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Trimetazidine Protects from Mercury-Induced Kidney Injury

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Keywords
Trimetazidine · Mercuric chloride · Nephrotoxicity · Renoprotection

Abstract

Introduction: The presence of mercury in the environment is a worldwide concern. Inorganic mercury is present in industrial materials, is employed in medical devices, is widely used in batteries, is a component of fluorescent light bulbs, and it has been associated with human poisoning in gold mining areas. The nephrotoxicity induced by inorganic mercury is a relevant health problem mainly in developing countries. The primary mechanism of mercury toxicity is oxidative stress. Trimetazidine (TMZ) is an anti-ischemic drug, which inhibits cellular oxidative stress, eliminates oxygen-free radicals, and improves lipid metabolism. The aim of this study was to evaluate whether the administration of TMZ protects against mercuric chloride ($HgCl_2$) kidney damage. ***Methods:*** Adult male Wistar rats received only $HgCl_2$ (4 mg/kg bw, sc) (Hg group, $n = 5$) or TMZ (3 mg/kg bw, ip) 30 min before $HgCl_2$ administration (4 mg/kg bw, sc) (TMZHg group, $n = 7$). Simultaneously, a control group of rats ($n = 4$) was studied. After 4 days of $HgCl_2$ injection, urinary flow, urea and creatinine (Cr) plasma levels, Cr clearance, urinary glucose, and sodium-dicarboxylate cotransporter 1 (NaDC1) in urine were determined. Lipid peroxidation (MDA) and glutathione (GSH) levels were measured in kidney homogenates. ***Results:*** Rats only treated with $HgCl_2$ showed an increase in urea and Cr plasma levels, urinary flow, fractional excretion of water, glucosuria, and NaDC1 urinary excretion as compared with the control group and a decrease in Cr clearance. TMZHg group showed a decrease in urea and Cr plasma levels, urinary flow, fractional excretion of water, glucosuria, NaDC1 urinary excretion, and an increase in Cr clearance when compared to the Hg group. Moreover, MDA and GSH levels observed in Hg groups were decreased and increased, respectively, by TMZ pretreatment. ***Conclusion:*** TMZ exerted a renoprotective action against $HgCl_2$-induced renal injury, which might be mediated by the reduction of oxidative stress. Considering the absence of toxicity of TMZ, its clinical application against oxidative damage due to $HgCl_2$-induced renal injury should be considered. The fact that TMZ is commercially available should simplify and accelerate the translation of the present data "from bench to bedside." In this context, TMZ become an interesting new example of drug repurposing.

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Introduction

Heavy metals are naturally occurring elements in earth's crust. Unfortunately, their natural biogeochemical cycles have been altered by uncontrolled anthropogenic activity [1]. Heavy metals are persistent in the environment since they are not degradable, exerting their toxic effects on microorganisms, plants, animals, and humans. Therefore, at present, heavy metal pollution has become one of the biggest environmental problems and is a growing concern for the future [1]. Mercury is between the first places in the priority list of these hazardous substances. Mercury concentration is rising continuously due to its increased industrial, medicinal, and domestic uses. Coal combustion in thermal power plants is the major anthropogenic source of mercury in the environment. Mercury is taken up by plant roots from contaminated soil and through bioaccumulation in the plant system that enters into the food chain, causing risks to health and ecology [2]. In addition, developing countries have also growing health problems and environmental risks due to the increased exposure of mercury in small-scale mining (mainly artisanal and illegal) [3].

As the kidney is involved in detoxification and excretion functions, most environmental toxins are concentrated by the kidney. Therefore, the kidney is highly vulnerable to environmental contaminants. Inorganic species of mercury are the most nephrotoxic species by far [4, 5]. After exposure to mercury compounds, inorganic mercury has a nonuniform distribution and mainly accumulates in the kidneys, causing acute renal injury [5]. Mercuric ions (Hg^{2+}) rapidly accumulate in renal proximal tubule cells [5]. In this sense, it has been described that the amount of mercury in the blood decreases rapidly after exposure to inorganic salts of mercury, suggesting that target cells such as proximal tubule cells are absorbing mercuric ions. Moreover, renal uptake and accumulation of mercury in vivo are very rapid. Up to 50% of an inorganic mercury dose has been shown to be present in the kidneys of rats within hours of exposure [4–6]. Furthermore, Blanuša et al. [7] reported a slow elimination of mercury from internal organs since the levels of mercury decreased in the blood and urine in a time-dependent manner, while the mass fractions of mercury in different organs, such as the kidney and the liver, remained relatively constant during 6 days following parenteral exposure to mercuric chloride ($HgCl_2$) in rats.

