Journal of Trace Elements in Medicine and Biology 45 (2018) 163–170

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Toxicology

Metallothionein 2A gene polymorphism and trace elements in mother-newborn pairs in the Croatian population

Ankica Sekovanić[a], Jasna Jurasović[a,*], Martina Piasek[a], Daria Pašalić[b], Tatjana Orct[a], Antonija Sulimanec Grgec[a], Sandra Stasenko[c], Karmen Branović Čakanić[d], Anamarija Jazbec[e]

[a] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[b] University of Zagreb, School of Medicine, Zagreb, Croatia
[c] Merkur University Hospital, Zagreb, Croatia
[d] Croatian Veterinary Institute, Zagreb, Croatia
[e] University of Zagreb, Faculty of Forestry, Zagreb, Croatia

ARTICLE INFO

Keywords:
Maternal blood
Placenta
Umbilical cord blood
Toxic metal
MT2A –5A/G polymorphism

ABSTRACT

The main source of exposure for all essential and toxic elements in the general population is diet. In smokers, the main route for cadmium (Cd) and lead (Pb) intake is the inhalation of tobacco smoke. Besides gender, age, nutrition, lifestyle, and physiological conditions such as pregnancy, specific genetic characteristics also influence individual element uptake. Metallothionein MT2 is a cysteine-rich low-weight protein found ubiquitously throughout the body. Specific gene polymorphism may influence MT2 expression and subsequent binding, transfer and organ accumulation of metals, though data on these influences are lacking, especially in human mother-newborn pairs. The objective of this study was to determine selected toxic (Cd, Pb, Hg) and essential (Fe, Zn, Cu, Se) elements in maternal blood, placenta, and cord blood (by ICP-MS), and MT2 levels in maternal serum (by ELISA) in relation to maternal *MT2A* –5A/G (rs28366003) polymorphism (by RFLP-PCR and electrophoresis). Study participants were healthy postpartum women in Croatia (n = 268, mean age 29 years) with term vaginal childbirth in a maternity ward assigned into two study groups by self-reporting about their smoking habit (by questionnaire). Smokers *vs*. non-smokers had increased levels of Cd and Pb in all measured samples, Fe and Cu in cord blood, Zn in placenta, and MT2 in maternal serum. Among subjects with AG/GG genotype, placental Fe was significantly lower only among non-smokers, while MT2 levels in serum were lower, though not significantly, regardless of maternal smoking habit. There was no impact of *MT2A* –5A/G SNP on any element in maternal or cord blood. In conclusion, the results confirmed maternal smoking-related increases in Cd and Pb levels in the maternal-placental-foetal unit. They also provided additional data on concomitant metal concentrations in representative samples of maternal blood, placenta, and cord blood, as well as increased cord blood Fe and Cu, placental Zn, and maternal serum MT2 in smokers. New evidence is that *MT2A* –5A/G SNP was associated with decreased placental Fe levels in non-smokers. For a final conclusion on the influence of the *MT2A* –5A/G polymorphism on toxic and essential element levels in mother-newborn pairs, further research would require a larger number of participants divided across subgroups defined by the main source of particular toxic metal exposure (such as specific food intake, cigarette smoking, air pollution and/or occupational exposure).

1. Introduction

The general population is exposed to essential and toxic metals and metalloids through the daily diet. In cigarette smokers, the main exposure route for cadmium (Cd) and lead (Pb) is the inhalation of tobacco smoke [1–3]. The absorption rate of both toxic metals is markedly higher when inhaled (5–50% for Cd and 30–50% for Pb, depending on particulate size and ventilation rate) than when ingested (1–10% for Cd and 3–10% for Pb) [4,5]. Mercury (Hg) is also contained in the tobacco plant and transferred to mainstream smoke at concentrations 2–3 orders of magnitude lower than Cd or Pb; hence, tobacco smoke is generally not considered to be a significant source of Hg exposure. Exposures to these three metals *via* tobacco smoke are shown to cause pro-inflammatory effects with metallothionein (MT) engagement [6].

