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Increased levels of metallothionein in placenta of smokers

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Abstract

Experiments were designed to evaluate and compare metallothionein (MT), zinc and cadmium levels in human placentas of smoking and non-smoking women. Smoking was assessed by self-reported cigarette consumption and urine cotinine levels before delivery. Smoking pregnant women with urine cotinine levels higher than 130 ng/ml were included in the smoking group. Determination of placental MT was performed by western blot analysis after tissue homogenization and saturation with cadmium chloride (1000 ppm). Metallothionein was analyzed with a monoclonal antibody raised against MT-1 and MT-2 and with a second anti mouse antibody conjugated to alkaline phosphatase. Zinc and cadmium were determined by neutron activation analysis and atomic absorption spectrometry respectively. Smokers showed higher placental MT and cadmium levels, together with decreased newborn birth weights, as compared to non-smokers. The semi-quantitative analysis of western blots by band densitometry indicated that darker bands corresponded to MT present in smokers' samples. This study confirms that cigarette smoking increases cadmium accumulation in placental tissue and suggests that this element has a stimulatory effect on placental MT production.

Keywords: Metallothionein; Placenta; Cadmium; Birth weight; Smoking

1. Introduction

Cigarette smoking is one source of human exposure to cadmium and causes increased levels of this toxic element in the body (Roquer et al., 1995). Smoking during pregnancy results in elevated cadmium concentrations in placental tissues (Piasek et al., 2001) and is known to cause a wide range of deleterious effects during fetal growth and development (Ferm, 1971) which leads to low birth weight (Abel, 1980). This latter effect has been subsequently related to the ratio of placental zinc to placental cadmium, (Kuhnert et al., 1987a), thereby linking both metals in the mechanisms involved in fetal growth. It has been suggested that the reduced birth weight found in neonates delivered by smokers may be related to a deficient trans-

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ference of zinc from the placenta to the fetus (Kuhnert et al., 1987b, 1988). Zinc is an essential micronutrient for fetal growth and for proper immune system function, stressing the importance of balanced zinc levels during pregnancy (Wellinghausen, 2001). When a mother has reduced zinc reserves, newborn zinc deficiency could occur during pregnancy and nursing due to reduced transference of this metal through the placenta and in breast-feeding respectively; therefore, improving maternal zinc nutrition is key for the infant's zinc nutritional status (Dorea, 2002). Zinc supplements during pregnancy do indeed reduce childhood morbidity in populations where zinc deficiency is common (Hamadani et al., 2002).

Different factors are known to influence the transference of zinc to the fetus including: gestational age, levels of maternal plasma zinc and zinc binding proteins in maternal and fetal circulation and in tissues (Simmer et al., 1985; Paterson et al., 1991). Interestingly, it has been reported that, in addition to having elevated placental cadmium concentrations, smokers also show increased placental zinc levels (Kuhnert et al., 1987a). Thus, the low birth weight of neonates born to smoking mothers could be due to placental zinc accumulation through a mechanism involving placental cadmium and metal binding proteins (Torreblanca et al., 1992).

At the cellular level, one of the most important bivalent metal (zinc, cadmium and copper) binding proteins is metallothionein (MT). This low molecular weight (6000–7000 Da), cysteine-rich protein may be crucial for the regulation of zinc homeostasis and metabolism (Richards, 1989; Kägi, 1991). It has been proposed that MT maintains zinc homeostasis by controlling cellular zinc uptake, distribution and excretion, and by acting as a short-term storage reservoir for this metal (Richards, 1989; Klaassen et al., 1999). Metallothionein synthesis may be induced by some of the metals to which it binds (Klaassen et al., 1999). Thus, cadmium and zinc, in addition of showing high affinities for MT, are potent inducers of MT synthesis (Harford and Sarkar, 1991). In this sense, high levels of cadmium in smokers' placentas and cadmium-zinc-induced MT synthesis in cultured human trophoblasts have been previously reported (Lehman and Poisner, 1984). The aim of this study was to evaluate and compare MT levels in placentas from smokers and non-smokers and correlate these results to placental zinc and cadmium concentrations. According to the present findings, a possible mechanism leading to low birth weight related to maternal smoking is suggested.

2. Materials and methods

2.1. Preparation of placentas

Placentas were obtained upon delivery in the maternity ward at Sótero del Río Hospital in southern Santiago. Inclusion criteria included healthy young parturients, with normal pregnancies and without history of alcohol or drugs. All mothers had normal nutritional status evaluated as previously described (Atalah et al., 1997). The ethical committee of our institution approved the research project and the questionnaire, which included medical and dietary history as well as data on occupational and possible environmental sources of metal exposure. The assessment of smoking was based on self-reported individual cigarette consumption and urine cotinine determination immediately before delivery. Parturients were divided into two groups: women who had never smoked (non-smokers) and women who smoked throughout the entire pregnancy (smokers).

Immediately after delivery, the entire placenta was weighted and placed in a plastic bag and frozen at −70 °C until laboratory transport. To determine trace elements, half of the partially thawed placenta was thoroughly washed and lyophilized using a programmed cycle of temperature and pressure: −25 °C; −20 °C (1.03 mbar); −10 °C; 20 °C (0.02 mbar) and 30 °C for 32 h (Christ Delta 1–20 KD). Finally, samples were ground and homogenized, constituting the stock placental material for metal element determinations.

2.2. Urine cotinine

A urine sample was taken just before delivery and maintained frozen until cotinine evaluation, which was carried out by radio-immunoanalysis (RIA; Diagnostic Product Company, Los Angeles, CA, USA).

2.3. Determination of placental levels of zinc and cadmium

Zinc was determined by instrumental neutron activation analysis (INAA) at the Neutron Activation Anal-