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Quantification of prenatal exposure and maternal-fetal transfer of nonylphenol

Mei-Lien Chen [a], Chi-Chang Chang [b], Yi-Ju Shen [a], Jeng-Hsiu Hung [c], Bey-Rong Guo [d], Hsin-Yi Chuang [a], I-Fang Mao [a,e,*]

[a] Institute of Environmental Health Sciences, College of Medicine, National Yang-Ming University, Shi-Pai, Taipei, Taiwan
[b] Department of Obstetrics & Gynecology, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan
[c] Department of Obstetrics & Gynecology, Taipei-Veterans General Hospital, Taipei, Taiwan
[d] Department of Pathology, Taipei Medical University, Taipei, Taiwan
[e] Department of Occupational Safety and Health and Graduate Program, Chung Shan Medical University, Taichung, Taiwan

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ABSTRACT

Nonylphenol (NP) is an environmental hormone commonly found in daily foodstuffs. This study examined maternal and umbilical-cord blood samples to explore prenatal exposure levels to nonylphenol and placental protection against NP exposure. One hundred and seventy-four mixed cord blood samples were collected. Among them, 42 pairs of expectant mothers and their prenatal fetus were matched to compare nonylphenol levels between mothers and fetuses. An additional 30 mother-infant dyads were chosen to give maternal, umbilical arterial and venous blood samples. Plasma samples were enzymatically deconjugated and then cleaned up with solid-phase extraction. After extraction, samples were analyzed with reversed-phase high-performance liquid chromatography coupled with fluorescence detection. Analytical results identified prenatal exposure to NPs and relatively high prenatal exposure levels in metropolitan areas. The concentrations ranged from undetectable (below 1.82 ng/g plasma) to 211 ng/g plasma. Concentrations of NP in mother-infant dyads showed the NP concentrations in maternal plasma were not definitely higher than that in fetal plasma. Still, 63.6% of NP detectable mother-infant dyads showed a higher concentration in umbilical venous plasma than those in umbilical arterial plasma. Through the repeated exposure from expectant mothers' dietary intake, fetuses could encounter high NP exposure level due to transplacental absorption, partitioning between the maternal and fetal compartments, as well as poor detoxification mechanisms of the developing organism. Some mechanisms may contribute to the reduction of NP levels in fetal blood circulation but those remain unclear.

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1. Introduction

Alkylphenol polyethoxylates (APEOs), which were introduced in the 1940s, are the second largest group of commercially produced nonionic surfactants. Annual global production of APEOs is approximately 650000 tons (Guenther et al., 2002). APEOs are widely used in detergents, paints, herbicides, pesticides, and numerous other formulated products. APEOs with 8–12 ethoxylate groups are commonly used, and nonylphenol polyethoxylates (NPEOs) represent about 80% of APEOs, while octylphenol polyethoxylates (OPEOs) make up most of the remaining 20% (White et al., 1994). NPEOs are used as emulsifying, dispersing, wetting and foaming agents in various industries in Taiwan. Ding et al. reported that NPE-type residues in Taiwanese rivers (0.6–2.4 μg/l) and sediments (250–8580 μg/kg dry wt) were higher than in other countries owing to the deficient municipal wastewater treatment in Taiwan (Ding and Tzin, 1998; Ding et al., 1999; Ding and Fann, 2000; Ding and Wu, 2000). They also found that NPEOs were detected in 41% of 90 household detergents at concentrations from 0.2% to 21% (Cheng and Ding, 2002). NP is a kind of alkylphenol. It exists mainly as intermediates in the manufacturing industry and is also degradation product of NPEOs used in industrial and institutional formulation. Mao et al. (2006) found that NP is ubiquitous in Taiwanese foods. Further, biological monitoring indicated that there were significant levels of NPs in both plasma and urine of textile and housekeeping workers in Taiwan (Chen et al., 2005).

