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Downregulation of placental S100P is associated with cadmium exposure in Guiyu, an e-waste recycling town in China

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ABSTRACT

Excessive release of heavy metals, especially cadmium (Cd) and lead (Pb), results from primitive electronic waste (e-waste) recycling activities in Guiyu, China, and has adverse effects on the health of local infants and pregnant women. We investigated the expression of placental S100P, a Ca^{2+}-binding protein, as a biological indicator of heavy-metal environmental pollution in pregnant women involved in these activities and constantly exposed to Cd and Pb. We included 105 pregnant women in the study: 55 from Guiyu and 50 from Shantou, an area not involved in e-waste recycling. The placental concentrations of Cd and Pb (PCCd, PCPb) after birth were measured by graphite-furnace atomic-absorption spectrometry. S100P mRNA expression was determined by semi-quantitative RT-PCR and real-time quantitative PCR. S100P protein expression was examined by western blot analysis and immunohistochemistry. The expression of metallothionein (MT), previously found upregulated after heavy metal contamination, was used for comparison. Placentas from Guiyu women showed 62.8% higher Cd concentrations, higher MT levels, and lower S100P protein levels than placentas from Shantou women. Furthermore, PCCd was negatively correlated with S100P protein expression and positively with MT expression, with no correlation between PCPb and S100P or MT expression. The PCCd-associated downregulation of S100P in placentas from Guiyu women suggests that S100P might be an effective biological indicator in the placental response to Cd toxicity in areas of e-waste recycling.

1. Introduction

Guiyu, a town in Guangdong province of south China, is a major processing center for electronic waste (e-waste) such as computers. Primitive recycling operations and disposal processes used for e-waste recycling cause serious heavy-metal contamination in the local environment (Guo et al., 2009). Excessive release of heavy metals, especially Cd and Pb, has adverse effects on human health, especially infants and pregnant women, who are more sensitive to heavy metal pollution (Jarup and Akesson, 2009; Olympio et al., 2009). In our previous study, we found blood levels of Cd and Pb higher in Guiyu children than in control children (Zheng et al., 2008). Another study found high levels of Pb in umbilical cord blood positively correlated with low behavioral neurological scores in Guiyu neonates (Li et al., 2008).

The placenta provides a connection between maternal and fetal circulation and is an important indicator organ of environmental pollution because of its central role as a barrier to the transfer of heavy metals to the fetus (Myllynen et al., 2005). Placentas are readily accessible tissues for research, and analysis of heavy-metal concentrations in placentas is commonly used for environmental monitoring (Falcon et al., 2002; Roels et al., 1978).

Because assessment based solely on chemical analysis of environmental or tissue samples cannot show the biological effects of pollutants, effective biological indices have been proposed as useful indicators of toxic chemical exposure and effects (Henderson et al., 1989). Intake of Cd and Pb modulates the activity or deregulates the expression of Ca^{2+}-binding proteins, some of which are indicators of heavy-metal pollution (Sun et al., 1999; Washko and Cousins, 1977). The S100 proteins, including S100A, B, and P, are the largest group of Ca^{2+}-binding proteins. These proteins can also bind divalent ions such as Cu^{2+}, Mg^{2+}, and Zn^{2+}. Each member of the S100 family has a tissue- or cell-type-specific expression and regulates intracellular processes, including Ca^{2+} homeostasis and protection against oxidative cell damage (Donato, 2001). We previously examined 11 members of S100 proteins in placentas from Guiyu women and those from an area not involved in e-waste recycling. Only S100P showed differential expression. S100P was first purified and found to be expressed at high levels in placenta (Becker et al., 1992).

In the present study, we evaluated the use of S100P as a potential stress indicator in the placental response to heavy metal pollution.

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We characterized S100P expression levels in placentas from women from Guiyu, a typical e-waste recycling site, and from Shantou city, an area located 50 km away not involved in e-waste recycling. We evaluated S100P expression in placentas and determined the placental concentrations of Cd and Pb (PCCd, PCPb) in pregnant women. We also correlated PCCd and PCPb with placental S100P levels, using expression of MT as a reference.

2. Experimental procedures

2.1. Subject recruitment

We recruited 105 pregnant women who were registered to give birth at hospitals in Guiyu ($n=55$) and in Shantou ($n=50$) between October 2008 and June 2009. The power of tests for each subgroup was more than 80% as calculated by NCSS-PASS 2005. The study protocol was approved by the Human Ethical Committee of Shantou University Medical College, China. All subjects gave their signed informed consent to be in the study. We gathered data by questionnaire on parental residence and dietary, occupational, and possible environmental sources of metal exposure.

2.2. Placenta collection

Immediately after delivery, we obtained 4 full-depth samples of placental tissue from the central region of each placenta. One piece (~0.3 g) was stored at −20 °C for measurement of PCCd and PCPb; 2 pieces were frozen at −80 °C for semi-quantitative RT-PCR, real-time quantitative PCR, and western blot analysis; and the fourth piece was washed with normal saline and fixed in buffered formalin for 24 h before sectioning for immunohistochemistry.

