



Cite this: *Metallomics*, 2015, 7, 267

The metallobiochemistry of ultratrace levels of platinum group elements in the rat

E. Sabbioni,^a S. Fortaner,^b S. Manenti,^{*c} F. Groppi,^c M. Bonardi,^c S. Bosisio^d and M. Di Gioacchino^a

The use of platinum, palladium and rhodium (Platinum Group Elements – PGEs) and the possibility of exposure to their ultratrace levels is increasing. In fact, the exponential development of metallic PGE-based nanoparticles (<100 nm in size) opens extraordinary perspectives in the areas of electrocatalysts and catalytic converters, magnetic nanopowders, polymer membranes, cancer therapy, coatings, plastics, nanofibres and textiles. Like other metal-based nanoparticles, exposure to PGEs nanoparticles may result in a release of ultratrace amounts of Pt, Pd, Rh ions in the body whose metabolic fate and toxicity still need to be evaluated. Furthermore, PGEs can act as allergic sensitizers by acting as haptens and inducing both type I and IV allergic reactions. In this work we studied the *in vivo* metabolic patterns of ultratrace levels of potent allergens and sensitizers PGE halogenated salts. ¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh radioisotopes were prepared via cyclotron irradiation and used for radiolabelling Na₂¹⁹¹PtCl₄, Na₂¹⁰³PdCl₄ and Na₂^{101m}RhCl₆ salts. These anionic chlorocomplexes were intraperitoneally injected into rats (114 ng Pt kg⁻¹ bodyweight; 24 ng Pd kg⁻¹ b.w.; 16 ng Rh kg⁻¹ b.w.). At 16 h post-exposure, PGEs were poorly but significantly retained in all tissues analysed. Kidneys, spleen, adrenal gland, liver, pancreas and small intestine were the organs with the highest Pt, Pd, Rh concentrations. In the blood 30–35% of ¹⁰³Pd and ¹⁹¹Pt and 10% of ^{101m}Rh were recovered in the plasma, mainly bound to albumin and to a less extent to transferrin. The hepatic and renal intracellular distribution showed the highest recovery of ¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh in the nuclear fraction (liver) and in the cytosol (kidney). Chromatographic separation and ultrafiltration experiments on kidney and liver cytosols showed the strong ability of biochemical macromolecules to bind ¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh, and being responsible for the retention of the three elements in the body. The link to macromolecules is the basis for the sensitizing capacity of PGEs.

Received 12th September 2014,
Accepted 22nd December 2014

DOI: 10.1039/c4mt00240g

www.rsc.org/metallomics

Introduction

Platinum (Pt), palladium (Pd) and rhodium (Rh) (Platinum Group Elements – PGEs) have historically been the metal components of the three-way catalytic converters of air pollution control devices mainly of cars and trucks.¹ In the form of highly dispersed particles with diameters in the 1–10 nm range² these auto-catalysts are unique in converting harmful hydrocarbons, carbon monoxide, and nitrogen oxide emissions from gasoline engines into less harmful CO₂, N₂ and H₂O.³ However, this type of device is not totally environmentally clean. The hot exhaust gases flowing through the catalytic converter cause abrasion

and ablation of these latter,⁴ leading to the release of micronic and nanosized PGEs particulates to air, roads, tunnels as well as roadside soil,⁵ representing anthropogenic environmental pollutants for the general population.⁶ Moreover, occupational exposure to PGEs occurs during mining, processing, refining and catalyst manufacture.⁷

PGEs have other applications, representing a further source for human exposure. Platinum is used in biomedicine in anti-cancer drugs,⁸ and as a component of pacemakers, catheters and defibrillators;⁹ in industrial processes as a catalyst as in the polymerisation of olefins, and the refining of petroleum and ammonia oxidation; in electronic industries and glass manufacturing; and in jewellery where it represents the second largest demand.¹⁰ Palladium is used in electronic applications; in dentistry for dental crowns and bridges though they have been increasingly replaced by all-ceramic; in jewelry; in the chemical industry, in fuel cell technology; for coinage purpose; and in hydrogen purification.¹¹ Although catalytic converters use about 80% of the world's rhodium production, this metal is applied in the glass industry, mostly for production of fiberglass

^a Aging Research Centre (Ce.S.I.), “G. d’Annunzio- University Foundation”, Via dei Vestini-21, 66100 Chieti, Italy

^b European Commission, IHCP, JRC-Ispra, Via Fermi, 2749, 21027 Ispra, Varese, Italy

^c UNIMI e INFN Sezione di Milano LASA, Via F.lli Cervi-201, 20090 Segrate, Milan, Italy. E-mail: simone.manenti@mi.infn.it

^d Department of Radiation Science and Technology, Delft University of Technology, Mekelweg 15, 2629 JB Delft, The Netherlands

and flat-panel glass; in the chemical industry and in jewelry.¹² Recently, a new impetus to the use of PGEs is coming from the exponential development of metallic PGEs as nanoparticles (<100 nm in size) to be used in key applications as electro-catalysts and catalytic converters, magnetic nanopowders, polymer membranes, cancer therapy, coatings, plastics, nanofibers and textiles.¹³

A certain number of *in vivo* studies have been carried out on the metabolic fate and toxicity of PGEs in mammals. However, they primarily refer to high-dose exposure, generally of the order of tens to hundreds mg kg⁻¹ b.w., falling in the category of acute/subacute exposure which is far from being predictive of the effects induced by low level exposure. The human dietary intake of PGEs have been estimated up to 2 µg per day (Pt and Rh)¹⁴ and 0.7 µg per day (Pd)¹⁵ while the estimated emissions from automobile catalysts are 100–800 ng km⁻¹ for Pt, 5–100 ng km⁻¹ for Pd and 1–10 ng km⁻¹ for Rh.¹⁶ The available emission data suggest that almost all Pt obtained from catalytic converters exists in metallic or oxidic forms although halogenated Pt salts may possibly be emitted or secondarily formed from monocrystalline Pt nanoparticles under environmental conditions in waters and sediments, and thereby may potentially enter the food chain.^{2,7} Moreover, Pt provides an excellent example of the significance of speciation in metal toxicity.² The metallic platinum (Pt) and platinum oxide (PtO₂) forms emitted from catalytic converters are considered to be inert as far as biological reactions are concerned.¹⁷ In contrast, soluble anionic halogenated compounds, such as hexachloroplatinum(IV) and tetrachloroplatinum(II) salts, are among the most potent allergens and sensitizers inducing type I hypersensitivity in refinery workers¹⁸ as also recently observed for hexachlororhodate(III).¹⁹ In contrast, non-halogenated (*e.g.* K₂[Pt(NO₂)₄]) and neutral complexes (*e.g.* the anti-cancer drug *cis*-[PtCl₂(NH₃)₂] cisplatin) are not allergenic, perhaps because they do not act as haptens.²⁰ There is a great need for *in vivo* studies on the metabolic fate and toxicity of PGEs at an exposure dose reflecting the human situation,²¹ estimated to be of the order of few tens of ng PGEs kg⁻¹ b.w.²²

This study was aimed at elucidating the metabolic patterns of ultratrace levels of PGEs in the rat at tissue, subcellular and molecular levels. Animals were exposed to a single intraperitoneal (i.p.) injection of Pt (114 ng Pt kg⁻¹ b.w.), Pd (24 ng Pd kg⁻¹ b.w.) and Rh (16 ng Rh kg⁻¹ b.w.) as soluble anionic radiolabelled ¹⁹¹Pt-tetrachloroplatinum(II), ¹⁰³Pd-tetrachloropalladium(II) and ^{101m}Rh-hexachlororhodium(III).

