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The association between some endocrine disruptors in human plasma and the occurrence of congenital hypothyroidism

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ABSTRACT

Congenital hypothyroidism is a common pediatric endocrine disease. Endocrine disruptors are indicated as a possible cause of congenital hypothyroidism. We investigated the associations between endocrine disruptors and the occurrence of congenital hypothyroidism and passage of target compounds from the mother. The levels of phthalates (DEHP, MEHP, DBP, MBP and PA), alkylphenols (n-NP and t-OP), bisphenol, and isoflavones (equol, daidzein and genistein) were determined by gas chromatography–mass spectrometry (GC–MS) in infants. t-OP and PA concentrations in the patient group were significantly higher than in normal infants. Genistein concentrations in normal infants were significantly higher than in patients. We compared the plasma levels of target compounds in infants with their mothers. There was no correlation with the passage of endocrine disruptors and isoflavones from the mothers, except for t-OP, which was weakly correlated between mother and infant.

1. Introduction

Endocrine disruptors are chemical substances or mixtures of substances that alter endocrine function and adversely affect a number of hormonal systems, including estrogen, androgens and thyroid hormones (Porterfield and Stein, 1994). There is also evidence that the thyroid is vulnerable to endocrine-disrupting effects in animal models and *in vitro* (Schmutzler et al., 2004; Tan et al., 2003). However, there is insufficient background data to assess whether the risk of endocrine disruptors in humans is a cause of congenital hypothyroidism, especially in infants, who are most vulnerable to EDCs. Endocrine disruptors may interfere with thyroid homeostasis through many mechanisms of action, i.e., at the receptor level, binding to transport proteins, cellular uptake mechanisms, or modifying the metabolism of thyroid hormones. Several endocrine disruptors are structurally similar to the thyroid hormones (THs) thyroxin (T4) and triiodothyronine (T3) and therefore interfere with TH binding to receptors or transport proteins (Morreale de Escobar et al., 2004).

Despite the fact that isoflavones are not endocrine disruptors, they influence the thyroid hormone system. Since the 1980s, it has been known from *in vitro* and animal studies that they also inhibit thyroid peroxidase (TPO) (Hampl et al., 2009). Soybeans contain 12 isoflavones, including genistein,

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daidzein, and glycitein. Isoflavones are goitrogenic substances that can interfere with iodine utilization or thyroid gland function, potentially causing thyroid problems in humans and animals. In particular, genistein and daidzein inhibit TPO-catalyzed iodination and coupling, thereby inhibiting thyroid hormone formation (Divi et al., 1997; Chang and Doerge, 2000; Hampl et al., 2009). Concern regarding thyroid hormone-disrupting chemicals has increased due to the critical role thyroid hormones play during development, especially in the nervous system (Zoeller and Crofton, 2000).

Congenital hypothyroidism occurs in 1 of 4000 infants and is one of the most frequently diagnosed pediatric endocrine diseases (La Franchi, 1999). Untreated congenital hypothyroidism produces neurologic deficits with predominantly postnatal origins. Untreated infants with severe congenital hypothyroidism can lose 3–5 IQ points per month if untreated during the first 6–12 months of life (Burrow et al., 1994). If the children are treated with thyroid hormones soon after birth, the more severe effects of thyroid deficiency are alleviated. However, these children remain at risk for mild learning disabilities (Fort et al., 1990). An altered thyroid hormone balance is considered a cause of congenital hypothyroidism (Rati and Newn, 1971). Recently, endocrine disruptors have been associated with cases involving an altered thyroid hormone balance (Crofton, 2008; Boas et al., 2010; Jugan et al., 2010).

In this study we determined the plasma levels in infants with congenital hypothyroidism and normal infants of the following target compounds: 2 alkylphenolic compounds, bisphenol A, 5 phthalates, and 3 isoflavones. A possible association between these substances and the occurrence of congenital hypothyroidism was investigated. This study also investigated passage of these target compounds from the mother.

