Inhibition of hydrogen sulphide formation reduces cisplatin-induced renal damage

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Abstract

Background. Cisplatin (CP)-induced renal damage is associated with inflammation. Hydrogen sulphide (H2S) is involved in models of inflammation. This study evaluates the effect of DL-propargylglycine (PAG), an inhibitor of endogenous H2S formation, on the renal damage induced by CP.

Methods. The rats were injected with CP (5 mg/kg, i.p.) or PAG (5 mg/kg twice a day, i.p.) for 4 days, starting 1 h before CP injection. Control rats were injected with 0.15 M NaCl or PAG only. Blood and urine samples were collected 5 days after saline or CP injections for renal function evaluation. The kidneys were removed for tumour necrosis factor (TNF)-α quantification, histological, immunohistochemical and Western blot analysis. The cystathionine γ-lyase (CSE) activity and expression were assessed. The direct toxicity of H2S in renal tubular cells was evaluated by the incubation of these cells with NaHS, a donor of H2S.

Results. CP-treated rats presented increases in plasma creatinine levels and in sodium and potassium fractional excretions associated with tubulointerstitial lesions in the outer medulla. Increased expression of TNF-α, macrophages, neutrophils and T lymphocytes, associated with increased H2S formation rate and CSE expression, were also observed in the outer medulla from CP-injected rats. All these alterations were reduced by treatment with PAG. A direct toxicity of NaHS for renal tubular epithelial cells was not observed.

Conclusions. Treatment with PAG reduces the renal damage induced by CP. This effect seems to be related to the H2S formation and the restriction of the inflammation in the kidneys from PAG+CP-treated rats.

Keywords: acute renal failure; cisplatin; DL-propargylglycine; hydrogen sulphide; inflammation

Introduction

Cisplatin (cis-diamminedichloroplatinum; CP), one of the most widely used anticancer drugs, is highly effective against many tumours [1]. However, the primary side effect of CP treatment, nephrotoxicity, has been reported to be a dose-limiting factor [1–3]. CP provokes necrosis and apoptosis of tubular epithelial cells, which is followed by inflammation and fibrosis [4–7]. CP is taken up by renal tubular cells, reaching its highest concentrations in proximal tubular cells of the inner cortex and the outer medulla, especially in the S3 segment, the major site of renal damage provoked by this drug [8–12]. The oxidative stress process that leads to inflammation contributes to the loss of renal function observed in CP-injected animals [5,6,13–15].

Hydrogen sulphide (H2S) is an important signalling molecule involved in inflammation [16–20]. H2S is an endogenous gaseous substance formed from the metabolism of L-cysteine by the action of two enzymes, cystathionine γ-lyase (CSE) and cystathionine β-synthase [21]. A third enzyme, 3-mercaptopyruvate sulphurtransferase (3-MST), along with cysteine aminotransferase, can produce H2S from cysteine in the presence of α-ketoglutarate [20,22]. It was observed that inhibition of CSE activity with DL-propargylglycine (PAG) reduced the intensity of the inflammation in pancreatitis and endotoxic shock induced by lipopolysaccharide (LPS) or cecal ligation and puncture, whereas sodium hydrosulphide, an H2S donor, intensified this process [19,23–25]. During the inflammatory response, H2S can provoke neutrophil adhesion and locomotion by a mechanism dependent on ATP-sensitive potassium channels [17]. However, the effect of H2S inhibitor has not been evaluated yet in CP-induced renal damage.
In this study, we evaluated the effects of the inhibition of H2S formation in CP-induced renal damage and its relationship with apoptosis and inflammation.

Materials and methods

Animals and experimental protocols

A total of 48 male Wistar rats (180–200 g) were provided by the Animal House of the Campus of Ribeirão Preto, University of São Paulo (Ribeirão Preto, SP, Brazil) and housed in polycarbonate cages (two to four animals per cage) under standard room temperature (25 °C) and 12 h light/dark cycle with free access to standard rat chow and water. The animals were divided into four groups: (i) Control (n = 12) received 250 μL/100 g of 0.9% saline, i.p. (the vehicle for cisplatin); (ii) CP (n = 8) received 250 μL/g of CP solution, i.p., twice a day (2 mg/mL diluted in 0.9% saline) for 4 days, starting 1 h before the single injection of 1 mL/100 g of 0.9% saline, i.p. (the vehicle for cisplatin); (iii) Cisplatin (CP; n = 14) received 1 mL/100 g of CP solution, i.p. (0.5 mg/mL diluted in 0.9% saline) (Quiral Química do Brasil, Juiz de Fora, MG, Brazil); (iv) CP+PAG (n = 14) received 250 μL/100 g of PAG solution, i.p., twice a day (2 mg/mL diluted in 0.9% saline) for 4 days, starting 1 h before CP injection. The dose of CP (5 mg/kg, i.p.) was selected on the basis of its effectiveness in inducing nephrotoxicity [13, 14]. The optimal dosages and timing for the PAG treatment were determined in previous studies in our laboratory where several doses of PAG were tested at different times. The current dose used provided protective effects in this model of renal injury and had no toxicity.

