Biometals
https://doi.org/10.1007/s10534-017-0068-0

Inorganic mercury in mammary cells: viability, metal uptake but efflux?

Mariángeles Ávila Maniero · Martin E. Guerrero-Gimenez · Mariel A. Fanelli · Rodolfo G. Wuilloud

Received: 29 September 2017 / Accepted: 20 November 2017

Abstract The viability, cellular uptake and subcellular distribution of heavy metal Hg, were determined in human mammary cell lines (MCF-7, MDA-MB-231 and MCF-10A). It was observed that Hg had the capacity of being excluded from the cells with a different type of possible transporters. MCF-7 cells showed the lowest viability, while the other two cell lines were much more resistant to Hg treatments. The intracellular concentration of Hg was higher at lower exposure times in MCF-10A cells and MCF-7 cells; but as the time was increased only MDA-MB-231 showed the capacity to continue introducing the metal. In MCF-7 and MCF-10A cells the subcellular distribution of Hg was higher in cytosolic fraction than nucleus and membrane, but MDA-MB-231 showed membrane and nucleus fraction as the enriched one. The analysis of RNA-seq about the genes or family of genes that encode proteins which are related to cytotoxicity of Hg evidenced that MCF-10A cells and MCF-7 cells could have an active transport to efflux the metal. On the contrary, in MDA-MB-231 no genes that could encode active transporters have been found.

Keywords Human mammary cell lines · Mercury · Transporters

M. Á. Maniero · R. G. Wuilloud (✉)
Laboratory of Analytical Chemistry for Research and Development (QUIANID), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Instituto Interdisciplinario de Ciencias Básicas (ICB), UNCUYO-CONICET, Padre J. Contreras 1300, 5500 Mendoza, Argentina
e-mail: rodolfowuilloud@gmail.com; rwuilloud@mendoza-conicet.gob.ar

M. Á. Maniero
Facultad de Farmacia y Bioquímica, Universidad Juan Agustín Maza, Av. Acceso Este, Lateral Sur 2245, Guaymallén, Mendoza, Argentina

M. E. Guerrero-Gimenez · M. A. Fanelli
Laboratorio de Oncología, Instituto de Medicina y Biología Experimental de Cuyo (IMBECU-CONICET), Av. Ruiz Leal, 5500 Mendoza, Argentina

Introduction

Mercury (Hg) is a harmful heavy metal that is widely distributed in earth's crust. It is involved in environmental toxicology and it has different sources, including volcanic activity, erosion of rocks and anthropogenic activity. Its toxicity is highly dependent on chemical speciation, with organic Hg species such as methyl- and ethyl-Hg being more toxic than inorganic species (Nabi 2014). It is already known that some organomercury compounds are stable in water, while others more soluble in lipids. Fish and shellfish can accumulate Hg and they are the lowest species in the food chain, in consequence some of the final fish in the food chain can contain high levels of Hg in their viscera and muscle tissue. This process is

Published online: 25 November 2017

known as biomagnification process. Nevertheless, inorganic Hg is the most common species and the main one in nature, and is considered as 3th class carcinogen by International Agency for Research on Cancer (IARC) (Aitio et al. 1993; Palacios and Capdevila 2013). Several groups have studied Hg toxicity in different organs such as kidney, brain or placenta, but a few ones focused on the mammary gland; although this is the source of nutrition for newborn (Lohren et al. 2015; Stacchiotti et al. 2009; Straka et al. 2016). Also, there is evidence about mammary cells susceptibility to Hg exposure as reported by Dos Santos et al. where they detected Hg in the milk of lactating women (Dos Santos et al. 2015). Moreover, Mohammadi et al. (2014) evaluated Hg concentrations in woman samples of mastectomy surgery. Likewise, it was reported that Hg could exert its toxic effects by mimicking or blocking the function of essential metals (Waalkes et al. 2000). In addition to these observations, Hg is considered an important endocrine disruptor, which is suggesting the occurrence of some pathways that might explain associations between metals and hormone related diseases, considering it as metalloestrogen (Byrne et al. 2013; Zhang et al. 2008). Environmental exposure to many of widespread metalloestrogens has increased significantly over the last 50–60 years, and could be accumulated in the body and in the breast. The mechanism of Hg toxicity is unknown, but the body of literature is growing up to understand how this metal could be transported into the cells and if the cells could efflux it (Bucio et al. 1999; Heggland et al. 2009). Furthermore, the literature has been mainly focused on possible transporters of Hg in target organs like kidney and brain, but also placental cells and umbilical cord have been studied (Bridges and Zalups 2010; Fujiyama et al. 1994; Patnaik and Padhy 2015; Straka et al. 2016). However, there are no reports on the role of Hg in the mammary gland, so further investigation is still needed in order to fully understand its mechanisms of toxicity. Evidence that exposed women may have accumulated Hg in their breast and that this is being able to pass through breast milk and finally infants, demonstrates the urgency of knowing the outlet mechanism that this metal use to get out from human mammary cell lines (Bose-O'Reilly et al. 2008). The chemistry of Hg in biological systems has the base in the high affinity of Hg(II) for cysteine residues which is thermodynamically extremely favorable (Parks and Smith 2016). Finally, at this point, it is also vital to identify potential target genes whose expression may be related with transporters regulating the Hg inlet and outlet from human breast cells. Whole-transcriptome deep-sequencing technologies (e.g., RNA-Seq), with their capacity to simultaneously assay the entire baseline transcriptome, of the cell lines, represent an excellent choice of tool in this regard (Costa et al. 2013).

In this work, new insights into the transport of Hg in human mammary cells are presented. Viability, cellular uptake and subcellular distribution have been evaluated in different cells by combining elemental-specific detection with molecular characterization tools. The sensibility of MCF-7, MDA-MB-231 (breast cancer cell lines) and MCF-10A nontumorigenic breast epithelial cell line, was assayed in the presence of different Hg concentrations and exposure times, evaluating also the subcellular distribution of the metal and RNA-Seq of gene expression from human breast cells.

Experimental

Chemicals and reagents

All the reagents were of analytical grade. Mercury(II) chloride was purchased from Anedra (Buenos Aires). Stock standard solutions of inorganic Hg(II) species (100 mmol l^{-1}) as $HgCl_2$ were prepared in ultrapure water (18.2 MΩ) obtained from a Milli-Q system (Millipore, Academic, USA) at 5% on HNO_3 purchased in Anedra (Buenos Aires).A working solution in the corresponding culture media was daily prepared at different concentrations needed, and sterilized using a 0.22 μm nylon filter (Sartorius, Germany).

