Smoking specifically induces metallothionein-2 isoform in human placenta at term

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Abstract

Recently, we reported the presence of higher levels of metallothionein (MT) in placentas of smokers compared to non-smokers. In the present study, we designed experiments to separate and evaluate two isoforms of MT (MT-1 and MT-2) in placentas of smokers and non-smokers. Metallothionein was extracted and separated by ion-exchange high performance liquid chromatography (HPLC), previous saturation with cadmium chloride. Two peaks eluting at 6 and 12.5 min, corresponding to MT-1 and MT-2, respectively, were obtained. Metallothionein present in both peaks was identified by Western blot analysis using a monoclonal antibody directed against MT-1 and MT-2. Each isoform concentration was calculated after measuring its cadmium content by atomic absorption spectrometry with inductively coupled-plasma. In placentas of smokers, MT-2 levels increased by seven-fold compared to non-smokers, whereas MT-1 was not changed. Total placental cadmium and zinc concentrations, determined by atomic absorption spectrometry and neutron activation analysis, respectively, were higher in smokers. Metallothioneins levels were clearly in excess to bind all cadmium ions present in placentas. However, most of placental zinc remains unbound to MTs, although as much as twice zinc ions could be bound to MT in smokers. In conclusion, MT-2 is the main isoform induced by smoking, suggesting that this isoform could be involved in placental cadmium and zinc retention. This fact, which could contribute to reduce the transference of zinc to the fetus, may be associated to detrimental effects on fetal growth and development.

Keywords: Smoking, Placenta, Cadmium, Zinc, Metallothionein isoforms

1. Introduction

Smoking during pregnancy increases the cadmium (Cd²⁺) body burden, including elevated concentrations of Cd²⁺ in the placenta (Ronco et al., 2005b). Smoking causes a wide range of deleterious effects during fetal growth and development (Ferm, 1971), which may result in low birth weight neonates (Abel, 1980; Ronco et al., 2005b).

Recent studies performed in our laboratory demonstrated that placentas of smoking mothers have increased levels of Cd²⁺, zinc (Zn²⁺) and the metal-binding protein metallothionein (MT) (Ronco et al., 2005a,b). These women also delivered neonates with lower birth weights than those delivered by non-smokers. In addition, placental Cd²⁺ levels were statistically and inversely correlated to birth weights (Ronco et al., 2005b). Several studies have reported effects of smoking on fetal growth, but at present, cellular and molecular mechanisms involved in reduced birth weight observed in infants of smokers are not completely understood (Kuhnert et al., 1988a; Roquer et al., 1995). It has been suggested that...
the reduced birth weight found in neonates delivered by smokers may be related to a deficient transfer of Zn\textsuperscript{2+} from placenta to the fetus (Kuhnert et al., 1987b, 1988a).

Zinc is an essential micronutrient for fetal growth and for proper immune system function, stressing the importance of Zn\textsuperscript{2+} during pregnancy (Wellinghausen, 2001). When a mother has reduced Zn\textsuperscript{2+} reserves, Zn\textsuperscript{2+} deficiency could occur during pregnancy and nursing due to reduced transfer of this ion through the placenta and breast-feeding. Therefore, maternal Zn\textsuperscript{2+} nutrition is crucial for the infant’s Zn\textsuperscript{2+} nutritional status (Dorea, 2002). It is also known that Zn\textsuperscript{2+} supplements reduce childhood morbidity in populations where Zn\textsuperscript{2+} deficiency is common (Hamadani et al., 2002).

Interestingly, it has been shown that smokers, in addition to having elevated placental Cd\textsuperscript{2+} concentrations, also show increased placental Zn\textsuperscript{2+} levels (Kuhnert et al., 1987a; Ronco et al., 2005b). Therefore, the low birth weight of infants born to smoking mothers may be due in part to placental Zn\textsuperscript{2+} accumulation through a mechanism involving placental Cd\textsuperscript{2+} and metal-binding proteins (Toreblanca et al., 1992).

At the cellular level, one of the most important divalent metal (Zn\textsuperscript{2+}, Cd\textsuperscript{2+} and Cu\textsuperscript{2+}) binding proteins is MT. In humans there are 14 MT isoforms with at least 10 functional isoforms (Chertan et al., 2003). Four MT isoforms are the most studied: MT-1 and MT-2 have ubiquitous tissue distribution being abundant in liver, kidney, intestine and pancreas, whereas MT-3 and MT-4 are mainly found in stratified squamous epithelium and brain (Davis and Cousins, 2000). All these four isoforms have been shown to be expressed in rodent placenta (Liang et al., 1996).