The rats were anaesthetized on Day 5 after saline or CP injection. The aorta artery was cannulated, the blood was collected, and the kidneys were perfused through the aorta with phosphate-buffered solution (PBS; 0.15 M NaCl and 0.01 M sodium phosphate buffer, pH 7.4). One kidney was removed for analysis of tumour necrosis factor (TNF-α) and platinum contents, CSE activity and expression. For these studies, after removing the capsule, the inner medulla was trimmed off with scissors, and the tissue from renal cortex and outer medulla was homogenized. The other kidney was perfused through the aorta with phosphate-buffered solution (PBS; 0.15 M NaCl and 0.01 M sodium phosphate buffer, pH 7.4). One kidney was removed for analysis of tumour necrosis factor (TNF-α) and platinum contents, CSE activity and expression.

All experimental procedures were conducted in accordance with the principles and procedures outlined in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and the Animal Experimentation Committee of the University of São Paulo at Ribeirão Preto School of Medicine approved the study protocol.

Renal function studies

On post-injection Day 4, the rats of all groups were placed in metabolic cages, and 24-h urine samples were collected to measure creatinine, sodium and potassium. Plasma and urine creatinine were measured by the Jaffe method [26], BUN by a urease Labtest Kit (Labtest Diagnostica S.A., Lagoa Santa, MG, Brazil), and sodium and potassium were determined in plasma and urine using flame photometry (model 262; Micronal, São Paulo, Brazil). The fractional excretions of sodium and potassium were calculated by dividing sodium or potassium clearance by creatinine clearance.

Light microscopy studies

Histological sections (4 μm thick) were stained with Masson’s trichrome and examined under light microscopy. Tubulointerstitial damage was defined as tubular necrosis, inflammatory cell infiltrate and tubular lumen dilation. Damage was graded on a scale of 0 to 4 (0 = normal; 0.5 = small focal areas; 1 = involvement of <10% of the cortex and outer medulla; 2 = 10–25% involvement of the cortex and outer medulla; 3 = 25–75% involvement of the cortex and outer medulla; 4 = extensive damage involving >75% of the cortex and outer medulla) [27]. We also determined the number of tubules with cellular necrosis in the renal cortex and outer medulla per grid field measuring 0.245 mm².

Antibodies

We used the following primary antibody: an anti-rat ED1 monoclonal antibody that only reacts with cytoplasmic antigen present in macrophages and monocytes [28], an anti-rat granulocytes monoclonal antibody for neutrophil infiltration evaluation [29] (Serotec, Oxford, UK) and an anti-rat T-lymphocyte monoclonal antibody (Accurate Chemical, Westbury, USA). The cystathionase (CTH) monoclonal antibody (Abnova, Taipei, Taiwan) was used for the detection of CSE [30,31].

Immunohistochemical studies

Kidney sections were submitted to immunohistochemical studies. The immunohistochemical studies with the anti-rat granulocytes were performed in frozen sections. After fixation in 4% paraformaldehyde, an appropriate portion of the kidney was cryopreserved in 30% sucrose. The 14-μm tissue sections were made and kept frozen (~70° C) until use. For the other immunohistochemical studies, we used paraffin-embedded sections. The kidney sections were incubated for 1 h with 1/1000 monoclonal anti-rat ED1, 1/20 monoclonal anti-rat granulocytes or 1/200 monoclonal anti-rat T-lymphocyte antibodies. The reaction product was detected with an avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA, USA). The colour reaction was developed with 3,3′-diaminobenzidine (DAB; Sigma Chemical Company, St. Louis, MO, USA). The sections were counterstained with methyl green (for ED1 and neutrophils) or Harris haematoxylin (for T lymphocytes), dehydrated and mounted. Negative controls were created by replacing the primary antibody with equivalent concentrations of normal mouse immunoglobulin G (IgG). For evaluation of immunoperoxidase staining, the ED1 (infiltrating macrophages and monocytes)-positive cells, neutrophils and T lymphocytes were counted in the renal cortex and outer medullary tubulointerstitium through examination of 35 grid fields, measuring 0.245 mm² each (15 from the outer medulla and 20 in the renal cortex), and the mean counts per area of 0.245 mm² per kidney were calculated [32].

