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The effects of rhodium on the renal function of female Wistar rats

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HIGHLIGHTS

- Rhodium exerts toxic effects on the renal function of female Wistar rats.
- Rhodium increases Retinol Binding Protein and β2-microglobulin urinary levels.
- These results define early biomarkers of effect for rhodium exposure.
- These results are relevant for general and occupational exposed populations.

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ABSTRACT

In recent years, the increased use of rhodium (Rh) as an active catalyst material in modern three-way automobile catalytic converters has led to a parallel rise in environmental levels of this metal. In spite of this, the literature contains few studies of the effects of Rh on human health.

The aim of this study is to assess the effects of Rh on the renal function of female Wistar rats.

Our findings show that sub-acute exposure to six increasing concentrations, ranging from 0.001 to 1 mg L^{-1}, of Rh (III) chloride hydrate in drinking water does not induce alterations in urinary albumin levels, while, at concentrations from 0.1 to 1 mg L^{-1}, a significant rise in urinary levels of Retinol Binding Protein is evident and an increasing trend in urinary β2-microglobulin, which becomes significant at 1 mg L^{-1}, is observed. These results therefore demonstrate a nephrotoxic action of Rh at tubular level in a wide range of doses. Interestingly, because of the recent increase in environmental Rh levels, these findings may have relevant implications both for occupationally exposed subjects and for the general population, especially children.

1. Introduction

Rhodium (Rh) is a noble metal that belongs to the platinum group elements (PGEs) and is naturally found at low concentrations in the earth's crust with an average level of 0.06 ng g^{-1} (Wedepohl, 1995). Rh finds industrial application as an alloying agent for hardening and improving the corrosion resistance of Platinum and Palladium (Lide, 2004). It is also increasingly employed in the glass manufacturing sector as well as in the electronic, chemical, jewellery and decoration fields (Morrissey, 2004; Smith, 2007; Johnson Matthey Publications, 2012). However, its principal application is as an active catalyst material in modern three-way automobile catalytic converters. This has determined an increasing gross demand for Rh over the past 5 years, accounting for 80% of the 2012 worldwide demand, forecast in 27584 kg (Johnson Matthey Publications, 2012).

The use of catalytic converters has resulted in a significant reduction in the emission into the atmosphere of hazardous pollutants from combustion engines, with more than 90% of carbon monoxide, hydrocarbons, and nitrogen oxides (NOx) being converted into less harmful carbon dioxide, water and nitrogen (Palacios et al., 2000; Merget and Rosner, 2001; Ravindra et al., 2004). Rh is therefore an excellent catalyst for the control of NOx (Johnson Matthey Publications, 2012).

Abbreviations: ELISA, enzyme-linked immunosorbent assay; NOx, nitrogen oxides; PGE, platinum group element; RBP, Retinol Binding Protein; Rh, rhodium; SE, standard error.

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Nevertheless, although these catalytic converters are beneficial for air quality, they have become a primary anthropogenic source of Rh which is released into the environment due to autocatalyst surface abrasion and deterioration. Once dispersed in the atmosphere, Rh can be adsorbed on air suspended matter (Gómez et al., 2002; Zereini et al., 2012) and subsequently accumulate in road dusts (Rauch et al., 2000; Jarvis et al., 2001; Djingova et al., 2003; Mathur et al., 2011) and in adjacent roadside soils (Ely et al., 2001; Fritsche and Meisel, 2004; Hooda et al., 2007; Zereini et al., 2007; Hooda et al., 2008), and/or be transported to aquatic systems (Moldovan et al., 2001; de Vos et al., 2002; Essumang et al., 2008). This may then lead to bioaccumulation in living organisms such as plants and animals (Djingova et al., 2003; Marcheselli et al., 2010; Pino et al., 2010).

Moreover, although literature studies have provided further evidence of a growing environmental presence of Rh (Ravindra et al., 2004; Wiseman and Zereini, 2009), and biological monitoring data have revealed higher Rh concentrations in urine and other biological matrices sampled from occupational exposed subjects compared to controls (Petrucci et al., 2004; Cristaudo et al., 2007; Iavicoli et al., 2007, 2008), the potential toxic effects on human health are still not fully understood. However, there is now a pressing concern regarding the potential health risks resulting from general and occupational Rh exposure, particularly for workers exposed to traffic emissions.

