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Pb enamel biomarker: Deposition of pre- and postnatal Pb isotope injection in reconstructed time points along rat enamel transect

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Abstract

Exposure to lead (Pb) as well as other heavy metals in the environment is still a matter of public health concern. The development of the enamel biomarker for heavy metal exposure assessment is designed to improve studies of dose–effect relationships to developmental anomalies, particularly embryonic dysfunctions, and to provide a time-specific recount of past exposures. The work presented in this paper demonstrates maternal transfer across the placental barrier of the enriched isotope ^{206}Pb tracer to the enamel of the rat pup. Likewise, injections of ^{204}Pb-eriched tracer in the neonate rat resulted in deposition of the tracer in the enamel histology as measured by secondary ion microprobe spectrometry. Through enamel, we were able to observe biological removal and assimilation of prenatal and postnatal tracers, respectively. This research demonstrates that enamel can be used as a biomarker of exposure to Pb and may illustrate the toxicokinetics of incorporating Pb into fetal and neonatal steady-state system processes. The biomarker technique, when completely developed, may be applied to cross-sectional and longitudinal epidemiological research.

Keywords: Isotopic tracer; Blood–enamel mineral exchange; Lead; Heavy metal biomarker; Amelogenesis

1. Introduction

Environmental exposure to lead (Pb) is a matter of continuing public health concern. Atmospheric Pb concentrations have decreased over the past 30 years due to reduced Pb production and elimination of Pb additives in gasoline in the USA, Europe, and other countries (Thomas et al., 1999; Nriagu, 1990). However the current use of leaded gasoline in other, often lesser-developed, countries throughout the world poses an important threat to the healthy development of children in those countries and also actively adds to the global Pb burden. The Center for Disease Control and Prevention (CDC) recommends that blood Pb levels not exceed 10 μg/dL (Lustberg and Silbergeld, 2002). Despite successful efforts to lower atmospheric Pb concentrations, the National Health and Nutrition Examination Survey (1999–2000) reports that approximately 434,000 children under 6 years old in the US have blood Pb levels of 10 μg/dL or more (Meyer et al., 2003).

Inhalation of Pb as airborne particles from automobile and industrial emissions used to be the dominant route of Pb exposure for children in the United States. As atmospheric Pb concentrations declined, ingestion of Pb from soil and food, in addition to inhalation from resuspended particulate matter (dust), has become the more prominent route of Pb exposure for children (Mielke et al., 1999; Mielke and Reagan, 1998; Lejano and Ericson, 2005).

Once deposited, Pb can remain trapped and concentrated in the upper horizons of undisturbed soil for up to 75 years (Bindler et al., 1999). Soil Pb concentrations accumulate due to resuspended particles from

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Pb-contaminated road services, continued automotive and industrial emissions, and soil erosion (Petrosyan et al., 2004; Kurkjian and Flegal, 2003). Pb washed into soil from painted surfaces and rooftops also contributes to the soil Pb burden near the perimeter of homes. This elevated level of Pb on surfaces near homes is often tracked into the home environment, adding to the total Pb exposure (Mielke and Reagan, 1998).

Fetal, neonatal, and adolescent development presents numerous time points during which organ-specific susceptibility to Pb exposure and toxicity may be critical. In evaluating the effects of Pb on health, it is important to determine when Pb exposure occurred, as this knowledge could greatly improve our ability to relate Pb exposures to specific neurological or physiological damage. Thus, reconstructing Pb exposure histories is important for assessing the ongoing potential health risks of Pb (Silbergeld, 2003, 1995). The current methods for measuring blood Pb and Pb-isotopic content from biological tissues are adequate for assessing recent exposures or for identifying source locations, but they are not adequate for reconstructing time-specific exposure histories. In this paper, we test the ability to reconstruct the time course of exposure to Pb during fetal and neonatal development using the enamel of the rat mandibular incisor.