The renal accumulation of Hg^{+2} causes mitochondrial dysfunction, DNA peroxidation, and metabolic disorders. Mercury can alter different signal pathways and cause the depletion of antioxidants which contain thiol, inducing structural and functional damage. The primary mechanisms of cytotoxicity during the development of cellular dysfunction are oxidative stress and cell apoptosis [5].

Trimetazidine (TMZ) is a cellular anti-ischemic agent which improves ischemic myocardial energy through inhibition of fatty acid oxidation secondary to a direct inhibition of long-chain 3-ketoacyl coenzyme A thiolase activity with the consequent reduction in oxygen consumption. This drug has a protective effect on the heart by inhibiting oxidative stress, eliminating oxygen-free radicals, and improving lipid metabolism [8]. Considering these pharmacological characteristics of TMZ, its protective effects on the kidney have been experimentally assessed in different models: cell culture, isolated and perfused kidneys, and in vivo models. These studies have demonstrated the potential of TMZ to prevent kidney damage from diverse etiologies such as ischemia and reperfusion, storage and transplantation, contrast-induced nephropathy, diabetic nephropathy, and nephrotoxic damage induced by cisplatin or cyclosporine A [8–12]. On that subject, it has been described that TMZ can prevent renal injury by ameliorating oxidative stress through inhibition of apoptosis and enhancement of antioxidant enzyme levels [9].

Mercury toxicity is an emerging problem in the world. The need for effective therapies in dealing with mercury intoxication associated with mercury exposure in human is an important concern. Since oxidative stress and endogenous thiol depletion are involved in Hg^{+2} toxicity, the aim of the present work was to study if the administration to rats of a single dose of TMZ (3 mg/kg, body weight (bw)) 30 min before a nephrotoxic dose of $HgCl_2$ (4 mg/kg bw) could contribute to protect against the renal deleterious effects of $HgCl_2$.

Methods

Reagents

Pure chemicals of analytical grades were acquired from Sigma-Aldrich (St. Louis, MO, USA). Noncommercial polyclonal antibody against sodium-dicarboxylate cotransporter (NaDC1) was generously provided by Prof. N. Anzai (Department of Pharmacology, Graduate School of Medicine, Chiba University, Japan).

Experimental Animals

Adult (90–120 days) male Wistar rats were used (n = 16). The animals had access to tap water and standard laboratory chow, ad libitum, with a temperature and humidity-controlled environment on a 12:12 h light cycles. All experiments were conducted

according to the National Institute of Health (NIH) Guide for the Care and Use of Laboratory and were approved by the Faculty of Biochemical and Pharmaceutical Sciences (UNR) Institutional Animal Care and Use Committee (Res No. 385/2017).

Experimental Protocols

Tablets containing 35 mg TMZ (Vastarel®, Servier) were crushed and suspended in saline solution (9 g/L NaCl) to yield a concentration of 3 mg/mL and appropriately warmed to body temperature before injection. The concentration of the solution was corroborated by the spectrophotometric method described by Chiş et al. [13]. The dose of TMZ and the time of its administration were chosen on the basis of previous studies [12, 14, 15].

Rats were randomly divided into 3 experimental groups: control ($n = 4$), rats treated with $HgCl_2$ (Hg) ($n = 5$), and rats treated with TMZ plus $HgCl_2$ (TMZHg) ($n = 7$). A single nephrotoxic dose of $HgCl_2$ (4 mg/kg bw, subcutaneous (sc), equivalent to 14.7 μmol/kg bw) was employed. TMZ was administered by an intraperitoneal (ip) injection as a single dose (3 mg/kg bw, equivalent to 8.8 μmol/kg bw) 30 min before the injection of $HgCl_2$ [5, 16]. Control rats received the corresponding vehicle injections (saline solution, 1 ip injection followed by 1 sc injection, both in a ratio of 1 mL saline/kg bw). After the $HgCl_2$ or vehicle injection, the animals were placed in individual metabolic cages where water and food were provided ad libitum. After 3 days, the animals were deprived of food, and urine was collected during 24 h. On the 4th day after $HgCl_2$ or vehicle administration, animals were anesthetized with an ip dose of sodium thiopental (70 mg/kg bw), and blood samples were obtained by cardiac puncture and both kidneys were removed. Finally, the rats were euthanized with an anesthetic overdose and thoracotomy, as previously reported [17].

