GENDER DIFFERENCES IN MERCURY-INDUCED HEPATOTOXICITY: Potential Mechanisms.

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Declaration of interest: none

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ABSTRACT

The accumulation of mercury in the liver causes hepatotoxicity. The organic anion transporter 3 (Oat3) and the multidrug-resistance associated protein 2 (Mrp2) are involved in the hepatic excretion of toxins and drugs and in the hepatic handling of mercury. The aim of this work was to study if there are gender-related differences in mercuric chloride ($HgCl_2$)-induced hepatotoxicity in rats. Total mercury levels and protein expressions of Oat3 and Mrp2 in liver samples were also assessed to clarify the mechanisms underlying mercury-induced liver damage in male and female rats.

Control and $HgCl_2$-treated male and female Wistar rats were used. Hepatotoxicity was evaluated by plasma activity of transaminases and alkaline phosphatase, as well as by histopathological analysis. Oat3 and Mrp2 expression was assessed by immunoblotting. Female rats displayed a higher $HgCl_2$-induced hepatotoxicity than male rats as demonstrated by the higher alterations in the plasma markers of liver damage and in the histopathology. The sex-related differences observed in the hepatic damage can be explained by the higher accumulation of mercury in liver from female rats. In this connection, after mercury treatment the expression of Mrp2 decreased in both sexes and the expression of Oat3 decreased only in males. The decreased in Oat3 abundance in the hepatocytes membranes in mercury-treated males would limit the uptake of mercuric ions into the liver protecting them from mercury hepatotoxicity.

Keywords: sex differences, mercuric chloride, Oat3, Mrp2, hepatotoxicity, rats.

1. Introduction

Mercury is a toxic heavy metal that can induce important alterations in a considerable variety of tissues and organs. All living organisms are in some way exposed to mercury because of their perpetual existence in the environment. Mercury causes several toxic effects in the central nervous system, the immune system, the kidneys and the liver. Mercury exposure has also been associated to gastrointestinal disturbances and cardiovascular diseases (Berlin et al., 2015). Moreover, mercury has been recognized as an endocrine disruptor (Iavicoli et al., 2009).

From a toxicological point, mercury compounds can be classified as organic and inorganic compounds. Inorganic divalent mercury (Hg^{+2}) salts are the compounds of more toxicological impact since other forms of mercury such as elemental mercury (Hg^{o}), mercurous mercury (Hg^{+1}) and organic compounds of mercury, can be converted to Hg^{+2} in the body (Zalups, 2000). Between Hg^{+2} and several protein and non protein thiols in the target cells and tissues there are complex binding interactions which determine the toxicological properties of mercury (Zalups, 2000; Berlin et al., 2015).

The liver is the major metabolic organ and is frequently a target for numerous toxic compounds. It has been described that the liver is the major site for mercury accumulation (Mieiro et al., 2011). Excessive accumulation of mercury in the liver can alter hepatic structure and function (Giari et al., 2008). Mercury can cause serious injury by protein damage and depletion of thiol-containing antioxidants and consequently oxidative stress-mediated liver injury (Gosh and Sil, 2008; Ung et al., 2010; Liu et al., 2017). The response of a single sex (male or female) to mercury exposure has been frequently evaluated, while studies comparing the hepatotoxic effects of mercury on females and males are uncommon.

The uptake of mercuric ions across the sinusoidal membrane of hepatocytes could involve specific mechanisms of active transport in the plasma membrane of hepatocytes (Bridges and Zalups, 2017). It has been reported that the Organic Anion Transporter 3 (*Slc22A8*, Oat3) is a membrane carrier that transport Hg^{+2} ions conjugated with cysteine or reduced glutathione (Aslamkhan et al., 2003; Bridges and Zalups, 2017). Oat3 is expressed in the sinusoidal membrane of hepatocytes, where it could uptake Hg^{+2} from blood to cytoplasm of liver cells. On the other hand, Bridges et al. (2011) have indicated that the transport of Hg^{+2} across the canalicular membrane could be mediated by the Multidrug resistance-associated protein 2 (*Abcc2*, Mrp2).