In situ detection of apoptotic cells

Kidneys were stained with terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine 5 triphosphate-biotin nick end labelling using a commercial kit (OncoGene Research Products, Boston, MA, USA) [33]. Tissues treated with DNase I were used as positive controls, and sections stained without terminal nucleotidyl transferase were used as negative controls. The apoptotic cells in the renal tubulointerstitium were counted in 35 grid fields (20 in renal cortex and 15 in outer medulla), measuring 0.245 mm², and the mean counts per kidney were calculated.

Measurement of TNF-α levels

TNF-α concentrations were measured in the cortex and outer medulla preparation from the kidneys of the animals using enzyme-linked immunosorbent assay (ELISA) [34]. One hundred milligrams of renal tissue was homogenized in 1 mL of PBS (0.4 mM NaCl and 10 mM NaPO4) containing antiproteases (0.1 mM phenylmethylsulphonyl fluoride, 0.1 mM benzonase, 10 mM EDTA and 20 kallikrein inhibitor units of aprotinin A) and 0.05% Tween 20. The samples were then centrifuged for 10 min at 3000 g and the supernatant immediately used for ELISA assay at a 1:5 dilution in PBS. ELISA plate (Nunc MaxiSorb) was coated with sheep anti-rat TNF-α polyclonal antibody (1–2 μg/mL) overnight. The plate was washed three times and then blocked with 1% bovine serum albumin. After a further wash, the plate was incubated with samples or recombinant rat cytokine and incubated overnight. The biotinylated polyclonal antibody was used at 1:1000 to 1:2000 dilutions, and the assay had a sensitivity of 16 pg/mL.

Renal platinum content

Renal tissue samples from rats killed 1 h after saline, CP and PAG+CP injections were analysed by graphite furnace atomic absorption spectrometry (GFAAS) using a Perkin Elmer 4100 ZL model in order to evaluate the renal platinum content [35]. Before platinum analysis, the samples were decomposed according to McGahan and Tyczkowska [36]. Briefly, concentrated nitric acid (0.5 mL) was added to 500 mg of renal tissue and incubated overnight at room temperature. It was then boiled for 3–5 min,
after which 30% v/v H$_2$O$_2$ (0.5 mL) was added, and the solution was boiled again. The resulting clear pale yellow solution was diluted to 50 mL with MilliQ water and analysed by GFAAS. It was found by Misrty et al. [37] that platinum levels in the kidney cortex and medulla and the cytosol reached maximal levels within 1 h of i.p. CP injection.

Assay of tissue H$_2$S synthesis—measurement of CSE activity

Kidney H$_2$S-synthesizing activity was determined as described previously [38]. Whole kidney cortex and outer medulla from the rats were homogenized in potassium phosphate buffer (100 mM; pH 7.4) using a polytron. Each sample (50% weight/volume; 100 μL) contained L-cysteine (10 mM; 20 μL), pyridoxal 5′-phosphate (2 mM; 20 μL) and PBS (30 μL). The reaction was performed in paraffilmed eppendorf tubes and initiated by transferring the tubes from ice to bath at 37°C. After incubation for 2 h, zinc acetate (1% weight/volume; 100 μL) was added to trap evolved H$_2$S followed by tricloroacetic acid (10% weight/volume; 100 μL) to precipitate protein and thus stop the reaction. After centrifugation, N,N-dimethyl-p-phenylenediamine sulphate (20 mM; 50 μL) in HCl 7.2 M followed by FeCl$_3$ (30 mM; 50 μL) in HCl 1.2 M was then added to 50 μL of the supernatant, and optical density was measured at 670 nm. The calibration curve of absorbance vs H$_2$S concentration was obtained using NaHS solution of varying concentration 0.1–100 μg/mL. NaHS was synthesized at the Prof. Dr. Alberto Federman Neto Laboratory (Faculty of Pharmaceutical Sciences of Ribeirão Preto) [16,17].