Tissue Zn\textsuperscript{2+} accumulation correlates well with MT synthesis (Davis and Cousins, 2000), suggesting that this protein is involved in Zn\textsuperscript{2+} homeostasis by controlling cellular Zn\textsuperscript{2+} uptake, distribution and excretion (Klaassen et al., 1999). Cadmium ions and Zn\textsuperscript{2+}, in addition to manifest high affinity for MT, are potent inducers of MT synthesis (Harford and Sarkar, 1991). Thus, high levels of Cd\textsuperscript{2+} and MT in smoker’s placentas (Ronco et al., 2005a) and Cd\textsuperscript{2+}–Zn\textsuperscript{2+}-induced MT synthesis in cultured human trophoblasts have been previously reported (Lehman and Poirier, 1984). In other species, particularly in invertebrates, it has been described that MT-2 is the sole Cd\textsuperscript{2+}-responsive MT and the major isoform involved in Cd\textsuperscript{2+} detoxification (Sturzenbaum et al., 2001, 2004). With all these antecedents, the aim of the present study was to separate and quantify placental MT-1 and MT-2 isoforms to determine whether they are differentially stimulated in smokers and non-smokers.

2. Materials and Methods

2.1. Preparation of placentas

Human placentas (n = 20) were obtained upon delivery in the maternity ward at the Sótero del Río Hospital located in southern Santiago. Inclusion criteria included healthy young parients, with normal pregnancies and without history of alcohol or drugs. All mothers had normal nutritional status and no evidence of smoking habit. Women who had smoked (non-smokers), and women who smoked during the entire pregnancy (smokers).

Immediately after delivery, the entire placenta was weighed and placed in a sterile plastic bag and frozen at −70°C. To determine metal elements, half of the partially thawed placenta was thoroughly washed and lyophilized as previously described (Ronco et al., 2005a). Finally, samples were ground and mixed, constituting the stock placental material for metal element determinations. For MT separation and analysis by HPLC, thawed placental tissue was weighted and homogenized.

2.2. Determination of placental levels of zinc and cadmium

In stock placental material, Zn\textsuperscript{2+} was determined by instrumental neutron activation analysis (INAA) at the Laboratories of the Chilean Commission for Nuclear Energy as previously described (Ronco et al., 2005a). The reference sample utilized for Zn\textsuperscript{2+} determinations was pig kidney (trace elements BCR 186, Certified Reference Material, Sigma–Aldrich, MO, USA) with a Zn\textsuperscript{2+} concentration of 128 \(\mu\)g/g.

Cadmium determinations were carried out by atomic absorption spectrometry with a graphite furnace device for solid samples (SS-GFAAS, Carl Zeiss Technology) at the Chilean Commission for Nuclear Energy, as previously described (Ronco et al., 2005a). The reference material used for Cd\textsuperscript{2+} determinations was dorm-2 dogfish muscle (National Institute for Standards and Technology, MD, USA) with a Cd\textsuperscript{2+} concentration of 44.3 \(\mu\)g/g.

