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Oral antimicrobial activity weakened in children with electronic waste lead exposure

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Abstract

Environmental lead (Pb) exposure can induce dysbacteriosis, impair oral health, and is associated with the development of dental caries. However, the mechanism is unclear. The aim of this study was to explore the effects of Pb toxicity on oral antimicrobial activity in children in an e-waste area. Results showed higher blood Pb levels in e-waste-exposed group children, accompanied by decreased saliva SAG (salivary agglutinin) concentrations, increased peripheral WBC (white blood cell) counts and monocyte counts, and elevated peripheral monocyte percentage. LnPb (natural logarithmic transformation of blood Pb level) was negatively correlated with saliva SAG concentration, while positively correlated with peripheral monocyte percentage. Saliva SAG concentration played a complete mediating role in the correlation of LnPb to peripheral monocyte percentage. To our knowledge, this is the first study on the relationship of environmental Pb exposure and oral antimicrobial activity in children, showing that environmental Pb exposure may weaken oral antimicrobial activity through reducing saliva SAG concentration, which may raise the risk of oral dysbacteriosis and ultimately pathogen infection.

Keywords Oral antimicrobial activity · Children · Lead exposure · Peripheral monocyte percentage · Saliva salivary agglutinin · Electronic waste

Abbreviations

Pb lead
LnPb natural logarithmic transformation
of blood Pb level

SAG salivary agglutinin
PM_{2.5} fine particulate matter
POPs persistent organic pollutants
BMI body mass index
AMP antimicrobial protein and peptide
WBC white blood cell
E-waste electronic waste
CI confidence interval

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Introduction

Exposure to environmental chemicals induces dysbacteriosis in the digestive tract, of which oral microbial variation may reflect a prospective risk for oral and general health, such as dental caries, periodontal disease, oral cancer, pancreatic cancer, esophageal cancer, and atherosclerosis (Aarabi et al. 2018; Fan et al. 2018; Gao et al. 2018; Hsiao et al. 2018; Peters et al. 2017; Sadykov et al. 2009; Sudhakara et al. 2018; Xia et al. 2018; Zhang et al. 2015; Jin et al. 2017). Rat experiment indicated that lead (Pb) toxicity can damage oral health including alveolar bone loss and periodontal prevalence (Terrizzi et al. 2014). Gil et al. (1996) identified that higher tooth Pb levels could increase dental

plaque, alter dental color, induce proliferation of cariogenic bacteria, and increase dental caries risk in people (≥ 10 years old). A cross-sectional study showed that, in Thailand, industry Pb contamination could increase caries in deciduous teeth of children (6–11 years old) with cariogenic bacteria increased (Youravong et al. 2006). In addition, epidemiology data also suggested that blood Pb levels were significant positive with dental caries (Gemmell et al. 2002; Kim et al. 2017; Moss et al. 1999). Our recent study also indicated that chronic Pb exposure can increase the potential risk of dental caries in deciduous teeth among pre-school children (Hou et al. 2020). Although Pb toxicity on oral dysbacteriosis and dental caries prevalence has been emphasized, the mechanism is unclear.

Pb, as a ubiquitous environmental chemical, imposes adverse effects on humans, especially for children in informal recycling areas (Dai et al. 2017; Heacock et al. 2018; Wang et al. 2018; Zeng et al. 2018b). Guiyu, a larger electronic waste (e-waste) dismantling area in the world, has witnessed ambient Pb contamination that has had negative health effects on local children, such as decreased olfactory memory, declined lung function, elevated cardiovascular risk, hearing loss, immune dysfunction, and endocrine disorder (Cao et al. 2018; Cong et al. 2018; Heacock et al. 2018; Li et al. 2018; Lin et al. 2017; Liu et al. 2018a, b; Lu et al. 2018; Zeng et al. 2017; Zhang et al. 2017; Zhang et al. 2016). Prior studies have indicated that ambient fine particulate matter (PM_{2.5}) can reduce airway antimicrobial proteins and peptides (AMPs) by oxidative stress injury and absorption on AMPs, which accelerates the risk of respiratory infection (Zhang et al. 2019a; Chen et al. 2018; Mushtaq et al. 2011; Rivas-Santiago et al. 2015; Vargas Buonfiglio et al. 2017). Pb, as a main component of PM_{2.5}, can also induce oxidative damage and apoptosis (Li et al. 2016; Vaziri 2008; Wannhoff et al. 2013; Xu et al. 2018; Zeng et al. 2018a; Zheng et al. 2016). However, whether Pb may inhibit AMPs has not been reported.