Western blot for CSE

The tissue from renal cortex and outer medulla was homogenized in lysis buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.1%
SDS, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 mM phenylmethylsulphonyl fluoride, 1 mM sodium orthovanadate, pH 10, 1 mM sodium pyrophosphate, 25 mM sodium fluoride, 0.001 M EDTA, pH 8) at 4°C. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, incubated for 24 h in 30 mL of blocking buffer (PBS, 7% skim milk), washed in buffer (PBS, 0.1% Tween 20, pH 7.6) and incubated with anti-CSE monoclonal antibody (1/3000 in 5% bovine serum albumin) for 3 h at 4°C. To adjust the equivalence of protein loading and/or transfer, the membranes were also incubated with anti-α-tubulin monoclonal antibody (1/15 000 in 5% bovine serum albumin) overnight at 4°C. Blots were washed and incubated with horseradish peroxidase-conjugated goat anti-mouse (1:20 000; Dako, Glostrup, Denmark) for 1 h at room temperature. The membrane-bound antibody was detected using the Supersignal West Pico Chemiluminescent substrate (Pierce, Rockford, IL, USA) and captured on X-ray film. The intensity of the identified lanes was quantified by densitometry using ImageJ NIH image software and was reported in arbitrary units [39].

**Cell culture and hyperosmotic stress induction**

Immortalized human proximal tubule epithelial cells (HK-2) were obtained from ATCC (Manassas, VA, USA) and passaged in the recommended growth medium keratinocyte-serum-free (KSF) medium containing 5 ng/mL recombinant human epithelial growth factor and 0.05 mg/mL bovine pituitary extract (Invitrogen, Carlsbad, CA, USA). Cells were used between passages 3 and 5. Cells were cultured in polystyrene bottles at 37°C in a humidified gas mixture (95% air and 5% CO2). HK-2 cells were treated with 0.9% saline or NaHS (30, 100 or 300 μM) for 24 h. The number of experiments for each group was 3. The cell viability was assessed by the dye exclusion method using trypan blue 0.4% (Sigma-Aldrich, UK). Cells were harvested with trypsin and then diluted 1:1 in trypan blue solution. All the cells which excluded the dye were considered viable. The results are expressed as percentage of viable cells [40].

**Statistical analysis**

Data concerning plasma creatinine and BUN levels, urine volume, scores for tubulointerstitial lesions and tubular cell necrosis were analysed statistically using the nonparametric Kruskal–Wallis test followed by the Dunn post-test. Those data are expressed as median and interquartile range (25–75%). For data related to other parameters studied, we used analysis of variance with the Newman–Keuls multiple comparisons test. Those data are expressed as mean ± SEM. The level of statistical significance was set at P < 0.05.

**Results**

**Renal function**

CP-treated rats presented increases in plasma creatinine and BUN levels, urinary volume and sodium and potassium fractional excretions. Treatment with PAG reduced all these alterations (Table 1). Rats treated with PAG alone did not present differences in any parameter of renal function studied, as compared with their respective controls.
Light microscopy studies

The score for tubulointerstitial lesions (tubular necrosis, interstitial inflammatory infiltrate, tubular lumen dilation) was increased on Day 5 after CP injection alone in the renal outer medulla [3.50 (3.00; 4.00)] compared with animals treated with PAG+CP [2.00 (0.00; 3.00)]. The number of tubules with cellular necrosis in the outer medulla was also lower in these animals, compared with CP-alone-treated rats (Figure 1). We did not observe any tubulointerstitial lesions or tubules with cellular necrosis in the kidneys of the rats injected with PAG only.

Immunohistochemical studies

The immunohistochemical studies showed increased numbers of ED1-positive cells (Figure 2), T lymphocytes (Figure 3) and neutrophils (Figure 4) in the renal cortex and outer medulla from animals injected with CP compared with controls. PAG treatment attenuated the number of ED1-positive cells and neutrophils in the outer medulla. It was also observed that the number of T lymphocytes was reduced in both renal cortex and outer medulla from PAG+CP-injected rats compared with CP group. There was no difference in the numbers of ED1-positive cells, T lymphocytes, or neutrophils in the kidney from PAG only-injected rats compared with controls.

In situ detection of apoptosis

CP-treated rats also presented a higher number of apoptotic cells in the outer medulla compared with the controls on Day 5 after CP injection. This alteration was attenuated by treatment with PAG (Figure 5). There was no difference in the number of apoptotic cells in the kidneys of PAG-only-injected rats compared with controls.