Hazelhoff et al. (2012) described gender related differences in the nephrotoxicity induced by $HgCl_2$ in rats, being females lesser affected by mercury than males. Authors concluded that the lower expression of mercury transporters in the basolateral membrane of proximal tubule cells in female rats restricts Hg^{+2} uptake into renal cells protecting them from mercury toxicity.

The purpose of this work was to determine if there are gender-related differences in the inorganic mercury-induced hepatotoxicity by evaluating the activity of plasma enzymes used as markers of hepatocellular damage, and liver histopathology in rats. Total mercury levels and protein expressions of Oat3 and Mrp2 in liver samples were also assessed in order to clarify the mechanisms underlying mercury-induced liver damage in male and female rats.

2. Materials and methods

2.1. Animals

Adult male and female Wistar rats (90-110 days old) were used in this study. All animals were provided a standard laboratory chow and tap water *ad libitum*. Animals were housed in a constant temperature and humidity environment with regular light cycles (12 h) in the course of the experiment. All experimental procedures were conducted following the Guide for the Care and Use of Laboratory Animals National Institutes of Health (NIH) and were approved by the Faculty of Biochemical and Pharmaceutical Sciences (UNR) Institutional Animal Care and Use Committee (Res Nº 484/2015).

2.2. Experimental design and groups

Four experimental groups were employed: Control Males (CM, n = 10), Control Females (CF, n = 9), Mercury-treated Males (Hg-M, n = 10) and Mercury-treated Females (Hg-F, n = 9). Hg-treated animals received a single intraperitoneal (i.p.) injection of $HgCl_2$ (4 mg/kg body weight) (*w*/*v* in 2 mL saline/kg) as previously reported (Torres et al., 2011; Hazelhoff et al., 2012, 2015; Trebucobich et al., 2014). Control groups were injected with the vehicle alone (2 mL saline/kg). The experiments were performed 18 h after the injection (Zalups, 2000; Hazelhoff et al., 2012, 2015).

Animals were weighed and then anesthetized with sodium thiopental (70 mg/kg body weight, i.p.) at the moment of the experiments. The collection and processing of hepatic tissue samples was different depending on the type of study to be performed. Different sets of experimental animals were used for biochemical determinations and histopathological studies (n=6 for CM and n = 6 for Hg-M; n=5 for CF and n = 5 for Hg-F), and for preparation of liver total plasma membranes for Western blotting assays (n=4 for each experimental group).

2.3. Determination of plasma markers of hepatocellular damage

Blood samples were obtained by cardiac puncture. Blood plasma was separated by centrifugation (1000 × *g* for 10 min). The plasma samples were used for the determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AP) employing commercial kits (Wiener Laboratory, Rosario, Argentina).

2.4. Histopathological studies

The liver from each experimental groups was briefly perfused with saline, followed by perfusion with periodate-lysine-paraformaldehyde solution (0.01 M $NaIO_4$, 0.075 M lysine, 0.0375 M phosphate buffer, with 2 % paraformaldehyde, pH 6.20), through a cannula inserted in the abdominal aorta. The slices were immersed in periodate-lysine-paraformaldehyde solution at 4 ºC overnight. After that, the tissue was embedded in paraffin. Paraffin sections were cut. After deparaffining, the sections were used for routine hematoxilin-eosin staining.

Histopathological preparations were randomly examined with 10 microscope fields for each sample by a blinded observed. The liver injury (parenchyma disorganization and fibrosis) was evaluated. The results from each observation were then combined for the overall results. Alterations were graded as follows: (-) not detected, (+) rarely detected, (++) frequently detected, (+++) very frequently detected.

