Triclosan/triclocarban levels in maternal and umbilical blood samples and their association with fetal malformation

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ABSTRACT

Triclosan (TCS) and triclocarban (TCC) are widely used as antimicrobial compounds in consumer products. TCS and TCC are frequently found in waste water and sewage. In this study, we investigate the potential impact of exposure to triclosan (TCS) and triclocarban (TCC) on fetal abnormalities. We measured TCS and TCC levels in maternal and umbilical cord blood samples from 39 pregnant women diagnosed with fetal or post-birth abnormalities at Beijing Obstetrics and Gynecology Hospital. 52 pregnant women who gave birth to healthy neonates during the same period of time were included as controls. Applying ultra-performance liquid chromatography-tandem mass spectrometry, TCS and TCC concentrations were measured in maternal and fetal sera. Significantly increased levels of TCS were detected in maternal sera from mothers with abnormal births. Similar levels of TCS or TCC were found in maternal and cord sera in control group. The concentrations of TCS or TCC in maternal sera correlated with those in umbilical cord sera (r = 0.649, P < 0.01). These observations suggest that maternal blood test could be a useful assay for detecting fetal exposure to TCS and TCC, and high exposure to TCS may be potentially associated with increased risk for fetal malformations.

1. Introduction

Fetal abnormalities/defects or birth defects include all abnormalities in the structure, function and/or metabolism of the fetus that develop prior to birth. The etiology of birth defects is generally thought to be a multifactorial process. Hereditary, environmental factors, or a combination of the two are often involved. In China alone fetal anomalies were found in 5.6% of all live births in 2012, with a total of 900,000 infants affected [1].

Triclosan (TCS) and triclocarban (TCC) are analogous in chemical structure but independent of each other. These chemicals have been widely used for >50 years as an antimicrobial component in consumer products such as toothpastes, soaps, shampoos, detergents, and medical disinfectants, for personal hygiene care, treatment of textiles and manufacturing of consumer plastics. TCS and TCC are also increasingly identified as contaminants in waste water and sewage material.

Untreated or incompletely treated waste water often contains detergent metabolites, pharmaceuticals, and plasticizers that pollute environment, which can adversely affect agriculture and different ecosystems [2–4]. TCS/TCC may act alone or interact with other contaminants to exert biological impacts on human health, especially on human reproduction [5].

Humans exposure to TCS and TCC usually occurs by dermal absorption or ingestion [6], or other environment exposures such as inhalation of contaminated indoor or outdoor air [7]. TCS has been detected in human breast milk [8], human plasma [9], urine [6], amniotic fluid [10], and umbilical cord blood [11]. Although these compounds are thought to be of low systemic toxicity in mammals, studies indicated that thyroxine homeostasis in weaning rats [12] and sheep could be disrupted by these compounds. Additionally it is thought that estrone sulfonation is inhibited by TCS and TCC [13]. These compounds were also found to interfere with blastocyst implantation in mice [14].

Although studies in animal models indicated that TCS and TCC could be endocrine disruptors, their impact on fetal health in human has not been determined. In this study, we measured the TCS and TCC levels in umbilical cord serum samples and analyzed their associations with fetal anomalies. Delineation of the association between fetal abnormalities and in utero exposure to TCS and TCC will help us to assess the risk...
of birth defects caused by these compounds, and build a foundation for the design of preventive procedures against these factors.

2. Materials and methods

2.1. Reagents and standards

Standard TCS (99.5%) and TCC (99%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Toronto Research Chemicals (North York, Ontario, Canada). 13C12-TCS (99%) and 13C6-TCC (99%) were obtained from Cambridge Isotope Laboratories (Andover, MA) and used as the internal standards. LC-MS grade methanol, acetonitrile, and water were purchased from Sigma-Aldrich (St. Louis, MO). Analytical-grade sodium acetate, and acetic acid (99.5%) were obtained from Beijing Chemical Works (Beijing, China). 13C6-TCC (99%) were obtained from Cambridge Isotope Laboratories (Andover, MA). Traceable and acetic acid (99.5%) were obtained from Beijing Chemical Works (Beijing, China).

