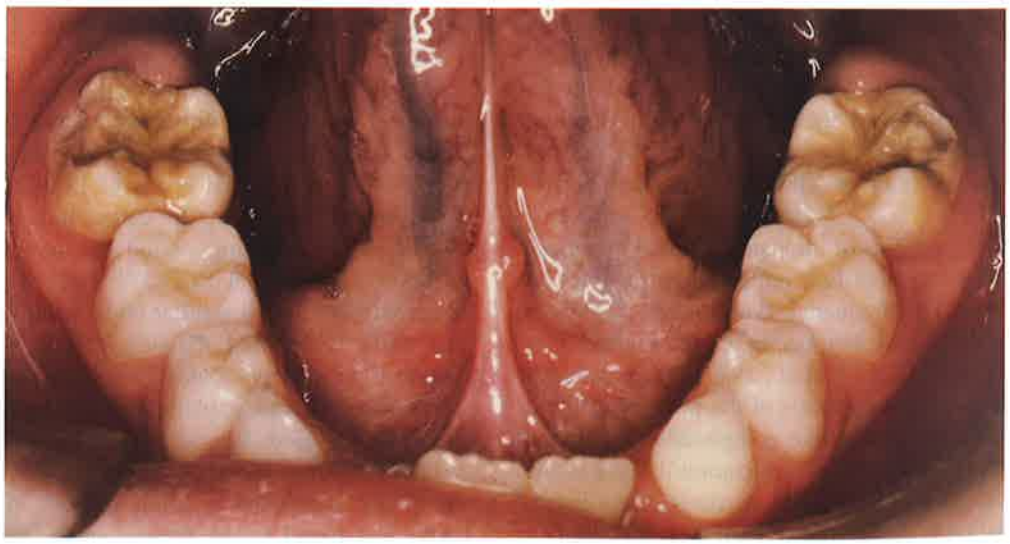

Fig. 1. Lower teeth of a 7-year-old girl. The permanent first molars have symmetrical mineralization defects which have developed in infancy.

had a history of cigarette smoking during the last 12 months before the delivery. All children of these mothers had normally mineralized permanent first molars.

4. Discussion

Mineralization of the permanent first molars starts at birth and at the age of two most of the crown is mineralized (Massler et al., 1941). Thus we presume that the defects seen in the crowns of the permanent first molars had mainly developed during the first 2 years of life. The data suggest that PCDD/Fs in mother's milk may be a causative agent of enamel hypomineralization among other known and unknown factors. As shown in Table 1, all nine children who had moderate or severe lesions or mild lesions in more than one of the four molars belonged to the moderate or high exposure groups while there was only one child who belonged to the low exposure group and had a mineralization defect (mild, in one tooth only).

Breast feeding is common in Finland and it lasts longer than in many other countries (Verronen, 1988). Therefore Finnish infants may be exposed to foreign compounds in milk to a greater extent than infants elsewhere. In the early eighties, two Finnish mothers in three nursed their children at least 6 months while at the same time only 5% of mothers in French- and German-speaking populations of Europe nursed their children 5–6 months (Salmenperä, 1986). In the present study population, the duration of breast feeding (mean 10.5 months) was longer than average. This resulted from the study protocol excluding mothers who were no more nursing 4 weeks after child birth.

Serum PCDF levels in children were shown to depend significantly on the duration of breast feeding in studies on the Yu-Cheng incident (Ryan et al., 1994) where the mothers had been exposed to contaminated rice oil. The levels were maintained long after the exposure. In the present study the occurrence of mineralization changes in the permanent first molars did not correlate significantly with the duration of breast feeding alone, but it did correlate with the total exposure which is a function of both the duration of the feeding and the PCDD/F concentration. This suggests that long-lasting breast feeding in Finland may increase the number of children with enamel hypomineralization.

In Sweden high numbers of idiopathic enamel hypomineralization in the permanent first molars were recorded in the late seventies. The frequency was highest (15%) in children born in 1970 and then decreased in children born in 1971, 1972 and 1974 (Koch et al., 1987). The reason for this remained unclear. On the basis of the present results it can be speculated that dioxin-like compounds might have been an etiological factor for mineralization defects in the teeth of children born around 1970. A retrospective investigation of breast milk in Sweden showed a clear decline in dioxin and PCB levels in the early seventies (Noren, 1993) which could have led to lower frequencies of mineralization defects in teeth after the peak prevalence in 1970.

Many children born around 1970 were also exposed to dioxin as an impurity of hexachlorophene. This was commonly used in Scandinavian countries as skin care antiseptic for newborn and infants. Its use increased until evidence on its adverse effects was published in the early seventies (Kimbrough, 1973). After over 20 years it may not be possible to confirm the role of dioxin for dental defects in Sweden. Nevertheless, the lesions that we found were similar to those presented by Koch et al. (1987) in their color prints and to those that we see when treating such lesions in the Department of Pedodontics and Orthodontics, University of Helsinki.

Prevalences of the mineralization defects in the permanent first molars in our study were on the same level as in a recent Danish study (14–25% depending on the district where the children lived) on over five thousand children born 1987 (International Association of Paediatric Dentistry Congress 1995, abstract no. 56). It is not clear whether the defects have increased in number during the last years, because no epidemiological data are available. It is possible that earlier high caries activity might have concealed the true prevalence of hypomineralization. In the present study group only five children (data not shown) had caries or fillings on one or two permanent first molars, which made it easy to establish the diagnosis of hypomineralization. In a population of high caries activity it is difficult to be sure whether a carious lesion was originally a mineralization defect and caries only a consequence in the defective enamel. Therefore mineralization defects may have been registered as caries. Such an explanation conforms with the observation of 'carious and broken teeth' in children after the exposure to PCB and PCDF in a mass poisoning (Yu-Cheng) in Taiwan (Rogan et al., 1988). According to the reported ages of the children the teeth were primary teeth. Since mineralization of primary teeth begins at the 14th week in utero and most primary teeth are mineralized by birth (Massler et al., 1941), the defects indicate transplacental exposure.

In our material the exposure to PCDD/F via mother's milk did not correlate either with caries or to the mineralization changes of the primary teeth (data not shown). At relatively low maternal levels of PCDD/F transplacental exposure is not likely to cause impairment. However, during a long nursing period the mother secretes even 20–30% of her total PCDD/F body burden which will distribute to a much smaller body volume and fat store of the child (Furst and Wilmers, 1992; Vartiainen et al., unpublished observations). This causes much higher concentrations perhaps explaining impairment of teeth during the critical period.

A correlation between dioxin exposure from mother's milk and hypomineralization defects in the developing teeth was found in the present study. As PCB and other chlorinated compounds found in human milk may also be

involved in disturbing tooth development, further studies are needed to evaluate their role.

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