2.5. Total Hg content analysis

Total mercury determination in plasma samples and liver tissue was performed by cold vapor atomic absorption as previously described by Trebucobich et al. (2014). Mercury determination was performed employing an Atomic Absorption

Spectrophotometer Perkin Elmer AAnalyst 300 measurement by cold vapor, Flow Injection Analysis System (FIAS) 100–Perkin Elmer. The Hg^{2+} was reduced by the treatment with stannous chloride to Hg°, and the Hg° was measured with cold vapor atomic absorption (λ= 254 nm) by a Hg monitor using argon as gas. Daily, standards for Hg^{2+} were prepared from a dilution of the stock solution generated by solving $HgCl_2$ p.a. in a nitric acid solution (1.354 g/L). Detection limit of the instrument: 1 g/L.

2.6. Preparation of plasma membranes from liver

Plasma membranes from liver were obtained as previously reported by our laboratory (Trebucobich et al., 2014). Animals were anesthetized and the liver was removed surgically. Briefly, the liver was immediately dissected out, rinsed in saline solution, and homogenized in 20 mL of 0.2 mM $CaCl_2$/0.25 M sucrose/0.1 mM PMSF/10 mM HEPES-Tris (pH 7.40). EDTA was added until reaching a final concentration of 1 mM. Afterward, the homogenate was diluted with homogenization buffer (+1 mM EDTA) to a final volume of 20 mL/2 g of tissue. All operations were performed at 0–4ºC. After centrifugation for 10 min at 1000 × *g*, the supernatant and upper fluffy layer of the pellet were collected and centrifuged later for 30 min at 20,000 × *g*. The resulting pellet, which represents crude membranes, was resuspended gently in 0.3 M mannitol/0.1 mM PMSF/10 mM HEPES-Tris (pH 7.50).

Aliquots of the membranes were stored immediately at −80 °C for 2 weeks. Each preparation represents the liver of one rat. For each experimental group, four different preparations were made.

2.7. Electrophoresis and immunoblotting

Plasma membranes (20 µg of protein/lane) samples (n = 4 for each experimental group) were boiled for 3 min in the presence of 1% 2-mercaptoethanol/ 2% sodium dodecyl sulphate (SDS). Proteins were separated through 8.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and then electroblotted to a pure nitrocellulose membrane (NC membrane) (Trans-Blot® Transfer Medium, Bio Rad Laboratories, Hercules, CA, USA). To verify equal protein loading and transfer between lanes, Ponceau Red and antibody against human β-actin were used as previously described (Hazelhoff et al., 2015). The NC membranes were incubated with 5% non-fat dry milk in phosphate-buffer saline containing 0.1% Tween 20 (PBST) (80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl, 0.1% Tween 20, pH 7.50) for 1 h. After being rinsed with PBST, the NC membranes were incubated overnight at 4 °C with a non-commercial rabbit polyclonal antibody against rat Oat3 or a commercial mouse monoclonal antibody against rat Mrp2 or a commercial mouse monoclonal antibody against human β-actin. The specificity of Oat3 antibody has been described elsewhere (Kojima et al., 2002). The NC membranes were incubated for 1 h with a peroxidase-coupled goat anti-rabbit IgG (Bio-Rad Laboratories, CA, USA) or peroxidase-coupled sheep anti-mouse IgG (Amersham, UK) after further washing with PBST. Blots were processed for detection using a commercial kit (Pierce™ ECL Western Blotting Substrate; Illinois, USA).

A densitometric quantification of the Western blotting signal intensity of membranes was performed using the Gel-Pro Analyzer (Media Cybernetics, Silver Spring, MD, USA) software. For densitometry of immunoblots, samples from Hg-treated rats were run on each gel with the corresponding control for each sex. The abundance of Oat3 and Mrp2 in the samples from Hg-treated rats were normalized to β-actin and considered as percentage of the mean control value of the same sex for that gel.

2.8. Materials

Chemicals were purchased from Sigma (St. Louis, MO, USA) and were analytical grade pure. The monoclonal antibody against Mrp2 was purchased from Abcam Inc. (Cambridge, MA, USA) and the monoclonal antibody against β-actin was purchased from Alpha Diagnostic International (San Antonio, TX, USA). The polyclonal antibody against Oat3 was kindly given by Prof. N. Anzai (Department of Pharmacology, Graduate School of Medicine, Chiba University, Japan).The Kaleidoscope Prestained Standards of molecular mass were purchased from Bio Rad Laboratories (Hercules, CA, USA).