Biochemical Determinations

Urine volume was estimated by gravimetry, and the urinary flow (Uf) was calculated and expressed as mL/min/100 g bw. Urine samples were centrifuged (1,000 *g* for 10 min) to remove cells and cell debris. They were used to determine glucose, Cr (Cr_u), and NaDC1 protein abundance. Blood samples obtained with heparinized syringes were centrifuged at 1,000 *g* (10 min) to obtain blood plasma. Plasma samples were used to measure levels of urea and Cr (Cr_p). Plasma urea, Cr_p, and Cr and glucose concentrations in urine were determined with commercial kits (Wiener Laboratory, Rosario, Argentina). Cr clearance (Cr_{Cl}) was calculated as $[(Cr_u \times Uf)/Cr_p]$. Fractional excretion of water (FE $\%_{H2O}$) was calculated employing the following formulae $[(Uf/Cr_{Cl}) \times 100]$.

Electrophoresis and Immunoblotting Studies

Electrophoresis and Western blotting were performed as previously described [16, 18, 19]. In a mix of 1% 2-mercaptoethanol and 2% sodium dodecyl sulfate, urine samples were boiled for 3 min. Proteins were separated through 8.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and then electroblotted to a pure nitrocellulose membrane (NC membrane) (Trans-Blot® Transfer Medium, Bio Rad Laboratories, Hercules, CA, USA). The membranes were incubated with noncommercial rabbit polyclonal antibody against rat NaDC1 overnight at 4°C. Specificity of NaDC1 antibody has been described elsewhere [19, 20]. The detection of blots was performed with commercial chemiluminescent reagents (Pierce™ ECL Western Blotting Substrate, IL, USA). The densitometric quantification of the chemiluminescent signals intensity was performed using the Gel-Pro Analyzer (Media Cybernetics, Silver Spring, MD, USA) software.

Assessment of Glutathione and Lipid Peroxidation Levels in Kidney

Lipid peroxidation (LPO) and glutathione (GSH) levels were measured in kidney homogenates as described by Montagna et al. [21]. On the day of the experiments, both kidneys were excised, decapsulated, washed in saline solution, and then were dried and weighed. Determination of GSH was carried out in renal homogenates prepared in a cold solution of 5% trichloroacetic acid in 0.01M HCl (0.2 g tissue/mL). The GSH content was measured at 412 nm using Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid)). The quantification of LPO was performed in homogenates prepared in 1.15% KCl (0.11 g/mL) by the reaction of malondialdehyde (MDA) with thiobarbituric acid. To perform these assays, both kidneys from each rat (4 rats for each group) were employed.

Statistics

ANOVA plus Newman-Keuls test was used to perform statistical comparisons between the 3 experimental groups. An unpaired Student's *t* test was employed for comparing 2 experimental groups. Differences were considered statistically significant when $p < 0.05$. Data were expressed as the means ± standard error (SE). The effect size was computed as partial eta-squared values (η_p^2; small, ≥ 0.01; medium, ≥ 0.06; large, ≥ 0.14) or as Cohen's *d* (*d*; small, = 0.2; medium, = 0.5; large ≥ 0.8) as appropriate [22, 23]. For statistics studies, a GraphPad 6 software (San Diego, CA, USA) was employed.

Results

Analyses of variance (ANOVA) revealed an overall statistically significant difference among the 3 groups for plasma urea levels ($F = 19.87$, $p < 0.0001$, $\eta_p^2 = 0.739$), plasma Cr levels ($F = 15.98$, $p = 0.0003$, $\eta_p^2 = 0.711$), and Cr clearance ($F = 51.75$, $p < 0.0001$, $\eta_p^2 = 0.888$). Newman-Keuls post-test identified the existent significant differences between groups as it is shown in Figure 1. In the Hg group, plasma urea and Cr levels were significantly higher than the control group. Cr clearance was decreased by $HgCl_2$. When rats received TMZ prior to $HgCl_2$, the increase in plasma urea and Cr concentrations was lower than in rats treated only with $HgCl_2$. The decrease in Cr clearance in TMZHg rats was significantly less marked than in Hg ones.