The estrogenic properties of *p*-alkylated phenolic compounds were recognized as early as 1938 (Dodds and Lawson, 1938; Dodds et al., 1938). Attention has been drawn since Soto et al. found that NP induced cell proliferation and bound to estrogen receptor in human estrogen-sensitive MCF7 breast tumor cells (Soto et al., 1991). White et al. (1994) also showed that some alkylphenols are estrogenic in fish, birds, and mammals. These environmental estrogenic chemicals may cause precocious sexual development (Perez-Comas, 1982); recently these chemicals have been hypothesized to

* Corresponding author. Present address: Department of Occupational Safety and Health and Graduate Program, Chung Shan Medical University, Taichung, Taiwan. Tel.: +886 2 28267057; fax: +886 2 28278254.
E-mail address: ifmao@ym.edu.tw (I-Fang Mao).

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account for the growing frequency of infertility and related disorders of the male reproductive system in humans (Sharpe and Skakkeback, 1993). The Japanese Environmental Agency listed nonylphenol as a suspected endocrine disruptor and initiated risk assessments in 1998. Several measures have been taken to reduce risk exposure in other countries. For example, the use of NPEOs has been banned or restricted in many European countries because of growing concern about the toxicity of NP in aquatic organisms. In spite of the ubiquity of NP in the environment, currently no restriction of NPEOs has been adopted by the Taiwan government.

Exposure to NP can be via ingestion of contaminated foods and drinking water, dermal absorption or inhalation (Gilbert et al., 1986; Ahel et al., 1993; Clark et al., 1996). The question arises whether NPs circulating in an expectant mother's body pass through the placenta, eliciting possible estrogenic effects on developing fetuses. It is expected that the placenta is an effective barrier against fetal exposure to certain harmful proteins and also protects the developing embryo against some hormones, including estrogen, circulating in maternal blood that adversely affect fetal development. The fetus may be exposed to chemicals through transplacental absorption by the diffusion, active transport and facilitated diffusion. Recent studies identified 2,3,7,8-TCDD, PCBs, bisphenol A, NP, octylphenol, and phthalates in cord blood (Tan and Mohd, 2003; Latini et al., 2003; Wang et al., 2004), indicating that almost all harmful chemicals in maternal circulation can pass to a fetus. Thus, it is worth knowing at what rate a chemical crosses the placenta and whether the fetal chemical levels will be higher than the maternal plasma levels. In the pharmacokinetic study of NP in humans by Müller et al. (1998), the half-life of NP in blood and the bioavailability (determined from oral and intravenous AUCs) were found to be 2–3 h and 20%. The biological half-life of NP is quite short thus the accumulation of NP may not be a significant issue. However, the repeated exposure of a fetus to NP due to dietary intake and the increased amounts taken by pregnant women is a concern if a placenta barrier to NP does not exist.

Müller et al. (1998) used gas chromatography/mass spectrometry to measure the parent and conjugated NP. Inoue et al. (2000) employed a high-performance liquid chromatograph with a multi-electrode electrochemical coulometric-array detector to measure NP and OP in human plasma. Tsuda et al. (1999) measured NP and other alkylphenols in fish and shellfish by high-performance liquid chromatography with fluorescence detection. Our analytical method utilized solid-phase extraction of nonylphenol in plasma and subsequent analysis by high-performance liquid chromatography with fluorescence detection. We compared human umbilical cord plasma samples collected from two geographic regions with different alkylphenol exposure levels. Comparisons between maternal venous blood and the umbilical arteries and veins samples were explored. The purposes of this study were to determine prenatal exposure levels in Taiwan and to determine the level of placental protection against NP exposure. The results will be useful for risk assessment of prenatal exposure to NP.

