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Prenatal triclosan exposure and cord blood immune system biomarkers

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ABSTRACT

Triclosan is widely used as an antimicrobial agent and preservative that has been hypothesized to play a role in asthma and allergic disease. The limited body of literature regarding the allergenicity of triclosan has not evaluated prenatal exposure and subsequent potential effects on the developing immune system. The objective of the present study was to determine the association between prenatal urinary triclosan concentrations and cord blood immune system biomarker concentrations. Umbilical cord blood samples were obtained from the Maternal-Infant Research on Environmental Chemicals (MIREC) Biobank and were tested for three immune system biomarkers: immunoglobulin E (IgE), thymic stromal lymphopoietin (TSLP), and interleukin-33 (IL-33). Triclosan concentrations were measured in urine at 6–13 weeks gestation. No statistically significant associations were observed between prenatal triclosan concentrations and elevated concentrations of any immune system biomarker (n = 1219 participants). Longitudinal studies are necessary to determine how the observed findings at birth translate into childhood.

1. Introduction

Triclosan is widely used as an antimicrobial agent and preservative. Common sources of exposure to triclosan include cosmetics, antibacterial soaps, and other household cleaning products (Health Canada and Environment Canada, 2012). Triclosan has been hypothesized to play a role in asthma and allergic disease (Anderson et al., 2013). The potential association between triclosan exposure and increased risk of allergic disease is consistent with the hypothesis that reduced microorganism exposure, due, in part, to the ubiquity of antibacterial soaps containing triclosan, may exacerbate susceptibility to childhood allergic disease (Calafat et al., 2008; Health Canada, 2015). Established risk factors, such as genetics and environmental tobacco smoke exposure, are likely involved in less than one half of childhood allergic disease cases (Backman et al., 2014; Cookson et al., 2011) and exposure to environmental contaminants has also been hypothesized to be responsible for some portion of these cases (Ho, 2010).

Authors of studies using US NHANES data (National Health and Nutrition Examination Survey) reported that urinary triclosan concentrations were positively associated with allergic sensitization in children between 6 and 18 years (Clayton et al., 2011; Savage et al., 2012). Parental-reported antimicrobial exposure has also been associated with a higher likelihood of wheezing and allergic rhinitis symptoms in children under the age of 13 (Hong et al., 2014). Urinary triclosan concentrations have been similarly associated with increased odds of allergic sensitization among Norwegian children (Bertelsen et al., 2013). These studies have not focused on exposure during pregnancy and subsequent effects on offspring immune system development.

Considering that the fetal time period is a critical window of immune system development and enhanced susceptibility to potential adverse effects of environmental exposures, we endeavored to determine the association between prenatal triclosan exposure and concentrations of cord blood immune system biomarkers. The biomarkers measured in this study included immunoglobulin E (IgE), Interleukin-33 (IL-33) and thymic stromal lymphopoietin (TSLP). IgE has been previously examined as a biomarker of immune system susceptibility to prenatal environmental contaminant exposures (Herr et al., 2011). IL-33 and TSLP have been recently recognized as being integral to the mechanisms underlying allergy (Bartemes and Kita, 2012), atopic dermati-

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Table 1
Adjusted[a] odds ratios (95% CI) of elevated cord blood immune system biomarkers and quartiles of urinary triclosan.

Triclosan (μg/L)	IgE	IL-33	TSLP	IL-33/TSLP
<2.30	1.0	1.0	1.0	1.0
2.30–<8.90	1.50 (0.95–2.38)	1.41 (0.92–2.17)	1.24 (0.82–1.89)	1.35 (0.85–2.15)
8.90–<77.09	1.29 (0.82–2.06)	1.21 (0.78–1.87)	1.13 (0.74–1.71)	1.21 (0.76–1.92)
≥77.09	1.03 (0.63–1.68)	1.32 (0.85–2.05)	1.08 (0.70–1.66)	1.17 (0.72–1.89)

[a] Adjusted for specific gravity, maternal age, and household income.

tis (Brandt and Sivaprasad, 2011), and asthma (Gauvreau et al., 2014). Further, a recent experimental study reported that triclosan induced TSLP and IL-33 expression in human skin (Marshall et al., 2015), suggesting that triclosan plays a role in triggering allergy response.