2.9. Data analyses

Statistical analysis was performed using the unpaired Student's *t*-test. When variances were not homogeneous a Welch's correction was employed. $p < 0.05$ was considered statistically significant. The values are expressed as the means ± standard error (S.E.M.). For these analyses, GraphPad software was used.

3. Results

3.1. Plasma markers of hepatocellular damage, liver weight and liver weight/body weight ratio

Enzymatic activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AP) in plasma, liver weight and liver weight/body weight ratio in control and Hg-treated male and female rats are shown in Table 1. AST activity increased in both genders with $HgCl_2$ treatment, but the increase was higher in females than in males. AP activity was lower in Mercury-treated Females (Hg-F), compared to Control Females (CF), and similar in Mercury-treated Males (Hg-M) when compared to Control Males (CM). ALT activity, liver weight and the liver weight/body weight ratio were not different between Hg-treated and control rats in both genders.

3.2. Histopathological observation

In the liver of control males and females, the hepatocytes showed normal polygonal structures and round nucleus with a prominent nucleolus (Fig. 1A, B, E and F). Histopathological alterations, such as parenchyma disorganization and fibrosis were observed in the liver of males and females treated with $HgCl_2$ (Fig. 1C, D, G and H). Changes in liver histology were more frequent in females than in males (Table 2).

3.3. Total Hg content analysis

The total amount of Hg in liver (µg/g liver) after the exposure to $HgCl_2$ (Figure 2) was higher in female rats compared to male rats. In addition, the liver burden of Hg 18 h after $HgCl_2$ injection (expressed as % of the administered dose) was a 60% greater

in female rats than male rats (10.13 ± 0.74 vs 6.3 ± 1.0, respectively. $p<0.05$). There was no significantly difference in the concentration of Hg in plasma (μg/mL) after $HgCl_2$ administration between male and female rats (1.93 ± 0.49 vs 2.59 ± 0.44, respectively).

3.4. Protein expression levels of membrane transporters

To study the effects of mercury exposure on liver Oat3 and Mrp2 protein expression by immunoblotting, liver plasma membranes were prepared from the four experimental groups of rats. As shown in Figure 3, Hg-M exhibited a significantly lower (26% lower) abundance of Oat3 compared to CM. On the contrary, the protein abundance of Oat3 in female rats was not affected by $HgCl_2$ treatment.

In this study, the Mrp2 protein expression was down-regulated by the dose of $HgCl_2$ employed in both genders (Figure 4). Hg-M presented approximately a 45% lower density of the Mrp2-related protein band, when compared with CM. In female rats, Hg-F showed a 42% lower density of the Mrp2-related protein band than CF.

4. Discussion

Mercury is a toxic element which differs from other metals, as cadmium and lead, because it is present in the environment in a number of different chemical forms that show a wide range of toxicological properties. Natural sources of mercury include earthquakes, volcanic eruptions and erosion from the earth's crust. It is also present in fossil fuels, metallic minerals and other minerals. Moreover, the numerous anthropological activities increase their release to the atmosphere, soils and water (Magos and Clarkson, 2006; Syversen and Kaur, 2012). Environmental pollution and occupational exposure are the primary forms of unintentional exposure to this metal. Anthropological uses include dental amalgams, incandescent lights, batteries and chlor-alkali and caustic soda industries (Magos and Clarkson, 2006; Berlin et al., 2015). While global use of metallic mercury has decreased over the past few decades, developing countries are facing growing health problems and environmental risks because of increased exposure from small-scale gold mining and combustion of fossil fuels (Magos and Clarkson, 2006; Berlin et al., 2015). Southeast Asia, Africa and South America are the main global emitters of mercury into the air. Peru, Brazil, Bolivia, Ecuador and Colombia are the main countries with artisanal mining to extract gold, usually illegally. On the other hand, there are some cultural and religious practices that use mercury (such as Palo Mayombé, Candomblé, Voodoo and Spiritism) (Magos and Clarkson, 2006; Berlin et al., 2015).