Statistically significant differences (using ANOVA test) were found among the 3 groups for urinary flow ($F = 16.35$, $p < 0.0003$, $\eta_p^2 = 0.715$) and for fractional excretion of water ($F = 7.36$, $p = 0.0073$, $\eta_p^2 = 0.530$). Figure 2 shows the results of multiple comparisons between groups using the post hoc test. Compared with the control group, the urinary flow and the fractional excretion

of water after 4 days of the $HgCl_2$ dose were significantly higher. In TMZHg rats, the increase in both the urinary flow and the fractional excretion of water was significantly lesser than the Hg group.

ANOVA test showed statistically significant difference between the 3 groups for glucose urinary excretion (F = 7.67, $p < 0.0056$, $\eta_p^2 = 0.523$) and for NaDC1 urinary excretion ($F = 28.55$, $p < 0.0001$, $\eta_p^2 = 0.864$). Post hoc analysis demonstrated significant differences between the mean of each group with the mean of every other group as it is observed in Figure 3. Urinary glucose and NaDC1 excretions were markedly increased 4 days after $HgCl_2$ injection when compared with the control group. The levels of these parameters were related to urinary Cr in order to correct variations in urine production as previously described [16, 18, 19, 24]. NaDC1 is expressed in the apical membrane of proximal tubule cells, and its main function is to reabsorb the filtered Krebs cycle intermediates [25]. Our investigation group has postulated the urinary excretion of NaDC1 as a biomarker of renal injury in different pathologies [19, 20]. NaDC1 urinary levels were determined by immunoblotting, and densitometric quantification of NaDC1 from urine was expressed as arbitrary units relative to urinary Cr concentration [19]. Pretreatment with TMZ significantly decreased glucosuria and NaDC1 urinary excretion as observed for rats treated with TMZ and $HgCl_2$ compared with rats only receiving $HgCl_2$.

Compared to the Hg group, LPO level was markedly decreased in the rats that received TMZ prior to the $HgCl_2$ dose (nmol MDA/g tissue; Hg: 4.77 ± 0.60, TMZHg: 2.90 ± 0.42, t = 2.553, p = 0.0433, d = 1.474, n = 4 for each group). Moreover, the corresponding levels of GSH in the Hg group and the TMZHg group were increased from 4.81 ± 0.32 μmol/g tissue to 6.37 ± 0.21 μmol/g tissue, respectively (t = 4.076, p = 0.0065, d = 2.3533, n = 4 for each group). The Cohen's d values represent a large effect size of TMZ pretreatment before $HgCl_2$ injection on LPO and GSH levels as compared with only $HgCl_2$ administration.