2.2. Sample collection

Two groups (fetal anomaly: N = 39; controls: N = 53) were included in this study. The fetal anomaly group comprised pregnant women who were diagnosed with fetal abnormalities during gestation or delivery of babies with malformations. Controls were pregnant women with normal fetuses and adverse neonatal outcomes at birth during the same period of time (form March 2013 to February 2014). Maternal venous (2 mL) and umbilical cord blood (2 mL) from all cases were obtained after patients’ admission to the Beijing Obstetrics and Gynecology Hospital. The study protocol was approved by the Institutional Review Board of Beijing Obstetrics and Gynecology Hospital. All study subjects have signed informed consent documents before participation.

2.3. Sample preparation and storage

Blood samples were centrifuged at 900 × g for 5 min and sera were transferred to polypropylene tubes. Serum samples were frozen and sent to Beijing Key Laboratory of Diagnostic and Traceability Technologies for Food Poisoning (Beijing Center for Disease Control and Prevention) where the samples were stored at −20 °C until measurement. For each sample (2 mL), concentrations of total (free and conjugated) TCS and TCC were measured using automated on-line solid phase extraction coupled with ultra-high-performance liquid chromatography-tandem mass spectrometry (on-line SPE UHPLC-MS/MS). Enzyme solutions were prepared daily by diluting 500 μL of β-glucuronidase/arylsulfatase (Helix pomatia, 100,000 units/mL) purchased from Roche Diagnostic GmbH (Mannheim, Germany) with 495 μL of acetonitrile was added to the sample. Following vigorous vortexing, the mixture was centrifuged at 14,000 rpm for 15 min. 400 μL of the supernatant was diluted with 400 μL water, and subjected to on-line SPE UHPLC-MS/MS analysis.

2.4. Instrument analyses

Chromatographic analyses of samples were performed using a Dionex Ultimate 3000 UHPLC system (Dunnyvale, CA) that consists of two binary pumps with degassers, an autosampler, and a column compartment with a 6-port switching valve. The samples (500 μL) were injected, and the analytes were extracted using a CAPCELL PAK MF C8 column (4.6 mm I.D. × 50 mm, 5 μm particle size, 8 nm pore size, Shiseido, Japan) operating at 2.0 mL/min. By switching the 6-port valve, the chemicals were back-flushed by a solvent stream of 0.3 mL/min into a Waters Acquity UPLCTM HSS T3 column (2.1 × 50 mm, 1.8 μm).

Liquid chromatography system was connected to a Waters XevoTM TQ-S triple-quadrupole mass spectrometer (Waters Corp., Milford, MA) operating with a negative electrospray ionization source. Multiple reaction monitoring mode was used to quantitatively measure the analytes. The instrumental parameters were set as following: capillary voltage = 1.0 kV; source temperature = 135 °C; desolvation temperature = 500 °C; desolvation gas (N2) = 1000 L/h; and collision cell pressure = 4.95 × 10⁻⁴ mbar.

2.5. Quality control

With every batch of samples analyzed, procedural blanks (n = 3) were included to confirm sample preparation and investigate instrumental system contamination. Approximately 0.005 μg/L of TCC in procedural sample blanks was observed, which was below the detection limit of the method and may be explained by the ubiquity and high sensitivity of TCC. Mean TCC and TCS concentrations measured in procedural blanks for each analysis batch were subtracted from concentrations detected in samples. Moreover, a calibration standard and solvent blank were injected after every tenth sample to monitor background TCC and TCS. Mean recoveries of both analytes were validated in six replicates by spiking at three fortification levels between 93.7 and 103.6% for samples. The intra-day variability was <20%, as represented by the relative standard deviation percent (RSD%) at each fortification level for each compound. The limit of detection (LOD) and limit of quantification (LOQ) were determined by signal-to-noise (S/N) ratios of 3:1 and 10:1, respectively. The LODs (LOQs) were 0.005 μg/L (0.01 μg/L) and 0.002 μg/L (0.006 μg/L) for TCS and TCC, respectively. For TCC, a 7-point calibration standard curve was obtained at concentrations between 0.005 and 100 μg/L (TCS was 10 times greater than TCC). Furthermore, concentrations measured in the samples were corrected for recoveries of internal standards (13C12-TCS and 13C6-TCC).