The main toxic metals of public concern, Cd, Pb and Hg, are among the most toxic agents known and may cause numerous adverse health

* Corresponding author at: Institute for Medical Research and Occupational Health, Ksaverska cesta 2, HR – 10000 Zagreb, Croatia.
E-mail address: jurasovic@imi.hr (J. Jurasović).

http://dx.doi.org/10.1016/j.jtemb.2017.10.011
Received 11 September 2017; Received in revised form 18 October 2017; Accepted 24 October 2017

effects [7,8]. Women during childbearing period and their offspring are population groups at a high risk of greater gastrointestinal absorption and body retention of toxic elements. This is due to specific maternal anatomical and physiological changes, which occur as a consequence of adaptation to the increased requirement for nutritional element intake *via* the gastrointestinal tract [9].

Most metals and metalloids induce the synthesis of MTs, low-molecular weight, cysteine-rich proteins required for the binding, transport and storage of metals and metalloids in the body [8,10–15]. In recent years, evidence has emerged to show that the genetic characteristics of MTs play an important role in physiological and pathological processes such as antioxidant defence, detoxification and cell proliferation, in addition to individual (hyper)sensitivity to toxic metals. It has been shown that *MT2A* rs28366003 polymorphism, an A/G substitution located in the 5′regulation region of MT2, may decrease the induction of *MT2A* gene transcription and expression, which may affect bodily element disposition and health effects including longevity, diabetes mellitus, breast cancer, and prostate cancer [15–17]. The data reported so far are inconsistent and more research on specific *MT2A* polymorphisms is needed.

The aim of this study was to determine selected toxic (Cd, Pb, Hg) and related essential (Fe, Zn, Cu, Se) elements in postpartum women and their offspring using maternal and cord blood and placental samples taken after term vaginal delivery. In addition, MT2 levels were determined in maternal serum. These biomarkers were compared to the maternal *MT2A* −5A/G single nucleotide polymorphism (SNP) to assess whether it had an impact on MT expression and element disposition in the maternal-placental-foetal unit.

2. Materials and methods

2.1. Study group and sample collection

The study was carried out in 268 mother-newborn pairs recruited during 2008–2010 in two Croatian cities, Zagreb (n = 172) and Zadar (n = 96). The subjects were healthy women (average age 29.3 ± 4.6 years) who had no chronic illness and/or major complications during pregnancy, and gave vaginal birth at term (between 37 and 42 weeks of pregnancy) in the maternity ward. All subject who agreed to participate in the study signed informed consent forms. Prior to the start of the study, the research protocol was reviewed and approved by the ethics committees at the participating institutions: Merkur University Hospital in Zagreb, General County Hospital in Zadar and the Institute for Medical Research and Occupational Health in Zagreb.

Relevant data regarding medical history, sociodemographic characteristics, dietary habit (with focus on seafood consumption), mineral-vitamin supplement intake, and cigarette smoking were collected by a questionnaire [2,18]. Personal data, maternal and neonatal clinical data, and biological samples were collected by trained medical personnel under the supervision of a gynaecology and obstetrics specialist. Based on self-reporting about cigarette smoking before and during pregnancy, the study subjects were assigned into two groups: smokers and non-smokers. Women who reported active smoking any time during pregnancy or within 12 months before the onset of the current pregnancy were considered “smokers”, while those who never smoked or who were former smokers who had quit smoking more than 12 months before the onset of the current pregnancy were considered “non-smokers”. The same approach to assigning study groups was applied in our previous study on elements and steroid hormones in the placenta of healthy postpartum women that discerned two cohorts with significantly different durations of active cigarette smoking and related Cd levels in the measured matrices [2,18–21].

Maternal and cord blood samples were collected in vacutainer tubes (Beckton-Dickinson) with K_2H_2-EDTA anticoagulant and whole placentas were placed in zip-lock polyethylene bags. The samples were then transported to the analytical laboratory and stored at −20 °C until analysis.