2.3. Separation of MT-1 and MT-2 isoforms by HPLC

Placental tissue was weighted (3 g), homogenized in buffer Tris–HCl (10 mM, pH 7.4) and centrifuged at 10,000 \(\times\) g for 15 min at 4°C and then at 100,000 \(\times\) g for 1 h (Nostelbacher et al., 2000). One milliliter aliquot was saturated with 50 \(\mu\)l of 0.11 M CaCl\(_2\) (1000 ppm or 5.2 mM CaCl\(_2\), final concentration in the placental sample) and heat denatured for 1 min in boiling
water. Subsequently, samples were centrifuged at 10,000 × g for 10 min, filtered through an ultra centrifugal filter (30 kDa MW cut-off, Millipore Co., MA, USA) and finally separated and analyzed by high performance liquid chromatography (HPLC) with a 1W detector (Merck Hitachi model L-4250). Experimental procedures were performed as briefly described: a 200 µl aliquot of the filtrated solution was injected onto an anion-exchange column EMD DEAE-650 (S) (Merck Fractogel EMD DEAE-650(S), 20–40 µm, 70 mm × 10 mm, with high protein binding capacity). Sample elution was carried out with 20 mM Tris–HCl, pH 7.4 (buffer A) and 200 mM Tris–HCl, pH 7.4 (buffer B) prepared with ultra pure deionized water. Two peaks sequentially corresponding to MT-1 and MT-2 were separated after elution with a continuous linear gradient of buffer B (0–60%) in buffer A, at a flow-rate of 1 ml/min during 25 min. Before any new sample injection, the column was rinsed with 1 M NaCl for 10 min and then with buffer A for 30 min. To quantify MT, different fractions from the HPLC column were collected by means of a fraction collector (Bio Rad model 2110), and Cd2+ concentrations of those fractions corresponding to MT-1 and MT-2, were measured by atomic absorption spectrometry with a continuous linear gradient of buffer B (0–60%) in buffer A, at a flow-rate of 1 ml/min during 25 min. Before any new sample injection, the column was rinsed with 1 M NaCl for 10 min and then with buffer A for 30 min. To quantify MT, different fractions from the HPLC column were collected by means of a fraction collector (Bio Rad model 2110), and Cd2+ concentrations of those fractions corresponding to MT-1 and MT-2, were measured by atomic absorption spectrometry with a continuous linear gradient of buffer B (0–60%) in buffer A, at a flow-rate of 1 ml/min during 25 min. Before any new sample injection, the column was rinsed with 1 M NaCl for 10 min and then with buffer A for 30 min.

MT-1 and MT-2 isoforms were separated by HPLC and identified by Western blot (Fig. 1). A typical chromatogram is shown in Fig. 1B. Chromatograms obtained either from placental samples of smokers or non-smokers, showed two main peaks eluting at 10.60 and 12.75 min and corresponding to MT-1 and MT-2 isoforms, respectively (Fig. 1B). In addition to MT-1 and MT-2 peaks, chromatograms always showed the presence of a smaller peak eluted at 16.5 min, and although we did not identify it, we cannot discard that it may correspond to another MT-related protein.

2.6. Statistics

Statistical analyses were performed using a statistical software package (Statistica for Windows Release 6, Statsoft Inc. 1984–2004, USA). Results obtained from 10 smokers and 10 non-smokers were expressed as median values. The non-parametric Mann–Whitney U-test was applied to compare data from both groups, and significance was assumed at p < 0.05.

3. Results

Both, MT-1 and MT-2 placental isoforms were separated by HPLC and identified by Western blot (Fig. 1). A typical chromatogram is shown in Fig. 1B. Chromatograms obtained either from placental samples of smokers or non-smokers, showed two main peaks

eluting at 6 and 12.5 min and corresponding to MT-1 and MT-2 isoforms, respectively (Fig. 1B). In addition to MT-1 and MT-2 peaks, chromatograms always showed the presence of a smaller peak eluted at 16.5 min, and although we did not identify it, we cannot discard that it may correspond to another MT-related protein.

The presence of MT in both peaks was confirmed by Western blot analysis performed in eluants from peaks 1 and 2 using a monoclonal antibody against both MTs (Fig. 1A).

A representative chromatogram of the Cd2+ content in fractions eluting from anion exchange HPLC and corresponding to MT-1 and MT-2 is shown in Fig. 2. It can be observed that Cd2+ content present in peak 1 (MT-1) from all analyzed samples (n = 20) is not significantly different when comparing both groups (median = 44.8 and 53.9 for smokers and non-smokers, respectively).
A.M. Ronco et al.

Fig. 1. Separation and identification of MT-1 and MT-2 isoforms from human placenta. (A) MTs Western blot: Eluants from peaks 1 and 2 (B) were freeze-dried, reconstituted in double distilled water and protein concentration determined by the Bradford method. SDS/PAGE was carried out with 20 μg of proteins. After transferrence of proteins to a PVDF membrane, it was incubated with anti-MT-1/MT-2 antibody as described in Section 2. The arrow shows a band corresponding to MTs contained in peaks 1 and 2. (B) Anion-exchange HPLC separation of human placental MT isoforms was performed with a Fractogel DEAE column (20–40 μm particle size) using a linear gradient from 20 to 200 mM Tris–HCl (0–60% B), pH 7.4 in 25 min at a flow rate of 1 ml/min. A 200 μl aliquot of the prepared sample was injected to the column. The efflux was monitored for UV absorbance at 254 nm.