Renal TNF-α content

Increase in TNF-α content in renal cortex and outer medulla was observed in CP-injected rats compared with control and PAG+CP rats (Figure 6). PAG-only injections did not affect the renal content of TNF-α in the animals.

Renal platinum content

Higher platinum renal contents, expressed as micrograms per gram of renal tissue, were observed in the kidneys from CP-injected rats at 1 h (10.94 ± 0.51) after CP injection, compared with controls (0 ± 0) (P < 0.001). This content was not affected by PAG injection (11.00 ± 0.21),
showing that this treatment did not interfere with CP uptake by renal tubular cells.

**Assay of H₂S synthesis**

Renal H₂S tissue concentration (μg/mg protein) was increased in the kidney from the animals injected with CP (51.60 ± 4.54), compared with controls (39.69 ± 0.69; P < 0.01), to PAG-injected (31.35 ± 3.14; P < 0.001) and to PAG+CP-injected rats (24.30 ± 1.95; P < 0.01) (Figure 7).

**Western blot for CSE**

Western blot analysis performed with anti-CSE antibody demonstrated the presence of a 44-kDa protein lane in the tissue samples from the renal cortex and outer medulla of all groups studied on Day 5. The increase in CSE expression induced by CP was prevented by treatment with PAG. There was no difference in the intensity of the lanes for α1-tubulin between the different groups, showing the equivalence of protein loading and transfer (Figure 8).

**Cell viability**

Exposure in vitro of tubular cells to NaHS (30, 100 or 300 μM) for 24 h did not significantly modify the cell viability compared with cells exposed to saline (Control). In the Control group, the cell viability was 89.25 ± 3.32%. In the groups treated with NaHS, the cell viability was: 95.13 ± 1.92% (for 30 μM NaHS), 95.88 ± 0.28% (for 100 μM NaHS) and 92.52 ± 2.69% (for 300 μM NaHS).

**Discussion**

Inflammatory events have an important role in CP-mediated renal damage [13,14]. Cisplatin can induce endothelial dysfunction and neutrophil infiltration, which can lead to release of cytokines [41,42]. The released cytokines activate the surrounding cells and induce the migration of leukocytes, enhancing the presence of these cells in the inflammatory site. Increase in pro-inflammatory cytokines such as TNF-α, regulated on activation, normal T cell expressed and secreted (RANTES), chemoattractant protein (MCP)-1 and intercellular adhesion molecule (ICAM) gene expression was observed in the kidneys from the CP-injected rats [41].

TNF-α-deficient mice were resistant to CP nephrotoxicity, and treatment with TNF-α inhibitors attenuates the renal damage induced by CP [42]. TNF-α is an important pro-inflammatory cytokine that can up-regulate its own expression and that of other genes involved in the inflamma-
tory response [41–43] and contributes to the increase in neutrophil accumulation in the kidneys from CP-treated rats. Neutrophil activation can lead to upregulation of cell surface integrin expression, adhesion to the endothelium, diapedesis and transmigration, and besides this, it can enhance tubular injuries via releasing oxidant products or elastase-like proteinases [44]. Neutrophils also release cytotoxic and pro-inflammatory mediators such as arachidonic acid metabolites, cytokines, oxygen and nitrogen-derived free radicals [45].

Brooks et al. [46] have recently shown that CP can induce apoptosis through mitochondrial fragmentation in...
proximal tubular cells. It was described that exogenous and endogenous H2S can very potently protect mitochondria [47] and the kidney [48,49] from oxidative stress and reduce the associated inflammation and apoptosis. On the other hand, our results show that H2S aggravates the renal functional and structural disturbances provoked by CP. However, despite the increased biosynthesis of H2S that has been demonstrated in several animal models of inflammatory disease [19,23,50,51] and the inhibition of the H2S formation that reduces the inflammation in these animals, the exact role of this gas in the inflammatory process has not been clarified yet [52,53]. There are also evidences that H2S in physiological concentrations presents anti-inflammatory effects, indicating a dual role of this gas in inflammation. Experimental studies show that the treatments with H2S donors can also reduce edema formation and leukocyte adherence to the vascular endothelium and inhibit pro-inflammatory cytokine synthesis [51]. Therefore, it has been suggested, based on these experimental evidences, that H2S in physiological concentrations has anti-inflammatory effects while in higher concentrations can present pro-inflammatory effects [51].