Materials and methods

Radiolabels

¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh for the radiolabelling of PGEs chloro-complexes were prepared at the Scanditronix MC40 cyclotron of the JRC-Ispra.²³

¹⁹¹Platinum. (*T*_{1/2} = 2.96 d) was produced *via* alpha irradiation of Os powder.²⁴ It was isolated by dissolving the Os target in *aqua regia* followed by distillation of OsO₄, reduction of ¹⁹¹Pt(IV) by SnCl₂ in 3 M HCl, solvent extraction of ¹⁹¹Pt-tetrachlorocomplex

by diethylether, evaporation of the organic phase to dryness and dissolution of the residue in physiological saline solution.²⁵ The characteristic of the separated ¹⁹¹Pt were: yield better than 90%; specific radioactivity 0.42 µCi (15.5 kBq) ¹⁹¹Pt per ng Pt (as determined using ICPMS); radioactive concentration: 40 µCi (1.5 MBq) ¹⁹¹Pt per mL; radionuclidic purity: detectable traces of ¹⁸⁸Pt (*T*_{1/2} = 10.2 d); chemical impurity: <0.05%; chemical form: Na₂¹⁹¹PtCl₄ in physiological saline solution, pH = 5.6.

¹⁰³Palladium. (*T*_{1/2} = 17 d) was produced *via* proton irradiation of the metallic Rh target. It was isolated by electrolytic dissolution of Rh²⁶ followed by the co-precipitation of ¹⁰³Pd with metallic Se, elimination of Se by distillation as SeO₂, evaporation to dryness and dissolution of the residue in physiological saline solution.²⁷ The characteristic of the separated ¹⁰³Pd were: yield close to 75%; specific radioactivity 2.5 µCi (925 kBq) ¹⁰³Pd per ng Pd (as determined using ICPMS); radioactive concentration: 150 µCi (5.6 MBq) ¹⁰³Pd per mL; radionuclidic purity: >96%; chemical impurities: <0.05%; chemical form: Na₂¹⁰³PdCl₄ in physiological saline solution, pH = 5.5.

^{101m}Rhodium. (*T*_{1/2} = 4.34 d) was produced *via* proton irradiation of metallic Pd foil. It was isolated by dissolving the Pd target in *aqua regia* followed by anion exchange chromatography, evaporation of the eluate to dryness and dissolution of the residue in physiological saline solution.²⁸ The characteristic properties of the separated ^{101m}Rh were: yield close to 80%; specific radioactivity 2.05 µCi (75.9 kBq) ^{101m}Rh per ng Rh (as determined using ICPMS); radioactive concentration: 82 µCi (3 MBq) ^{101m}Rh per mL; radionuclidic purity: the presence of detectable ¹⁰⁰Rh (*T*_{1/2} = 20.8 h); chemical impurities: <0.04%; chemical form: Na₂^{101m}RhCl₆ in physiological saline solution, pH = 5.6.

Radioactive counting

Counting of ¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh in tissues, subcellular fractions and chromatographic fractions from gel filtration was carried out *via* integral gamma-counting using a Wizard 3 Gamma Counter (Perkin Elmer, Life Sciences) apparatus equipped with a well-type 3.15" × 3" NaI(Tl) size crystal (4π counting geometry for optimal counting efficiency), and performance of up to 2048 keV. Since ¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh were not in the radioisotope library for automatic calibration a manual calibration was carried out. In this context, samples of the three radiotracer solutions as obtained from the radiochemical separations to be injected into animals (mother solutions) were placed in the counter to obtain the gamma ray spectra. From the spectra and for the main gamma peaks the lower and upper energy levels were determined. These values were used to set appropriate energy threshold and windows for our experimental conditions: 100–700 keV (¹⁹¹Pt); 50–600 keV (¹⁰³Pd); 200–700 keV (^{101m}Rh). Each time radioactivity measurement of ¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh was interpreted in terms of exogenous metal concentrations by comparison with ¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh reference solutions. These latter were aliquots of the same radioactive mother solutions administered to animals whose specific radioactivity was previously determined using ICPMS (see the above section Radiolabels). In addition, the linearity of counting related to the dead time was verified by

counting five diluted mother radiotracer solutions covering the range of the expected radioactivity in the samples.

Animals and treatment

Male Sprague–Dawley rats (230–250 g, Harlan Nossan, Correzzana, Italy) were individually kept in metabolic cages (200 cm², Techniplast, Gazzada, Italy) in temperature, humidity, ventilation and light–dark-controlled rooms (temperature: 23 ± 2 °C; humidity: $50 \pm 10\%$). The animals were maintained with commercial food in pellets and natural mineral water (Acqua Panna, Tuscania, Italy) *ad libitum*. Housing conditions and experimental procedures were in strict accordance with the European Community regulations.²⁹ All animals were accustomed to our housing conditions for one week before being used.

¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh-chlorocomplex exposure. Fifteen rats were used. They were divided into three groups of five animals each (hereinafter referred to as the Pt-, Pd- and Rh-groups). Animals of the Pt- group were i.p. injected with 0.3 mL solution of Na₂¹⁹¹PtCl₄ (12 µCi (444 kBq) per rat, 28.5 ng Pt per rat); the animals of the Pd- and Rh-groups received 0.1 mL solution of Na₂¹⁹¹PdCl₄ (15 µCi (555 kBq) per rat, 6.2 ng Pd per rat) and Na₃^{101m}RhCl₆ (8.2 µCi (303 kBq) per rat, 4 ng Rh per rat), respectively.

Retention in tissues. After 16 h animals were anaesthetised using Nembutal (40 mg kg⁻¹ wet wt) and sacrificed *via* cardiac puncture. Blood was collected by heparinized syringes and centrifuged at 2500g for 10 minutes to separate plasma and red blood cells (RBC). The tissues were dissected out, washed and perfused with saline solution, weighted and counted for the ¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh content.

Fractionation of plasma components. Plasma was fractionated using size exclusion chromatography (SEC) on a Sephadex G-150 column (2.5 × 100 cm) previously equilibrated with 10 mM ammonium acetate buffer, pH 7.2 and precalibrated with blue dextran and a kit of standard proteins with known molecular weight: horse spleen apoferritin (440 kDa); bovine serum albumin (66 kDa), bovine erythrocytes carbonic anhydrase (29 kDa); horse heart cytochrome C (12.4 kDa) and bovine lung Aprotinin (6.5 kDa) (Fluka, Milan, Italy). The UV profile of the eluate at 280 nm was recorded on a Lambda 25 spectrometer (Perkin-Elmer, Milan Italy) and each fraction counted for the ¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh content. The ¹⁹¹Pt-, ¹⁰³Pd- and ^{101m}Rh-containing fractions in the eluates corresponding to the molecular weight of about 70 kDa (peaks II, Fig. 1) were pooled, desalted by SEC on a Sephadex G-25 column, freeze-dried, dissolved in 10 mM Tris buffer, pH 8.0, and submitted to ion exchange chromatography on a DEAE-Sephadex A-50 column (1.5 × 25 cm)³⁰ previously equilibrated with the same buffer. The absorbed components were eluted with a linear saline gradient composed of 10 mM Tris, 0–0.5 M NaCl, pH 8 to separate transferrin and albumin fractions. Protein profiles and ¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh in the eluates were monitored spectrophotometrically at 280 nm and by integral gamma counting, respectively.