Cell culture conditions

Tumor (MCF-7 and MDA-MB-231) and non-tumor (MCF-10A) human breast epithelial cell lines were used. MCF-7, MDA-MB-231 and MCF-10A were kindly provided by Dr. Pistone Creydt V. (IMBECU-CONICET) and were originally obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA), MCF-7 and MDA-MB-231 cells lines were routinely cultured in DMEM high glucose: Dulbecco's Eagle Medium (GIBCO, Invitrogen

Corporation, Argentina), containing 10% fetal bovine serum (Internegocios, Buenos Aires, Argentina) and, 100 IU ml^{-1} penicillin and 100 μg ml^{-1} streptomycin (GIBCO). The non-tumorigenic cell line, MCF-10A, was cultured in DMEM-F12: Dulbecco's modified eagle medium: nutrient mixture F-12 (Ham) (GIBCO) containing 10% fetal bovine serum (GIBCO) and, 100 IU ml^{-1} penicillin and 100 μg ml^{-1} streptomycin (GIBCO), supplemented with 2 μg ml^{-1} insulin (Lilly, Humalog, Italia), 0.5 μg ml^{-1} cortisol (Sigma-Aldrich, St. Louis, USA) and 20 ng ml^{-1} epidermal grown factor (Sigma-Aldrich). The three cell lines were maintained at 37 °C in an incubator with 5% CO_2 and 100% humidity. Sub-confluent cells were split twice a week at a ratio of 1:20.

Toxicity of Hg

The viability of three human mammary cell lines after Hg treatment, was evaluated using Trypan blue staining (Sigma-Aldrich); and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich). Experiments were repeated three times for each concentration.

MTT assay

To carried out the standard procedure for the estimation of the cell number based on metabolic activity, cells were seeded in 96-well plates (10,000 MCF-7 cells, 7000 cells MCF-10A and 10,000 MDA-MB-231 cells per well) and grown for 48 h in complete medium. When cells reached 85–95% confluence, the medium was aspirated and cells were washed twice with tempered PBS. Then, the cells were exposed to 0, 0.5, 5, 10, 25, 50, 100, 150, 200 and 250 μM $HgCl_2$ in fresh culture medium (without fetal bovine serum) during 3, 12 and 24 h. Following drug exposure, MTT was added to the cells at a final concentration of 0.5 mg ml^{-1} and incubated for 3 h at 37 °C, after that the MTT was discarded and the violet formazan was dissolved by the addition of 200 μl of DMSO (Sigma-Aldrich). The optical density was measured at 570 nm wavelength using a microtiter plate reader Multiskan EX (Thermo Fisher Scientific, Vantaa, Finland) and the percentage of surviving cells was calculated from the ratio of absorbance of treated to untreated cells. Cells without any treatment used as the untreated control were set to 100% of viability.

Trypan blue staining

The percentage of viability cells was determined after the exposure time to Hg by Trypan blue exclusion. After 3 h of incubation with Hg concentrations of IC_{25}, IC_{50} and IC_{75}, the medium was removed and the cells were detached with trypsin. After 5 min centrifugation at 1500 rpm, the cells suspension was mixed with the Trypan blue solution (0.4% (w/v)) at the 1:1 ratio. Cells were counted in Neubauer's chamber with an inverted Zeiss 47 12 02-9901 microscope (West Germany). As this stain is a membrane permeable dye, all cells that exclude the dye are viable and those blue cells are non viable ones. The viability was obtained as a percentage between dead and alive cells.

Hg cellular uptake

For cellular uptake studies MCF-7, MCF-10A and MDA-MB-231 were seeded in 6-well plates, grown to approximately 80% confluence and incubated at 37 °C with 0.5% CO_2. The cells were treated with 10, 25, 50, 75 and 100 μM $HgCl_2$ (daily prepared in the incubation medium) for 3, 12 and 24 h. At the end of the treatment, the cells were washed three times with 1 ml of template PBS per well. Then, 500 μl of MiliQ water were added with protease inhibitor (Roche, Indianapolis, USA) and cells were detached using a scraper, 6-well plates were on ice bath. After harvested, samples were divided in two aliquots, one of them was stored at 5% (v/v) of HNO_3 for Hg determination by cold vapor atomic fluorescence spectrometry (CV-AFS), and the other was analyzed to determine their protein content with the bicinchoninic acid (BCA) assay (Sigma Aldrich).

Hg determination

Hg determination was performed with a Rayleigh AF-640A atomic fluorescence spectrometer (Beijing Rayleigh analytical Instrument Corp., Beijing, China). Instrumental conditions of CV-AFS are shown in Table 1. Concentration of Hg was obtained after sample digestion as follows. Three cells lines (20 μl)

Table 1 Instrumental conditions for Hg determination

HG-AFS	
Carrier gas and flow rate	Ar, 800 ml min^{-1}
Auxiliar gas and flow rate	Ar, 400 ml min^{-1}
Atomization temperature	200 °C
Lamp and wavelength	Hg high intensity hollow cathode lamp, 253.65 nm
Main current	40 mA
Auxiliary current	0 mA
Reductant	0.14% (w/v) KBH_4 in 0.02% (w/v) NaOH
Carrier	5% (v/v) HNO_3

were digested with 125 µl of concentrated nitric acid (96%) (Anedra), 250 µl of sulphuric acid (98%) (Merck, Darmstadt, Germany), 400 µl of 1% (w/v) potassium permanganate (Sigma-Aldrich), 400 µl of 5% (w/v) potassium persulfate (Sigma-Aldrich) and filled to a total volume of 3 ml with ultrapure water. The set of vials was placed in a thermostatic bath at a temperature of 90 °C for 2 h, with vigorous shaking each 30 min. Then, the flasks were cooled to room temperature and 100 µl of 12% (w/v) hydroxylamine hydrochloride (Sigma-Aldrich) were added to reduce excess of $KMnO_4$. In order to evaluate possible matrix effects caused by cell material, standard addition method was performed by spiking MCF-7, MCF-10A and MDA-MB-231 cells at 2, 4 and 10 µg l^{-1} Hg. No significant differences in the slopes of the calibration curves obtained by external and standard addition methods were observed.

