

Zinc Supplementation Ameliorates Biochemical Changes and Hg Intestinal Deposition Caused by Inorganic Mercury Intoxication

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Received date: November 21, 2017; Accepted date: January 31, 2018; Published date: February 07, 2018

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Abstract

Mercury is a toxic metal used in industries and in the process of gold extraction. The inorganic form of mercury, Hg^{2+} , is known to cause alterations in the renal system. In this context, this work evaluated the effects of oral $HgCl_2$ exposure in markers of nephrotoxicity and hepatotoxicity and gastrointestinal Hg levels. Moreover, it evaluated the preventive effects of $ZnCl_2$. Male Wistar rats were exposed orally for five days to $ZnCl_2$ (27 mg/kg/day) and subsequently for five days to $HgCl_2$ (5 mg/kg/day). Rats were sacrificed 24 h after last $HgCl_2$ administration. $HgCl_2$ exposure caused a significant increase in serum urea levels and a decrease in serum lactate dehydrogenase activity without altering gastrointestinal δ -aminolevulinic acid dehydratase activity. Zn pre-exposure avoided the increase in urea levels. Still, Zn supplementation increased stomach Hg accumulation and decreased Hg intestinal burden. These results suggested that the oral exposure to five doses of $HgCl_2$ is nephrotoxic, and the preventive effect of zinc can be related to the lower intestinal Hg absorption due to stomach Hg retention.

Keywords: Nephrotoxicity; Inorganic mercury; δ -ALA-D; Lactate dehydrogenase

Introduction

Mercury toxicity, as well as its deposition in different tissues depends on the chemical form (inorganic or organic), time and route of exposure [1]. The main route of exposure to mercury is oral via, in view of the large consumption of contaminated food and water [2,3]. The oxidized form of mercury, Hg^{2+} , presents as the first target the renal system [4,5]. $HgCl_2$ exposure causes a high Hg deposition in the kidney as well as renal failure characterized by an increase in serum urea and creatinine levels, proteinuria, histological alterations and inhibition of sulfhydryl enzymes [5-15]. In fact, toxicological properties of Hg are directly related to the affinity of this metal for sulfhydryl groups (-SH), which can interfere in membrane structure and functions as well as in enzyme activities [1,16]. For example, shortly after exposure to inorganic mercury most of the Hg present in plasma is bound to SH-containing proteins [4]. This interaction Hg-SH may cause an alteration in several organs function [1].

Several drugs and compounds such as vitamin E, selenium, and melatonin have been tested in an attempt to reduce the damages caused by mercury exposition [6,17-19]. Moreover, previous studies from our research group have shown the importance of zinc against the toxicity of $HgCl_2$ in rats with different ages [7-10,20].

Zinc (Zn) is an essential metal, which is involved in many enzymatic activities, cellular growth and gene expression and its absorption occur mainly in the small intestine [15]. Several studies have shown that subcutaneous Zn exposure attenuates or protects renal alterations induced by Hg [8,11,21], as well as Zn supplementation in drinking water, ameliorates the antioxidant system alterations induced by malathion [22] and chlorpyrifos [23]. Still, pre

and post oral treatment with zinc were efficient against alterations in stomach and intestine induced by ethanol [24].

Thus, the present work aims to investigate the effects of oral mercury chloride exposure on markers of renal, hepatic and gastrointestinal toxicity. Besides, to evaluate the preventing role of zinc chloride against alterations induced by mercury.

Material and Methods

Chemicals

Mercuric chloride, zinc chloride, sodium chloride, potassium phosphate monobasic and dibasic, absolute ethanol, sodium hydroxide, trichloroacetic acid, o-phosphoric acid, perchloric acid, and glacial acetic acid were purchased from Merck (Darmstadt, Germany). Bovine serum albumin and Coomassie brilliant blue G were obtained from Sigma (St Louis, MO, USA). ρ -Dimethylaminobenzaldehyde was obtained from Riedel (Seelze, Han, Germany). The commercial kits for biochemical dosages were obtained from Kovalent do Brasil Ltda. (São Gonçalo/ RJ/ Brazil) or Labtest Diagnóstica S.A. (Lagoa Santa/ MG/ Brazil).

Animals

Sixteen adult male Wistar rats (200-220 g) (N=4/group) obtained from Animal House of Federal University of Santa Maria were transferred to our breeding colony and maintained on a 12 h light/dark cycle and at a controlled temperature ($22 \pm 2^\circ C$). Animals had free access to water and commercial food (GUABI, RS, Brazil) and they were used according to guidelines of Committee on Care and Use of Experimental Animal Resources, Federal University of Santa Maria, Brazil (096/2011).

Exposure to metals

Animals were distributed in four groups (N=4/group), pretreated by gavage for five days with 0.9% NaCl (saline solution) or ZnCl₂ (27 mg/kg/day), and after, treated by gavage for more five subsequent days with saline or HgCl₂ (5 mg/kg/day). Metals were dissolved in saline solution and administered by gavage at a volume of 1 mL/kg body weight. Zn and Hg doses were selected according to previous studies performed by our research group [7-13,20].