Subcellular distribution in kidney and liver homogenates. Subcellular fractions of liver and kidney were obtained from homogenized tissues in sucrose–cacodylate medium (0.25 mol L⁻¹ sucrose, sodium cacodylate–HCl buffer, pH = 7.2) *via*

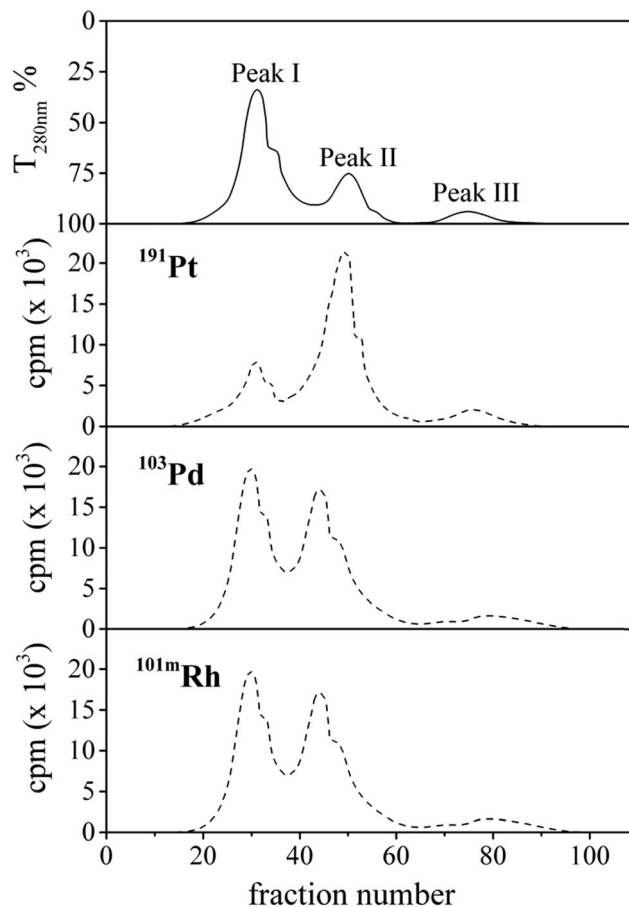


Fig. 1 Size exclusion chromatography of plasma proteins from rats 16 h after i.p. injection of 28.5 ng Pt per rat, 6.2 ng Pd per rat and 4 ng Rh per rat as Na₂¹⁹¹PtCl₄, Na₂¹⁰³PdCl₄ and Na₃^{101m}RhCl₆. Sephadex G-150 columns: 2.5 × 100 cm. The loaded samples were eluted with 10 mM ammonium acetate buffer, pH 7.2. In the collected fractions ¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh were analysed by integral gamma counting.

differential centrifugation. The homogenates were centrifuged at 700g for 10 min to sediment the nuclear fraction; at 9000g for 15 min to yield the mitochondrial fraction; at 30 000g for 25 min to sediment the lysosomal fraction. The lysosome-free supernatant was then centrifuged at 105 000g for 110 min to yield microsomes and organelle-free cytosol (TL-100 ultracentrifuge, Beckmann Instrument, Palo Alto, CA).³¹ All crude fractions were washed once with the same buffer and the washing was added to the following centrifugation steps. Each subcellular fraction was counted for the ¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh content.

Ultrafiltration experiments. 1 mL of ¹⁹¹Pt or ¹⁰³Pd or ^{101m}Rh-containing liver or kidney cytosols was inserted into a Centricon (YM-10) Centrifugal Filter device (Amicon, Millipore, nominal molecular weight cut-off 10 kDa) previously equilibrated with 10 mM sodium cacodylate–HCl buffer, pH 7.2. The filter devices were centrifuged at 500g for 30 min and the retentate was washed 3 times with the same cacodylate buffer. The ¹⁹¹Pt, ¹⁰³Pd and ^{101m}Rh contents were monitored in the retentates and in the filtrates.

The same ultrafiltration experiments were carried out on the extracts from nuclei (bulk chromatin fraction) and mitochondria (soluble subfraction) prepared as previously reported.³²

Table 1 Distribution of Pt, Pd, Rh in rat tissues after 16 h i.p. injection of 28.5 ng Pt per rat, 6.2 ng Pd per rat and 4 ng Rh per rat as $\text{Na}_2^{191}\text{PtCl}_4$, $\text{Na}_2^{103}\text{PdCl}_4$ and $\text{Na}_3^{101\text{m}}\text{RhCl}_6$ ^a

Tissue	Pt		Pd		Rh	
	%dose per g	pg g ⁻¹	%dose per g	pg g ⁻¹	%dose per g	pg g ⁻¹
Kidney	2.55 ± 0.19	730 ± 41	2.2 ± 0.26	130 ± 14	3.30 ± 0.19	132 ± 15
Adrenal gland	1.10 ± 0.15	460 ± 18	0.31 ± 0.002	25 ± 10	0.31 ± 0.035	12.4 ± 2.5
Spleen	1.25 ± 0.025	360 ± 16	2.02 ± 0.28	120 ± 10	1.5 ± 0.28	60 ± 4.6
Liver	0.85 ± 0.11	240 ± 30	0.81 ± 0.18	49 ± 6.1	0.94 ± 0.05	38 ± 1.6
Pancreas	0.67 ± 0.12	190 ± 30	0.54 ± 0.16	33 ± 9.3	0.70 ± 0.16	28 ± 4.8
Lung	0.31 ± 0.06	90 ± 18	0.31 ± 0.09	19 ± 4.2	0.54 ± 0.16	22 ± 5.5
Testis	0.31 ± 0.022	88 ± 6.1	0.14 ± 0.07	8.4 ± 0.7	0.30 ± 0.04	12 ± 1.3
Heart	0.22 ± 0.04	63 ± 12	0.31 ± 0.08	18 ± 4.5	0.55 ± 0.12	22 ± 2.7
Femur	0.20 ± 0.05	57 ± 14	0.10 ± 0.03	6 ± 2.4	0.30 ± 0.09	12 ± 3.8
Fat	0.18 ± 0.07	51 ± 20	0.31 ± 0.08	19 ± 4.3	0.16 ± 0.10	6.4 ± 3.5
Muscle	0.10 ± 0.05	26 ± 13	0.21 ± 0.04	13 ± 2.2	0.09 ± 0.015	3.6 ± 0.4
Skin	0.04 ± 0.01	11 ± 2.5	0.06 ± 0.017	3.6 ± 0.9	0.07 ± 0.03	2.8 ± 0.9
Brain	0.027 ± 0.001	2.0 ± 1.1	0.031 ± 0.016	1.9 ± 0.4	0.008 ± 0.02	0.3 ± 0.1
Thyroid	0.02 ± 0.002	5.7 ± 0.6	0.025 ± 0.011	1.4 ± 0.6	0.008 ± 0.0009	0.3 ± 0.1
Thymus	0.012 ± 0.004	3.4 ± 1.0	0.011 ± 0.005	0.7 ± 0.2	0.022 ± 0.006	0.9 ± 0.2
Trachea	0.05 ± 0.01	14 ± 3.1	0.12 ± 0.04	7.2 ± 1.5	0.17 ± 0.10	68 ± 2.4
Duodenum	0.075 ± 0.016	21 ± 4.2	0.07 ± 0.012	4.2 ± 0.6	0.1 ± 0.03	4 ± 0.9
Stomach	0.15 ± 0.09	43 ± 2.4	0.078 ± 0.003	4.7 ± 0.2	0.22 ± 0.011	9 ± 1.7
Intestine small	0.41 ± 0.12	120 ± 32	0.39 ± 0.04	23 ± 2.6	0.63 ± 0.14	25 ± 4.1
Intestine large	0.10 ± 0.02	28 ± 5.4	0.09 ± 0.02	5.4 ± 1.5	0.08 ± 0.02	32 ± 0.6
Blood ^a	1.0 ± 0.18	280 ± 44	0.8 ± 0.18	48 ± 5.5	0.6 ± 0.17	24 ± 5.2
Plasma	76.6 ± 2.3	214.5 ± 11	72 ± 3.8	34.6 ± 4.2	89 ± 5.1	21.4 ± 3.3
RBC	23.4 ± 1.2	65.5 ± 3.4	28 ± 1.1	13.4 ± 0.8	11 ± 0.6	2.6 ± 0.10

^a % or pg mL⁻¹.

Fractionation of liver and kidney cytosols. The liver and kidney cytosols were submitted to SEC at 4 °C on Sephadex G-150 columns (2.5 e 5 × 100 cm) (Pharmacia, Uppsala, Sweden) previously equilibrated with 10 mM ammonium acetate buffer, pH 7.2 and precalibrated with the same blue dextran and the kit of standard proteins with known molecular weights as described in the section fractionation of plasma proteins. The UV profile of the eluate at 280 nm was recorded spectrophotometrically and the ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ were monitored in each fraction.