Inorganic mercury salts are found in daily use products such as cosmetics (mainly whitening creams), teeth whitening powders, laxatives, antiseptics and diuretics (Magos and Clarkson, 2006; Syversen and Kaur, 2012; Berlin et al. 2015). These salts are also of toxicological interest since other forms of mercury (Hg^{o}, Hg^{+1} and organic

mercury) are transformed to Hg^{+2} in the body (Zalups, 2000; Torres, 2013).The administration of a single dose of mercuric chloride ($HgCl_2$) in rats is a classic experimental model of mercury induced nephrotoxicity and hepatotoxicity (Zalups, 2000; Gosh and Sil, 2008; Torres et al., 2011; Bashandy et al., 2011; Hazelhoff et al., 2012, 2015; Trebucobich et al., 2014; Joshi et al., 2017; Liu et al., 2017; Zhang et al., 2017). The liver is the primary site of metabolism for mercury. Mercuric ions (Hg^{+2}) accumulates in the liver causing hepatocellular injury with oxidative stress, oxygen species overproduction, depolarization of the mitochondria with the consequent ATP depletion, formation of intracellar vacuoles and cell death (Gosh and Sil, 2008; Ung et al., 2010; Bashandy et al., 2011; Liu et al., 2017). Moreover, $HgCl_2$ has been reported to cause histopathological damage and ultrastructural lesions in the liver due to degeneration of fatty acids and cellular necrosis (Gosh and Sil, 2008; Joshi et al., 2017; Zhang et al., 2017).

In the present study, both liver weight and the ratio between liver weight and body weight were not affected by the administration of $HgCl_2$, as observed by other authors (Abarikwu, et al., 2017; Liu et al., 2017). The relation between the hepatic tissue damage and the elevation of the relevant plasma enzymes is well documented and serum activities of AST, ALT and AP are used as markers of hepatocellular damage (Reichling and Kaplan, 1988). In the present study, the $HgCl_2$ treatment increased plasma activity of AST in both male and female rats. The elevation of plasma activity of AST was higher in $HgCl_2$-treated females than in treated males (360 % vs 128 %, respectively). An elevation of plasma activity of AST, a liver mitochondrial and cytoplasmic enzyme, indicates structural lesions in the liver causing increase in the permeability of the hepatocytes membranes leading to the release of the enzyme in the blood. After $HgCl_2$ exposure a decreased plasma activity of AP was observed in both

296 sexes, being statistically significant only in female rats. AP is expressed in the
297 hepatocyte canalicular membrane and its decreased activity in plasma has been
298 associated with damage caused by the lipid peroxidation of membranes (El-Demerdash,
299 2001; Reus et al., 2003; Wadaan, 2009). ALT plasma activity was not modified after 18
300 h of $HgCl_2$ administration. ALT is found in the cytosol of the liver cells and the release
301 of ALT from damaged hepatocytes increases its activity in plasma. It has been reported
302 that plasma ALT levels rise at the initial stages of hepatocellular injury and as fibrosis
303 progresses ALT levels declines (Kim et al., 2008). In the present work, the
304 histopathological analyses showed that $HgCl_2$ induced damage in liver tissue with the
305 presence of isolated focus of fibrosis in both sexes, being the female rats more affected
306 than male rats. Thus, the present study demonstrates a gender-related difference in the
307 hepatotoxicity induced by inorganic mercury. Mercury exposure caused alterations in
308 liver function markers (such as AST and AP activities) as well as increase in
309 histopathological lesions of the liver, which were more severe in females than in males.

310 In order to clarify, at least in part, the mechanisms underlying the sex-dependent
311 effects of $HgCl_2$ on liver function and structure, we assessed total mercury levels in liver
312 from male and female rats.