Subcellular fractionation

For subcellular fractionation, the three cell lines were grown in 75 cm^2 flasks, at approximately 85% confluence and incubated with IC_{25} concentration for 3 h. At the end of the treatment, cells were washed with template PBS before being detached using mQ water with protease inhibitor (Roche) and scraper. Fractionation was achieved using the Subcellular Protein Fractionation Kit for Cultured Cells (purchased at Thermo Scientific, Rockford, USA). All samples (total cells and fractions) were analyzed for their protein content prior to CV-AFS determination using the BCA assay (Sigma Aldrich).

Mercury efflux

The three cell lines where individually seeded in a 6-well plate, after 3 h incubation at 37 °C with $HgIC_{25}$, the cells were washed with template PBS and 2 mL of fresh medium free of Hg were added to each well. After 3, 12 and 24 h the medium was removed and centrifuged at 1500 rpm by 5 min. The cells were washed three times with PSB and detached with mQ water with protease inhibitor (Roche) and scrapper. The intracellular and expelled Hg from the cells was determined using CV-AFS. Protein concentration in the cells was evaluated with BCA (Sigma Aldrich).

In silico analysis

Human breast cells RNA-Seq of gene expression from human breast cells database generated by Genentech/gRED were used (Klijn et al. 2015). RNAseq raw fastq files were obtained for three cell lines (MCF-7, MDA-MB-231, MCF-10A) from the European Genome-phenome Archive (EGA) (accession number EGAD00001000725) with permission from the Genentech Data Access Committee. Briefly, data quality control was performed using FastQC reads that were aligned with HISAT with default parameters against GRCh38.87 reference genome (Kim et al. 2015; Andrews 2010). The per-gene counts were retrieved using HTseq algorithm in unionmode (Anders et al. 2015). All the data was analyzed using Galaxy platform and the workflow can be accessed at https://usegalaxy.org/u/martinguerrero/w/hisat-htseq-rnaseq-protocol-1 (Afgan et al. 2016).

Results and discussion

Evaluation of Hg toxic effects on human mammary cells

In order to determine the impact of different Hg concentrations and incubation times (3, 12 and 24 h) on cell viability of the three human mammary cell lines, MTT assay and Trypan blue exclusion were performed. The MTT assay was used to measure metabolic activity and Trypan blue was used to determine membrane integrity of the cells. The cell line with the highest IC_{50} was considered the most tolerant to Hg exposure. The data showed that the metabolic activity of the cell lines studied decreased as the Hg concentration was increased. Mercury cytotoxicity effects were determined in the three cell lines comparing the IC_{50} values (nonlinear regression, with 95% confidence interval Fig. 1) that are shown in Table 2. In this study, the IC_{50} values obtained for MCF-10A and MDA-MB-231 cells were not significantly modified by increasing the exposure time to Hg. On the contrary, the metabolic activity of MCF-7 cells at 12 h decreased more abruptly than the rest of the cells that was demonstrated by a reduction of IC_{50} value in almost fifty percent. Moreover, MCF-7 cells did not survive after the 24 h treatment even at the lowest concentration caused by the cytotoxicity of Hg. On the other hand, there were no significant differences in the results obtained by MTT and Trypan blue assays (data not shown), indicating that Hg was not only decreasing the metabolic activity but also the cell viability. Based on the results of the MTT and Trypan Blue, it was possible to determine that the cytotoxicity of the cell lines was different between them and they were influenced by concentration and exposure time. This differential response to Hg is in agreement with previous studies performed in lymphocytes (Patnaik and Padhy 2015). However, there does not seem any study carried out in these three human mammary cell lines evaluating Hg cytotoxicity. Therefore, the results obtained in the present work are indicating the occurrence of different mechanisms of Hg toxicity involved in these cell lines.

Uptake of Hg

In order to understand the intracellular toxicity of Hg, experiments with cells were performed at different concentrations and exposure time in the three mammary cell lines. It has to be mentioned at this point, that no transporters have been designed specifically for the entry of most toxic metal species into mammalian cell. Instead, it has been described that some of these metals gain entry into target cells following the mechanisms of ionic and/or molecular mimicry, at the site of transporters of essential elements and/or molecules

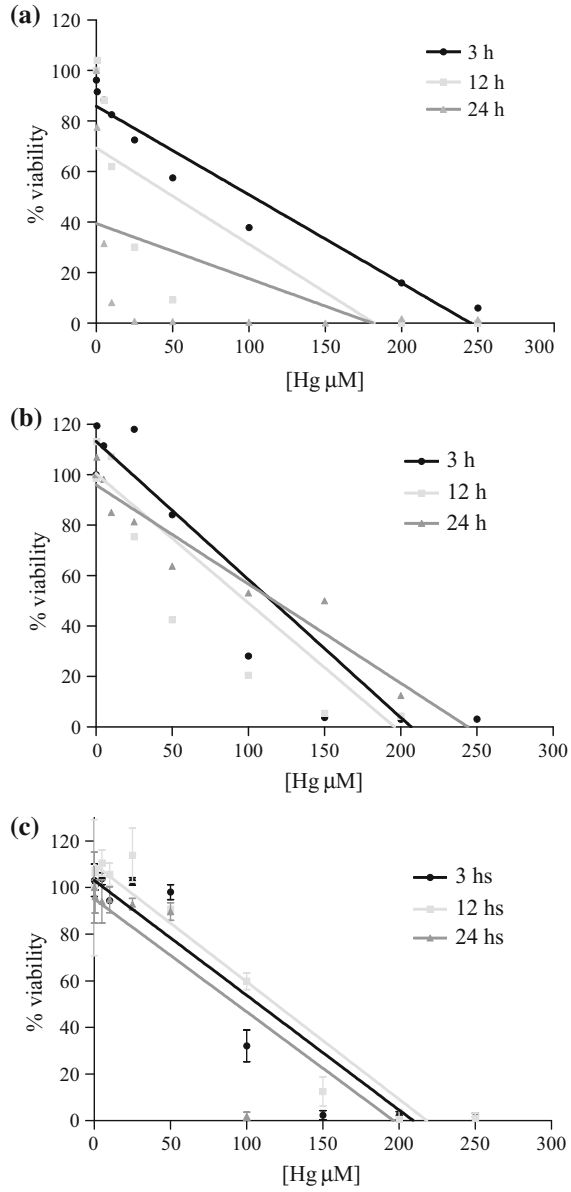

Fig. 1 Viability by MTT assay, non linear regression. **a** MCF-7, **b** MCF-10A and **c** MDA-MB-231 exposed to different concentrations of Hg for 3, 12 and 24 h. Graphpad Prism 5 was used to make graphics