2. Material and methods

Data and biospecimens were obtained from the Maternal-Infant Research on Environmental Chemicals (MIREC) Study, a *trans*-Canada cohort study of 2001 pregnant women from ten Canadian cities recruited between 2008 and 2011 (Arbuckle et al., 2013). The study population for the MIREC Study and the sub-cohort for the present analysis have been described previously (Arbuckle et al., 2013; Ashley-Martin et al., 2015). Umbilical cord blood samples that were determined to be contaminated with maternal blood based on an elevated immunoglobulin A (IgA) concentration (≥10 μg/mL) were excluded from the analysis (Ownby et al., 1996). As pre-term and multiple birth infants had lower cord blood concentrations of the immune system biomarkers and may have a differing allergic disease risk profile (Algert et al., 2011; Been et al., 2014), these subjects were excluded from this study. This study received ethical approval from Health Canada, St. Justine's Hospital (Montreal, QC) and the IWK Health Centre (Halifax, NS) and all participants provided consent.

Immune system biomarkers were measured in the plasma of umbilical cord blood samples (details in Ashley-Martin et al., 2015). TSLP concentrations were determined using a commercial antibody kit (Biolegend, San Diego, CA, USA). IL-33 concentrations were analyzed using antibodies from an R & D systems duoset (Minneapolis, MN, USA). ELISA kits (EBioscience, San Diego, CA, USA) were also used to assess both total IgE and IgA concentrations.

Urinary concentrations of free and conjugated forms of triclosan were measured in the first trimester (6–13 weeks) in MIREC study participants. Details regarding triclosan measurement methods and descriptive results are presented elsewhere (Arbuckle et al., 2015). In this study, triclosan concentrations are reported as total triclosan and represent the sum of free and conjugated forms. The limit of detection (LOD) for total triclosan was 0.12 μg/L, which is the more conservative LOD of the two triclosan components. Values below the LOD are reported as machine readings.

The immune system biomarkers were dichotomized because of the high percentage of samples that were below the LOD. A composite variable was developed to identify samples with elevated concentrations of both TSLP and IL-33 (IL-33/TSLP) as these cytokines are highly correlated (Spearman correlation coefficient (rs) = 0.8). Because there are no pre-existing thresholds for TSLP and IL-33, they were categorized at the 80th percentile (TSLP = 554 pg/mL; IL-33 = 879 pg/mL) to allow for a sufficient sample size for comparisons. Elevated concentrations of the composite IL-33/TSLP variable were defined as those samples that had elevated concentrations (≥80% ile) of both TSLP and IL-33. The cut-off percentile for IgE was defined at 1.2 ng/mL (0.5 kU/L), a cut-off point previously used in studies of cord blood IgE (Sadeghnejad et al., 2004).

We performed logistic regression to calculate the odds of elevated biomarker concentrations according to quartiles of triclosan concentrations. Logistic regression models were adjusted for maternal age and household income as these variables are potential predictors of both urinary triclosan concentrations (Calafat et al., 2008) and the cord blood biomarkers measured in this study (Ashley-Martin et al., 2015). Specific gravity was also included as a covariate in the model to account for urinary dilution heterogeneity (Arbuckle et al., 2015). We evaluated the relationship between log-transformed triclosan as a continuous variable and the binary immune system biomarkers using restricted cubic spline curves. Effect modification by sex was also evaluated by examining the significance of the sex*triclosan product term.

3. Results

For a total of 1219 participants, both cord blood samples and triclosan data were available and these data were included in the present analysis. As previously reported, the majority of participants had a normal pre-pregnancy BMI, never smoked, were over 30 years of age, and had a household income greater than $50,000 (Ashley-Martin et al., 2015). Median specific gravity-adjusted triclosan concentrations were 9.52 μg/L with an interquartile range of 10.24 μg/L. Nearly all participants (99.4%) had triclosan values above the LOD (>0.12 μg/L).