The rat mandibular incisor is a continuously growing, occlusal tissue. Enamel only forms along the labial edge of the incisor where enamel at the incisor tip represents first formed enamel and enamel near the gum line (gingival margin) indicates the most recently formed enamel (see Fig. 1). The final stages of enamel maturation occur at the gingival margin just prior to eruption into the oral cavity (Sato et al., 1996) and the enamel is fully mature at time of eruption. Before eruption, mineral exchange can occur between the blood and the forming enamel, allowing minerals to incorporate into the maturing enamel crystalline structure. This blood–enamel mineral exchange decreases as the enamel matures, and after eruption there is minimal to no mineral exchange between the secreted enamel and the surrounding environment. Therefore, the Pb content of mature enamel should reflect the Pb concentration in the blood while that enamel was developing.

We therefore tested the hypothesis that we could expose pregnant animals at specific times and then reconstruct the time course of Pb exposures by determining Pb concentrations at specific locations along the tooth enamel that represent the developing enamel and the enamel–mineral exchange that occurred at the time of exposure. To test this hypothesis we exposed rats prenatally and postnatally to Pb by injection under controlled conditions using two different nonradioactive isotopically-enriched Pb tracers, ^{206}Pb and ^{204}Pb, to unambiguously differentiate between prenatal and postnatal exposures, respectively. Secondary ion mass spectrometry was used to longitudinally scan rat tooth enamel and to determine the concentration of these Pb isotopes at histological locations representing enamel formed at the time of prenatal and postnatal Pb exposure and that could be characteristic of different time points of the rats' development.

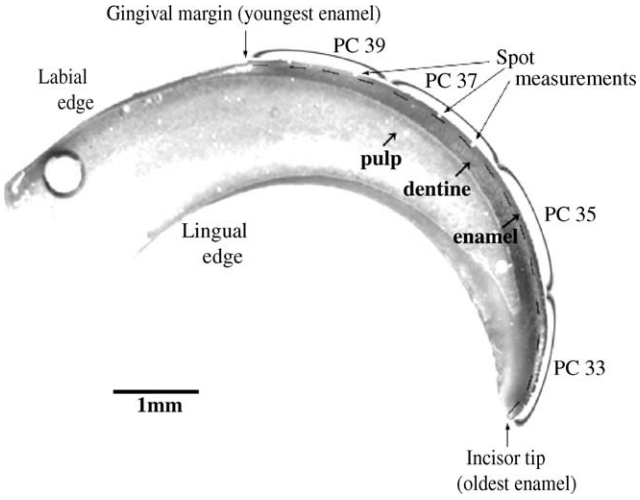

Fig. 1. Cross-section of a rat mandibular incisor (unstained, gold-coated, thick-sectioned specimen photographed under reflected light). The enamel transect is situated along the labial edge of the incisor. The dashed line indicates the histological division between enamel and dentine. The incisor tip, representing the first erupted and oldest enamel, and the gingival margin, representing the most recent erupted and youngest enamel, are located at the beginning and end of the enamel transect, respectively. The mandibular incisor is continuously growing outward from the gingival margin and enamel maturation is completed just prior to erupting into the oral cavity. According to respective proximity, spot measurements (white dots indicated by arrows) along the enamel transect were averaged into four equidistant locations corresponding to the postconception age (PC 33, 35, 37, and 39), in days, of enamel eruption for each spot. The mandibular incisor first erupted at PC 33 and enamel growth continued through PC 39, when the tooth was excised for data acquisition.

2. Materials and methods

Four timed-pregnant Sprague Dawley rats (Charles River, Inc., Hollister, CA) were randomized into control and exposure groups. Litters ranged from 10 to 13 pups, though only male progeny were used. The rats and dams with littermates were individually housed under barrier conditions in an AAALAC-accredited vivarium using a 12-h light/dark cycle. Standard rat chow and distilled water were provided ad libidum. Animal husbandry was conducted in strict accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and the research protocol was approved by the Institutional Animal Care and Use Committee at the University of California, Irvine (Protocol No. 1998–1992).