Results

The distribution of ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ in tissues of rats exposed i.p. to a single dose of $\text{Na}_2^{191}\text{PtCl}_4$, $\text{Na}_2^{103}\text{PdCl}_4$ and $\text{Na}_2^{101\text{m}}\text{RhCl}_6$, expressed as a % of the dose per g and pg per g of tissue wt w. (wet weight), is reported in Table 1. At 16 h post-treatment the three radioisotopes were poorly but significantly retained in all tissues analysed. In terms of concentrations, the highest amounts of Pt, Pd, Rh were present in the kidney, followed by spleen, adrenal glands, liver, pancreas and small intestine (from 28 pg g⁻¹, Rh in the pancreas to 730 pg g⁻¹, Pt in the kidney). In all other tissues analysed concentrations of the three elements were <100 pg g⁻¹, with the lowest concentrations being recovered in the brain, thyroid and thymus.

PGEs in the blood

The concentrations of Pt, Pd and Rh in the blood were 280, 48 and 24 pg mL⁻¹, respectively (Table 1). The binding of PGEs to the erythrocytes accounted for about 70–75% of the total

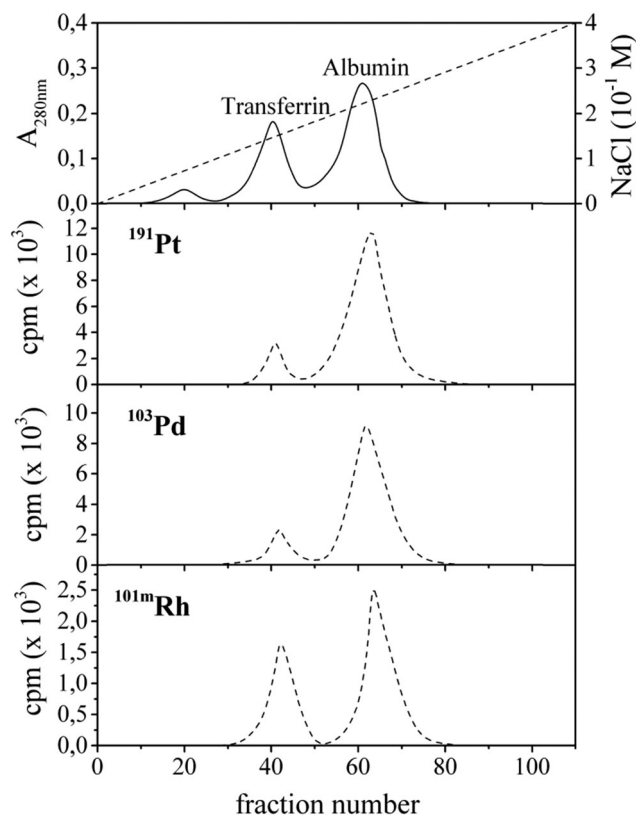


Fig. 2 Anion exchange chromatography on a DEAE A-50 column of the pooled ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ peaks II using size exclusion chromatography (see Fig. 1). Column dimensions 1.5 × 25 cm. The loaded samples were eluted with a linear saline gradient composed of 10 mM Tris, 0–0.5 M NaCl, pH = 8. The collected fractions were analysed for the ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ content.

radioactivity in whole blood for ^{103}Pd and ^{191}Pt , respectively, while 30–35% of the two radiotracers were recovered in the plasma. The corresponding values of $^{101\text{m}}\text{Rh}$ were significantly ($p < 0.05$) different (10% found to be associated with red blood cells and 90% recovered in the plasma).

Fig. 1 shows the UV and ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ elution profiles of plasma proteins obtained by SEC on G-150 resin. In all the cases, the radioactivities were recovered in the chromatogram as three peaks associated with UV absorbing components. Peak I (about 20, 40 and 45% of the total ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$, respectively) emerged after the void volume (molecular weight $> 300\,000$ kDa) while peak II was eluted in the region with a molecular weight of about 70 kDa. A third small peak (peak III, $< 2\%$ of the total radioactivity) appeared in the chromatogram in association with UV absorbing components with < 5 kDa.

Resolution of the ^{191}Pt , ^{103}Pd - and $^{101\text{m}}\text{Rh}$ -containing peak II into components using ion exchange chromatography on DEAE A-50 (Fig. 2) indicated that approximately 90% of ^{191}Pt and ^{103}Pd in the eluate was associated with the albumin fraction and 10% with transferrin. The percentage bound to the transferrin fraction increased to about 30% in the case of $^{101\text{m}}\text{Rh}$.

Subcellular distribution in kidney and liver homogenates

The hepatic and renal subcellular distributions of ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$, expressed as percentages of the total homogenate, are shown in Fig. 3. In the liver of the Pt-group (Fig. 3a) most of the ^{191}Pt was recovered in the nuclear fraction (about 60% of the homogenate), followed by cytosol (21.3%) and mitochondrial (10.8%) fractions. Similar trends were observed for Pd- and Rh-groups, although the percentages of radioactivity incorporated into the nuclear fractions were significantly higher ($p < 0.05$) than the corresponding values for the Pt-group (Fig. 3b and c).

In the kidneys, the subcellular distribution pattern of ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ was different. The highest percentage of ^{191}Pt and $^{101\text{m}}\text{Rh}$ were recovered in the cytosol (more than 50% of the total homogenate), the nuclear fractions retaining about 20% of the two radiotracers (Fig. 3d and f). It is noteworthy that the corresponding percentage of ^{103}Pd in the nuclear fraction was approximately double (40% of the total homogenate, Fig. 3e).

In both liver and kidneys small percentages ($< 5\%$) of ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ were constantly found to be associated with lysosomal and microsomal fractions.

Ultrafiltration of liver and kidney cytosols as well as of liver nuclear and mitochondrial extracts from Pt-, Pd- and Rh-treated groups (Fig. 4) shows that the PGEs were mostly retained on the