Saliva, contains several AMPs, plays a pivotal role in maintaining oral microbial balance and contributing to human health (Fabian et al. 2012; Pedersen et al. 2018). Salivary agglutinin (SAG), a major AMP in saliva, promotes microbial agglutination to facilitate microbial clearance, which provides protection against pathogen infection (Gunput et al. 2016; Reichhardt et al. 2017; Reichhardt and Meri 2016; Sonesson et al. 2011; Zhang et al. 2019b). Moreover, SAG can also elevate pathogen removal by competitively inhibiting microbial colonization to host cells (Boks et al. 2016). In addition, SAG is able to bind endogenous molecules, such as lactoferrin, secretory immune globulin (Ig) A, and surfactant proteins A and D, to increase antimicrobial effects in a synergistic action (Hartshorn et al. 2006; Madsen et al. 2010; Reichhardt et al. 2017). Collectively, the critical role of SAG in sustaining microbiota balance has been highlighted.

Peripheral leukocytosis, which is a biomarker of infection and inflammation, is associated with clinical outcomes (including sepsis, cancer, and tissue injury) and is an ordinary finding

in clinical laboratory (Chabot-Richards and George 2014; Fabian-Jessing et al. 2018; Fessler et al. 2017). Notably, the absolute count of peripheral white blood cell (WBC) is emphasized in predicting human all-cause mortality (de Labry et al. 1990). Previous studies have suggested that dengue virus infection can induce monocyte activation, although only the increase is significant in peripheral monocyte percentage (Naranjo-Gómez et al. 2018; Castillo and Urcuqui-Inchima 2018). Reactive monocyte can also be induced by activated neutrophils, and may reflect pathogen chronic infection (Chabot-Richards and George 2014; Kumar and Sharma 2010).

This study aims to investigate the association of Pb toxicity and oral antimicrobial activity in children. We hypothesize that exposure to Pb may weaken oral antimicrobial activity through reducing saliva SAG concentration, ultimately raising the risk of oral dysbacteriosis and pathogen infection.

Materials and methods

Study population

A cross-sectional study, recruiting totals of 207 healthy children (3–7 years old) from Guiyu (exposed group, $n = 101$) and Haojiang (reference group, $n = 106$), was performed in 2017. Except for e-waste contamination, these two groups were similar including cultural background, dietary habit, and ethnicity (Xu et al. 2015; Zhang et al. 2017). In addition, our prior studies had indicated that air pollution in Guiyu was worse than in Haojiang, with higher levels of heavy metals (such as Pb and cadmium) in Guiyu PM_{2.5} (Cong et al. 2018; Zeng et al. 2016; Zheng et al. 2016). All children have lived in local areas exceeding 1 year and are free of any known medical condition, and their guardians signed the informed consent form. A questionnaire on basic population information, dwelling environment, and children's physical condition was collected. All proposals of this study were approved by the Human Ethics Committee of Shantou University Medical College, China.

Sample collection and biological measurements

Fasting venous blood (2 mL), obtained by nurses, was applied to determine blood Pb levels by graphite furnace atomic absorption spectrophotometry (Jena Zeenit 650, Germany) and measure peripheral WBC counts and monocyte counts as well as the ratio of monocyte by automatic hematology analyzer (Sysmex XE-2100, Japan), the detection methods were described elsewhere in detail (Fessler et al. 2017; Guo et al. 2010; Huo et al. 2007; Zeng et al. 2017). During blood Pb level analysis, linearity of the standard curve was good ($r^2 = 0.993$), the detection limit was 0.53 $\mu\text{g/L}$, the accuracy was 95–107%, and the precision was less than 15%.