Table 2 IC_{50} and IC_{25} for Hg exposure in MCF-7, MCF-10A and MDA-MB-231 human mammary cell lines

	MCF-7			MCF-10A			MDA-MB-231		
	3 h	12 h	24 h	3 h	12 h	24 h	3 h	12 h	24 h
IC_{50}	102.5	50.9	#	115.5	98.5	117.1	107.7	119	93.2
IC_{25}	31	#	#	70	49.7	53.4	57	69	41

Values represent averages of three independent experiments in μM. Bars indicate standard deviations. #: data could not be obtained

(Bridges and Zalups 2005). Recently, Vázquez et al. (2015) described that the intracellular Hg accumulation may be mediated by the divalent cations transporter DMT1 in the intestinal epithelium. Although some mechanisms responsible for the transport of Hg inside the cells have been described in vitro models, no information has been reported about Hg accumulation in human mammary cell lines. Thus, the results obtained in the present work are the first evidences of Hg accumulation in this type of human cells.

The results obtained after Hg cellular uptake experiments performed at different concentrations and exposure times in the three mammary cell lines are shown in Fig. 2. It can be observed that after an incubation period time of 3 and 12 h, in MCF-10A cells the intracellular concentration of Hg was higher than the rest of the cells, demonstrating to possess a higher incorporation capacity at short exposure times. On the other hand, at 24 h, an elevated amount of Hg was observed to be accumulated in MDA-MB-231 cells, much more, than the others. Therefore, it seems that in MDA-MB-231 cells, Hg is taken up continuously but much more slowly, suggesting a time-dependent process. On the other hand, MCF-7 showed a lower intracellular concentration of Hg at all exposure times evaluated in this study. Moreover, slightly lower concentrations of Hg were detected at 10 μM and after 24 h in MCF-7 in comparison to 3 h incubation, and the same behavior was found in the non tumorigenic cell line. Thus, the exposure time is inversely proportional to the intracellular concentration of Hg (Fig. 3). From the results obtained after viability and intracellular accumulation experiments, it was observed that MCF-10A and MDA-MB-231 cells were the most resistant and/or tolerant to Hg exposition, perhaps due to different uptake mechanisms (one slower and other faster). Secondly, MCF-7 cells may have a lower capacity to accumulate Hg, possibly because of a saturable, detoxification dose-dependent mechanism.

Subcellular fractionation and Hg concentration in the different cells fractions

The intracellular distribution of Hg was studied using a subcellular fractionation technique which separates, in a first step, the cytosolic and soluble cytoplasmic fractions using a selective cell membrane permeabilization buffer (Baghirova et al. 2015). In a second step, membranes were dissolved and separated by centrifugation, while in a final step soluble nucleus was extracted. For all samples, the concentration of Hg was determined and standardized against the amount of protein determined in each fraction.

Figure 4 shows Hg distribution among cytoplasmic, membrane and nuclear fractions for MCF-7, MDA-MB-231 and MCF-10A cell lines and after 3 h incubation at each IC_{25} concentration. Distinct differences between enriched fraction in MDA-MB-231 and the two others cells were observed. For MCF-10A, there was no difference in Hg concentration found in the membrane and nucleus fraction; whereas the cytoplasmic fraction was enriched in Hg. Similar results were found in MCF-7, where the most enriched fraction was the cytoplasmic one. On the other hand, the highest Hg concentrations in MDA-MB-231 were found in nuclear and membrane fractions. It has to be mentioned that distribution of Hg in subcellular fractions was described in human hepatic non tumorigenic cell lines in the past, with the highest Hg concentrations found in the nucleus and mitochondria (Bucio et al. 1999). Furthermore, it was reported that accumulation in the nucleus could produce damage at the DNA level. Therefore, in MDA-MB-231 nucleus fraction, where the highest concentration of Hg was observed, accumulation could be accompanied by possible damage to DNA. In MCF-10A and MCF-7, Hg could be attached to the membrane during a process of Hg efflux. In human mammary cell lines, there are not previous reports about Hg enriched in subcellular fractions.

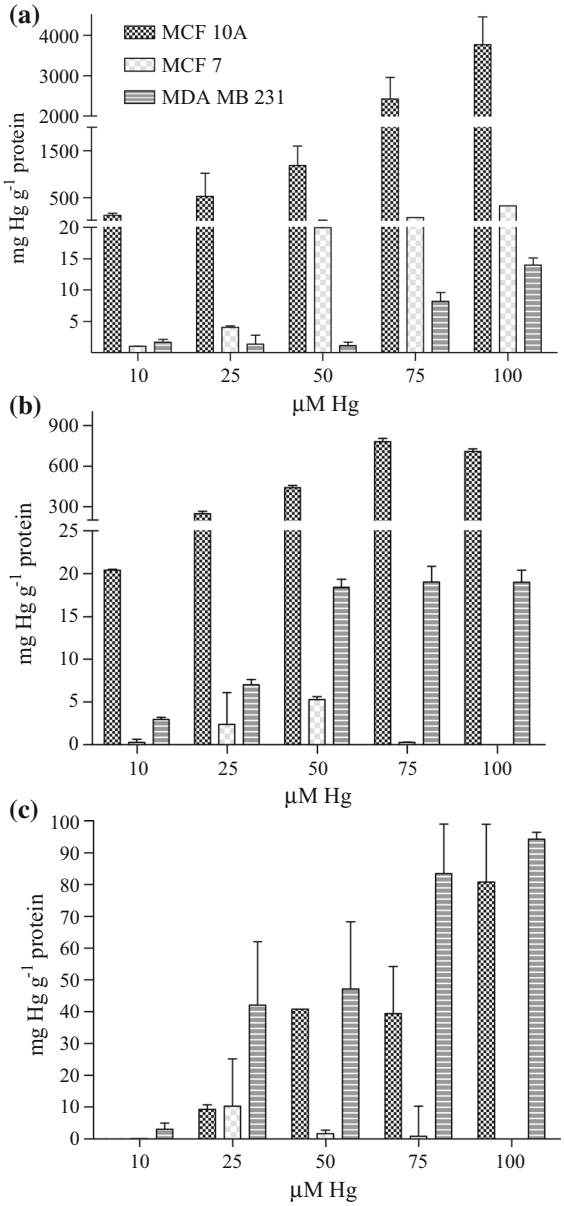

Fig. 2 Hg uptake in MCF-7, MCF-10A and MDA-MB-231 cells. The concentration of Hg was determined by CV-AFS after incubation times of **a** 3 h, **b** 12 h and **c** 24 h. Experiments were performed at five different concentration of Hg (10–100 µM). Graphpad Prism 5 was used to make graphics

