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Ligustrazine modulates renal cysteine biosynthesis in rats exposed to cadmium

Wenhua Kuang[a], Xu Zhang[b], Wufu Zhu[c], Zhou Lan[c,*]

[a] School of Pharmaceutical Sciences, Tsinghua University, Beijing 100016, China
[b] School of Life Sciences, Tsinghua University, Beijing 100084, China
[c] Jiangxi Provincial Key Laboratory of Drug Design and Evaluation, School of Pharmacy, Jiangxi Science & Technology Normal University, Nanchang 330013, China

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ABSTRACT

The objective of this study was to determine the effect of ligustrazine (TMP) on cadmium (Cd)-induced nephrotoxicity and its relevant mechanism. TMP (50 mg/kg) was injected intraperitoneally (i.p.) into rats 1 h prior to $CdCl_2$ exposure (at a Cd dose of 0.6 mg/kg). TMP reversed Cd-induced nephrotoxicity, evidenced by the relatively normal architecture of the renal cortex. Additionally, TMP alleviated renal oxidative stress of rats that were exposed to Cd, evidenced by the decreased levels of malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), elevated levels of glutathione (GSH) and GSH/GSSG (glutathione disulfide) ratios. Furthermore, TMP also raised the decreased levels of S-adenosylmethionine (SAM) and cystathionine involved in cysteine biosynthesis in rats exposed to Cd. Further analysis revealed that TMP treatment upregulated expression of several proteins involved in cysteine biosynthesis including methionine adenosyltransferases (MATs) and cystathionine-beta-synthase (CBS). Taken together, these results suggest that TMP remodeled metabolomics of cysteine biosynthesis in rat kidneys and attenuated Cd-induced nephrotoxicity.

1. Introduction

Cadmium (Cd) is a well-known toxicant which has multi-biological toxicities on multiple organ systems such as the liver, kidneys, lungs and reproductive systems (Go et al., 2014). Cd-induced nephrotoxicity has been extensively studied and widely reported; its mechanisms are involved in overproduction of reactive oxygen species (ROS), lipid metabolism disorder, glutathione (GSH) depletion, protein cross-linking, DNA damage and ultimately oxidant-induced cell death (Matović et al., 2015).

Ligustrazine (tetramethylpyrazine, TMP) is a major constituent of *Ligusticum wallichii*, which has been used for treating cardiovascular disease, headaches and vertigo in traditional Chinese medicine for hundreds of years (Tan et al., 2009). Effects of TMP on organ damage induced by oxidative stress have been widely reported in recent years. These studies clearly indicate that the ways in which TMP regulates oxidative stress-related enzymes are key mechanisms by which it protects against oxidative damage, leading to alterations in upregulating superoxide dismutase (SOD) and glutathione reductase (GR) activities, upregulating glutathione-*S*-transferase (GST) activity, downregulating xanthine oxidase (XO) activity and decreasing malondialdehyde (MDA) generation, phosphorylating endothelial nitric oxide synthase (eNOS) and also interrupting protein kinase (ERK) and p38 pathways (Zhao et al., 2016).

Glutathione (GSH) is an endogenous antioxidant found in living organisms such as humans, animals, plants, fungi and some bacteria and archaea. It is capable of preventing oxidative damage to important cellular components induced by heavy metals due to their thiol groups (Paolicchi, 2015). GSH is predominantly synthesized in the liver and transported to the kidney through blood circulation. The kidney is the major organ for blood GSH uptake; approximately 80% of blood GSH is absorbed through the kidney. Of this, approximately 30% of GSH is re-synthesized from cysteine through two adenosine triphosphate-dependent steps by the effect of several enzymes including gamma-glutamylcysteine synthetase and glutathione synthetase (Lash et al., 1997). Most importantly, cysteine is generally the limiting amino acid for GSH biosynthesis in humans and rodents (Yoganandarajah et al., 2017). Thus, factors such as insulin and chemical drugs that stimulate cysteine uptake or biosynthesis generally increase intracellular GSH concentrations (Green et al., 2014).

Our previous study demonstrated that TMP treatment upregulated activities of renal SOD and GR. Furthermore, it decreased renal MDA production and accelerated the urinary excretion of Cd (Lan et al., 2014). What is more, TMP also attenuated 1-methyl-4-phenyl-1,2,3,6-

* Corresponding author.
E-mail address: A83875785@126.com (Z. Lan).

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tetrahydropyridine (MPTP)-induced decreased GSH level in rats of substantianigra through regulating the activities of enzymes involved in cysteine-mediated GSH biosynthesis, including GCL and nuclear factor erythroid 2 p45-related factor 2 (Nrf2), a regulator for the expression of a battery of antioxidant enzymes including GCL (Lu et al., 2014). Thus, we conjecture that TMP may have similar effects on the kidneys of the rats exposed to Cd. In addition, although it has been reported that Cd exposure dysregulates cysteine biosynthesis and further reduces GSH production in rat testes (Sugiura et al., 2005), it is unknown whether there is a similar effect in rat kidneys under Cd exposure. Thus, the present study was designed to investigate the effect of TMP on renal cysteine biosynthesis and GSH contents and its relevant mechanisms under Cd exposure.