313 Inorganic mercury has a highly differentiated distribution in the body to specific
314 organs and to specific cells inside such organs. In spite of the dose, the route of
315 administration and the elapsed time after absorption, inorganic mercury accumulates
316 mainly in the kidneys but the second larger pool of mercuric ions is found in the liver.
317 The amount of a metal accumulated at the target organ has been described as causing its
318 toxicity (Ekstrand et al., 2010; Joshi et al., 2014; Berlin et al., 2015). Moreover, it was
319 described that $HgCl_2$ exposure causes a dose-dependent accumulation in the liver and
320 consequently, a dose-dependent damage in both hepatic tissue and function (Merzoug et

al., 2009; Liu et al., 2017). In the present study, female rats presented higher total content of mercury in the liver than males. In this connection, in mice treated with $HgCl_2$ in drinking water for 6 weeks, female mice have a higher hepatic Hg concentration than male mice (Ekstrand et al., 2010). On the contrary, in a model of liver injury induced by chronic exposition of inorganic mercury in zebrafish, Chen et al. (2017) described that males accumulated more mercury in liver and were more vulnerable to $HgCl_2$ exposure than females. The results of the present work on a whole show that female rats display a higher $HgCl_2$-induced hepatotoxicity than male rats as consequence of the higher accumulation of this metal in liver cells.

As previously described, Hg^{+2} have affinity for sulphydryl groups (-SH) from proteins, cysteine and reduced glutathione (GSH) (Berlin et al., 2015; Bridges and Zalups, 2017). The Organic anion transporter 3 (Oat3) could transport mercuric ions from blood to the cytoplasm of hepatocytes, since Oat3 is involved in the renal uptake of –SH conjugated Hg^{+2} ions (Bridges and Zalups, 2017). Oat3 mediates the transport of: bioenergetic compounds (such as compounds of the tricarboxilic acid cycle), prostaglandins, vitamins, steroids, uremic toxins and the most commonly prescribed drugs such as penicillins, nonsteroidal anti-inflammatory drugs, cephalosporins, angiotensin converting enzyme inhibitors, diuretics, HIV antivirals, methotrexate, and statins (Burckhardt, 2012). On the other hand, the Multidrug resistance-associated protein 2 (Mrp2) would participate in the efflux of mercury conjugates at the canalicular membrane (Bridges et al., 2011). Most of the Mrp2 substrates are conjugated (with reduced glutathione or glucuronide) and no-conjugated organic anions (like bilirrubin glucuronide and conjugated acetaminophen) and organic cations (such as vinblastine, cisplatin and fluoroquinolones) by cotransport with glutathione (Keppler, 2011).

Hazelhoff et al. (2012) described gender related differences in the nephrotoxicity induced by $HgCl_2$ in rats. In female rats, kidney function was observed lesser affected 18 h after a single dose of $HgCl_2$ (4 mg/kg, body weight, i.p.) than in male rats. In kidney, the uptake of -SH conjugated Hg^{+2} ions at the basolateral membrane is mediated by Oat3 and the Organic anion transporter 1 (Oat1) (Bridges and Zalups, 2017). Torres et al. (2011) have described that mice deficient in Oat1 are protected from $HgCl_2$-induced nephrotoxicity. Moreover, Oat1 and Oat3 are expressed in a lower abundance in kidney from female rats as compared with male ones (Cerrutti et al., 2002; Ljubojevic et al., 2004). Hazelhoff et al. (2012) corroborated the gender related differences in Oat1 and Oat3 renal expression previously reported and concluded that the minor expression of Oat1 and Oat3 in kidney from females restricts the uptake of mercury into renal cells protecting them from the toxicity of $HgCl_2$.

In order to define the mechanisms involved in the higher accumulation of inorganic mercury in female livers as compared with male, we decided to evaluate the protein expressions of Oat3 and Mrp2 in liver from both sexes after a single dose of $HgCl_2$.