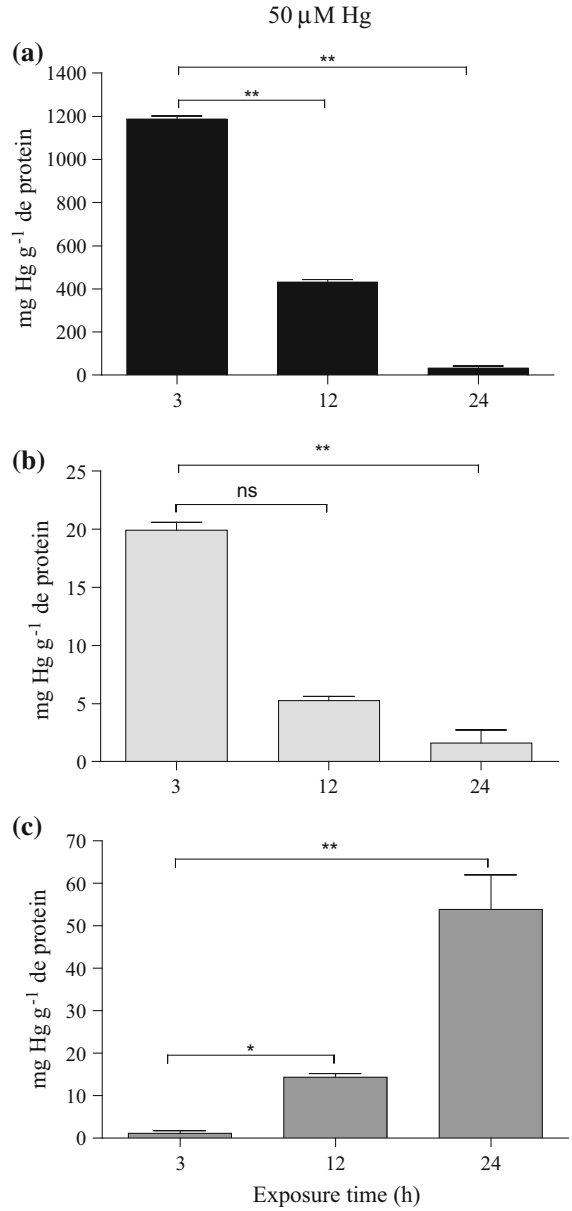

Fig. 3 Intracellular Hg after 50 µM of metal treatment, during 3, 12 and 24 h. **a** MCF-10A, **b** MCF-7 and **c** MDA-MB-231. *P < 0.05 versus 3 h, **P < 0.01 versus 3 h. Graphpad Prism 5 was used to make graphics

Study on the differential efflux of Hg from the cells

At a first glance, the amount of Hg seemed to be higher in the culture medium across the time, indicating that the cells could be releasing the metal to the medium. This result was remarkable, especially in MDA-MB-231 because of its high capacity to accumulate Hg following a slow path depending on Hg concentration and exposure time. Likewise, the non tumorigenic cell line and MCF-7 showed the same behavior. After these findings, it was evaluated the intracellular concentration of Hg in the three cell lines when they were in contact with an Hg-free medium. The results

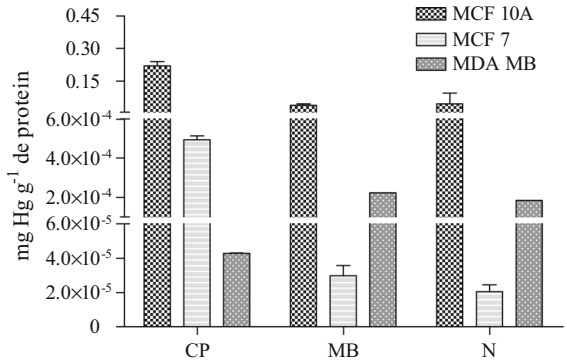

Fig. 4 Subcellular distribution of Hg after 3 h incubation at IC_{25} concentration in MCF-7, MCF-10A and MDA-MB-231 cells. Graphpad Prism 5 was used to make graphics

showed a decrease in the intracellular Hg content that was accompanied by the increase of Hg concentration in the extracellular medium (Fig. 5). The MDA-MB-231 cell lines accumulated Hg during the 3, 12 and 24 h. Nevertheless, the same capacity than the others two cells to efflux Hg was found. This particular behavior in MDA-MB-231 cells could indicate that Hg maybe using a passive transport mechanism to exit the cells. On the other hand, MCF-7 and MCF10A cell lines may use an active transport as they showed an efflux capacity of Hg during their contact with an extracellular medium containing high concentrations of this metal.

This kind of behavior induced us to wonder about the baseline expression levels of many active or passive transporters that could be used by Hg in the three cell lines. Llop et al. had evaluated the state-of-the-art of genes or gene families which may have a relationship with Hg toxicokinetics and established a list of genes that could be involved in the process (Llop et al. 2015). Taking into account the genes proposed in the list, it was first analyzed the baseline mRNA expression levels of these genes in the three cell lines, determining which genes were expressed in the different lineages. Differences in the expression levels of the genes were considered compared to the other cell lines and correlated to the Hg efflux behavior observed in the experiments to grasp a better understanding of the complex regulation involved in the transport of Hg observed in the different cell types.