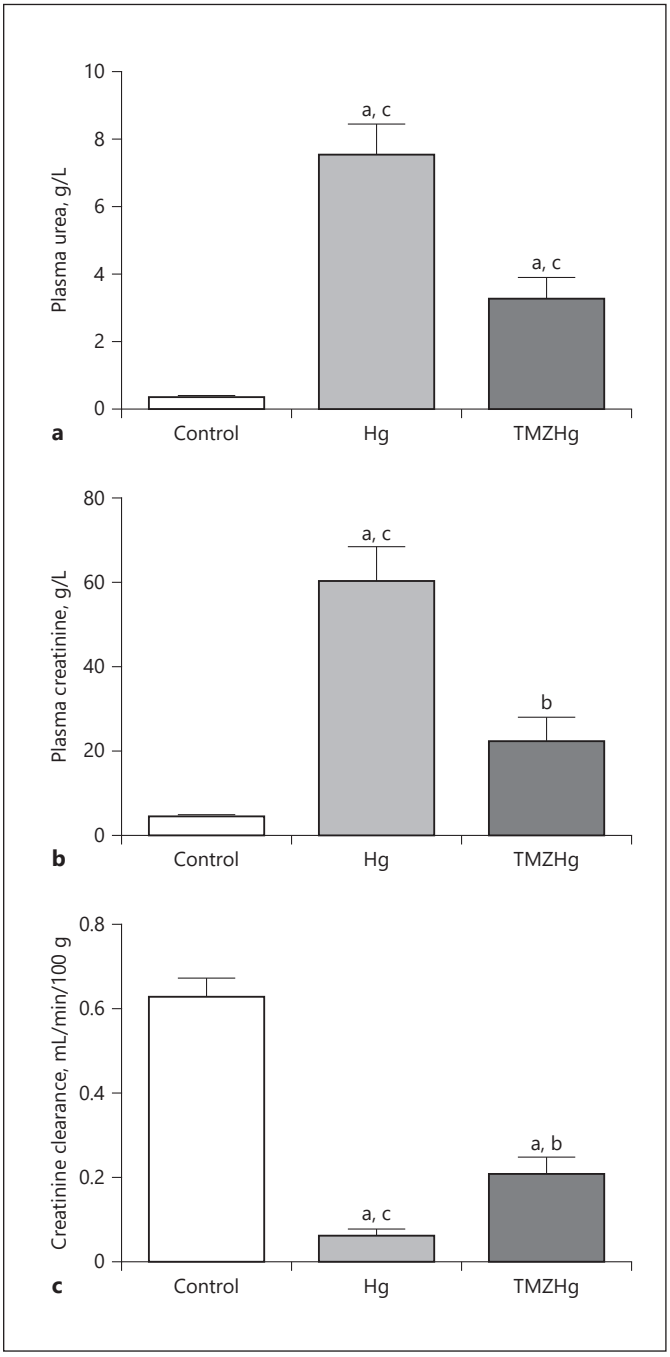

Fig. 1. Plasma urea (**a**), plasma Cr (**b**), and Cr clearance (**c**) in control, Hg, and TMZHg groups. Control = control rats (n = 4), Hg = rats treated with $HgCl_2$ (4 mg/kg bw, sc, n = 5), TMZHg = rats treated with TMZ (3 mg/kg bw, ip) 30 min before $HgCl_2$ administration (4 mg/kg bw, sc) (n = 7). Studies were performed 4 days after $HgCl_2$ injection. Results are expressed as mean values ± SE $p < 0.05$. [a]Versus control, [b]versus Hg, [c]versus TMZHg. TMZ, trimetazidine; $HgCl_2$, mercuric chloride; SE, standard error.

Discussion

The presence of mercury in the environment remains a complicated health problem, mainly in developing countries. With regard to the toxicity of mercury, 3 major chemical forms of the metal must be distinguished: elemental mercury, salts of mercury, and organic mercurials. It is well known that all forms of mercury damage the kidney and both inorganic and organic forms of mercury

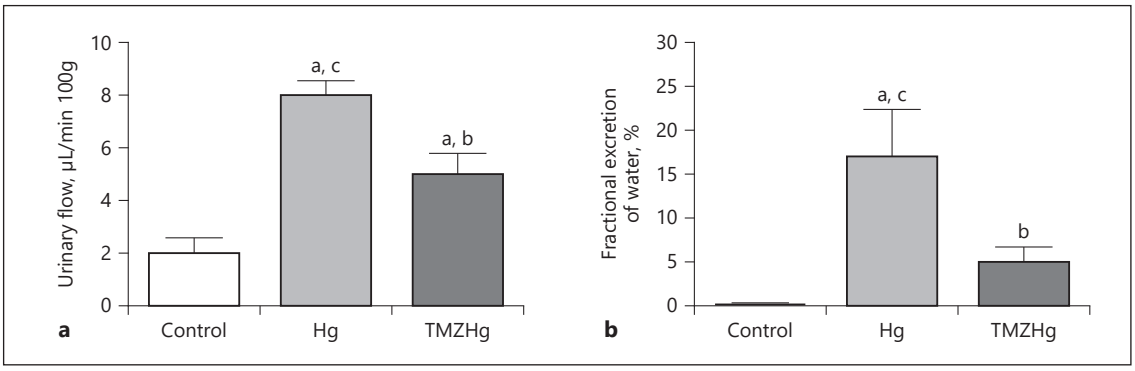

Fig. 2. Urinary flow (**a**) and fractional excretion of water (**b**) in control, Hg, and TMZHg groups. Control = control rats ($n = 4$), Hg = rats treated with $HgCl_2$ (4 mg/kg bw, sc, $n = 5$), TMZHg = rats treated with TMZ (3 mg/kg bw, ip) 30 min before $HgCl_2$ administration (4 mg/kg bw, sc) ($n = 7$). Studies were performed 4 days after $HgCl_2$ injection. Results are expressed as mean values ±SE $p < 0.05$. [a]Versus control, [b]versus Hg, [c]versus TMZHg. TMZ, trimetazidine; $HgCl_2$, mercuric chloride; SE, standard error.