Saliva, collected as previously described, was tested for SAG detection by enzyme linked immunosorbent assay (ELISA) with a quantitative sandwich ELISA kit (ELISAGenie Inc., UK) (Gunput et al. 2016). According to the manufacture's technical manual, saliva had cellular debris removed and collected supernatant by centrifugation (1000g for 20 min, 4 °C). The relevant parameters of this kit were as follows: measuring range was 0.312–20 ng/mL, sensitivity was 0.188 ng/mL, and the intra- and inter-assay accuracies were 8% and 10%, respectively. The remainder blood and saliva were then aliquoted and stored at −80 °C.

Statistical analysis

To compare the differences of two groups, independent-sample *t*-test, Mann-Whitney U test, and Pearson chi-square test were performed appropriately. The associated factors of blood Pb level were determined by Pearson correlation analyses. Simultaneously, Kolmogorov-Smirnov test was conducted to estimate the data distribution, and natural logarithmic transformation of blood Pb level (LnPb) was used to estimate the correlations of LnPb to saliva SAG concentration by univariate linear regression analysis. Additionally, the mediation effect of saliva SAG concentration on the correlation between LnPb and the ratio of monocyte was conducted by covariate adjusted mediator analysis as prior described (Lu et al. 2018; Preacher and Hayes 2008). All analyses were performed with GraphPad Prism 7.0 (GraphPad, CA) and SPSS 19.0 (IBM Corporation, USA), and statistical significance was a $P < 0.05$ in a two-tailed test.

Results

General characteristics of children in this study

As shown in Table 1 and Table S1, totals of 207 children were recruited in this study. There were no significant differences on gender, age, and body mass index (BMI) of the two groups (all $P > 0.05$). Exposed group children had a lower chest

circumference than reference group children ($P < 0.01$). Compared with reference group children, exposed group children were more likely to eat preserved egg ($P < 0.01$), and they had unhealthy living habits such as pencil biting ($P < 0.001$), moreover, their dwelling environment (namely, family member daily smoking and distance away from road) was worse (all $P < 0.01$), and their parental educational level and family socioeconomic status was lower (all $P < 0.001$).

There was a lower saliva SAG concentration in exposed group children than reference group children ($P < 0.001$) (Table 1). In addition, peripheral cell counts of WBC and monocyte in exposed group children were more than reference group children (all $P < 0.05$) (Fig. 1a), although the values were within the biological reference range (WBC $3.5\text{--}9.5 \times 10^3/\mu\text{L}$ and monocyte $0.1\text{--}0.6 \times 10^3/\mu\text{L}$). Moreover, exposed group children had a higher peripheral monocyte percentage than reference group children ($P < 0.001$) (Fig. 1b).

Blood Pb level and associated factors

The median blood Pb level in exposed group children was higher than reference group children ($P < 0.001$) (Table 1). Additionally, the blood Pb level percentage, exceeding $5 \mu\text{g/dL}$, was higher in exposed group children than in reference group children ($P < 0.001$) (Table 1). Pearson correlation analysis suggested that blood Pb level was positively correlated with e-waste contacting, family member daily cigarette consumption, and residence within 50 m from an e-waste site ($r_s = 0.138$, $r_s = 0.143$, and $r_s = 0.189$, respectively, all $P < 0.05$), while negatively associated with parental educational level (father/mother, $r_s = -0.211$ and $r_s = -0.190$, respectively, all $P < 0.01$) (Fig. 2 and Table S2). The high blood Pb level, take together, may be blamed on unhealthy hygiene and habits, poor dwelling environment, and low parental educational level.