Radar plot in Fig. 6 shows the different expression levels of mRNA between the three cell lines, where MCF-10A was considered as the baseline level. The

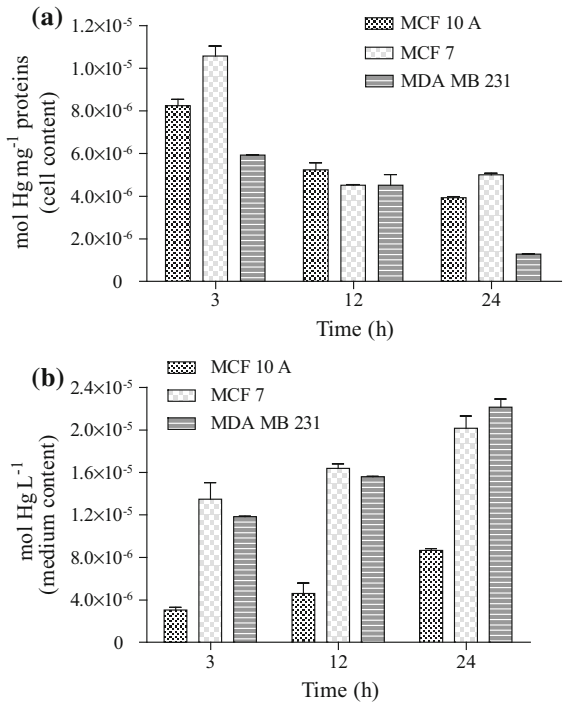

Fig. 5 Efflux assay. **a** Hg accumulation in human mammary cell lines at 3, 12, and 24 h of recovery time with Hg free medium. In all three cases, cell lines were previously exposed with IC_{25} concentration by 3 h. **b** Extracellular Hg in medium after 3, 12 and 24 h in contact with treated cells. Graphpad Prism 5 was used to make graphics

genes that revealed differences between MCF-10A and MCF-7 over MDA-MB-231 were: ABCB6, GSTO2, SLCO5A1, GSTM1, GSTM2, GSTM4, MT1A and SLCO1B7. These genes encode proteins with different functions like membrane protein ATP-binding cassette transporters, glutathione S-transferase, solute carrier anion transporter and mammalian metallothioneins (Board and Menon 2016; Ho and Kim 2010; Irvine et al. 2016; Kiss et al. 2015).

Only ABCB6, SLCO5A1 and GSTO2 where found diminished in MDA-MB-231 compared with the other cells as it was expected. The first gene codifies an active transporter, indicating that MCF-7 and MCF-10A could be using this pathway for Hg efflux. The same behavior was also described by Straka et al. in trophoblast cells, where they described an efflux of Hg conjugated to glutathione (GSH) in fetal endothelial cell (Straka et al. 2016). They could determine that when this transporter was down regulated, intracellular Hg concentration was higher than the controls. Although this gene has been found with a mayor

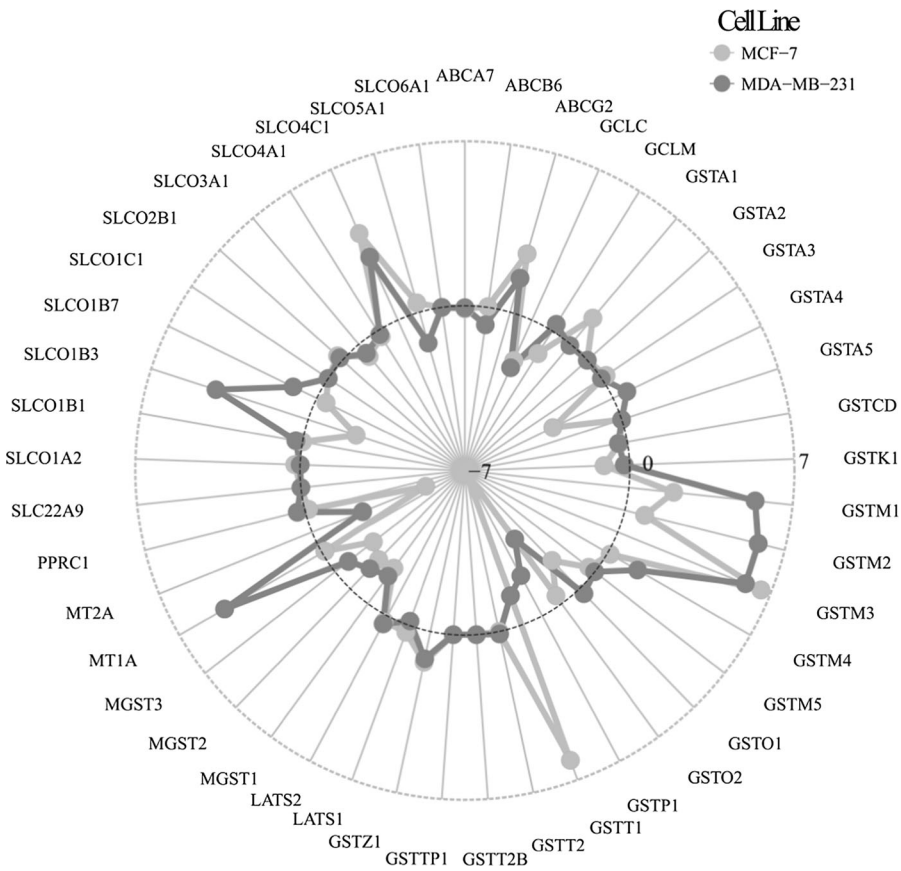

Fig. 6 Radar plot showing the different expression level of mRNA expressed in three cell lines. The dotted line represents baseline level from the non tumorigenic cell line (MCF-10A). The peaks outwards represent positive expression of mRNA and the inwards represent the less expression of mRNA. Illustrator 14 was and R 3.2.3 used to make graphics

expression in MCF-10A and MCF-7, it is necessary to perform specific assays to further evaluate this hypothesis. The second gene SLCO5A1, codifies a plasma membrane protein, OATP5A1, that is expressed in normal and tumor mammary tissue samples (Kindla et al. 2011). Ding J. et al. have demonstrated that OATP5A1 may play the role of mediate the uptake of toxins by neurons, these findings suggest that this protein could act as a transporter (Ding et al. 2017). In a topology analysis carried out on the SLCO5A1 protein, ten cysteine residues were predicted to be located in the extracellular loop (Sebastian et al. 2013). Conjugation of Hg with Cys may promotes the transport of low concentrations of this metal through the luminal membrane (Zalups and Lash 1997). The third gene GSTO2 gives rise to a homodimeric protein GSTO2-2, in which 11 Cys residues are present (Zhou et al. 2012). The protein encoded by this gene may contribute to the maintenance of cellular redox balance because of its action as a dehydroascorbate reductase but the precise role remains unclear. Also, it has been demonstrated that this protein could catalyze the reduction of other elemental species than those containing Hg, such as inorganic As (arsenate and arsenite), monomethylarsenoate and dimehylarsenoate (Board and Menon 2016).