In contrast, Cd2+ content in peak 2 (MT-2) is significantly higher in placentas of smokers (median = 7.4 and 58.1 ng for non-smokers and smokers, respectively; Fig. 2). Total Cd2+ concentrations from peaks 1 and 2 were used to calculate MT-1 and MT-2 concentrations according to the equation given in Section 2 (Table 1). Results show that placental MT-1 concentrations present in smokers are not different from those found in non-smokers. However, placental MT-2 concentrations are higher in smokers than in non-smokers; thus, total placental MT concentrations present in smokers are increased by two-fold in comparison to non-smokers (Table 1). Total concentration of Cd2+ in the whole placental tissue was also higher in smokers than in non-smokers being 4 and 9 ng (36 and 80 pmol/g) of wet tissue, respectively (Table 2). To estimate the concentration of total MTs bound to Cd2+ present in placentas, a maximal binding of 7 mol of Cd2+ per mol of MT molecules was considered (Table 2).

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>MT-1 (nmol/g wet tissue)</th>
<th>MT-2 (nmol/g wet tissue)</th>
<th>Total MT (nmol/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers</td>
<td>0.90</td>
<td>0.15</td>
<td>1.1</td>
</tr>
<tr>
<td>Smokers</td>
<td>1.06</td>
<td>1.16</td>
<td>2.2</td>
</tr>
</tbody>
</table>

a Median, n = 10 placentas. MT-1 and MT-2 concentrations were calculated from the equation given in Section 2 according to the Cd2+ content present in the eluants corresponding to peaks 1 and 2.
Table 2
Concentrations of Cd\textsuperscript{2+} in placentas of non-smokers and smokers and estimation of metallothioneins bound and unbound to Cd\textsuperscript{2+}

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cd\textsuperscript{2+} (pmol/g wet tissue\textsuperscript{a})</th>
<th>Total MTs (pmol/g wet tissue\textsuperscript{b})</th>
<th>MTs bound to total Cd\textsuperscript{2+} (pmol/g wet tissue\textsuperscript{c})</th>
<th>MTs unbound to Cd\textsuperscript{2+} (pmol/g wet tissue\textsuperscript{d})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers</td>
<td>36 (27–46)</td>
<td>1100</td>
<td>5.1</td>
<td>1095</td>
</tr>
<tr>
<td>Smokers</td>
<td>80* (62–103)</td>
<td>2200</td>
<td>11.4</td>
<td>2189</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Median, \(n=10\) placentas of non-smokers and 10 of smokers. Initial values of 4 and 9 ng of total Cd\textsuperscript{2+} per gram wet tissue corresponding to non-smokers and smokers, respectively, were converted to pmol considering a Cd\textsuperscript{2+} MW of 112.3. Total Cd\textsuperscript{2+} was determined by AAS.

\textsuperscript{b} Same values as Table 1 expressed as pmol/g wet placental tissue.

\textsuperscript{c} Potential pmol of MTs bound to total Cd\textsuperscript{2+} considering a maximal binding capacity of 7 mol of Cd\textsuperscript{2+} per mol of MT.

\textsuperscript{d} Values in \textsuperscript{b} minus values in \textsuperscript{c}.

\* \(p < 0.05\) Mann–Whitney U-test.

result, which assumes that all MT molecules would be completely saturated with Cd\textsuperscript{2+}, indicates that only 0.5% of total MT is bound to Cd\textsuperscript{2+}. Consequently, as high as 99.5% of total MT are unbound to Cd\textsuperscript{2+} and thus are available to bind other divalent ions, such as Zn\textsuperscript{2+} or Cu\textsuperscript{2+}. It is important to mention that percentage of MT unbound to Cd\textsuperscript{2+} is similar in both, the non-smokers and smokers group (Table 2).

Total placental levels of Zn\textsuperscript{2+} were higher in smokers than in non-smokers being 9.2 and 8.4 \(\mu\)g/g of wet tissue, respectively. These values were converted to nmol (140 and 128 nmol for smokers and non-smokers, respectively; Table 3) to calculate the maximal Zn\textsuperscript{2+} concentrations that could be bound to MT-1 and MT-2 in both groups. These estimations considered a maximal binding capacity of 7 mol of Zn\textsuperscript{2+} per mol of MT-1 or MT-2. Results of Table 3 show that similar concentrations of Zn\textsuperscript{2+} could be potentially bound to MT-1 in both groups. Conversely, higher concentrations of Zn\textsuperscript{2+} could be potentially bound to MT-2 in smokers than in non-smokers. Although Zn\textsuperscript{2+} bound to total MTs may increase by two-folds in smokers, most of placental Zn\textsuperscript{2+} would remain not associated to MTs in both groups since total placental levels of Zn\textsuperscript{2+} are too high in comparison to MT concentrations (Table 3).