2. Materials and methods

2.1. Chemicals and animals

Ligustrazine hydrochloride (purity > 98.0%) was bought from Weifang Fine Chemical Co., Ltd. (Shanghai, CN). It is a white crystalline powder which is soluble in PBS (solubility > 2 g/100 ml). Cadmium chloride is a white crystalline powder (purity > 99.0%) which was bought from Sigma–Aldrich Biotechnology (St. Louis, MO, USA). It is also soluble in PBS (solubility > 10 g/100 ml). Phosphate-Buffered Saline (PBS, pH = 7.4) was bought from Beyotime Biotechnology (Beyotime, Wuhan, CN). Solvents used as eluents for LC–MS/MS were HPLC grade methanol, acetonitrile from Fisher Scientific (FairLawn, NJ, USA) and distilled water. Ammonium acetate and ammonium hydroxide were bought from Kermel Chemical Reagents (Kermel, Tianjin, CN). Polyvinylidene fluoride membrane and western horseradish peroxidase substrate were obtained from Millipore (Bed-ford, MA, USA). All other reagents were analytical grade. Redistilled water was used throughout this study. Metformin hydrochloride (internal standard, IS) is a white crystalline powder (purity > 98.0%) with a high solubility in water that was bought from Yuanye Biotechnology (Shanghai, CN).

Male Sprague-Dawley (SD) rats (7 weeks of age) were obtained from Vital River Laboratory (Animal Technology Co., Ltd., Beijing, CN), initially weighing about 230 g each. All animals were maintained in a specific pathogen free (SPF)-conditioned room with a 12 h light/dark cycle. The ambient air temperature and relative humidity was set to 22 ± 2 °C and 50 ± 10%, respectively. All animals were checked for any overt signs of illness and only healthy animals were selected before the experiment. Tap water and rodent chow were given ad libitum. The rats were acclimatized and habituated to the laboratory for at least 1 week before being subjected to tests and were used only once throughout the experiments. The protocol was approved by the Institutional Animal Care and Use Committee of Jiangxi Science and Technology Normal University (No. 2013-012).

2.2. Experimental design

All animals were randomly assigned into four groups containing eight rats per group as described below. The volume of each dose injected was adjusted to 5 ml/kg per rat on the basis of the daily body weights of the rats just before dosing. The animals in each group were randomly divided into the four groups listed below:

I: Control group. Rats were treated with 1 ml PBS by an i.p. injection at 2.00 pm followed by a subcutaneous (s.c.) injection of the same 1 h later once a day, 5 days/week over six weeks;

II: TMP group. Rats were treated with TMP (50 mg/kg) by an i.p. injection at 2.00 pm each day, 5 days/week over six weeks;

III: Cd group. Rats were exposed to 0.6 mg/kg Cd (s.c. injections) at 3.00 pm each day, 5 days/week over six weeks;

IV: TMP + Cd group. Rats were treated with TMP daily by an i.p. injection at 2.00 pm followed by an s.c. injection of Cd at a dose of 0.6 mg/kg 1 h later in the same manner described in “group II” and “group III”, 5 days/week over six weeks.

The dose and administration route of Cd used in this study is based on a previous report (Prozialeck et al., 2016) which successfully induced renal tubule damage in rats. The dose and administration route of TMP is based on our previous study and other reports (Lan et al., 2014; Liu et al., 2008) in which TMP showed beneficial effects on oxidative stress-induced injuries. After the final treatment, all rats were immediately put into individual metabolic cages to collect urine for 24 h. All urine samples were immediately centrifuged at 15000*g* for 10 min in a 4 °C centrifuge and supernatants were stored at −80 °C in a freezer for the analysis of urinary biochemical parameters. The rats were then killed and blood samples were collected from the arteria femoralis and centrifuged at 850*g* for 10 min. The serum obtained was frozen at −20 °C until the biochemical analysis was performed. Kidney tissues were excised and weighed immediately. A small portion of the left kidney was fixed in 10% neutral buffered formalin for histopathologic use, while the other portion was assigned for renal ROS and GSH/GSSG analysis. The right kidney was frozen in liquid nitrogen and stored at −80 °C for LC–MS/MS and western blot analyses.

2.3. Urinalysis and serum biochemical parameters

Serum biochemical parameters including blood urea nitrogen (BUN) and creatinine were analyzed using an automated Hitachi Analyzer (7020 Clinical Analyzer; Hitachi, Tokyo, Japan). Urinary total protein was determined by the protein assay reagent (BioRad, Hercules, CA, USA), and urinary creatinine and alkaline phosphatase (ALP) were determined using a urine chemistry analyzer (TBA-200FR NEO, Toshiba, Tokyo, Japan).

2.4. Histological evaluations

For hematoxylin and eosin (H & E) staining, tissue samples of kidney were dissected and fixed in a 10% neutral formalin solution, dehydrated in serial alcohol solutions and embedded in paraffin. All tissues were cut to a thickness of 5 μm thick sections and stained with H & E. All slides were viewed with an optical microscope (Olympus Optical, Tokyo, Japan).

2.5. Determination of oxidative damage markers

Lipid peroxidation is a well-known consequence of reactive oxygen species (ROS), and 4-hydroxynonenal (4-HNE) and MDA are two by-products of lipid peroxidation widely regarded as biomarkers of oxidative stress (Guo et al., 2013). In the current study, 4-HNE and MDA were measured in frozen samples of renal tissue from rats using an ELISA kit (Cell Biolabs, San Diego, CA, USA) and an MDA quantification kit (Beyotime, Wuhan, CN) respectively. Renal samples were processed according to the manufacturer’s protocol. All assays were performed in triplicate.

2.6. GSH and GSSG assay

The rat kidneys frozen in liquid nitrogen were homogenized in ice-cold 5% sulfosalicylic acid and centrifuged at 8000*g* for 10 min. The supernatant of total GSH and GSSG concentration was measured using a GSH/GSSG assay kit (Beyotime, Wuhan, CN) following the given protocol. The total GSH level was measured in the supernatant with a SpectraMax 190 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at room temperature at 412 nm. The GSH levels were calculated from the difference between the total GSH and the GSSG concentration.

2.7. Quantification of metabolites involved in cysteine biosynthesis in kidneys

Kidney samples frozen in liquid nitrogen were cut to 0.1 g, spiked