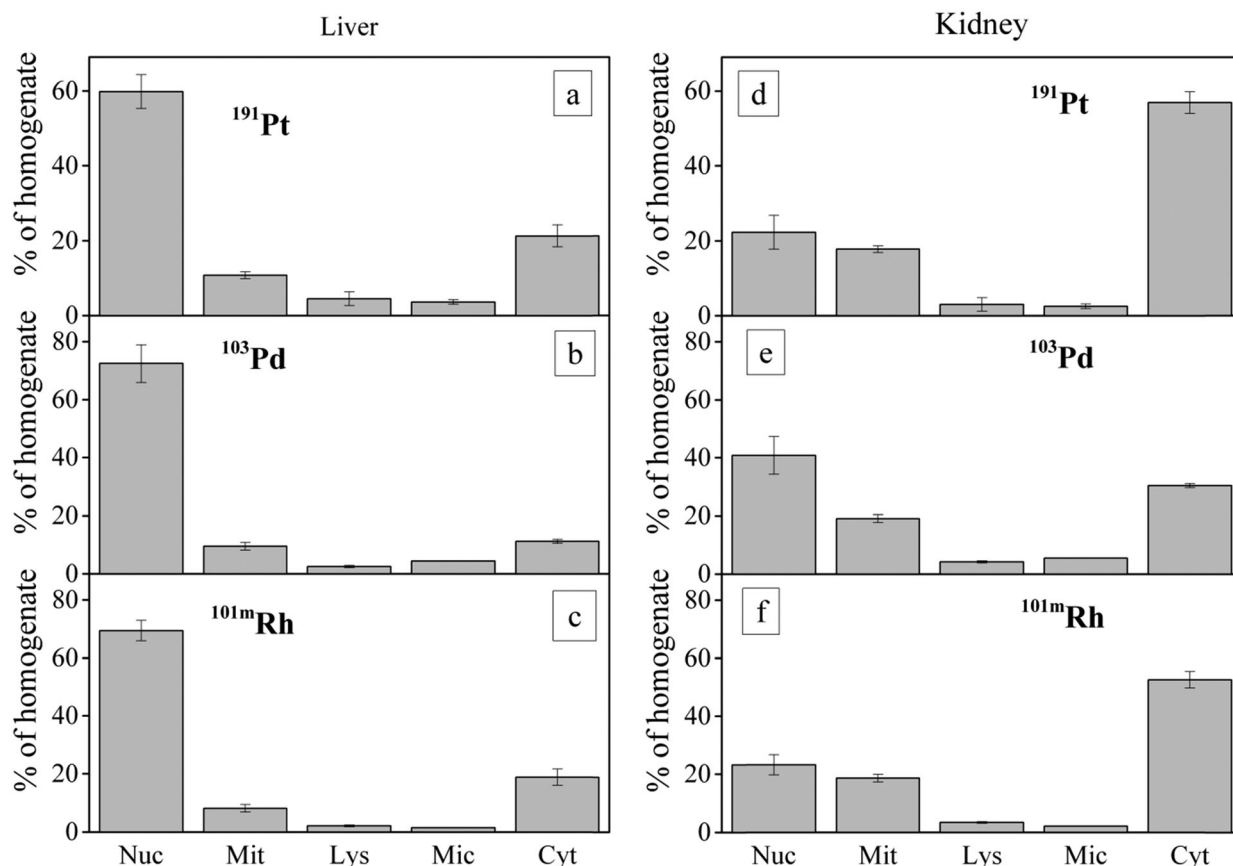


Fig. 3 Intracellular distribution of Pt, Pd and Rh in liver and kidneys of rats 16 h after i.p. injection of 28.5 ng Pt per rat, 6.2 ng Pd per rat and 4 ng Rh per rat as $\text{Na}_2^{191}\text{PtCl}_4$, $\text{Na}_2^{103}\text{PdCl}_4$ and $\text{Na}_3^{101\text{m}}\text{RhCl}_6$. The subcellular fractions were isolated by differential centrifugation and counted for the ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ content. The results, expressed as the percentage of the total ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ in the homogenate, are the mean of individual tissues of five rats. Nuc (nuclear), Mit (mitochondrial), Lys (lysosomal), Mic (microsomal), Cyt (cytosol) fractions.

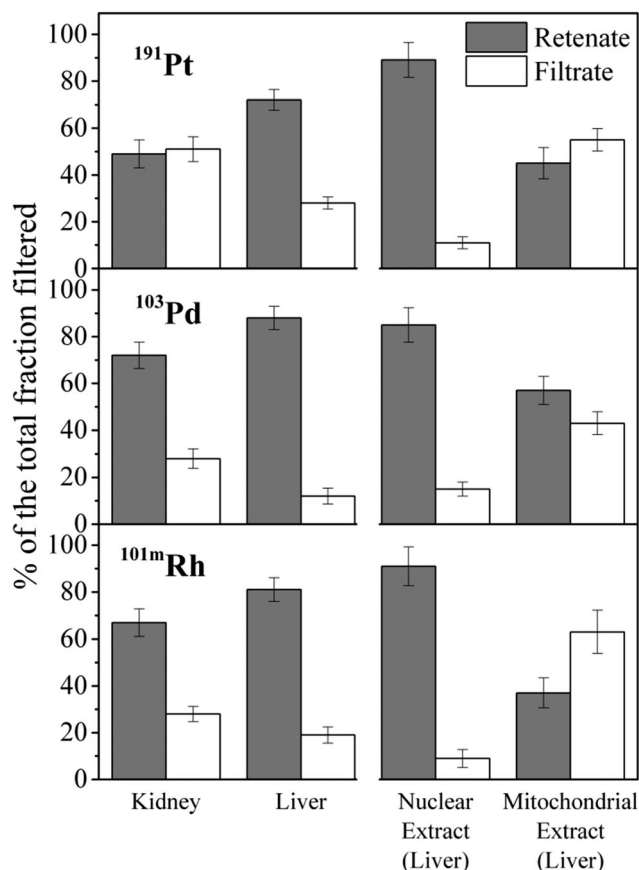


Fig. 4 Ultrafiltration of ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ renal and hepatic cytosol as well as of the liver nuclear and mitochondrial extracts of rats 16 h after i.p. injection of 28.5 ng Pt per rat, 6.2 ng Pd per rat and 4 ng Rh per rat as $\text{Na}_2^{191}\text{PtCl}_4$, $\text{Na}_2^{103}\text{PdCl}_4$ and $\text{Na}_3^{101\text{m}}\text{RhCl}_6$. Samples were inserted into a Centricon (YM-10) centrifugal filter device (nominal molecular weight cut-off 10 kDa) previously equilibrated with 10 mM cacodylate-HCl buffer, pH 7.2, followed by centrifugation and three washings of the retentate with the same buffer. Retentate and filtrate were analysed for the ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ content.

ultrafiltration membrane (retentate), particularly in the liver, in association with components with molecular weights > 10 kDa. The binding of ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ to the retentate components was also obvious when nuclear and mitochondrial extracts were ultrafiltered.

Fractionation of liver and kidney cytosols

Fig. 5 shows the results of the SEC on G-150 resin of rat liver and kidney cytosols 16 h after i.p. injection of ^{191}Pt -, ^{103}Pd - and $^{101\text{m}}\text{Rh}$ -hexachlorocomplexes.

In the liver cytosol ^{191}Pt was mainly eluted in three main peaks. The first peak (about 25% of the cytosolic ^{191}Pt) emerged after the void volume in association with high molecular weight components (Pt-HMWC, > 300 kDa); the second peak (about 50% of the cytosolic ^{191}Pt) appeared in the 70 kDa region of the chromatogram; the third peak (25% of the cytosolic ^{191}Pt) was recovered in association with low molecular weight components (Pt-LMWC, < 10 kDa). The corresponding profiles of ^{103}Pd and $^{101\text{m}}\text{Rh}$ were qualitatively similar to that of ^{191}Pt , showing,

however, obviously different proportions among the relative areas of the three peaks.

In the kidney cytosol, the elution profile of ^{191}Pt was similar to that in the liver, showing, however, a different proportion between the first two peaks (^{191}Pt -HMWC vs. ^{191}Pt -LMWC). The corresponding chromatographic profiles of ^{103}Pd and $^{101\text{m}}\text{Rh}$ -containing renal cytosols were similar, although ^{103}Pd showed an obviously different proportion among the relative areas of the peaks compared to those of ^{191}Pt and $^{101\text{m}}\text{Rh}$.

Discussion

For the first time we have carried out a comparative study in the rat on the biodistribution and retention of ultratrace levels of ^{191}Pt -, ^{103}Pd - and $^{101\text{m}}\text{Rh}$ -radiolabelled soluble anionic tetrachloroplatinum(II), tetrachloropalladium(II) and hexachlororhodium(III) salts. We have considered this halogenated class of PGEs because it is known to comprise potent respiratory allergens leading to dermatitis, rhinitis, conjunctivitis, asthma and urticaria,³³ and because the production of “no carrier added” ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ radiotracers²⁵ provided the analytical sensitivity needed for *in vivo* studies at the tissue, subcellular and molecular levels.³⁴

The bulk of literature concerning metabolic patterns and biochemical effects of PGEs refers primarily to studies with experimental animals after exposure to relatively high doses of their salts or complexes. In this context, the toxicological effects induced by PGEs are unlikely to be really representative of the environmental human exposure. This latter is much lower than those generally used in laboratory animal experiments (of the order of micrograms or less), particularly in relation to studies of PGEs as anticancer drugs (some mg kg^{-1} b.w.).^{35,36} On the other hand, the mechanistic understanding of the interaction of PGEs with biomolecules at concentrations far from saturation phenomena at the binding sites is a key precondition to improve the scientific basis of risk assessment of PGEs. Such experimentation poses severe analytical difficulties that may explain the very low quantity of experimental data in the literature on the metabolic fate of environmental levels of PGEs. We have overcome these difficulties using radiotracing techniques producing ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ radioisotopes of high specific radioactivity at the cyclotron that allowed the detection and measurements of ng–pg concentrations of Pt, Pd, Rh in rat tissues, subcellular fractions and molecular components. Notably, the knowledge of the metabolic fate of ultratrace soluble ions in mammalian systems is also of growing importance in nanotoxicology research, an emerging discipline for the safety and sustainable production of nanomaterials.³⁷ In particular, the very promising metallic PGEs-based nanoparticles may be a source of ions in biological fluids that may translocate to different tissues, introducing complications in the interpretation of the toxic responses induced by exposure to the original nanoparticles.³⁸