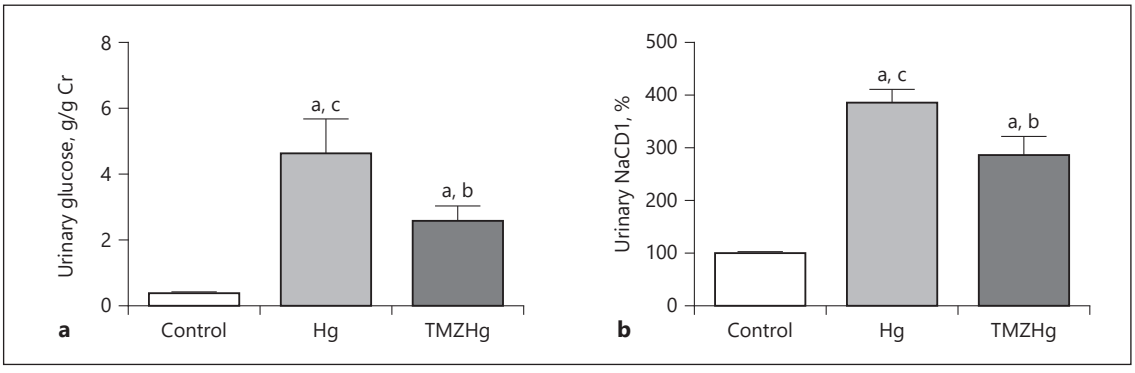

Fig. 3. Urinary glucose (**a**) and NaDC1 abundance in urine (**b**) in control, Hg, and TMZHg groups. Control = control rats ($n = 4$), Hg = rats treated with $HgCl_2$ (4 mg/kg bw, sc, $n = 5$), TMZHg = rats treated with TMZ (3 mg/kg bw, ip) 30 min before $HgCl_2$ administration (4 mg/kg bw, sc) ($n = 7$). Studies were performed 4 days after $HgCl_2$ injection. Results are expressed as mean values ±SE $p < 0.05$. In Fig. 3b, the results are expressed as percentages, and the mean of control levels was set as 100%. $p < 0.05$. [a]Versus control, [b]versus Hg, [c]versus TMZHg. TMZ, trimetazidine; $HgCl_2$, mercuric chloride; NaDC1, sodium-dicarboxylate cotransporter 1; SE, standard error.

accumulate readily in the kidney. Moreover, elemental mercury, methylmercury, and ethylmercury, which mainly cause toxicity to the central nervous system, are also fully or partially converted to the inorganic form which is highly nephrotoxic [5, 26–28]. The most common route of human exposure to mercury is via the ingestion of food, primarily fish, contaminated with methylmercury. Nevertheless, the inorganic forms of mercury such as $HgCl_2$ are by far more acutely nephrotoxic, and the use of $HgCl_2$ is an established chemical model for toxic kidney responses [4, 5]. Mercury salts are currently used as catalyst or reagents in several chemical reactions in chlor-alkali and caustic soda industries and the manufacturing of electrical switches use mercury electrodes. Moreover, inorganic mercury is a common ingredient in soaps and creams intended to lighten the skin. On the other hand, there are some cultural and religious practices that use mercury as well as the use of Ayurvedic medicines (Indian herbal-metallic preparations). By last, artisanal and small-scale gold mining employ mercury to extract gold [4, 5]. In connection with this, $HgCl_2$ intoxications are observed today in emergency departments and morgues, representing a challenge for the specialists involved (forensic toxicologists and pathologists) [6]. For

these reasons, there is an emerging field of mercury research in the preventive and therapeutic role of different compounds to counteract $HgCl_2$ poisoning in the kidney [24, 29–36].