4. Discussion

In a recent publication (Ronco et al., 2005a), we reported higher levels of total placental MTs in smokers than in non-smokers. In the present study, we show results demonstrating that the increased MT concentrations are specifically due to the smoking-induced placental MT-2 isoform. This finding suggests that elevated placental MT-2 may be one of the factors involved in the reduced birth weight generally observed in neonates born to smoking mothers.

Metallothioneins are proteins highly conserved through evolution; their presence has been documented throughout the animal kingdom, in higher plants, in eukaryotic organisms and in many prokaryotes (Kagi, 1993; Ridley, 1996). These low molecular weight cysteine-rich proteins belong to a super family of intracellular metal-binding proteins. Their functions include involvement in cell protection against heavy metal toxicity and oxidant damage, and metabolic regulation of Zn\textsuperscript{2+} homeostasis via Zn\textsuperscript{2+} donation, sequestration

Table 3
Concentrations of Zn\textsuperscript{2+} in placentas of non-smokers and smokers and estimation of Zn\textsuperscript{2+} bound and unbound to metallothioneins

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Zn\textsuperscript{2+} (nmol/g wet tissue\textsuperscript{a})</th>
<th>Zn–MT-1 (nmol/g wet tissue\textsuperscript{b})</th>
<th>Zn–MT-2 (nmol/g wet tissue\textsuperscript{b})</th>
<th>Zn\textsuperscript{2+} bound to total MTs (nmol/g wet tissue\textsuperscript{c})</th>
<th>Zn\textsuperscript{2+} unbound to MTs (nmol/g wet tissue\textsuperscript{d})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers</td>
<td>128 (79–179)</td>
<td>6.3</td>
<td>1.05</td>
<td>7.35</td>
<td>120.7</td>
</tr>
<tr>
<td>Smokers</td>
<td>140 (111–157)</td>
<td>7.4</td>
<td>8.1</td>
<td>15.5</td>
<td>124.5</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Median, \(n=10\) placentas of non-smokers and 10 of smokers. Initial values of 8.4 and 9.2 \(\mu\)g of total Zn\textsuperscript{2+} per gram wet tissue corresponding to non-smokers and smokers, respectively, were converted to nmol considering a Zn\textsuperscript{2+} MW of 65.5. Total Zn\textsuperscript{2+} was measured by INAA.

\textsuperscript{b} Potential pmol of Zn\textsuperscript{2+} bound to MT-1 and MT-2 considering a maximal binding capacity of 7 mol of Zn\textsuperscript{2+} per mol of MT-1 or MT-2. Values for MT-1 and MT-2 are those from Table 1.

\textsuperscript{c} Zn–MT-1 + Zn–MT-2.

\textsuperscript{d} Values in \textsuperscript{a} minus values in \textsuperscript{c}.

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In a recent publication (Ronco et al., 2005), we reported higher levels of total placental MTs in smokers than in non-smokers. In the present study, we show results demonstrating that the increased MT concentrations are specifically due to the smoking-induced placental MT-2 isoform. This finding suggests that elevated placental MT-2 may be one of the factors involved in the reduced birth weight generally observed in neonates born to smoking mothers.

Metallothioneins are proteins highly conserved through evolution; their presence has been documented throughout the animal kingdom, in higher plants, in eukaryotic organisms and in many prokaryotes (Kagi, 1993; Ridley, 1996). These low molecular weight cysteine-rich proteins belong to a super family of intracellular metal-binding proteins. Their functions include involvement in cell protection against heavy metal toxicity and oxidant damage, and metabolic regulation of Zn\textsuperscript{2+} homeostasis via Zn\textsuperscript{2+} donation, sequestration.
Metallothioneins have also been shown in human placenta and fetal membranes (Wier and Miller, 1987), being mainly located in fetal amniotic cells, syncytial trophoblasts and villous interstitial cells and in maternal decidual cells (Goyer et al., 1992). The presence of MT at those specific sites suggests that it may be implicated in the regulation of transplacental transport of metals such as Zn\(^{2+}\), Cd\(^{2+}\) and Cu\(^{2+}\).