We observed positive associations between triclosan and each biomarker but none of these associations reached statistical significance. The magnitude of the effect was greatest in the second quartile of exposure for each biomarker; for example, compared to women with triclosan concentrations below 2.30 μg/L, women with concentrations in the second quartile (2.30 to <8.90 μg/L) had a 50% increase in odds of high IgE cord blood levels (OR = 1.50; 95% CI:0.95,2.38) (Table 1). The magnitude of this effect was attenuated in the third and fourth quartiles. There were no significant associations in the spline analysis of log transformed triclosan modeled as a continuous variable (data not shown). No effect modification by sex was observed (all p-values for the product terms were >0.1).

4. Discussion

We observed neither statistically significant associations nor dose-response relations between prenatal triclosan exposure and cord blood levels of IgE, TSLP or IL-33, although the direction of effect was positive. It is possible that the lack of statistical significance reflects (1) a true lack of effect, (2) residual confounding or (3) limited study power to observe the small effect sizes we detected. Direct comparison with previous epidemiological literature is not straightforward due to differences in study design and population. Authors of cross-sectional studies have reported that triclosan exposure is associated with certain measures of allergic sensitization or allergies in children 6–18 years of age (Clayton et al., 2011; Savage et al., 2012). Median concentrations in the MIREC Study (8.90 μg/L) are comparable to those reported among females in NHANES (8.5 μg/L) (Calafat et al., 2008), suggesting that the differences in results are not due to lower exposures in MIREC.

Recent experimental evidence suggests that local triclosan exposure can induce TSLP and IL-33 activity in human skin (Marshall et al., 2015). Results from a mouse model suggest that triclosan may exacerbate allergic responses in the presence of co-occurring allergenic exposures, such as ovalbumin, but not in absence of these co-exposures (Anderson et al., 2013). It is possible, therefore, that triclosan may enhance the effects of co-occurring allergens. To our knowledge, there has not been an animal model that has determined the relation between maternal-fetal triclosan transfer and resulting immune response; therefore, it is not possible to know whether triclosan exposure would elicit a maternal immune response that, in turn, influences fetal immune responses or whether offspring are influenced by the direct effect of triclosan that has crossed the placenta. The limited epidemiological literature of maternal-fetal triclosan exposure has reported a poor correlation between maternal urinary triclosan and cord blood plasma concentrations (Pycke et al., 2014); however, animal studies have demonstrated that maternal triclosan exposure does influence offspring outcomes such as thyroid levels (Paul et al., 2010).

The generalizability of the findings is limited by the fact that the MIREC cohort is largely Caucasian with higher income and education levels compared to the overall Canadian population of women in this age group. Our findings are limited by the use of a single measurement of triclosan during the first trimester. Triclosan is rapidly eliminated with an estimated half-life in plasma of 21 h (Sandborgh-Englund et al., 2006). A Canadian study of four prenatal and one postpartum serial triclosan measurements reported an intraclass correlation coefficient (ICC) of 0.50 over the entire study period (Weiss et al., 2015). A study of six serial urinary triclosan measurements in children over the course of six months reported an ICC of 0.4(Teitelbaum et al., 2008). These values imply that first trimester triclosan concentrations may be reflective of exposure throughout pregnancy, though serial measurements would be necessary to confirm such reproducibility. We explored the possibility of selection bias in the participants of this study. Since 1363 women from the MIREC cohort did not provide a cord blood sample, we compared the distribution of factors between women who did, and did not, provide a cord blood sample and found similarities between these two groups. Therefore, we do not think our results are compromised by selection bias. The lack of clinical outcomes in children is another limitation. Given, the lack of literature regarding correlation between TSLP and IL-33 at birth and in childhood, we cannot predict the extent of allergic disease among children with high cord blood cytokine levels; however, our study benefits from the comprehensive nature of the MIREC data. Further longitudinal studies are necessary to determine how the observed findings at birth translate into childhood.

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