The effects of mercury on the protein expression of Oat3 and Mrp2 were evaluated in liver from male and females rats after 18 h of $HgCl_2$ single injection (4 mg/kg body weight, i.p.). In males, the Oat3 expression significantly decreases in liver plasma membranes after mercury treatment. These results suggest that mercury could be mediating a decrease in its synthesis, an increase in its degradation, a decrease in the recruitment rate of the protein transporter into the membranes or an increase in the removal rate of the protein transporter from the membranes into the cytoplasm. On the other hand, no modifications were observed in the protein abundance of Oat3 in liver cell membranes following $HgCl_2$ treatment in female rats. Thus, the decrease in Oat3

abundance in the hepatocytes membranes in mercury-treated males would limit the uptake of mercuric ions into the liver protecting them from mercury hepatotoxicity.

The liver expression of Mrp2 in cell membranes was significantly decreased in a similar percentage in both sexes, 18 h after the $HgCl_2$ dose. This behaviour suggests, as described above for Oat3, a decrease in the synthesis, an increase in the degradation of the protein, or modifications in the intracellular traffic of the protein. On the contrary, it was reported that the liver Mrp2 mRNA expression after 7 days of treatment with $HgCl_2$ (33,6 mg/kg b.w.) in drinking water was increased in male mice (Xu et al., 2016). Differences between the different studies could be attributed to the fact that mercury toxicity is highly dependent of the animal species, the dose, the route and the time of administration.

A number of studies indicated that members of the solute carrier (SLC) and the ATP-binding cassette (ABC) transporter families (to which belong Oat3 and Mrp2, respectively) have an important role in moving metabolites, drugs and toxins between tissues and interfacing body fluids. In this sense, it was hypothesized that these family transporters are part of a large interorgan small molecule communication network that supports homeostasis in the different tissues and body fluid compartments. This hypothesis (*"the Remote Sensing and Signaling hypothesis")* was first proposed in 2007 (Kaler et al., 2007) and later elaborated (Ahn and Nigam, 2009; Ahn et al., 2011; Wu et al., 2011, 2013; Wang and Sweet, 2013; Nigam et al., 2015; Nigam, 2015, 2018; Bush et al., 2017). In this connection, several transporters in different tissues appear to be regulated by injury to the same or another tissue in order to help and coordinate the restoration to the original state (Torres, 2008; Brandoni et al., 2012; Nigam et al., 2015, Bhatnagar et al., 2016).

The present work shows that in liver tissue from male rats, mercuric ions accumulation was lesser than in liver from female rats. The decrease in the hepatic expression of Oat3 and Mrp2 observed in male rats could limit mercury hepatic uptake and mercury biliary excretion, and in consequence, could increase the bioavailability of mercuric ions to the kidneys. The later could explain, at least in part, why after the administration of $HgCl_2$, male rats presented a lesser hepatotoxicity than female rats. Moreover, it would also provide an additional explanation to the greater mercury induced nephrotoxicity previously described in male rats as compared with female ones (Hazelhoff et al., 2012). Mrp2 decreased in a similar percentage both in male and female livers after mercury treatment. On the contrary, liver Oat3 showed a higher decrease in males than in females which was consistent with the relevant role for Oat3 in the regulation of cellular metabolism and remote communication as was recently reported by Wu et al. (2013) and by Bush et al. (2017). Altogether, these results would support the remote sensing and signalling theory and could open a new and interesting gender-related angle on remote sensing to be explored.

The changes induced by mercury on the expression of Oat3 and Mrp2 in liver of male and female rats are of physiological, pharmacological and toxicological importance. Main functions of the liver are the metabolic transformation of endogenous or xenobiotic compounds to favour its elimination and the metabolic activation/inactivation of compounds of pharmacological interest. Moreover, metabolomic studies in Oat3 knock-out mice, demonstrated that Oat3 plays a critical role for the handling of phase I and phase II metabolites, dietary flavonoids and antioxidants and it is also important in modulating the levels of metabolites flowing through the gut-liver-kidney axis (Wu et al., 2013; Bush et al., 2017). Therefore, in individuals poisoned with mercury, mercury-induced modulation in the hepatic protein

