SCIENCE OF THE TOTAL ENVIRONMENT 389 (2008) 253–258

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Chemical composition of enamel and dentine in primary teeth in children from Thailand exposed to lead

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ARTICLE INFO

Article history:
Received 28 May 2007
Received in revised form
30 August 2007
Accepted 31 August 2007
Available online 29 October 2007

Keywords:
Lead
Primary teeth
Enamel
Dentine
SIMS
XRMA

ABSTRACT

Enamel and dentine in teeth of children with high blood levels of lead were analyzed by means of secondary ion mass spectrometry (SIMS) and X-ray micro-analyses (XRMA) and compare with teeth from children with low blood levels of lead. The SIMS analysis revealed detectable levels of Pb in dentine close to the pulp. The XRMA analyses could not detect any lead. There were no differences found in lead level in enamel of high lead level exposed teeth from low level exposed. The results confirm that children with high blood levels of lead have an uptake of lead in dentine close to the pulp.

1. Introduction

Human exposure to lead due to environmental factors is known to affect several tissues in the body, especially organs under development. Lead is neurotoxic and particularly harmful to the developing nervous system. If children are exposed to lead, even in low doses, they may suffer from mental retardation, coma, seizures and even death. The risk for long-term effects on the brain is well known. However, low levels of lead may cause a number of problems such as learning disabilities and impaired growth (Fosse and Justesen 1978; van Wyk and Grobler 1983;Haavikko et al., 1984; Anttila and Anttila 1987; Anttila 1986, 1987a,b; Frank et al., 1988; Heilmann et al., 1990; Frank et al., 1990; Cleymaet et al., 1991a,b,c,d,e; Needleman and Bellinger 1991; Needleman and Bellinger 2001; Choi et al., 2004; Hernández-Guerrero et al., 2004; Gomes et al., 2004).

The source of lead may vary considerably. It may emanate from areas with industries without treatment of waste water, from paint, plumbing, food and gasoline. Despite great efforts made to prohibit the use of lead, children are still exposed due to e.g. residual lead contamination in soil.

Heavy metals are known to be incorporated into dental hard tissues both during their mineralization and also posteruptively. Lead is regarded being an important biomarker and it

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doi:10.1016/j.scitotenv.2007.08.053

has been shown that the spatial distribution of lead in primary enamel and dentine reflect the blood lead levels (Arora et al., 2006). A recent study of primary teeth have shown statistical differences in lead content in the surface enamel between an uncontaminated and a lead contaminated child population (Costa de Almeida et al., 2007). In some studies, a correlation between lead exposure and higher caries prevalence has been shown (Bowen, 2001; Bud et al., 2000; Brudevold et al., 1977; Derise et al., 1974).

Since heavy metals from the environment may inflect on the development of the nervous system, exposure is a matter of public health concern. Therefore, the incorporation of lead in dental hard tissues makes teeth suitable for cross-sectional and longitudinal epidemiological research (Needleman and Bellinger 1991; Rabinowitz et al., 1993; Bellinger et al., 1994; Rinderknecht et al., 2005).

In southeast Thailand, in the Singhanakorn district, many families live close to small shipyards which still use lead-containing paint. Blood samples from children living in the district have revealed higher levels of lead in their blood serum compared with children living in non-shipyard areas (Youravong et al., 2006).

The aim of this study was to investigate the lead content in the enamel and dentine of primary teeth of children with high levels of blood-lead and compare with a group of children with low levels of blood-lead.

2. Materials and methods

2.1. *Subjects*

The patients live in the Huakao subdistrict, Singhanakorn district, Songkhla Province of Southern Thailand. The Huakao subdistrict consists of eight villages where one of the income sources is fishing and boat repair in small shipyards. This area is known as an endemic area for lead exposure. Lead has been environmentally contaminated both in soil and dust in the area. The shipyard industry involves the use of Pb_3O_4 as a material for repairing. Three primary schools are located in the subdistrict; Banbosub School, Banhuakao and Bankaodang School. The two first schools are located close to the two shipyards. Teeth were collected from the two schools from children who gave consent to participate in the clinical study. Consent was also given by their parents.

A detailed background of caries and blood lead levels was presented in a clinical paper. Therefore, only a brief description of the children (aged 6–11 years), represented by their primary teeth, will be given here (Youravong et al., 2006). The number of children with blood lead levels <10 μg/dL is 57 and for levels ≥ 10 μg/dL the number is 23. For the original study population (292 children) the *df* (*d* = decayed; *f* = filled; primary teeth) values were significantly higher ($p<0.05$) in the high blood lead level group compared with the low-level group (Youravong et al., 2005, 2007).

2.2. *Tooth material*

The teeth for the SEM/XRMA analyses derive from a tooth material of exfoliated primary teeth collected for morphological studies of enamel, therefore, only a short résumé will be given here (Youravong et al., 2006). Eighty-seven exfoliated primary teeth, from children attending the Banbosub and Banhuakao schools in the Huakao subdistrict were collected and prepared for the investigations. Twenty-three of these teeth originated from children who had been exposed to lead and known to have high blood lead levels (Youravong et al., 2006). The remaining 64 teeth originated from children who regarded as a low-exposure group. After being embedded in an epoxy resin, central bucco-lingual sections were prepared from each tooth and examined in polarized light, micro radiography and SEM. Since de-ionized water was used for the cutting procedure and no lead containing instruments and materials were used the risk for lead contamination of the samples was minimal.

For the XRMA analyses, either an un-decalcified section or the remaining part of a tooth was used. The bulk of the enamel appeared morphologically normal, irrespective of Pb exposure or not. In all of the teeth a porous, hypomineralized, enamel surface could be seen, possibly reflecting a highly caries active situation. The number of teeth used for the elemental analyzes were limited since both methods are time consuming and especially SIMS very costly. For the SIMS and XRMA analysis only primary incisors were used. The teeth from the lead exposed children were selected from those subjects having the highest levels of lead in the blood. The selection of the control teeth was based on having a normal morphological appearance as seen in the polarized light microscope.

2.3. *SIMS analyses*

After mounting in a sample holder for the ion probe, the specimens were polished and then coated with a thin layer of gold by vapor deposition, to avoid build-up of surface charge during analysis.

The analysis was performed using an ion probe at Chalmers University of Technology (Cameca IMS 3F, Paris, France). The principles for the ion probe have been described in detail elsewhere (Lodding et al., 1982, Lodding 1983) wherefore only a brief outline will be given here. The surface of the specimen was bombarded with negative oxygen ions. Positive secondary ions were then collected from the center of the bombarded area. The secondary ions were passed to a magnetic analyzer and separated according to mass/charge ratio. The counts of the different ion species were then registered. Since no quantitative measurements were performed no hydroxyapatite standard doped with lead was used. However, the SIMS-laboratory has a long history of measurements of dental hard tissues and the mass-resolution of the instrument is valid for semi-quantitative measurements.

For a first analysis, two teeth were selected representing one child with high blood lead levels and one child with low lead-levels. In each tooth, mass spectra in enamel and dentine were taken in up to eight different locations, in order to establish possible variations of the Pb content. The measured mass numbers were the Pb isotopes 204, 205, 206, 207 and 208. The mass spectrum range was limited from 202 to 213, since Pb and its isotopes are found at this interval. In these locations, Pb-peaks of 206, 207 and 208 showed the correct relationship. In-depth profiles were made, i.e. continuous sputtering into