2. Materials and methods

2.1. Materials

n-Octylphenol (n-OP), bisphenol B (BPB), terephthalic acid (t-PA), benzyl butyl phthalate (BBP) and 6-hydroxyflavone were used as internal standards, while n-nonylphenol (n-NP), 4-tertiary-octylphenol (t-OP), bisphenol A (BPA), phthalic acid (PA), mono-n-butyl-phthalate (MBP), mono-(2-ethylhexyl) phthalate (MEHP), di-n-butyl-phthalate (DBP), di-(2-ethlyhexyl) phthalate (DEHP), equol, diadzein, and genistein were used as recovery standards; these compounds were obtained from Sigma–Aldrich (St. Louis, MO, USA). All solvents including acetonitrile, diethyl ether, and methanol were HPLC grade (J.T. Baker Co., Phillipsburg, NY, USA). β-glucuronidase/arylsulfatase was purchased from Roche Diagnostics (Mannheim, Germany). N,O-bis-(trimethylsilyl)-trifluoroacetamide + 1% trimethyl chlorosilane (BSTFA + 1% TMCS) was also purchased from Sigma–Aldrich. Extractions were performed with SPE cartridges (HLB OASIS®, 3 mL) by Waters (Milford, Massachusetts, USA).

2.2. Sample collection

Plasma samples were collected at Soonchunhyang Hospital, Seoul, South Korea. They were obtained from 39 infants with congenital hypothyroidism and their mothers and from 20 normal infants and their mothers, making the total number of collected plasma samples 118. All 59 mother–infant pairs provided informed consent prior to participate. The ages of the infant donors ranged from 1 to 54 months. Infant body weight ranged from 2.50 to 18 kg. All samples were moved from the hospital to the laboratory in an ice-packed box and stored at −20 °C.

2.3. Sample preparation

Plasma samples (0.5 mL-aliquots) were treated as follows. After adding 50 μL (10 ng/mL) t-PA, BzBP, n-OP, and 6-hydroxyflavone as internal standards, 5 mL acetonitrile was added for protein removal. The organic layers were collected and completely evaporated with nitrogen gas. The sample was diluted with pH 3.0 phosphate buffer (1 mL) and 50 μL β-glucuronidase/arylsulfatase from Helix pomatia were added. The resulting mixture was incubated at 55 °C for 90 min. The sample was loaded onto an HLB Oasis cartridge that had been preconditioned with 3 mL methanol:water (9:1). The cartridge was rinsed with 2 mL water and eluted with 6 mL methanol. The eluate was evaporated under a stream of nitrogen and the residue reconstituted in 1 mL phosphate buffer (0.2 M, pH 3.0). This solution was extracted 3 times with diethyl ether (4 mL, each time for 10 min.). The organic solvent was evaporated with nitrogen gas and dried in a vacuum desiccator over P_2O_5–KOH for more than 30 min. Finally, the dried residue was derivatized with BSTFA containing 1% TMCS (50 μL) at 65 °C for 30 min, after which 2 μL of the resulting solution was injected into the GC–MS in selected-ion monitoring (SIM) mode.

2.4. Instrumentation

Analysis was performed on an Agilent 6890 gas chromatograph coupled to a 5975 mass selective detector (Palo Alto, CA, USA) with EI ionization at 70 eV. The column was an HP-5MS 25 m × 0.2 mm capillary column with a film thickness of 0.25 μm (J&W Scientific; Folsom, CA, USA). The ion source and transfer line temperatures were set to 230 and 300 °C, respectively. The injector was set to split injection at a temperature of 280 °C with a split ratio of 5:1. The temperature program was set to begin at 150 °C for 5 min, elevated at 15 °C min^{-1} to 240 °C, and finally increased by 1 °C/min to 300 °C (maintained for 2 min). The carrier gas was ultra-high-purity helium (99.999%) at a flow rate of 1 mL min^{-1}.

2.5. Statistical analysis

Values below the limit of detection (LOD) were valued as zero for calculating the total concentrations of chemicals. p values less than 0.05 were considered statistically significant. Correlation (r) between the levels of investigated compounds in mother and infant was tested using Pearson's coefficient of determination.