These results indicate that MCF-10A and MCF-7 may efflux Hg using transporters or enzymes which generate a new molecule that bind Hg to their structures. However, specifics assays are necessary to characterize the mechanisms involved in the efflux of Hg by these two cell lines.

On the other side, a few genes were found to be higher in MDA-MB-231 than the other cells as GSTM1, GSTM2, GSTM4, MT1A, SLCO1B3 and SLCO1B7. The family of GSTs encodes a glutathione S-transferase that belongs to the mu class. This class of enzymes has functions in the detoxification of electrophilic compounds, including environmental toxins and therapeutic drugs, by conjugation with glutathione (Hollman et al. 2016). In fact, it is already known that

Hg could conjugate with this molecule, and they could leave the cells using different transporters as proved by Straka et al. (Straka et al. 2016). However, there is no evidence of these enzymes in the breast cells acting as transferase in presence of Hg. The other gene MT1A, encodes the apo-MT1a protein.

Metallothioneins have the function to bind essential or toxic metals for different purposes. Irvine et al. had demonstrated that the concept of the roll of MTs as a detoxification protein is not correct. They used Cd as the toxic metal, and they could prove that the structure of metallothioneins binding to Cd is resistant to degradation and could provide the mechanism by which Cd(II) persists in lower pH compartments in renal cells for many years, provoking more damage at this target organ (Irvine et al. 2016). In MDA-MB-231 cell line these protein could be binding Hg to diminish its possible release from the cell. The family of genes SLCO (encodes solute anion carrier transporters family) encodes families comprising passive transporters, ion-coupled symporters, and antiporters in the plasma membrane and other cellular membrane compartments. The SLCO1B7 gene encodes a putative protein (OAT1B7) which function is unknown and poorly characterized (Legge et al. 2016). The other gene of the family of solute anion carrier transporters is SLCO1B3. This gene codifies a protein which can act as a hepatic bile acid efflux transporter, protecting the liver from accumulation of toxic intracellular solutes (Ho and Kim 2010).

Conclusions

In this study, it has been demonstrated that different human mammary cell lines have specific behaviors regarding Hg viability, uptake and subcellular distribution, which is also proposing different efflux mechanisms for this element. The complementary use of the three areas of knowledge—analytical chemistry, biology and bioinformatics—allows obtaining reliable data about possible transports of Hg in three human mammary cell lines. The Hg concentration in the extracellular medium of MCF-10A and MCF-7 provides insight into the active transport that these two cells have. On the other hand, MDA-MB-231 had a different behavior when they were exposed to Hg. The efflux assay allowed us to shuffle the possibility to indicate that this cell line could have passive transport to the Hg. That was lightly support about the different mRNA founded in the data base that shown mayor presence of gens that could encode proteins that may be passive transporters, or binding proteins that could be retaining the metal inside de cell. To verify the efflux mechanisms performed by these three cell lines, it is necessary to develop specific assays. Thus, everything is an important step to elucidate the possible mechanism of toxicity generated by Hg in human breast cell lines.

Acknowledgements This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (FONCYT) (PICT-BID), Universidad Nacional de Cuyo (Argentina) and Instituto Nacional del Cáncer (INC). We gratefully acknowledge access to RNAseq data base from Genentech.

References

Afgan E, Baker D, van den Beek M et al (2016) The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. Nucleic Acids Res 44(W1):W3–W10. https://doi.org/10.1093/nar/gkw343

Aitio A, Alessio L, Axelson O et al (1993) IARC monographs on the evaluation of carcinogenic risks to humans: beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. IARC monographs on the evaluation of carcinogenic risks to humans, vol 58. IARC, Lyon, pp 1–415

Anders S, Pyl PT, Huber W (2015) HTSeq-A python framework to work with high-throughput sequencing data. Bioinformatics 31(2):166–169. https://doi.org/10.1093/bioinformatics/btu638

Andrews S (2010) FastQC: a quality control tool for high throughput sequence data (Galaxy version 0.65). Babraham Institute, Cambridge

Baghirova S, Hughes BG, Hendzel MJ, Schulz R (2015) Sequential fractionation and isolation of subcellular proteins from tissue or cultured cells. MethodsX 2:440–445. https://doi.org/10.1016/j.mex.2015.11.001

Board PG, Menon D (2016) Structure, function and disease relevance of Omega-class glutathione transferases. Arch Toxicol 90(5):1049–1067. https://doi.org/10.1007/s00204-016-1691-1

Bose-O'Reilly S, Lettmeier B, Roider G, Siebert U, Drasch G (2008) Mercury in breast milk—a health hazard for infants in gold mining areas? Int J Hyg Environ Health 211(5):615–623. https://doi.org/10.1016/j.ijheh.2007.09.015

Bridges CC, Zalups RK (2005) Molecular and ionic mimicry and the transport of toxic metals. Toxicol Appl Pharmacol 204(3):274–308. https://doi.org/10.1016/j.taap.2004.09.007

Bridges CC, Zalups RK (2010) Transport of inorganic mercury and methylmercury in target tissues and organs. J Toxicol Environ Health B 13(5):385–410. https://doi.org/10.1080/10937401003673750

Bucio L, García C, Souza V et al (1999) Uptake, cellular distribution and DNA damage produced by mercuric chloride in a human fetal hepatic cell line. Mut Res 423(1–2):65–72. https://doi.org/10.1016/s0027-5107(98)00226-7

Byrne C, Divekar SD, Storchan GB, Parodi DA, Martin MB (2013) Metals and breast cancer. J Mammary Gland Biol Neoplasia 18(1):63–73. https://doi.org/10.1007/s10911-013-9273-9

Costa V, Aprile M, Esposito R, Ciccodicola A (2013) RNA-Seq and human complex diseases: recent accomplishments and future perspectives. Eur J Hum Genet 21(2):134–142. https://doi.org/10.1038/ejhg.2012.129