Association of LnPb and saliva SAG concentration

A linear regression model suggested a statistically significant, negative correlation of LnPb to saliva SAG concentration [B

Table 1 General characteristics of the study volunteers

	Reference group (<i>n</i> = 106)	Exposed group (<i>n</i> = 101)	<i>P</i> value
Gender (boys/girls)	54/52	50/51	0.836 ^a
Age (years)	4.82 ± 0.79	4.71 ± 0.82	0.323 ^b
BMI (kg/m ²)	15.39 ± 1.40	15.12 ± 1.18	0.139 ^b
Saliva SAG concentration (ng/mL)	8.62 (10.99)	4.40 (5.94)	< 0.001 ^c
Blood Pb level (μg/dL)	3.47 (1.09)	4.89 (2.64)	< 0.001 ^c
> 5 μg/dL [<i>n</i> (%)]	11 (10.38)	48 (47.52)	< 0.001 ^a

BMI, body mass index. SAG, salivary agglutinin. Statistical significance, $P < 0.05$. ^a Analysis by Pearson Chi-square test. ^b Mean ± standard deviation, analysis by independent-sample *t*-test. ^c Median (interquartile range), analyzed by Mann-Whitney U test.

Fig. 1 Peripheral cell counts and the monocyte percentage in children (Data are presented as mean $\pm$ standard deviation, obtained with an independent-sample *t*-test, * $P < 0.05$, *** $P < 0.001$)

(95% CI) = -1.813 (-3.490 , -0.136), $P < 0.05$], illustrating that with each one-fold increases in LnPb, the saliva SAG concentration reduces by 1.813 ng/mL. In brief, the higher the blood Pb level, the lower the saliva SAG concentration.

Mediation effect analysis

Mediation model analysis showed a statistically significant result that saliva SAG concentration was a mediator in the correlation of LnPb and peripheral monocyte percentage [B (95% CI) = 0.081 (0.016 , 0.201), $P < 0.05$] (Fig. 3). A one-fold increase in LnPb was estimated to be correlated with a 0.081% increase in peripheral monocyte percentage by decreasing saliva SAG concentration. In addition, the direct effect of LnPb on peripheral monocyte percentage was not

statistically significant in mediation effect analysis [B (95% CI) = 0.193 (-0.190 , 0.577), $P > 0.05$], indicating that saliva SAG concentration plays a complete mediating role.

Discussion

The present study showed that e-waste-exposed group children have higher blood Pb levels, decreased saliva SAG concentrations, and increased peripheral WBC counts and monocyte counts as well as monocyte percentage than reference group children. LnPb was negatively correlated with saliva SAG concentration, while positively correlated with peripheral monocyte percentage. Saliva SAG concentration played a complete mediating role in the correlation of LnPb to

Fig. 2 Pearson correlations (r_s) of blood Pb level and associated factors (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Fig. 3 Mediation effect of saliva SAG concentration on the relationship between LnPb and the ratio of peripheral monocyte (LnPb, natural logarithmic transformation of blood Pb level; SAG, salivary agglutinin; BMI, body mass index; B, unstandardized coefficient; CI, confidence interval. Analyses are controlled for age, gender, BMI, chest circumference, and pencil biting; 5000 bootstrap samples; $n = 195$. Statistical significance, $P < 0.05$)

peripheral monocyte percentage. Collectively, the present study indicates that environmental Pb exposure may weaken oral antimicrobial activity through reducing saliva SAG concentration in children.

Despite a reduction in Pb exposure, as a result of concerted government and public efforts, Pb remains the most ubiquitous environmental toxicant in Guiyu, which constitutes a detrimental public health problem (Cao et al. 2018; Huo et al. 2007; Lu et al. 2018; Zhang et al. 2017). The results showed the median blood Pb levels of exposed group children were 4.89 $\mu\text{g/dL}$, with 47.52% of children exceeding 5 $\mu\text{g/dL}$. In addition, the blood Pb level was affected by hygiene and living habits, dwelling environment, and parental educational level, which is consistent with prior findings (Lu et al. 2018; Zhang et al. 2017). As a whole, through hand/object-to-mouth activities, children are more vulnerable to environmental Pb exposure, especially in e-waste dismantling areas (Lu et al. 2018; Xue et al. 2010; Zahran et al. 2013).

