Fluoride increases lead concentrations in whole blood and in calcified tissues from lead-exposed rats

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Higher blood lead (BPb) levels have been reported in children living in communities that receive fluoride-treated water. Here, we examined whether fluoride co-administered with lead increases BPb and lead concentrations in calcified tissues in Wistar rats exposed to this metal from the beginning of gestation. We exposed female rats and their offspring to control water (Control Group), 100 mg/L of fluoride (F Group), 30 mg/L of lead (Pb Group), or 100 mg/L of fluoride and 30 mg/L of lead (F + Pb Group) from 1 week prior to mating until offspring was 81 days old. Blood and calcified tissues (enamel, dentine, and bone) were harvested at day 81 for lead and fluoride analyses. Higher BPb concentrations were found in the F + Pb Group compared with the Pb Group (76.7 ± 11.0 μg/dL vs. 22.6 ± 8.5 μg/dL, respectively; p < 0.001). Two- to threefold higher lead concentrations were found in the calcified tissues in the F + Pb Group compared with the Pb Group (all p < 0.001). Fluoride concentrations were similar in the F and in the F + Pb Groups. These findings show that fluoride consistently increases BPb and calcified tissues Pb concentrations in animals exposed to low levels of lead and suggest that a biological effect not yet recognized may underlie the epidemiological association between increased BPb lead levels in children living in water-fluoridated communities.

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1. Introduction

Low-level environmental exposure to lead has been associated with subclinical deficit in neurocognitive function in young children (Needleman et al., 1979) and in adolescents (Needleman et al., 1990), as well as with numerous other conditions prevalent in industrialized societies, such as attention deficit hyperactivity disorder (ADHD) (Braun et al., 2006), delinquent behavior (Dietrich et al., 1991, 2001; Needleman et al., 2002; Wright et al., 2008), hearing impairment (Rothenberg et al., 2000), spontaneous abortions (Borja-Aburto et al., 1999), periodontal disease (Saraiva et al., 2007), decreased renal function (Muntner et al., 2003), and hypertension (Hu et al., 1996). Interestingly, a strong association has been described between preschool blood lead levels and crime rates in many countries (Nevin, 2007). Together, these findings indicate that low-level exposure to lead is still a matter of concern.

Lead enters the human body by inhalation or by gastrointestinal absorption, and a single dose remains in the blood for a short time (35 days) (Rabinowitz, 1976). Thereafter, lead is stored in calcified tissues (Barbosa et al., 2005), thus allowing primary teeth dentine to be used to assess subclinical past exposure to lead (Needleman et al., 1972), as it was done in the seminal studies which showed an association between exposure to lead and adverse effects to children’s intelligence (Needleman et al., 1979, 1990). Since then, prospective studies that collected blood at birth and in the first school years showed that early exposure of children to lead results in lower IQ scores (Dietrich et al., 1991, 1993; Bellinger et al., 1992). In this regard, while the U.S. Centers for Disease Control and Prevention (CDC) established in 1991 a blood lead (BPb) concentration of 10 μg/dL as a concentration that should prompt public health actions (U.S. CDC, 1991), there is now clear evidence that BPb concentrations < 10 μg/dL cause cognitive impairment (Lanphear et al., 2000; Canfield et al., 2003; Hu et al., 2006). Indeed, BPb as low as 2.5 μg/dL reduces a child’s IQ measurably (Lanphear et al., 2000), and there is no evidence of a threshold under which lead levels appear to be safe in terms of neurobehavioral outcomes (Chiodo et al., 2000).
the administration of fluosilicic acid (H2SiF6) could increase BPb and accumulation. Therefore, this study aimed at testing whether they might have an effect on each other’s absorption, metabolism, and mineralized tissue lead concentrations in rats exposed to low levels of lead from the beginning of gestation.

2. Materials and methods

2.1. Animals

This study was approved by the Ethical Committee for Animal Research of the University of São Paulo (Campus of Ribeirão Preto, Protocol 07.1.346.53.3), and conformed to the guidelines established by this Committee. Animals were handled humanely, and in accordance with the guidance principles published by the National Institutes of Health. Twenty-eight Wistar rats (190–210 g, 24 females and 4 males) were obtained from the University’s colony. The rats were randomly divided into 4 groups of 6 females and 1 male, according to the amount of fluoride and lead in drinking water. Mating began at the same time that the animals received different water treatments, and lasted for 1–2 weeks maximally.

Food was provided ad libitum, and animals were maintained under 12-h light/dark cycle. Rat chow, as pellets, was purchased from Nuvital (Nuvilab CR-1, Colombos, SP, Brazil). Pb and F concentrations did not exceed 0.05 mg/kg, according to the manufacturer. Animal room temperature and humidity were 24–26 °C, and 40–60%, respectively. Maximally 12 offspring were housed per cage until weaning, and 4 animals were housed per cage after weaning. Plastic cages with fitted stainless steel wire lids, whose dimensions were 41 cm × 34 cm × 16 cm (height), giving a floor space of 1394 cm2. All rats were checked daily for health, feed and water, and clean cages. Rooms were stocked with in-date supplies. Water was provided ad libitum via glass bottle, stopper and sipper tube. Three times a week bottles were cleaned with soap and copious washing with tap water.