From a toxicokinetic point of view, Rh metal is poorly absorbed due to its low solubility, while Rh organic salts can be adsorbed by the gastrointestinal tract or, in the case of occupational exposure, via inhalation (Schroeder and Mitchener, 1971; Czerczak and Gromiec, 2001). Rh can be distributed via blood circulation and mainly excreted through the urinary system as demonstrated in Wistar rats orally treated with Rh chloride ($RhCl_3$) for 14 d (Iavicoli et al., 2012a). A significant Rh accumulation has also been detected in the kidneys of wood mice exposed to urban environmental levels of the metal (Marcheselli et al., 2010). Limited information is available concerning Rh toxicity in *in vitro* and *in vivo* models. Cytotoxic effects were observed when potassium pentachlororhodate and ammonium hexachlororhodate were applied to mouse fibroblasts (L929) and human embryonal lung cells (L132) (Bünger et al., 1996). Comparably, $RhCl_3$ reduced viability in human monocyte-derived dendritic cells, human bronchial epithelial cells (BEAS-2B) and rat embryo fibroblasts (RAT-1) (Paolucci et al., 2007; Schmid et al., 2007; Iavicoli et al., 2012b). This compound was found to have genotoxic properties as demonstrated by the induction of micronuclei and oxidative DNA damage in human lymphocytes and rat fibroblasts (Migliore et al., 2002; Iavicoli et al., 2012b). Moreover, Rh is able to functionally modulate the activation state of the immune system *in vitro*, affecting cytokine production and costimulatory molecule expression in peripheral blood mononuclear and dendritic cells, respectively (Boscolo et al., 2004; Paolucci et al., 2007; Bordignon et al., 2008). This immuno-modulating role was confirmed *in vivo* by the reduction in serum cytokine levels following $RhCl_3$ administration (Iavicoli et al., 2012a). Moreover, chronic treatment with drinking water spiked with 5 ppm $RhCl_3$ induced an increase in lymphoma–leukemias or adenocarcinomas of the lung in Swiss mice (Schroeder and Mitchener, 1971).

As regards human health effects, knowledge has principally focused on Rh-induced hypersensitivity reactions (Vilaplana et al., 1994; Vilaplana and Romaguera, 2000; Stingeni et al., 2004). Allergic contact dermatitis due to Rh salts has been reported in subjects working in the jewelry trade and in a worker in a precious metal refinery (Bedello et al., 1987; de la Cuadra and Grau-Massanés, 1991; de la Fuente et al., 2003; Forte et al., 2008; Goossens et al., 2011). Moreover, skin prick tests for a series of PGE salts including Rh salts have proven positive in refinery and catalyst production workers, with or without symptoms of rhinitis, asthma and urticaria, (Murdoch et al., 1986; Murdoch and Pepys, 1987; Santucci et al., 2000; Cristaudo et al., 2005, 2007). A case of immediate-type allergic asthma due to Rh sulphate has been recently described in an electroplater (Merget et al., 2010).

Thus the increase in Rh levels in living and working environments as well as the limited knowledge of its kinetics and toxic effects seem to call for greater efforts on the part of the scientific community to examine the potentially harmful effects this metal may have on a broader range of organs. Considering the key role the renal system plays in Rh kinetics (Iavicoli et al., 2012a) and the adverse effects on renal function we previously detected for iridium (Ir), another PGE element with similar physico-chemical properties (Iavicoli et al., 2011, 2012c), an evaluation of Rh toxicity on this apparatus would appear to be extremely relevant. Therefore, the aim of the present study is to investigate urinary excretion of Retinol Binding Protein (RBP), β2-microglobulin and albumin, respectively low and high molecular weight proteins, so as to assess the toxicological effects of Rh on the kidneys of female Wistar rats.

2. Materials and methods

2.1. Animal husbandry

Thirty-five three-month-old female Wistar rats were supplied from the Experimental Animal Production Plant of the Catholic University of Sacred Heart (Rome, Italy) and allowed to acclimatize for two weeks before starting the experiment. Animals were maintained during the entire experiment in Macrolon cages (Tecniplast S.p.A., Buguggiate, Italy) at a room temperature of 23.1 °C, a relative humidity of 55% and a 12-h light/dark cycle. Animals had a mean weight of 265 ± 26 g and were fed with the solid maintenance diet R (Altromin Rieper A. S.p.A., Vandoies, Italy). Diet was available to rats, as well as purified water (described below) or Rh (III) chloride hydrate–spiked drinking water *ad libitum*. No significant changes in body weight were observed at the end of the experiments.

2.2. Sub-acute exposure

The experimental protocol as described below was approved by the Ethical Committee of the Catholic University of Sacred Heart (Rome, Italy). Thirty-five female Wistar rats were obtained and randomly divided into seven groups of five rats each. A double demineralization system with a mixed bed Culligan cartridge (Cadriano di Granarolo Emilia, Italy) and a MilliQ A10 apparatus (Millipore, Bedford, Massachusetts, USA) were used to obtain high-purity deionized water for the Rh solutions. The concentrations of Rh (III) chloride hydrate (Alfa Aesar GmbH & Co., Karlsruhe, Germany) (as Rh) in the solutions used for Rh administration were: 0 (control group), 0.001, 0.01, 0.1, 0.25, 0.5, and 1 mg L^{-1}. The water solubility of the Rh salt used was sufficient to yield clear and homogeneous solutions. The actual concentration of the stock solution was checked by analyzing three replicates and results gave a maximum loss of the expected actual concentration <10% that was satisfactory for the aim of this study. The maximum concentration employed (1 mg L^{-1}) was the highest that could ensure a stable solution in water.

Water was given *ad libitum* to the animals in each group for the entire period of Rh administration, resulting in a daily ingestion of 19 ± 5 mL of drinking water spiked with Rh (averaged over 14 d) for each rat. Consequently, rats in the different groups of exposure ingested doses of approximately 19, 190, 1900, 4750, 9500 and 19 000 ng Rh d^{-1}, respectively.