419 expression of Oat3 and Mrp2 could alter the elimination of toxic xenobiotics agents,
420 drugs of pharmacological importance and endogenous compounds that are substrates of
421 Oat3 or/and Mrp2 and are excreted to the bile, present liver metabolic transformation, or
422 flow through intestine, liver and kidney.
423
424 **5. Conclusion**
425 In summary, the results of the present work showed that female rats display a
426 higher $HgCl_2$-induced hepatotoxicity than male rats as demonstrated by the higher
427 alterations in the plasma markers of liver damage and in the histopathology. The sex-
428 related differences observed in the hepatic damage produced by inorganic mercury can
429 be explained by the higher accumulation of this metal in liver from females. In this
430 connection, after mercury treatment the liver expression of Oat3 in plasma membranes
431 of hepatocytes was decreased in males but in females no significant changes were
432 observed. The decreased in Oat3 abundance in the hepatocytes membranes in mercury-
433 treated males would limit the uptake of mercuric ions into the liver protecting them
434 from mercury hepatotoxicity.
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Conflict of interest

The authors have declared no conflict of interest.

Acknowledgements

This work was supported by the following grants: Agencia Nacional para la Investigación Científica y Tecnológica (ANPCyT) (PICT 2012: Nº 0225); Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (PIP 2015-2017: Nº 00460); and Universidad Nacional de Rosario (UNR) (PIP 2016-2019, BIO 479). The authors thank to Prof. N. Anzai (Department of Pharmacology, Graduate School of Medicine, Chiba University, Japan) for kindly providing Oat3 specific antibody, to Prof. A. Chevalier (GIHON Laboratorios Químicos SRL, Facultad de Ciencias Exactas. Universidad Nacional de Mar del Plata, Argentina) for mercury determinations, and to Lic. Mara Trebucobich (Area Farmacología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina) for technical assistance. The authors also thank Wiener Lab Argentina for analytical kits.

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609 **FIGURE LEGENDS**

610 **Figure 1:** Representative micrographs of liver sections with hematoxylin/eosin-
611 stained from Male (A, B, C and D) and Female (E, F, G and H) rats. After 18 h of a
612 dose of $HgCl_2$ (4 mg/kg body weight, i.p), females showed a notable disorganization on
613 the radial pattern of hepatocytes and dispersed areas of fibrosis (arrow). In mercury-
614 treated males, the microscopic changes were fewer compared to mercury-treated
615 females. Magnifications of 100X and 200X. **CM**: Control Males, **Hg-M**: Mercury-
616 treated Males, **CF**: Control Females, **Hg-F**: Mercury-treated Females.

617 **Figure 2:** Content of total mercury in liver from Mercury-treated Males (**Hg-M**)
618 and Mercury-treated Females (**Hg-F**). Each column represents the mean ± SEM. $^{*}p<$
619 0.05.

620 **Figure 3**: Immunobloting analyses for Oat3 in liver total plasma membranes
621 from Control Males (**CM**), Mercury-treated Males (**Hg-M**), Control Females (**CF**) and
622 Mercury-treated Females (**Hg-F**). Each column represents the mean ± SEM.$^{*}p< 0.05$ vs
623 respective control. Kaleidoscope Prestained Standards of molecular mass corresponding
624 to bovine serum albumin (89.4 kDa) and to carbonic anhydrase (38.9 kDa) are indicated
625 in the right of the immunobloting bands.

626 **Figure 4**: Immunobloting analyses for Mrp2 in liver total plasma membranes
627 from Control Males (**CM**), Mercury-treated Males (**Hg-M**), Control Females (**CF**) and
628 Mercury-treated Females (**Hg-F**). Each column represents the mean ± SEM.$^{*}p< 0.05$ vs
629 respective control. Kaleidoscope Prestained Standards of molecular mass corresponding
630 to myosin (206.4 kDa), β-Galactosidase (127.5 kDa) and to carbonic anhydrase (38.9
631 kDa) are indicated in the right of the immunobloting bands.

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