It has been recognized that cigarette smoke increases placental levels of Cd\(^{2+}\) (Piasek et al., 2001; Ronco et al., 2005a,b). Results obtained in the present study show elevated levels of placental Cd\(^{2+}\) and Zn\(^{2+}\) in smokers in concordance with previous reports (Kuhnert et al., 1987b).

Therefore, one of the numerous factors involved in the reduced birth weight of infants delivered by smokers could be related to placental Zn\(^{2+}\) retention, an effect that may be an indirect consequence of the high levels of Cd\(^{2+}\) present in this tissue (Kuhnert et al., 1988b). Maternal MT induction by several chemicals – including toxic heavy metals such as Cd\(^{2+}\) – contributes to cause developmental toxicity by a chain of events leading to an adverse decrease in Zn\(^{2+}\) bioavailability to the embryo (Daston et al., 1991), a fact which in turn may be involved in low birth weight (Kuhnert et al., 1987b).

In this study, placental MT-1 and MT-2 from smokers and non-smokers were separated by HPLC as previously described for rat liver (Klaassen and Lehman-McKeeman, 1991). In our experimental protocol, MT-1 and MT-2 retention times were 6 and 12.5 min, respectively, values closely related to rat liver MT-1 and MT-2 retention times, as already shown by Klaassen and Lehman-McKeeman (1991), but different to those retention times described by Nostelbacher et al. (2000). Differences may be attributed to different experimental conditions and tissues used. It is important to mention that all the analyzed samples (n = 20) showed the same elution profile for both isoforms. The presence of MTs in both elution peaks was corroborated by Western blot analysis. Results demonstrated that in non-smoker’s placentas, MT-1 was expressed by six-fold over MT-2 isoforms. We used HPLC coupled to Cd\(^{2+}\) measurements to separate and quantify MT-1 and MT-2; followed by Western blot analysis for identification. In the study reported by Milnerowicz (1997), identification of MT-1 and MT-2 was based on different electrophoretic mobility in SDS-PAGE, a method which may not be accurate enough to separate and identify different protein isoforms.

Since MTs have high affinity for Cd\(^{2+}\) \((K_d = 10^{-17} \text{ to } 10^{-22})\) (Klaassen et al., 1999; Croyte et al., 2002) and placental MT concentrations observed are high compared to total Cd\(^{2+}\), all Cd\(^{2+}\) ions would be bound to MTs. Furthermore, assuming a maximal binding of 7 mol of Cd\(^{2+}\) per mol of MT, most of MTs (99.5% either in smokers or in non-smokers) should remain unbound to Cd\(^{2+}\) and thus available to bind other divalent ions. In our case, placental MT-2 resulted elevated in smokers, and as a consequence, the percentage of Zn\(^{2+}\) that could be bound to total MTs increased from 5.7% in non-smokers to 11.1% in smokers. Therefore, although Zn\(^{2+}\) unbound to MTs is similar in both groups (Table 3), the increased amount of Zn\(^{2+}\)–MT-2 complex present in smokers could be relevant enough to interfere with the dynamic of the mechanisms involved in the transference of placental Zn\(^{2+}\) to the fetus. This fact could explain, at least in part, the reduced levels of Zn\(^{2+}\) previously found in red blood cells of umbilical cord of newborn delivered by smokers (Kuhnert et al., 1987b).

An additional role for the increased placental MT-2 levels found in smokers could be related to fetus protection against Cd\(^{2+}\) toxicity. Previous research in non-vertebrate organisms showed that MT-2 expression is the primary response to Cd\(^{2+}\) exposure, and established that MT-2 is the sole Cd\(^{2+}\)-responsive MT isoform (Sturzenbaum et al., 2001). Furthermore, there is evidence that MT-2 rather than MT-1 is the major isoform implicated in Cd\(^{2+}\) detoxification in these organisms (Sturzenbaum et al., 2004). Thus, the elevated levels of MT-2 present in placentas of smokers may have a positive specific function by sequestering and compartmentalizing Cd\(^{2+}\).
ing Cd\(^2+\) inside the placenta, avoiding Cd\(^2+\) transport to the fetus (Boardi et al., 1991).

In summary, results presented in this study show that MT-2 is specifically induced in smoker’s placentas, suggesting that this isoform could be involved in placental Cd\(^2+\) and Zn\(^2+\) retention. This fact could contribute to reduce the transference of Zn\(^2+\) to the fetus, which in turn, may be associated to detrimental effects on fetal growth, finally leading to the reduced birth weights observed in neonates born from smoking mothers.

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