2.6. Data analyses

All statistical analyses were performed using SPSS 17.0 (IBM, Chicago, IL). Concentrations below the LOD were assigned a value of half of the LOD [15]. Detection rate, mean ± standard deviation (SD), geometric mean (GM), median, and range were used to describe the TCS and TCC in samples. Detection rate was analyzed using Chi-squared test. TCS and TCC data were assessed with rank sum tests due to the skewed distribution. Differences and correlations were tested with non-parametric tests: Mann-Whitney U test, Wilcoxon signed rank test, and Spearman rank correlation. Significance level was set at 0.05.

3. Results

This study covers 39 cases in the fetal-defect group (40 samples; one case was twins), and 52 cases in the control group (Table 1). There was no significant difference in mothers’ age and gestational age between the two groups. Fetal defects of the study subjects were classified according to ICD-10 guidelines. The distribution of defects is shown in Table 2. Congenital malformations of the circulatory system, eye, ear, face, neck, urinary system and musculoskeletal system were among the most frequent abnormalities.

Table 1: Detailed sample characteristics in this study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Fetal defect group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>40</td>
<td>52</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>32.2</td>
<td>29.5</td>
</tr>
<tr>
<td>Average gestation week</td>
<td>25.4</td>
<td>39.2</td>
</tr>
</tbody>
</table>
The concentrations of the detectable samples below the limit of quantification (LOD) were assigned a value of half the LOD.

a Values above the limit of detection.

b SD: standard deviation.

c GM: geometric mean.

d ND: not detected.

The detection rates of TCS in maternal sera were significantly different between the two groups (P < 0.01).
disrupt intrauterine blastocyst implantation in mice [14], and TCS may have estrogenic function [20]. On the other hand, TCS may inhibit estrogen sulfotransferase in sheep placenta [13], resulting in reduced total placental estrogen secretion and activity in target tissues. Uterine blood flow is regulated to an extent by estrogen, and it has been shown that placental estrogen biosynthesis is required for fetal development. Moreover, a series of studies in rats indicated that TCS could lower maternal thyroid hormone (T4) levels [12,21]. Thyroxine is a critical hormone for fetal development, especially for brain maturity. It was reported that a slight decrease in maternal T4 level could cause adverse effects on childhood cognition and motor function [22,23]. Although the mechanisms underlying the TCS effects on human endocrine system are not fully understood, it is clear that through these effects, intrauterine exposure of TCS could impose a threat to the growth and differentiation of fetal cells/tissues, leading to various fetal malformations. The complex nature of the relationship between teratogen exposure and fetal malformation makes it difficult to identify the true culprit out of all the potential factors, and we could not exclude the contribution by other factors to the malformation observed in this study.

We found that the detection rate of TCS is significantly higher in the fetal anomaly group than normal controls. However, the quantitative analysis demonstrates no significant difference between the levels of TCS in the two groups. The divergent results of the two compounds could be caused by their differential absorption, stability, and/or metabolism in vivo. Although the median concentrations of TCS in maternal (0.215 ng/mL) and cord sera (0.07 ng/mL) in the fetal anomaly group were several folds higher than those of controls (0.055 versus 0.029 ng/mL), the difference did not reach a statistically significant level. Data shows that median TCC concentrations in maternal (0.048 ng/mL) and cord sera (0.03 ng/mL) of the fetal anomaly group was lower than in controls (0.065 versus 0.03 ng/mL). Further studies using larger sample size are required to accurately assess the risk for fetal malformation by these chemicals.

We combined the data from the anomaly group and the control group and asked whether the concentrations of TCS and TCC in maternal blood could be associated with levels in fetal blood. Further analysis of the data showed a significant positive correlation in TCS (r = 0.649, P < 0.01) and TCC (r = 0.683, P < 0.01) levels of maternal and cord sera. Thus, the levels of compounds in maternal sera may predict those in cord sera, and consequently fetal exposure. These observations indicated that although the levels of these compounds are not necessarily correlated with the malformation, monitoring the maternal blood levels of these compounds could be used as a screening tool for fetal exposure to TCC/TSC in pregnancy.

In summary, TCS and TCC can disrupt intrauterine blastocyst implantation, inhibit estrogen sulfotransferase and reduce the thyroxine (T4) level. Our suggested a possible association of TCS and TCC levels in maternal and cord sera with fetal anomalies. Future research efforts in this area are required to confirm this observation. Additionally, further investigation is needed to determine the dose/time effects of TCS and TCC on fetal anomalies and to investigate the underlying molecular mechanisms by which TCC/TCS exposure may lead to fetal anomalies.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

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