In our *in vivo* study, the administration of ultratrace levels of halogenated Pt, Pd and Rh showed kidneys, spleen and adrenal glands as target tissues with concentrations higher than those

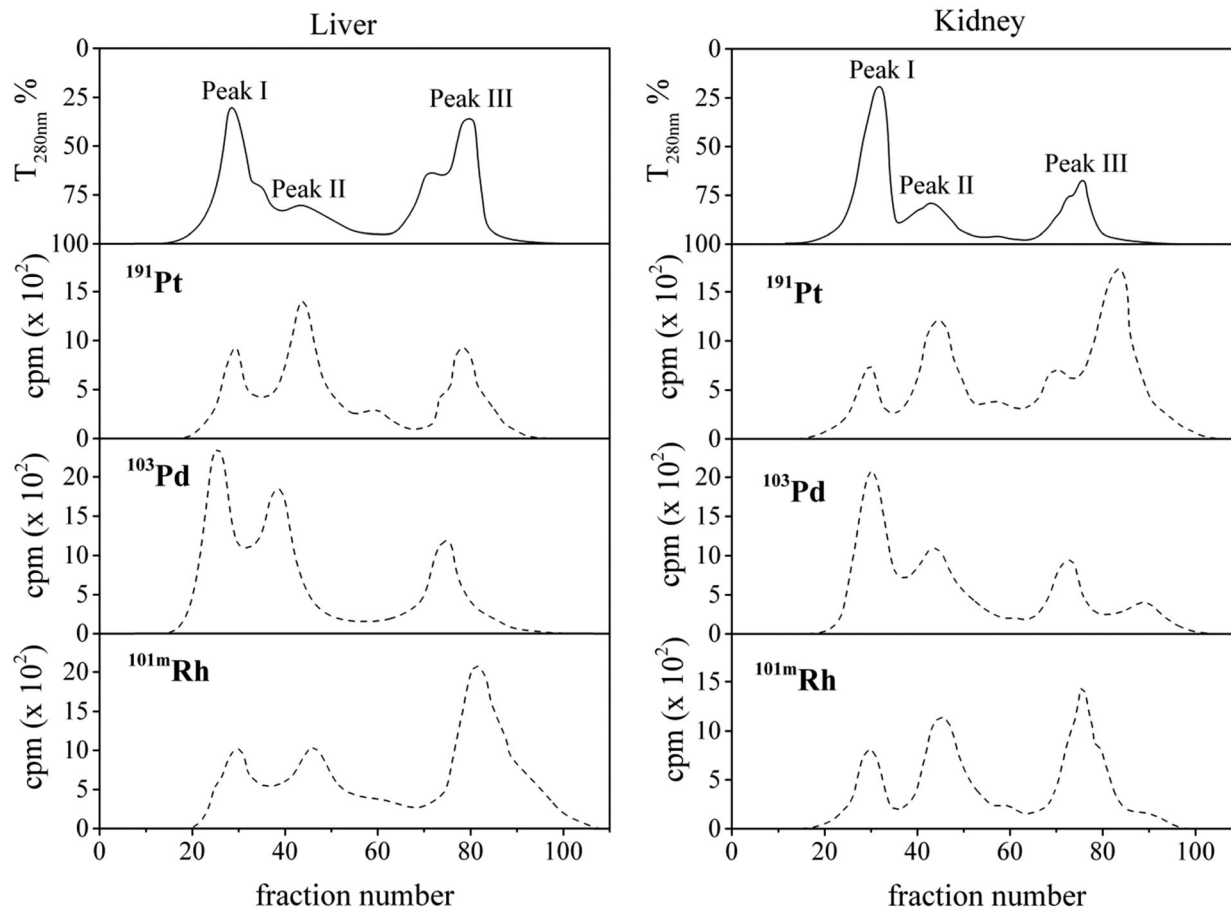


Fig. 5 Size exclusion chromatography of hepatic and renal cytosols of rats 16 h after i.p. injection of 28.5 ng Pt per rat, 6.2 ng Pd per rat and 4 ng Rh per rat as $\text{Na}_2^{191}\text{PtCl}_4$, $\text{Na}_2^{103}\text{PdCl}_4$ and $\text{Na}_3^{101\text{m}}\text{RhCl}_6$. Sephadex G-150 columns: 2.5 or 5×100 cm. The loaded samples were eluted with 10 mM ammonium acetate buffer, pH 7.2. The collected fractions were analysed for the ^{191}Pt , ^{103}Pd and $^{101\text{m}}\text{Rh}$ content.

in the blood. This is in agreement with the pioneering data of Durbin³⁹ and Moore.⁴⁰ Interestingly, the target tissues of ^{191}Pt identified in the present study are the same as those of a study in rats injected with a single dose of cationic tetravalent $^{191}\text{PtCl}_4$ in rats (59 ng Pt per rat).²⁰ Since our study involved the exposure to anionic divalent $^{191}\text{PtCl}_4^{2-}$ the similarity in the tissue distribution of ^{191}Pt suggests that cationic or anionic forms of Pt(II) and (IV) soluble salts would undergo a biotransformation process *in vivo* leading to a species with the same oxidation state, probably Pt(II). Pt(IV) complexes used as anticancer drugs are reduced intracellularly giving cytotoxic Pt(II) compounds.^{41–43}

In the present work, extremely low but significant concentrations of PGEs were detected in the brain. However, knowing the hematic concentrations of Pt, Pd and Rh (Table 1) and assuming the blood residue of the rat brain to be $49.7 \mu\text{L g}^{-1}$ wet⁴⁴ the concentrations would almost due to Pt, Pd and Rh in the blood residue. This suggests that under “physiological conditions” the blood–brain barrier would be impermeable to PGEs administered in the form of halogenated salts. In the circulatory system, for the first time we showed that exposure to PGEs halogenated salts leads to the presence of Pt, Pd and Rh mainly in the plasma (Table 1) in association with albumin and to a lesser extent transferrin (Fig. 2). Until now, these associations

were mainly proved by *in vivo* and *in vitro* studies only in non-halogenated and neutral platinum and rhodium complexes such as anticancer drugs (oxaliplatin, *cis*-platin).⁴⁵ Moreover, a complex of potassium tetrachloroplatinum(II) with human transferrin was also observed *in vitro*.⁴⁶

Our results at the subcellular level suggest that the hepatic and renal retention of ultratrace levels of PGEs in the rat would be due to their interaction with intracellular components. However, significant differences between hepatic and renal intracellular distributions of Pt, Pd and Rh emerged. In the liver the nuclear fraction contained higher concentrations of the elements than that in the kidney (Fig. 5), mostly (approx. 90%) protein-bound as shown by ultrafiltration. This finding is of particular interest, considering that Pt, Pd and Rh can interact with DNA, binding both with phosphate and the bases.^{43,47,48} Moreover, the presence of PGEs in the liver and particularly in the kidney mitochondria may be of interest in relation to the altered mitochondrial structure and function observed in mice exposed to Pt antitumor drugs.⁴⁹

Gel filtration experiments further confirmed the ability of Pt, Pd and Rh to interact strongly with proteins and small biomolecules. It is reported that the high affinity of Pt(II) for sulfur-containing biomolecules and the stability of such Pt(II)-complexes

would be responsible for the observed toxic side effects.⁵⁰ Moreover, potassium tetrachloroplatinum(II) binds to pyruvate kinase changing its structure and activity⁵¹ and induces a conformational distortion of albumin.⁵² In addition, chromatographic fractionation of the blood plasma, liver and kidney cytosols shows that only a very small fraction of PGEs was unbound to plasma proteins (peaks III, Fig. 1). Differently, in liver and kidney cytosols the binding of PGEs to small biocomponents (peaks III, Fig. 5) is a relevant biochemical pool of PGEs. Pt, Pd and Rh can interact with amino acids and peptides.^{53–55}

Moreover, our findings on the strong ability of PGEs to form intracellularly complexes with proteins also seem to be of particular interest regarding the potent allergenic action of soluble chlorinated PGEs. These latter are able to induce the production of antibodies of the E class,^{56–58} so acting as sensitising allergens. This can be due to the ability of PGEs to form intracellular complexes with proteins, becoming complete antigens. In the present work in all tissues and in the blood PGEs are bound to macromolecules, in agreement with the fact that PGEs can act as haptens and so explaining the sensitizing capacity of these chlorinated salts.