2.2. Trace element analysis in blood and placental tissue samples

All samples were prepared and analysed in a laboratory operating under positive pressure maintained by a HVAC (Heating, Ventilating and Air Conditioning) system combined with HEPA filters. Nitric acid (65% p.a., Merck, Germany) purified by sub-boiling distillation in the duoPUR system (Milestone, Italy), ultrapure water (GenPure, TKA Wasseraufbereitungssysteme GmbH, Germany), and high purity chemicals were used throughout the analysis.

Maternal and umbilical cord blood were prepared by dilution (1:60) with a solution containing 0.7 mmol L^{-1} ammonia, 0.01 mmol L^{-1} EDTA, 0.07% (v/v) Triton X-100, and 2 μg L^{-1} of internal standard (Ge, Rh, Lu and Ir) in ultrapure water before analysis, and measured applying essentially the same method as described in our lab [22].

Whole placentas were thawed, residues of umbilical cord and extraembryonic membrane trimmed away, organs washed in saline solution (0.9% NaCl), remaining fluids blotted on filter paper, and fresh placental mass weighted. To assure representative samples for element analyses, three full-thickness samples were cut using a ceramic knife; one sample from a section from the midline of the placental disc, avoiding the region of umbilical cord insertion, and two peripheral samples from between the central region and periphery, the outermost 3 cm within the placental disc edge. In each sample, 1–2 mm thin layers were cut from both the foetal and maternal parts, including the chorionic membrane and decidua basalis to obtain mostly trophoblastic tissue (chorionic villi). The samples were washed with ultraclean water and the remaining fluid blotted. Sub-boiled concentrated nitric acid (4 mL) and ultrapure water (2 mL) were added to tissue samples (1–2 g) weighed in PTFE vessels and digested in the UltraCLAVE IV microwave digestion system (Milestone, Italy). After digestion, samples were adjusted to 8 g with ultrapure water, transferred into polypropylene tubes, and stored at +4 °C until further 20-fold dilution before analysis. Element levels were measured in the samples from three locations and placental element concentrations expressed as average values. Our previously published results [18] showed no effect of sampling site (with locations chosen the same way) for most of the elements analysed here.

Concentrations of Cd, Pb, Hg, Fe, Zn, Cu, and Se were measured in maternal blood, umbilical cord blood, and placental samples using an inductively coupled plasma − mass spectrometer (ICP-MS) Agilent 7500cx (Agilent Technologies, Tokyo, Japan). The ICP-MS collision/reaction cell, pressurized with He or H_2, was used to remove the plasma and matrix-based interference: He mode was used for ^{68}Zn, ^{63}Cu, ^{114}Cd, and ^{208}Pb measurements, H_2 mode for ^{54}Fe and ^{78}Se, while ^{202}Hg was analysed in no-gas mode. Sample introduction was performed with a MicroMist nebulizer combined with a Peltier standard quartz spray chamber (Scott–type) cooled at 2 °C and a quartz torch with a 2.5 mm diameter injector with a Shield Plate system. Ni sampler and skimmer cones were used. The instrument was optimized daily with a tuning solution containing 1 μg L^{-1} ^{7}Li, ^{59}Co, ^{89}Y, ^{140}Ce, and ^{205}Tl. Matrix matched calibration was used for the quantification of metal concentration in blood, while the calibration curves of standards prepared in 1% HNO_3 were used for the quantification of element concentration in digested placental tissue. Internal standards used (^{74}Ge, ^{103}Rh, ^{175}Lu, ^{193}Ir) were chosen to match the ionization energy or atomic mass of the analysed metal as closely as possible to correct for instrument signal instabilities.

Commercially available reference materials were used to verify the accuracy of analytical measurements: Seronorm™ Trace Element Whole Blood Level I, and Level II (Sero AS, Norway), pig kidney BCR 186R (IRMM, Belgium), and bovine liver 1577b (NIST, USA). Details on the analysis results of the certified reference materials used are shown in the Supplementary Material (Table S1). The laboratory also