Tissue preparation

Twenty-four hours after the last administration of saline or HgCl₂, rats were weighed and killed by decapitation. Total blood samples were collected from the body and centrifuged at 1,050 g for 10 min at 4°C to obtain the serum, which was used for determination of urea and creatinine levels and alanine aminotransferase (ALT), alanine aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activity. For the δ-aminolevulinic acid dehydratase (δ-ALA-D) activity assay, stomach and intestine were quickly removed, placed on ice and homogenized in 5 and 7 volumes of NaCl (150 mM, pH 7.4), respectively. The homogenate was centrifuged at 8,000 g for 30 min at 4°C and the supernatant fraction (S1) was used in the enzyme assay. Furthermore, the stomach and intestine were used in the determination of mercury levels.

ALT, AST and LDH activity and creatinine and urea levels

Enzymes activities and creatinine and urea levels were determined by using a Labtest commercial kit as describe in Peixoto and Pereira [11].

δ-ALA-D activity

Enzymatic activity was assayed according to Sassa [25] by measuring the rate of product (porphobilinogen - PBG) formation, as previously described by Peixoto et al. [12]. Enzyme activity was expressed as nmol PBG/h/mg protein. Protein concentration was determined by Bradford method [26] using bovine serum albumin as a standard.

Determination of metal levels

Metal analyses were carried out using a Model AAS EA 5 atomic absorption spectrometer (Analytik Jena, Jena, Germany) equipped with a transversely heated graphite tube atomizer with pyrolytic coated tubes as described by Peixoto et al. [13] and Oliveira et al. [27].

Statistical analysis

Results were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test when appropriate. A value of $p \leq 0.05$ was considered significant.

Results

ALT, AST and LDH activity and creatinine and urea levels

Markers of renal damage (urea and creatinine levels) and hepatic damage (AST, ALT and LDH activities) are shown in Table 1. One way ANOVA shows the significant effects of treatment on serum urea levels [F(3,14)=4.364, $p=0.022$] and on LDH activity [F(3,14)= 4.845, $p<0.016$]. HgCl₂ exposure caused an increase in serum urea levels and

a decrease in LDH activity when compared to control group ($p<0.05$, Duncan's multiple range test). ZnCl₂ pre-treatment protected the increase in urea levels but it did not protect the LDH activity inhibition.

ALT and AST activities were not altered by treatments.

Serum assays	Sal-Sal	Zn-Sal	Sal-Hg	Zn-Hg
Nephrotoxicity markers				
Urea (mg/dL)	42.9 ± 4.1 ^a	51.6 ± 4.7 ^a	79.4 ± 9.9 ^b	55.8 ± 7.7 ^a
Creatinine (mg/dL)	1.04 ± 0.11	1.05 ± 0.04	1.36 ± 0.36	1.02 ± 0.04
Hepatotoxicity markers				
ALT (U/mL)	45.1 ± 8.7	54.6 ± 7.3	43.2 ± 4.4	47.9 ± 1.8
AST (U/mL)	106.0 ± 2.8	105.5 ± 4.8	96.7 ± 6.5	95.2 ± 4.3
LDH (U/L)	1906 ± 155 ^a	1521 ± 279 ^{a,b}	1036 ± 144 ^b	1199 ± 107 ^b
Data are expressed as mean±S.E.M. (n=4) and the values followed by different letters in the same line are statistically different ($p<0.05$)				

Table 1: Serum nephrotoxicity and hepatotoxicity markers from male Wistar rats exposed orally to ZnCl₂ (27 mg/kg/day) or saline for five consecutive days and exposed to HgCl₂ (5 mg/kg/day) or saline on the five subsequent days.

δ-ALA-D activity

Stomach and intestine δ-ALA-D activities are shown in Table 2. One way ANOVA shows an absence of treatment effects on gastrointestinal δ-ALA-D activity.

Hg levels

Stomach and intestine Hg levels are shown in Table 2. One way ANOVA shows significant effect of treatment on stomach [F(3,12)=8.324, $p=0.003$] and intestine [F(3,12)=10.24, $p=0.001$] Hg levels. Both groups exposed to Hg (Sal-Hg and Zn-Hg) presented stomach and intestinal Hg levels greater than the control group ($p<0.05$, Duncan's multiple range test). Moreover, Zn-Hg group presented an increase in Hg stomach levels (~81%) and a decrease in Hg intestine levels (~21%) compared with the Sal-Hg group, though these changes were not significant.