Control animals received water containing maximally 0.1 mg/L of F and 0.5 μg/L of lead. Animals in the Fluoride group (F Group) received water containing 100 mg/L of fluoride as fluosilicic acid (H2SiF6). Animals in the group exposed to lead (Pb Group) received water containing 30 mg/L of lead as lead acetate (Pb(CH3 COO)2). Pb and F concentrations did not exceed 0.05 mg/kg, according to the manufacturer. Animal room temperature and humidity were 24–26 °C, and 40–60%, respectively. Maximally 12 offspring were housed per cage until weaning, and 4 animals were housed per cage after weaning. Plastic cages with fitted stainless steel wire lids, whose dimensions were 41 cm × 34 cm × 16 cm (height), giving a floor space of 1394 cm2. All rats were checked daily for health, feed and water, and clean cages. Rooms were stocked with in-date supplies. Water was provided ad libitum via glass bottle, stopper and sipper tube. Three times a week bottles were cleaned with soap and copious washing with tap water.

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2.8. Statistical analysis

The results were analyzed for normality, and the data were normally distributed. Comparison between groups was performed using ANOVA, and a $p$ value < 0.05 was accepted as significant. Differences between groups were analyzed using the Bonferroni Test, and differences were considered significant when a $p$ value of 0.008 was reached, since 6 comparisons were performed.

3. Results

We found no bodyweight differences at the end of the study (data not shown). Fig. 1 shows 3.4-fold higher BPb concentrations in the F+Pb Group ($76.7 \pm 11.0 \mu g/dL$) compared with the Pb Group ($22.6 \pm 8.5 \mu g/dL$) ($p < 0.001$). BPb concentrations were below 0.5 $\mu g/dL$ both in the Control and in the Fluoride groups (Fig. 1).

Fig. 2 displays lead concentrations in the calcified tissues, and shows higher lead levels in the F + Pb Group vs. the Pb Group for all tissues examined in this study. Higher superficial enamel lead concentrations were found in the (F + Pb) group compared to the Pb Group ($4369 \pm 1353 \mu g/g$ vs. $1768 \pm 1892 \mu g/g$, respectively) ($p < 0.001$), thus indicating a 2.5-fold increase in the amounts of lead in the F + Pb Group. Similarly, higher lead concentrations were found in the F + Pb Group compared with the Pb Group in dentine samples ($8.5 \pm 2.0 \mu g/g$ vs. $4.9 \pm 1.7 \mu g/g$, respectively; $p < 0.001$; Fig. 2b). Whole bone lead concentrations doubled in the F + Pb Group ($14.2 \pm 2.6 \mu g/g$) compared with those found in the Pb Group ($6.8 \pm 1.7 \mu g/g$) ($p < 0.001$). Lead concentrations in the bone surface were also 3 times higher in the F + Pb Group compared with those found in the Pb Group ($28.0 \pm 10.6 \mu g/g$ vs. $9.0 \pm 3.7 \mu g/g$, respectively; $p < 0.001$). The amount of bone dissolved by etching ranged from 1.5 to 10 mg, indicating that the superficial dissolution of bone (on average) ranged from 0.01 to 0.14 $\mu m$ of superficial bone, with no difference among groups in the etching depth (not shown).

Dentine was the calcified tissue that exhibited the lowest lead concentrations found in this work. Dentine was followed by whole bone and then by surface bone. The calcified tissues with similar amounts of minerals and with a collagenous organic matrix (dentin and bone) showed 5–9 $\mu g/g$ of lead in the Pb Group, whereas the same tissues showed 8.5–28 $\mu g/g$ of lead in the F + Pb Group. Enamel, on the other hand, which was sampled by a superficial etch technique, contained very high concentrations of lead, which reached approximately 500 times as much as those found in dentine in the F + Pb Group. Superficial enamel is known to accumulate...
lead in the range of hundreds to thousands of μg/g of lead (Robinson et al., 1995; Gomes et al., 2004; Costa de Almeida et al., 2007).

As shown in Fig. 3, significantly higher fluoride concentrations were found in the F and F + Pb Groups as compared with those found in the Control or in the Pb Groups (p < 0.001 for all comparisons). However, no significant differences in the fluoride concentrations in the tested calcified tissues were found when the F and F + Pb Groups were compared (p > 0.05 for all comparisons).