Ding J, Wang J, Xiang Z et al (2017) The organic anion transporting polypeptide 1a5 is a pivotal transporter for the uptake of microcystin-LR by gonadotropin-releasing hormone neurons. Aquat Toxicol 182:1–10. https://doi.org/10.1016/j.aquatox.2016.11.005

Dos Santos FA, Cavecci B, Vieira JCS et al (2015) A metalloproteomics study on the association of mercury with breast milk in samples from lactating women in the Amazon Region of Brazil. Arch Environ Contam Toxicol 69(2):223–229. https://doi.org/10.1007/s00244-015-0161-8

Fujiyama J, Hirayama K, Yasutake A (1994) Mechanism of methylmercury efflux from cultured astrocytes. Biochem Pharmacol 47(9):1525–1530. https://doi.org/10.1016/0006-2952(94)90527-4

Heggland I, Kaur P, Syversen T (2009) Uptake and efflux of methylmercury in vitro: comparison of transport mechanisms in C6, B35 and RBE4 cells. Toxicol In Vitro 23(6):1020–1027. https://doi.org/10.1016/j.tiv.2009.06.018

Ho RH, Kim RB (2010) Uptake transporters comprehensive toxicology, vol 4, 2nd edn. Vanderbilt University School of Medicine, Nashville, pp 519–556

Hollman AL, Tchounwou PB, Huang HC (2016) The association between gene-environment interactions and diseases involving the human GST superfamily with SNP variants. Int J Environ Res Public Health 13(4):379. https://doi.org/10.3390/ijerph13040379

Irvine GW, Pinter TBJ, Stillman MJ (2016) Defining the metal binding pathways of human metallothionein 1a: balancing zinc availability and cadmium seclusion. Metallomics 8(1):71–81. https://doi.org/10.1039/c5mt00225g

Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. Nat Methods 12(4):357–360. https://doi.org/10.1038/nmeth.3317

Kindla J, Rau TT, Jung R et al (2011) Expression and localization of the uptake transporters OATP2B1, OATP3A1 and OATP5A1 in non-malignant and malignant breast tissue. Cancer Biol Ther 11(6):584–591. https://doi.org/10.4161/cbt.11.6.14533

Kiss K, Kucsma N, Brozik A et al (2015) Role of the N-terminal transmembrane domain in the endo-lysosomal targeting and function of the human ABCB6 protein. Biochem J 467(1):127–139. https://doi.org/10.1042/BJ20141085

Klijn C, Durinck S, Stawiski EW et al (2015) A comprehensive transcriptional portrait of human cancer cell lines. Nat Biotechnol 33(3):306–312. https://doi.org/10.1038/nbt.3080

Legge SE, Hamshere ML, Ripke S et al (2016) Genome-wide common and rare variant analysis provides novel insights into clozapine-associated neutropenia. Mol Psychiatry. https://doi.org/10.1038/mp.2016.137

Llop S, Ballester F, Broberg K (2015) Effect of gene-mercury interactions on mercury toxicokinetics and neurotoxicity. Curr Environ Health Rep 2(2):179–194. https://doi.org/10.1007/s40572-015-0047-y

Lohren H, Bornhorst J, Galla HJ, Schwerdtle T (2015) The blood-cerebrospinal fluid barrier—first evidence for an active transport of organic mercury compounds out of the brain. Metallomics 7(10):1420–1430. https://doi.org/10.1039/c5mt00171d

Mohammadi M, Riyahi Bakhtiari A, Khodabandeh S (2014) Concentration of Cd, Pb, Hg, and Se in different parts of human breast cancer tissues. J Toxicol. https://doi.org/10.1155/2014/413870

Nabi S (2014) Toxic effects of mercury. Springer, New Delhi

Palacios Ò, Capdevila M (2013) Toxicology (Pb, Hg, Cd, As, Al, Cr, and Others) comprehensive inorganic chemistry II: from elements to applications, vol 3, 2nd edn. Pergamon Press, Oxford, pp 51–63

Parks JM, Smith JC (2016) Modeling mercury in proteins. Methods Enzymol 578:103–122. https://doi.org/10.1016/bs.mie.2016.05.041

Patnaik R, Padhy RN (2015) Cellular and nuclear toxicity of HgCl < inf > 2</inf > to in vitro grown lymphocytes from human umbilical cord blood. Proc Natl Acad Sci India Sect B 85(3):821–830. https://doi.org/10.1007/s40011-015-0503-3

Sebastian K, Detro-Dassen S, Rinis N et al (2013) Characterization of SLCO5A1/OATP5A1, a solute carrier transport protein with non-classical function. PLoS ONE. https://doi.org/10.1371/journal.pone.0083257

Stacchiotti A, Morandini F, Bettoni F et al (2009) Stress proteins and oxidative damage in a renal derived cell line exposed to inorganic mercury and lead. Toxicology 264(3):215–224. https://doi.org/10.1016/j.tox.2009.08.014

Straka E, Ellinger I, Balthasar C et al (2016) Mercury toxicokinetics of the healthy human term placenta involve amino acid transporters and ABC transporters. Toxicology 340:34–42. https://doi.org/10.1016/j.tox.2015.12.005

Vázquez M, Vélez D, Devesa V, Puig S (2015) Participation of divalent cation transporter DMT1 in the uptake of inorganic mercury. Toxicology 331:119–124. https://doi.org/10.1016/j.tox.2015.03.005

Waalkes MP, Fox DA, States JC, Patierno SR, McCabe MJ Jr (2000) Metals and disorders of cell accumulation: modulation of apoptosis and cell proliferation. Toxicol Sci 56(2):255–261

Zalups RK, Lash LH (1997) Binding of mercury in renal brush-border and basolateral membrane-vesicles: implication of a cysteine conjugate of mercury involved in the luminal uptake of inorganic mercury in the kidney. Biochem Pharmacol 53(12):1889–1900. https://doi.org/10.1016/S0006-2952(97)00138-X

Zhang X, Wang Y, Zhao Y, Chen X (2008) Experimental study on the estrogen-like effect of mercuric chloride. Biometals 21(2):143–150. https://doi.org/10.1007/s10534-007-9102-y

Zhou H, Brock J, Liu D, Board PG, Oakley AJ (2012) Structural insights into the dehydroascorbate reductase activity of human omega-class glutathione transferases. J Mol Biol 420(3):190–203. https://doi.org/10.1016/j.jmb.2012.04.014