Concomitantly, compared with reference group children, exposed group children had a lower saliva SAG concentration (median 4.40 ng/mL vs. 8.62 ng/mL). Sensitivity analysis indicated that the higher the blood Pb level, the lower the saliva SAG concentration. Previous studies have determined that exposure to ambient $\text{PM}_{2.5}$ can downregulate expression of AMPs by oxidative stress damage (Chen et al. 2018; Mushtaq et al. 2011; Rivas-Santiago et al. 2015). Pb, a component of $\text{PM}_{2.5}$, can also induce oxidative damage and apoptosis (Li et al. 2016; Vaziri 2008; Wannhoff et al. 2013; Xu et al. 2018; Zeng et al. 2018a; Zheng et al. 2016). In addition, our prior studies have indicated the $\text{PM}_{2.5}$ -bound Pb concentration is higher in Guiyu than most other cities in Asia, accompanied by increased blood Pb levels in Guiyu children (Zeng et al. 2016, 2018a; Zheng et al. 2016). On the other hand, mounting studies implicated Pb exposure may be associated with dental caries prevalence, while saliva SAG can affect dental caries prevalence by interacting with *Streptococcus mutans* (Moss et al. 1999; Gemmel et al. 2002; Youravong et al. 2006; Jonasson et al. 2007). In

addition, our recent study also showed that Guiyu children had an increased risk of dental caries in deciduous teeth (Hou et al. 2020). Therefore, the phenomenon of blood Pb level and saliva SAG concentration might be blamed on oxidative stress damage of Pb toxicity (Vaziri 2008; Wannhoff et al. 2013; Xu et al. 2018). Additionally, saliva SAG concentration played a pivotal role in the correlation of LnPb to peripheral monocyte percentage.

Peripheral leukocytes are pathogen infection biomarkers, and monocyte may reflect chronic pathogen infection (Chabot-Richards and George 2014; Fessler et al. 2017). The present study showed that exposed group children had more peripheral cell counts of WBC (mean: $7.25 \times 10^3/\mu\text{L}$ vs. $6.82 \times 10^3/\mu\text{L}$) and monocyte (mean: $0.41 \times 10^3/\mu\text{L}$ vs. $0.35 \times 10^3/\mu\text{L}$), and a higher ratio of monocyte (mean: 6.26% vs. 5.03%) than reference group children. Previous studies have suggested, in occupationally exposed workers, that short-term Pb exposure increases peripheral WBC counts, whereas this change was not observed in chronic Pb exposure (Chwalba et al. 2018; Dobrakowski et al. 2016). In addition, other prior study showed that environmental Pb exposure did not affect child peripheral WBC counts (Zeng et al. 2017). Although Pb toxicity on peripheral WBC counts is inconsistent, that Pb can alter the proportions of leukocyte subtypes has been demonstrated (Chwalba et al. 2018; Dobrakowski et al. 2016). On the other hand, even if the difference is relatively small in peripheral WBC counts ($0.43 \times 10^3/\mu\text{L}$), it has been estimated that a 20% difference in all-cause mortality is caused by each $1 \times 10^3/\mu\text{L}$ change in peripheral WBC counts even within the normal biological reference range (de Labry et al. 1990).

Several limitations in this study should be noted. Firstly, we did not evaluate the status of salivary microbes which may directly reflect the variation of oral microbiota. In addition, we just estimated Pb toxicity on saliva SAG concentration because of limited blood volume. Other environmental chemicals, such as benzo[a]pyrene, cadmium, mercury, and persistent organic pollutants (POPs) also can induce oxidative

stress damage, which might suppress expression of SAG in saliva. Therefore, in future studies, more attention should be paid to the combined effects of multiple environmental toxicants on the alteration of oral antimicrobial activity and the variation of oral microbiota.

Conclusion

To our best knowledge, this is the first time to investigate the association of environmental Pb exposure and oral antimicrobial activity in children. The present results show the increased blood Pb levels, decreased saliva SAG concentration, and elevated peripheral cell counts in e-waste-exposed group children. This available data supports the hypothesis that environmental Pb exposure might weaken oral antimicrobial activity by decreasing saliva SAG concentration, which may raise the risk of oral dysbacteriosis and ultimately pathogen infection. Despite recent declines, Pb is still the most ubiquitous and prominent environmental pollutant in e-waste dismantling areas, posing a serious threat to the health of children. Therefore, in the future, to keep children away from the toxic effect of environmental Pb exposure, more effective and stronger management measures should be carried out.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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