Delineation of the metabolic patterns of ultratrace PGE levels is important to assess effects of their environmental exposure. Without such data, the mechanistic interpretation of toxicological data is difficult, as exposure determines whether or not, or to which extent, a hazard constitutes a risk. In this context, we are aware that the present study is far from being exhaustive, representing, however, a good basis for continuing the work on key merging aspects. Our study has been carried out at a single short exposure time and further studies at different exposure intervals are necessary, primarily to determine the toxicokinetic parameters. In addition, studies on the effects of occupational exposure to PGEs involved exposure for longer periods.¹⁸ Therefore long term experiments with regard to the exposure to ultratrace PGE levels is another important aspect to be considered for future studies.

Acknowledgements

We are grateful to Mrs J. Edel for her excellent technical assistance with the work on laboratory animals and gel filtration experiments. We would also like to thank Messrs R. Pietra and G. Tettamanti (Ascom, Milan) for their assistance with the radioactive countings. Grateful acknowledgements are due to the health physics personnel of JRC and LASA-INFN for their technical assistance. The experiments carried out in the present study comply with the Council Directive 603/2010/EEC (8 September 2010) on the approximation of laws, regulation and administrative provisions of the Member States regarding the protection of animals used for experimental and scientific purposes.

References

- 1 J. Hoffmann, Precious metal technologies, in *Process metallurgy*, ed. A. E. Thermae and I. H. Gondolier, Elsevier, Amsterdam, The Netherlands, 1989, vol. 5.
- 2 K. Ravindra, L. Bencs and R. Van Grieken, Platinum group elements in the environment and their health risk, *Sci. Total Environ.*, 2004, **318**, 1–43.
- 3 H. P. Konig, R. F. Hertel, W. Koch and G. Rosner, Determination of the platinum emissions from three-way catalyst-equipped gasoline engine, *Atmos. Environ.*, 1992, **26**, 741–745.
- 4 E. Helters, Platinum emission rate of automobiles with catalytic converters, *Environ. Sci. Pollut. Res.*, 1997, **4**, 100–103.
- 5 H. M. Prichard and P. C. Fisher, Identification of Platinum and Palladium Particles Emitted from Vehicles and Dispersed into the Surface Environment, *Environ. Sci. Technol.*, 2012, **46**, 3149–3154.
- 6 S. Rauch and G. M. Morrison, Environmental Relevance of the Platinum-Group Elements, *Elements*, 2008, **4**, 259–263.
- 7 R. Merget and G. Rosner, Evaluation of the health risk of platinum group metals emitted from automotive catalytic converters, *Sci. Total Environ.*, 2001, **270**, 165–173.
- 8 D. Wang and S. J. Lippard, Cellular processing of platinum anticancer drugs, *Nat. Rev. Drug Discovery*, 2005, **4**, 307–320.
- 9 A. Cowley and W. Brian, A Healthy Future: Platinum in Medical Applications, *Platinum Met. Rev.*, 2011, **55**, 98–107.
- 10 Johnson Matthey, Platinum 2013 Interim review, Johnson Matthey Public Limited Company, published in November 2013 by Johnson Matthey.
- 11 PAI (Palladium Alliance International), Palladium metal of 21st century, Uses of palladium, 2004.
- 12 T. Bell, 2014 Metal Profile: Rhodium About.com.
- 13 AZoNano Platinum nanoparticles-Properties-Applications Sept 10, 2013, www.AZoNano.com.
- 14 G. Ysart, P. Miller, H. Crews, P. Robb, M. Baxter, C. De L'Argy, S. Lofthouse, C. Sargent and N. Harrison, Dietary exposure estimates of 30 elements from the UK Total Diet Study, *Food Addit. Contam.*, 1999, **16**, 391–403.
- 15 M. Rosel, M. Baxter, N. Brereton and C. Baskaran, Dietary exposure to metals and other elements in the 2006 UK. Total Diet Study and some trends over the last 30 years, *Food Addit. Contam., Part A*, 2010, **27**, 1380–1404.
- 16 C. Fortin, F. Wang and D. Pitre, Critical Review of Platinum Group Elements (Pd, Pt, Rh) in Aquatic Ecosystems EC Contribution Agreement with the CNTC for 2010/2011 Research Rep. R-1269 April 2011.
- 17 M. E. Farago, P. Kavanagh, R. Blanks, J. Kelly, G. Kazantzis, I. Thornton, P. R. Simpson, J. M. Cook, H. T. Delves and G. E. Hall, Platinum concentrations in urban road dust and soil, and in blood and urine in the United Kingdom, *Analyst*, 1998, **123**, 451–454.
- 18 P. J. Linnett and E. G. Hughes, 20 years of medical surveillance on exposure to allergenic and non-allergenic platinum compounds: the importance of chemical speciation, *Occup. Environ. Med.*, 1999, **56**, 191–196.
- 19 R. Merget, I. Sander, V. van Kampen, M. Raulf-Heimsoth, H. M. Ulmer, R. Kulzer and T. Bruening, Occupational immediate-type asthma and rhinitis due to rhodium salts, *Am. J. Ind. Med.*, 2010, **53**, 42–46.