Parameters	Sal-Sal	Zn-Sal	Sal-Hg	Zn-Hg
δ-ALA-D activity (nmol PBG/h/mg protein)				
Stomach	5.25 ± 0.68	4.29 ± 0.91	5.21 ± 0.36	4.75 ± 0.64
Intestine	6.50 ± 0.49	6.36 ± 0.60	6.79 ± 0.16	6.19 ± 0.40
Hg levels (mg Hg/g)				
Stomach	n.d. ^a	0.97 ± 0.20 ^{a,b}	1.54 ± 0.46 ^{b,c}	2.79 ± 0.62 ^c
Intestine	n.d. ^a	0.28 ± 0.07 ^{a,c}	0.71 ± 0.13 ^{b,c}	0.56 ± 0.11 ^c
Data are expressed as mean±S.E.M. (n=4) and the values followed by different letters in the same line are statistically different ($p<0.05$). The samples whose mercury concentrations were below the detectable limit (non-detected, n.d.) of				

the technique were considered, for statistical analysis, as containing 0.05 µg of metal/g of tissue, which was the minimum measurable quantity

Table 2: δ-ALA-D activity and Hg level in stomach and intestine of male Wistar rats exposed orally to ZnCl₂ (27 mg/kg/day) or saline for five consecutive days and exposed to HgCl₂ (5 mg/kg/day) or saline on the five subsequent days.

Discussion and Conclusion

This work evaluated the effects of oral exposure to 5 mg of HgCl₂/kg/day during five days on markers of toxicity and Hg levels. Besides, we evaluated if ZnCl₂ pretreatment avoids the HgCl₂ effects.

Serum urea and creatinine levels are used as markers of renal damage; these metabolites are increased in serum of animals who present a decrease in kidney excretory function [28,29]. Mercury entry in renal cells causing or predisposing these cells to oxidative stress and consequent cellular death [4]. In our work, orally HgCl₂ exposure is nephrotoxic, once animals presented an increase in serum urea and creatinine levels.

Regarding hepatotoxic markers, both groups exposed to HgCl₂ presented a decrease in LDH activity. This inhibition in LDH activity is not associated with hepatic damage since in cases of cellular and tissue injury the enzyme activity is increased in serum [30]. However, it is not the first time that our research group observes serum LDH inhibition induced by HgCl₂ [7,11] without hepatic LDH activity alterations [20]. Peixoto and Pereira [11] and Franciscato et al. [7] reported an inhibition about 20% and 32%, respectively, of serum LDH from young rats killed 24h or 21 days after HgCl₂ exposure. Moraes-Silva et al. [20] showed that HgCl₂ inhibits serum and hepatic LDH activity *in vitro*. It is probable that LDH inhibition is caused by an interaction between Hg and sulfhydryl groups (SH groups) present in the enzyme structure. In this line, Lugokenski et al. [31] and Pamp et al. [32] showed that compounds with sulfhydryl affinity inhibited the LDH activity proving the importance of SH groups to keep the enzyme conformational structure and activity.

δ-ALA-D has been used as a marker of toxic metal exposition. Divalent metals are known to inhibit δ-ALA-D activity from different tissues [33]. In our work, we verified that the HgCl₂ exposure induced a stomach and intestine Hg accumulation without altering δ-ALA-D activity. These results suggest that gastrointestinal δ-ALA-D activity is less sensitive to Hg and the enzyme requests higher Hg levels to present a significant inhibition. Although Zn pretreatment increases still more the stomach Hg levels, it is possible that Zn is protecting the enzyme sulfhydryl groups from Hg, since δ-ALA-D activity has not been altered.

These findings are important since the main Hg exposition via is through contaminated food and water (oral form) [2,3]. Still, the protection exhibited by ZnCl₂ pre-exposure using different exposure models [7-12], also was verified when it was administrated by oral via. One of the mechanisms suggested to Zn protective effects is related to a concomitant increase in metallothionein (MT) synthesis in liver and intestine [34]. MTs are important metalloproteins, rich in SH groups; it is an endogen scavenger molecule that binds to metals, forming a complex and making it unable to cause harm to the organism [35]. In this way, since Hg bind to metallothionein in intestine or liver, it can be carried to kidney and be released through the urine. Another possible protective mechanism in which Zn can act is in stomach mucus secretion decreasing Hg absorption. Cho and Ogle [36] showed that

Zn exposure caused a dose-dependent increase in gastric wall mucus content in rats, and Vázquez et al. [37] observed an increase in mucus Hg levels in models of Caco-2 and HT29-MTX cells. In our work, animals pre-exposed to ZnCl₂ presented ~81% more Hg in the stomach than animals that received only HgCl₂, probable avoiding its absorption.

These results suggest that ZnCl₂ can be used as promising supplementation to people who work with mercury.

Acknowledgements

M.E.P. is the recipient of CNPq fellowship (503867/2011-0; 311082/2014-9), C.S.O. R.P.I. and V.A.O. are recipients of CAPES fellowships.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Standards

The study was approved by the Committee on Care and Use of Experimental Animal Resources, Federal University of Santa Maria, Brazil (096/2011).

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