In the present renal model of mercury nephrotoxicity, renal function was impaired after 4 days of administration of the $HgCl_2$ dose (4 mg/kg bw, sc) as assessed by the increased urea and Cr plasma levels and the decrease in Cr clearance (which is used to estimate glomerular filtration rate). The intense efferent arteriolar vasoconstriction and/or the impaired tubular solute and fluid reabsorption induced by $HgCl_2$ could result in a rise in tubular hydraulic pressure and may contribute to decrease the glomerular filtration rate as previously described [5, 37]. The urinary flow, the fractional excretion of water, the urinary excretion of glucose, and NaDC1 protein abundance in urine were altered the 4th day after $HgCl_2$ dose, evidencing a still impaired reabsorptive capacity of the nephrons at this point of time, as a functional consequence of the tubular injury-induced by $HgCl_2$. Acute kidney injury can be defined as an abrupt reduction in glomerular filtration rate that can be observed through the marked increase in plasma concentrations of urea and Cr and may or may not be accompanied by a decrease in urine production [38]. Previous studies reported by Hazelhoff et al. [16] described the decrease in urinary flow 18 h after the administration of $HgCl_2$ (4 mg/kg bw). These data would indicate that the renal damage induced by this dose of $HgCl_2$ would progress from a serious oliguria during the 1st hours after injury to a state of polyuria 4 days post-injection.

TMZ is an anti-anginal and anti-ischemic drug that was originally developed to improve myocardial energy metabolism. It has the potential to be used to treat the metabolic remodeling of heart failure (HF). TMZ can inhibit the excessive release of oxygen-free radicals, increase glucose metabolism, limit intracellular acidosis, and prevent the accumulation of protons, sodium, and calcium in muscle cells during cardiac ischemic [9, 39, 40]. Besides beneficial effects on the heart, several studies have shown the protective effects of TMZ on kidney injury [8–12, 14, 15, 40].

The pretreatment with TMZ improved the $HgCl_2$-induced renal injury. The TMZHg group showed a reduced plasma urea and Cr levels and an increased Cr clearance with respect to Hg group. In addition, TMZ restored the fractional excretion of water and reduced urine flow. The last one is an important point because polyuria can be incapacitating for patients as it can disrupt their daily activities and sleeping. Moreover, an important polyuria can result in volume depletion, fluctuations in serum sodium levels, and distension of the renal tract [41]. In addition, pretreatment with TMZ improved the tubular damage induced by $HgCl_2$, as assessed by glucosuria and the urinary excretion of the proposed renal damage biomarker NaDC1. Glucosuria was shown as a greater sensitive indicator of $HgCl_2$ exposure than the other standard clinical urinary indices reported by Kyle et al. [42]. The urinary excretion of NaDC1 was postulated as an early biomarker of obstructive nephropathy that also gives information about the duration of the obstruction because of its increase after short times of ureteral obstruction and the positively correlation with the time elapsed after obstruction [19].

TMZ exerts its functions on myocardial energy metabolism through several mechanisms. The mechanism that has been most studied and described has to do with its role as a reversible competitive inhibitor of mitochondrial 3-ketoacyl coenzyme A thiolase (3-KAT), an enzyme that catalyzes the terminal reaction of fatty acid beta-oxidation. Different studies showed that 3-KAT in the mitochondrial matrix inhibit pyruvate dehydrogenase. Thus, by inhibiting 3-KAT, TMZ could increase pyruvate dehydrogenase activity, which in turns substantially increase myocardial glucose oxidation and increase the oxygen use during myocardial hypoxia [40, 43]. In addition, Li et al. [43] proposed that a potential mechanism for the cardioprotective effect of TMZ linked with the regulation of adenosine monophosphate-activated protein kinase expression, where activation of adenosine monophosphate-activated protein kinase in turns increase glucose uptake via glucose transporter 4, and increase fatty acid uptake and oxidation by increasing the activity of carnitine palmitoyltransferase 1. Besides, it has also been observed that TMZ modifies the production and utilization of ketone bodies, which in turns improves cardiac function with an enhancement of ATP production. It has also been suggested that TMZ could exert a cardioprotective effect through decreasing reactive oxygen species (ROS) production. About that, Dedkova et al. [44] have shown in a rabbit model of HF that TMZ protects myocytes against mitochondrial permeability transition pore opening by attenuation of ROS generation by the mitochondrial electron transport chain (ETC) and uncouples mitochondrial nitric oxide synthase. Most of ROS generated by the ETC in HF emerge from enhanced complex II-mediated electron leak. TMZ seems to inhibit the elevated electron leak at the level of mitochondrial ETC complex II and to improve impaired activity of mitochondrial complex I, by that means restoring redox balance and mitochondrial membrane potential in HF [44].