2.3. Analysis of Cd and Pb

To determine PCCd and PCPb contents, 0.3 g wet placental tissue was placed in pre-weighed vessels and dried in a microwave oven at 110 °C for 1 h. An amount of 350 μ HNO_3 was added for digestion for 1 h in a water bath at 80 °C, then 350 μL H_2O_2 was added. The vessels were cooled and distilled, and deionized H_2O was added to a total volume of 3 mL. Cd and Pb levels were determined as described (Guo et al., 2010).

2.4. Quantification of S100P mRNA expression in placenta

Total RNA was isolated from placental tissue by the Trizol method (GIBCO-BRL, New York, NY) and quantified by measuring absorbance ratios at 260/280 nm. cDNA was prepared by reverse transcription of 1 μg total RNA with an oligo dT primer and Mo-MLV reverse transcriptase (Invitrogen) according to the manufacturer's protocol. cDNA was amplified with forward and reverse specific primers for S100P (Table 1). β-Actin was an internal control.

Semi-quantitative RT-PCR and real-time quantitative PCR were performed as described (Scacco et al., 2003). The conditions for semi-quantitative RT-PCR conditions were 95 °C for 5 min, 30 cycles of denaturation at 95 °C for 30 s, annealing temperatures as shown in Table 1 for 30 s, and extension at 72 °C for 30 s. Real-time quantitative PCR involved the 7300 Real-Time PCR System (Applied Biosystems, CA, USA) for conditions of 5 min at 95 °C, then 40 cycles of amplification (15 s at 95 °C, 30 s at 60 °C or 52 °C, 40 s at 72 °C). A melting curve was generated at the end of each run to ensure product quality. All samples were amplified in triplicate under the same amplification conditions. Relative gene expression was calculated by the $2^{-\Delta Ct}$ method (Livak and Schmittgen, 2001). PCR products were electrophoresed on a 1.5% agarose gel stained with SYBR Green I Nucleic Acid Gel Stain and photographed by use of a Fluor-S MultiImager (Bio-Rad, CA, USA).

Table 2
Characteristics of pregnant women and infants from Guiyu and Shantou ($n=105$).

General characteristics	Guiyu ($n=55$ mothers)	Shantou ($n=50$ mothers)
Maternal age, years	25.75 ± 5.29	27.80 ± 5.51
Gestational age, weeks	39.27 ± 1.48	38.86 ± 1.47
Infant length, cm	49.91 ± 0.73	49.96 ± 1.01
Infant weight, g	3120.91 ± 364.73	3194.40 ± 441.49
Apgar scores, no. (%)	9.85 ± 0 .85	9.98 ± 0.14
Male	29 (52.7)	30 (60.0)
Female	26 (47.3)	20 (40.0)

Values are mean ± SD unless indicated.

2.5. Western blot analysis of S100P

Western blot analysis involved use of frozen tissue (100 mg) homogenized in 400 μL ice-cold RIPA buffer (Solarbio, Beijing) supplemented with phenylmethanesulfonyl fluoride (100 μg/mL, Sigma). After overnight incubation at 4 °C, the insoluble material was removed by centrifugation at 12,000 rpm for 20 min at 4 °C. The protein concentrations of the lysates were measured with the BCA Protein Assay Kit (Beyotime, Jiangsu, China). Aliquots corresponding to 50 μg of protein were separated by SDS–PAGE and transferred to a nitrocellulose membrane (Hybond-C Extra, Amersham). The membrane was blocked for 1 h at room temperature with 5% nonfat dry milk in Tris-buffered saline (TBS)-Tween buffer. Membranes were incubated with anti-human S100P monoclonal antibody (R&D Systems) at 1:500 dilution in 5% nonfat dry milk in phosphate-buffered saline (PBS) buffer overnight at 4 °C, then horseradish peroxidase-conjugated anti-mouse antibody, and signals were detected by enhanced chemiluminescence with Kodak XAR5 film. Equal loading of proteins was confirmed by incubation with anti-β-actin antibody (1:5000, Sigma).

2.6. Immunohistochemical determination of S100P and MT expression

Formalin-fixed samples were cut into cubes (1 × 1 × 0.5 cm), dehydrated, paraffin-embedded, serially sectioned at 4 μm, and mounted on slides. Sections were subsequently deparaffinized in xylene, rehydrated through a graded series of alcohol, and washed in distilled water. For quenching endogenous peroxidase activity, sections were

Table 1
Primers for PCR.

	Semi-quantitative RT-PCR			Real-time quantitative PCR		
	Primer sequence (5′ to 3′)	Size (bp)	Annealing Tm (°C)	Primer sequence (5′ to 3′)	Size (bp)	Annealing Tm (°C)
S100P	F: AGCACGCAGACCCTGACCAAG R:GGCTCTGCCAGGAATCTGTGAC	253	63	F:GGAGAAGGAGCTACCAGG R: GCCACGAACACGATGAAC	126	60
β-actin	F: GGCTACAGCTTCACCACCAC R:CGGACTCGTCATACTCCTGCT	331	58	F:TTAGTTGCGTTACACCCTTTC R:ACCTTCACCGTTCCAGTTT	150	52

F: forward. R: reverse. Tm: temperature.