DEVELOPMENTAL DENTAL DEFECTS AFTER THE DIOXIN ACCIDENT IN SEVESO

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Introduction

The compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), or dioxin, is a widespread environmental toxicant that arises from combustion and as a by-product from various industrial processes. It is extremely stable with a half-life in humans ranging from 7 to 9 years.

Developmental toxicity of TCDD is well established and constitutes a major field of interest. Teeth like many other organs (such as lungs, mammary glands or kidneys) develop as a result of inductive interactions between the ectoderm and the subjacent mesenchyme. Development proceeds via consecutive morphogenetic stages to the differentiation of tooth-specific cells and the subsequent deposition and mineralization of the dental matrices[1]. Tooth development is genetically regulated but sensitive to environmental disturbances. Once teeth have formed, they do not undergo remodeling and therefore, any disturbances in morphogenesis or function of dentin-forming ameloblasts or enamel-forming odontoblasts lead to permanent defects.

Findings from many experimental studies have suggested that developing organ systems are more susceptible to TCDD than the organs of animals that have reached maturity. Accordingly we have shown that lactational exposure of rats to TCDD results in the arrest of early third molar development[2, 3] and impaired mineralization and arrested root formation of the more advanced first and second molar teeth. TCDD also causes defective dentin and enamel formation in the continuously erupting rat incisor teeth in vivo[4].

We have further shown that dioxins in mothers' milk may cause mineralization defects in children's permanent first molar teeth undergoing mineralization during the first two years of life[5, 6]. To our knowledge, this is the only developmentally toxic effect of dioxins established thus far in humans in the absence of heavy accidental or occupational exposure. In limited data of two

episodes of epidemic PCB and PCDF poisoning in Japan and Taiwan, excess of ectodermal defects and developmental delay, including a variety of dental and oral changes were reported in children. At birth natal teeth and oral pigmentation were prevalent and later, missing permanent teeth, delayed eruption of permanent teeth, and disturbed root development were observed[7-9]. A report from Slovenia also suggested that children who were pre- and postnatally exposed to PCBs in the contaminated region of Bela Krajina had a high prevalence of developmental dental defects[10].

The best-known dioxin accident took place in Seveso, Italy, in 1976. A trichlorophenol production reactor in a chemical factory blew up and released kilogram quantities of dioxins, mainly TCDD, into the environment[11]. Serum samples collected soon after the accident rendered it possible to quantify individual TCDD exposures. It was found that the explosion resulted in the highest known TCDD exposure in human residential populations. Measurements two decades after the accident showed that serum levels of TCDD in subjects who lived in the most polluted areas were persistantly high[12].

Twenty-five years after the Seveso accident we invited subjects exposed in 1976 to dental examination. Our aim was to determine the dental and oral consequences after heavy exposure to TCDD. We hypothesized that TCDD causes developmental dental defects that can still be seen in adulthood, since the defects are permanent in nature.

Subjects

We invited 48 subjects who at the time of the accident had been younger than 9.5 yrs. Plasma, collected in 1976, had been previously analysed for the TCDD concentration[13]. In addition, we included subjects (N=65) who lived outside the contaminated area. Demographical information was officially obtained through the different municipalities' censuses and consisted of date of birth and town of residence in July 1976. The controls were similar as regards age, gender, education and smoking habits. At the time of the dental and oral examination the exposed subjects were from 25.4 to 34.0 yrs old (mean 29.1) and the controls from 24.6-34.1 yrs old (mean 29.2).

All permanent teeth were recorded for developmental dental defects using the Developmental Defects of Enamel (DDE) Index (FDI)[14] with the modification that hereditary defects in tooth structure and tetracycline staining were recorded but not included in the analysis. Demarcated opacities less than 2 mm in diameter were not recorded. One examiner (PC) undertook the examinations. During the dental and oral examination, he had no knowledge whether the subject belonged to the control or the study group or of the serum TCDD concentration.

Caries status was recorded according to WHO[15]. The following periodontal variables were examined: the presence or absence of visible plaque, gingival bleeding on probing, suppuration, supragingival and subgingival calculus and pocket depth. Pigmentation of gingiva, palate, buccal mucosa and tongue were recorded. For the measurement of the salivary flow, paraffin stimulated saliva was collected for 5 min. The clinical examination was supplemented by radiographic examination using panoramic tomography. Alveolar bone loss, root deformities and the presence of jaw cysts were recorded. In association with the dental examination the subject or the mother of the subject was interviewed on medical and dental history, education, smoking habits and natal teeth.

Written informed consent was obtained and approval given by the local institutional review board.

Statistical analysis
Pearson's correlations were computed on the entire study population and for both genders separately to examine associations between different variables. When necessary, logarithmic transformation of the data was performed to obtain a normal distribution. Comparisons between the categorized variables were evaluated by $\chi 2$ test and Mantel Hänszel $\chi 2$ test. In all statistical tests, probability of equal to or less than 0.05 was considered statistically significant.

Results
TCDD concentrations of the exposed subjects ranged from 23 to 26.000 (median 476) ng/kg in serum lipid. A total of 33% of them had developmental dental defects in at least one permanent tooth compared with 17% of the controls. Subjects with higher serum TCDD levels had more often developmental dental defects than those with lower TCDD levels or the controls.

A total of 12.5% of the exposed subjects had congenitally missing permanent teeth (excluding wisdom teeth) compared to 3.1% of the controls. Subjects with higher serum TCDD levels had more often congenitally missing permanent teeth than those with lower TCDD levels or the controls. There was no difference in the prevalence of missing wisdom teeth in the exposed subjects and in the controls (31% and 29%, respectively).

Other dental and oral aberrations were few. Two exposed subjects had gingival pigmentation. Associations between caries, periodontal disease or salivary flow rate and dioxin exposure levels were not found.

Discussion
The development of the human permanent dentition extends from birth to the completion of adolescence. All teeth including the wisdom teeth normally start to develop before the age around 9 years and during that time they are targets to external modification. Congenital lack of a tooth traces back to disturbances during early stages of tooth development while enamel defects result from aberrations during secretory stages of enamel formation and during enamel mineralization and maturation.

Our results showed that the prevalence of developmental enamel defects and missing permanent teeth increased with the exposure to TCDD. The result is consistent with our earlier finding in a normal child population exposed via mother's milk and in line with our animal experiments.

Prevalence figures for nonsyndromatic hypodontia (1 to 6 teeth congenitally missing) in the permanent dentition in a normal population have some variation in different countries but most studies show prevalences of 3-8%. In the majority of cases hypodontia has a genetic basis. However, environmental factors may arrest tooth development although known causative factors are few. Among them are different kinds of trauma as well as multiagent chemotherapy and radiation therapy. In our study the prevalence was 3% in the controls compared to 19% of the subjects in the highest tertile of the serum concentrations. This result suggests that we can add TCDD to those agents that can be associated with hypodontia.

Most developmental defects of enamel are idiopathic. Still almost one hundred different factors have been listed as being responsible for developmental defects of enamel. The prevalence varies

greatly depending on the population studied and method used for the screening. In our study the prevalence of 33% in the exposed subjects was not exceptionally high when compared with the results from other prevalence studies on a normal child population. However, it was remarkable that the prevalence was related to serum TCDD levels, which indicates that TCDD may be a causative agent of developmental defects of enamel.

Taken together the results support the hypothesis of human developing tissues being sensitive to dioxins.

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