2. Materials and methods

2.1. Study subjects

The Ethics Committee of the Veteran General Hospital, Taipei (VGH) approved this study. Before delivery, expectant mothers gave written, informed consent. A total of 174 human mixed umbilical cord blood (mixed arterial and venous blood) were collected. Among these specimens, 124 originated from the Chiayi Chang-Qung Memorial Hospital (CCQMH) and 50 from the VGH in metropolitan Taipei. The CCQMH is in the Chiayi county. The county is located in central Taiwan with a population of around 0.55 millions. It is an agricultural region. The metropolitan Taipei is located in northern Taiwan and is the capital of Taiwan, ROC. It is one of the most crowded cities in the world with a population reaching 2.63 million. Forty-two of the 174 expectant mothers agreed to provide both maternal venous blood as well as umbilical cord blood and also to complete a questionnaire of demographic data. Therefore, we had 42 pairs of maternal and fetal samples to study the placenta transfer of NP. Further, thirty mother-infant dyads were chosen from the E-Da Hospital in southern Taiwan to collect maternal, umbilical arterial and venous blood for comparison of NP levels. The hospital is located in a county blended with activities of industry, agriculture, and fishery-Kaohsiung county. It is a county with 60% of petrochemical plants of Taiwan and over 5000 factories. The total population is around 1.1 million.

Cord blood was collected in a 10 ml glass K_3 EDTA Vacutainer (BD, Franklin Lakes, NJ) upon delivery at the hospital and the mother's blood was obtained by venipuncture puncture before delivery. All samples were immediately chilled and transported to the laboratory. Plasma was fractioned by centrifugation at 1800 rpm for 15 min and kept frozen until analysis.

2.2. Samples extraction and analysis

2.2.1. Reagents

Analytical grade acetic acid, acetonitrile, ammonium acetate, ammonium solution, hydrochloric acid, methanol, β-glucuronidase/arylsulfatase (5.2 U/ml/2.1 U/ml) were purchased from Merck (Darmstadt, Germany) and 4-nonylphenol (*p*-isomers > 85%) were purchased from Fluka (USA). A Millipore water purification device (Millipore, Bedford, MA) supplied ultrapure water. All water was prepared freshly before use and collected in a glass container.

2.2.2. Instrumentation

A Hitachi (Tokyo) LC system was used for NP analysis. This system comprised an L-6200 intelligent pump, L-7200 auto-sampler, F-4010 fluorescence detector, L-6100 interface for linking the detector, and D-6000 data management software. The software ran on a Copam computer (Taiwan) for online recording of output.

2.2.3. Sample pretreatment

This study adopted the sample cleanup procedure of Inoue et al. (2000) as well as the enzymatic deconjugation of Müller et al. (1998), with some modifications (Chen et al., 2005). Plasma samples were homogenized using a XL 2020 sonicator (USA) for 10 min. One gram (Precisa, 40SM-200A, Switzerland) of homogenized plasma was diluted with 5 ml of ultra pure water in a 10 ml beaker. The pH value of the diluted sample was determined and were then adjusted to 5.5 (Hanna model 8520, Italy) with acetic acid. Then 1 ml of 1 M ammonium acetate solution (pH 5.3) and 125 μl β-glucuronidase/arylsulfatase were added. The mixture was incubated for 15 h at 37 °C in a shaker bath (Kodman, USA) and acidified to pH 3 using hydrochloric acid.

2.2.4. Sample cleanup

Following enzymatic deconjugation, samples were cleaned up with 3 ml Varian PH solid phase extraction (SPE) cartridges. The SPE cartridges were inserted with 2 cm of silanized glasswool and washed with 20 ml methanol. After conditioning the cartridges with 3 ml of pure water adjusted to pH 3.0 using 1.0 M HCl, the deconjugated samples were passed through. Sample application was followed by washing with 5 ml of pure water, and adsorbed compounds on the cartridge were eluted with 3 ml of methanol. Conditioning and elution were performed under a vacuum manifold. To extend HPLC column lifespan, all samples were filtered through a 5 μm PTFE membrane filter (Titin, USA).

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