Our results also show that the role of H2S in the inflammatory process cannot be direct, since the incubation of tubular epithelial cells with NaHS (a donor of H+ and S2− for synthesis of H2S) did not modify the cell viability. However, the role of endogenous H2S in the recruitment of neutrophils has been demonstrated in many inflammatory models [17,23,24]. It has been observed that increased production of H2S during inflammation modulates neutrophil rolling and adhesion as well as their locomotion. Dal-Secco et al. [17] showed that H2S mediates the increase of ICAM-1 in the endothelial cells of mesenteric vessels induced by LPS challenge in the peritoneal cavity. Although the exact mechanism by which PAG reduces the neutrophil infiltration in the kidneys of CP-treated animals is not known yet, the study of Dal-Secco et al. suggests that the PAG effect can be related with neutrophil adhesion and locomotion by a mechanism dependent on ATP-sensitive potassium channels [17].

The administration of NaHS in mice increased the activity of myeloperoxidase (MPO) (a marker for neutrophil infiltration) in liver and kidney, promoted the accumulation of neutrophils in tissues and increased the concentrations of plasma TNF-α [19]. In a model of sepsis in mice, Zhang et al. [24] observed that H2S may lead to increased tissue levels of adhesion molecules and promote leukocyte–endothelium interaction in sepsis through a mechanism involving the activation of NF-κB. According to these authors, the H2S also facilitates the migration of polymorphonuclear cells through the activation of receptors for chemokines. However, the mechanism may be indirect, by the interception of peroxynitrite (formed by the interaction of NO and superoxide anion). As NO and superoxide anion are important regulators of leukocyte function, it is possible that the main effect of H2S is to modulate the tissue concentrations of these mediators [54].

In the present study, H2S was shown to play a role in the renal damage induced by CP. The animals treated with PAG+CP presented less renal damage compared with those injected with CP alone. This protective effect seems to be related with the inflammatory process that was less intense in the PAG+CP group. We observed that treatment with PAG reduced the increase of T lymphocytes induced by CP in the renal cortex and outer medulla. The increases in the number of macrophages, neutrophils and apoptotic cells were less intense in the cortex, where the damage was less intense. Cisplatin is taken up by renal tubular cells, reaching its higher concentrations in the tubular proximal cells of the inner cortex and outer medulla, especially in the S3 segment. Therefore, the main site of CP-induced damage in the kidney is the outer medulla where the in-
flamatory process and the apoptosis were more intense, while a smaller damage was observed in the renal cortex. Besides this, in a recent study [55], we observed that the number of apoptotic cells was higher 2 days after CP treat-
ment, while its population progressively decreased by the fifth day. Apoptotic cells and bodies are rapidly phagocy-
tosed and destroyed by macrophages and neighbouring
cells [56]. Therefore, apoptosis is usually inconspicuous in tissue sections even when it is responsible for extensive and rapid cell loss [5].

The structural alterations observed in the outer medulla in CP-treated rats can lead to increase in plasma creatinine and urea levels and in the fractional excretion of sodium and potassium that were attenuated by PAG treatment. PAG decreases H2S formation through the inhibition of CSE activity [57], which was confirmed in our study by the quantification of H2S formation rate in renal tissue. According to Whiteman and Moore [58], it is possible that this spectrophotometric assay measures the sum of H2S-derived species such as HS− and S2− that exist at physiological pH rather than H2S itself. The increased expression of CSE induced by CP injection was also decreased by PAG treatment. However, we cannot exclude in our study the interactions with other inflammatory mediators, such as NO or NF-κB, despite several studies that showed that PAG provokes specific blockage in CSE activity [23–25].

Our data also showed that platinum concentration in the kidney tissue from PAG+CP group was not different from the rats treated with CP alone, although they presented a reduction in renal damage. Therefore, this effect of PAG was not associated with changes in the renal CP handling. This hypothesis was supported by the fact that the levels of platinum in renal tissue were similar in these groups 1 h after CP injection, when the platinum in the renal cortex and medulla reached maximal levels [37].

Taken together, these data show that treatment with PAG, an inhibitor of endogenous H2S formation, reduces the renal damage induced by CP in rats. This effect might be related, at least in part, to the reductions in the apoptosis and inflammatory response observed in PAG+CP-treated animals. These data also suggest that the reduction on H2S formation may represent a novel approach to reduce CP-induced renal damage.

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