- 20 WHO, 1991 Environmental health criteria 125 platinum, first draft prepared by G. Rosner, H. P. König and D. Coenen-Stass, WHO, Geneva, 1991.
- 21 Z. E. Gagnon, C. Newkirk and S. Hicks, Impact of platinum group metals on the environment: a toxicological, genotoxic and analytical chemistry study, *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.*, 2006, **41**, 397–414.
- 22 E. Sabbioni, L. Goetz, C. Birattari and M. Bonardi, Environmental biochemistry of current environmental levels of heavy metals: preparation of radiotracers with very high specific radioactivity for metallobiochemical experiments on laboratory animals, *Sci. Total Environ.*, 1981, **17**, 257–276.
- 23 K. Casteleyn, M. Castiglioni, L. Manes and B. Weckermann, Production of radionuclides at the JRC-Ispra cyclotron, *J. Trace Microprobe Tech.*, 1993, **11**, 13–27.
- 24 C. Birattari, M. Bonardi, F. Groppi and L. Gini, in *Cyclotrons and Their Applications, Sixteenth International Conference*, ed. F. Marti, American Institute of Physics, 2001.
- 25 M. Bonardi, C. Birattari, M. Gallorini, F. Groppi, D. Arginelli and L. Gini, Cyclotron production, radiochemical separation and quality control of platinum radiotracers for toxicological studies, *J. Radioanal. Nucl. Chem.*, 1998, **236**, 159–164.
- 26 V. I. Levin, M. D. Kozlova, A. B. Malinin and A. B. Zalesskaya, Production of palladium-103 without a carrier and preparation of a radioactive colloidal preparation of palladium for medical purposes, *Sov. Radiochemistry*, 1971, **13**, 637–642 (*Radiokhimiya*, 1971, **13**, 622–627).
- 27 W. M. Garrison and J. G. Hamilton, Production and isolation of carrier-free radioisotopes, *Chem. Rev.*, 1951, **49**, 237–272.
- 28 M. C. Lagunas-Solar, S. R. Wilkins and D. W. Paulson, Cyclotron production of rhodium-101m from natural palladium radiochemical methods and preliminary biological studies, *J. Radioanal. Nucl. Chem.*, 1982, **68**, 245–260.
- 29 EU, Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes, *Official Journal of the European Community*, 1986, **L358**, 1–29.
- 30 B. Gelotte, P. Flodin and J. Killander, Fractionation of human plasma proteins by gel filtration and zone electrophoresis or ion-exchange chromatography, *Arch. Biochem. Biophys.*, 1962, (suppl 1), 319–326.
- 31 J. Edel, G. Pozzi, E. Sabbioni, R. Pietra and S. Devos, Metabolic and toxicological studies on cobalt, *Sci. Total Environ.*, 1994, **150**, 233–244.
- 32 E. Sabbioni and E. Marafante, Identification of lead-binding components in rat liver: *in vivo* study, *Chem.-Biol. Interact.*, 1976, **15**, 1–20.
- 33 R. Merget, A. Schulte, A. Gebler, R. Breitstadt, R. Kulzer, E. D. Berndt, X. Baur and G. Schultze-Werninghaus, Outcome of occupational asthma due to platinum salts after transferral to low-exposure areas, *Int. Arch. Occup. Environ. Health*, 1999, **72**, 33–39.
- 34 E. Sabbioni, R. Pietra, M. A. Serra and S. Fortaner, Application of Nuclear, Radiochemical and Spectrochemical Techniques in Metal Toxicology, *J. Trace Microprobe Tech.*, 1991, **11**, 217–233.
- 35 H. Sajjad, Toxicity effect of cisplatin-treatment on rat testis tissue, *Ann. Biol. Res.*, 2012, **3**, 2297–2303.
- 36 M. Sakurai, N. Egashira, T. Kawashiri, T. Yano, H. Ikesue and R. Oishi, Oxaliplatin-induced neuropathy in the rat: involvement of oxalate in cold hyperalgesia but not mechanical allodynia, *Pain*, 2009, **147**, 165–174.
- 37 G. Bernardini, A. G. Cattaneo, E. Sabbioni, M. Di Gioacchino, M. Chiriva-Internati and R. Gornati, Toxicology of engineered metal nanoparticles, in *Handbook of system toxicology*, ed. S. C. Sahu and D. Casciano, Wiley & Sons Ltd, Chichester, 2011, vol. II, pp. 729–742.
- 38 F. Benetti, L. Bregoli, I. Olivato and E. Sabbioni, Effects of Metal(loid) Nanomaterials on Essential Element Homeostasis: The Central Role of Nanometallomics for Nanotoxicology, *Metallomics*, 2014, **6**, 729–747.
- 39 P. W. Durbin, K. G. Scott and J. G. Hamilton, The distribution of radioisotopes of some heavy metals in the rat, *Univ. Calif. Publ. Pharmacol.*, 1957, **3**, 1–34.
- 40 W. Moore Jr, D. Hysell, W. Cocker and J. Stara, Biological fate of a single administration of ^{191}Pt in rats following different routes of exposure, *Environ. Res.*, 1975, **9**, 152–158.
- 41 C. S. K. Pillai and U. S. Nandi, Interaction of palladium(II) with DNA, *Biochim. Biophys. Acta*, 1977, **474**, 11–16.
- 42 R. Sasi and U. S. Nandi, Interaction of rhodium(III) with DNA, *Biochim. Biophys. Acta*, 1979, **563**, 527–533.
- 43 I. Turel and J. Kljun, Interactions of metal ions with DNA, its constituents and derivatives, which may be relevant for anticancer research, *Curr. Top. Med. Chem.*, 2011, **11**, 2661–2687.
- 44 A. Keyeux, D. Ochymowicz-Bemelmans, C. Van Eyll and A. A. Charlier, Total cerebral blood volume calculated from a model of $^{99\text{m}}\text{Tc}$ pertechnetate distribution in the head, *J. Appl. Physiol.*, 1985, **74**, 2886–2895.
- 45 A. Martinčič, M. Cemazar, G. Sersa, V. Kovač, R. Milačič and J. Ščančar, A novel method for speciation of Pt in human serum incubated with cisplatin, oxaliplatin and carboplatin by conjoint liquid chromatography on monolithic disks with UV and ICP-MS detection, *Talanta*, 2013, **116**, 141–148.
- 46 R. Stjernholm, F. W. Warner, J. W. Robinson, E. Ezekiel and N. Katayama, Binding of platinum to human transferrin, *Bioinorg. Chem.*, 1978, **3**, 277–280.
- 47 E. Tselepi-Kalouli and N. Katsaros, The interaction of Rh(II) and Rh(III) with DNA, *J. Inorg. Biochem.*, 1990, **40**, 95–102.
- 48 A. G. Tikhomirov, N. A. Ivanova, O. S. Erofeeva, L. B. Gorbacheva and I. A. Efimenko, Interaction of Palladium(II) Acido Complexes with DNA, *Russ. J. Coord. Chem.*, 2003, **29**, 489–493.
- 49 M. Rosen, M. Figliomeni and H. Simpkins, The interaction of platinum antitumour drugs with mouse liver mitochondria, *Int. J. Exp. Pathol.*, 1992, **73**, 61–74.
- 50 D. Petrović, B. Stojimirović, B. Petrović, Z. M. Bugarcic and Z. D. Bugarcic, Studies of interactions between platinum(II) complexes and some biologically relevant molecules, *Bioorg. Med. Chem.*, 2007, **15**, 4203–4211.

- 51 L. Trynda, D. Kwiatkowska and W. Tyran, Platinum complexes and pyruvate kinase activity, *Gen. Physiol. Biophys.*, 1998, **17**, 25–36.
- 52 E. N. Zhmareva, G. D. Zegzhda, G. B. Kas'ian and O. A. Livenskaia, Interaction of proteins with platinum and palladium compounds with differing biological activities, *Ukr. Biokhim. Zh.*, 1996, **68**, 74–79.
- 53 A. Iakovidis and O. Hadjiliadis, Complex compounds of platinum(II) and (IV) with amino acids, peptides and their derivatives Coordination, *Chem. Rev.*, 1994, **135–136**, 17–63.
- 54 T. G. Appleton, D. R. Bedgood Jr. and J. R. Hall, Amino Acid Complexes of Palladium(II). 2. Reactions of the Diaqua-(ethylenediamine)palladium(II) Cation with N-Acetylglycine, Glycinamide, and N-Glycylglycine, *Inorg. Chem.*, 1994, **33**, 3834–3841.
- 55 J. F. Waller, Amino Acid Complexes of Rhodium(III), UNT Digital Library, Denton, Texas, 2014, <http://digital.library.unt.edu/ark:/67531/metadc130648/>.
- 56 R. D. Murdoch, Pepys J Immunological responses to complex salts of platinum. Specific IgE antibody production in the rat, *Clin. Exp. Immunol.*, 1984, **57**, 107–114.
- 57 P. Boscolo, L. Di Giampaolo, M. Reale, M. L. Castellani, A. Ritavolpe, M. Carmignani, J. Ponti, R. Paganelli, E. Sabbioni, P. Conti and M. Di Gioacchino, Different effects of platinum, palladium, and rhodium salts on lymphocyte proliferation and cytokine release, *Ann. Clin. Lab. Sci.*, 2004, **34**, 299–306.
- 58 M. Di Gioacchino, N. Verna, L. Di Giampaolo, F. Di Claudio, M. C. Turi, A. Perrone, C. Petrarca, R. Mariani-Costantini, E. Sabbioni and P. Boscolo, Immunotoxicity and sensitizing capacity of metal compounds depend on speciation, *Int. J. Immunopathol. Pharmacol.*, 2007, **20**, 15–22.