$HgCl_2$ can induce the production of peroxides and superoxide anion radicals causing oxidative stress that can lead to the peroxidation of membrane lipids, protein denaturation, DNA damage, and cellular injury. Moreover, it has been demonstrated that mercury induces alterations in the mitochondrial inner membrane, which would lead to an increase in H_2O_2 in the mitochondria ETC and consequently a decrease in mitochondrial levels of GSH. These mercury-induced changes in the normal functioning of the mitochondria would lead to cells death by apoptosis and/or necrosis [4, 5]. In the present work, TMZ treatment, prior to the $HgCl_2$ administration, increased GSH levels in a 23% and decreased MDA values in a 60%. In this sense, other than the beneficial effects on the heart, protective effects of TMZ on kidney injuries have also been observed by other authors. Fu et al. [45] speculate that by enhancing mitochondrial activity in the kidney, TMZ reduces oxygen-free radical release, and reduces the toxicity of compound such as contrast agents to renal tubular epithelial cells. Also, TMZ could increase kidney glucose metabolism, reduce fatty acid oxidation, and also could have a protective effect on kidney-free radical damage. Amini et al. [9] have demonstrated that TMZ administration (5 mg/kg) before the beginning of reperfusion in a rat model of renal ischemia/reperfusion (I/R) injury increased antioxidant enzymes in the kidneys improving renal function. Mahfoudh-Boussaid et al. [12] have shown that pretreatment with TMZ reversed the oxidative stress caused by warm renal I/R finding a significant lower MDA and higher GSH level as consequence of an increase in antioxidant enzymes activities in TMZ + I/R group regarding I/R group and also demonstrating that such protection implicates an activation of Akt/eNOS signaling pathway. Singh et al. [14] have reported that pretreatment of rats with 3 mg/kg of TMZ 30 min before ferric nitrilotriacetate administration (which induces acute tubular necrosis) markedly attenuated renal dysfunction, reduced elevated MDA levels, and prevented the severe depletion of renal antioxidant enzyme pool. Thus, based on these previous works, where there is a clear evidence on the renoprotective effects of TMZ on kidney injuries of different etiologies and the results of the present study, we could suggest that the improvement of renal functions in the $HgCl_2$-induced renal injury model could be due, at least in part, to the renal oxidative stress-lowering effect mediated by TMZ described in other kidney injury models. Nevertheless, other possible mechanisms could be implicated in this renoprotective action of TMZ in $HgCl_2$-induced renal injury that were described for the cardioprotection TMZ action. Further studies are needed in order to confirm that possibility.

Conclusion

As a whole, these findings demonstrate that TMZ is a renoprotective agent against $HgCl_2$-induced renal injury as it improved plasma urea and Cr levels, Cr clearance, the urinary flow, the fractional excretion of water, the glucosuria, and the urinary biomarker of renal injury NaDC1, as well as GSH and MDA tissue levels. Considering the apparent virtual absence of either acute or chronic toxicity of TMZ, its clinical application against oxidative damage due to $HgCl_2$-induced renal injury should be considered. The fact that TMZ is commercially available should simplify and accelerate the translation of the present data "from bench to bedside." In the next future, TMZ could become an interesting new example of drug repositioning.

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Statement of Ethics

All experiments were conducted according to the National Institute of Health (NIH) Guide for the Care and Use of Laboratory and were approved by the Faculty of Biochemical and Pharmaceutical Sciences (UNR) Institutional Animal Care and Use Committee (Res No. 385/2017).

Conflict of Interest Statement

All the authors declare no conflicts of interest.

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Author Contributions

M.H.H. and R.P.B. performed the experiments, analyzed and interpreted experimental data, and contributed in writing the manuscript. A.M.T. designed the work, interpreted the results, and contributed in writing the manuscript. All authors read and approved the final manuscript.

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