4. Discussion

This study shows that co-exposure to fluoride and lead from the beginning of gestation consistently increases the concentrations of lead in whole blood and in calcified tissues of 81-day-old animals, with no changes in the concentrations of fluoride. Lead concentrations were found to be 2.5 times higher in the superficial enamel, 3 times higher in surface bone, 2 times higher in whole bone, and 1.7 times higher in the dentine when the animals were co-exposed to fluoride, thus indicating a consistent rise in the amounts of lead found in whole blood and calcified tissues in the F + Pb Group. This is the first study to show that fluoride affects lead concentrations during lead exposure, and our findings may have serious implications for populations exposed to increased amounts of both lead and fluoride, particularly young children.

Decreased learning ability and low hippocampus glutamate has been recently shown in offspring rats exposed to fluoride and lead (Niu et al., 2009). Essential and toxic metal concentrations in the body are regulated by gastrointestinal absorption together with urinary excretion (Barbier et al., 2005). Lead (Pb2+) and many other non-essential divalent metals are transported by a protein transporter known as divalent metal transporter 1 (DMT-1) (Garrick et al., 2003), which is expressed both at the intestinal brush border, and at the renal tissue (Barbier et al., 2005). Fluoride is known to inhibit enzymes (Marquis et al., 2003 discuss some examples of inhibition), and sodium fluoride (NaF) is a potent, rapid, and reversible activator of the regulatory heterotrimeric GTP-binding proteins in virtually all in vitro systems (Chabre, 1990). Thus, we speculate that the unknown mechanism that explains the increased lead levels found in the blood and in the calcified tissues may involve the effect of fluoride on the control of lead absorption in the intestine or excretion in the kidney. This effect may be direct, by a direct effect of fluoride on the DMT-1 protein, or indirect, since changes in iron metabolism, for example, will increase DMT-1 expression, thus increasing the absorption of toxic metals. It is of note that changes in the structure of DMT-1 induced by mutations have been shown to completely change the capability of this transporter to recognize metals, changing it from a major transporter of Fe into a Ca transporter (Xu et al., 2004). Therefore, further multidisciplinary physiological, biochemical, and molecular studies are needed to support our findings and provide mechanistic insight.

Lead and fluoride share a common distribution, at least in some calcified tissues such as dental enamel (Robinson et al., 1995). Lead shows a high degree of accumulation in the very first micrometers of superficial (outer) enamel, particularly in the first 6 μm in humans (Brudevold et al., 1975; Purchase and Fergusson, 1986; Cleymaet et al., 1991; Gomes et al., 2004; Costa de Almeida et al., 2007; De Almeida et al., 2008), which correspond to the same accumulation site for fluoride (Robinson et al., 1995). Studies using unerupted molars from rats clearly show that accumulation of lead in the superficial enamel is a pre-eruptive event (Arora et al., 2005, 2007). Therefore, this particular overlap in the distribution of both elements in dental enamel suggests a biological interaction between lead and fluoride, possibly involving the precipitation
ties using H2SiF6 (fluosilicic acid) to fluoridate the drinking water (Coplan, 1999; Masters et al., 2000). Children living in communities of fluorosis in front teeth and first molars (Ismail et al., 1990), and producing more lead toxicity. High levels of fluoride, which may cause their BPb levels to increase, should be avoided (Binns et al., 2007). While the benefits of water fluoridation for caries prevention are unquestionable (Kumar and Moss, 2008), concerns have recently been raised regarding the association of fluoride in the drinking water with increased BPb levels in large populations (Masters and Coplan, 1999; Masters et al., 2000). Children living in communities using H2SiF6 (fluosilicic acid) to fluoridate the drinking water have the highest BPb levels (Masters et al., 2000). Consistent with these epidemiological findings, our results showed that fluoride (as H2SiF6) increases BPb levels and lead concentrations in calcified tissues. These findings suggest a possible biological effect of the co-exposure to lead and fluoride that deserves further studies using different doses and forms of fluoride to better characterize this effect. Furthermore, such studies are needed to establish safety guidelines for the use of topical, mouth rinse of toothpaste fluoride for children under 5 years, since so far the fluoride doses of concern are based on the “probable toxic dose” (Shulman and Wells, 1997).

The concerns about the effects of fluoride may also apply to fluoride–polluted areas, where the exposure of the populations and animals to other toxic metals, such as lead, may be increased. In addition, it is possible that fluoride may affect the concentrations of other metals that are found in calcified tissues and share a similar distribution with fluoride and lead, such as zinc and cadmium (Cleymaet et al., 1991; Robinson et al., 1995). As pointed out by Bellinger (2004) “Co-exposure to other toxicants is another candidate explanation for individual differences in susceptibility (to lead), although greater attention has been paid to the potential of co-exposures to be confounders than to be effect modifiers”.

In conclusion, this study showed that co-exposure to fluoride increases lead concentrations in the blood and in calcified tissues in animals exposed to lead from the beginning of gestation. These findings suggest that a biological effect not recognized so far may underlie the epidemiological association between increased BPb levels in children and water fluoridation.

**Conflict of interest**

None.

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