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Original Research Article

Hyperhomocysteinemia exacerbates cisplatin-induced acute kidney injury in mice by upregulating the expression of endoplasmic reticulum stress protein

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Abstract

Purpose: *To determine the effect of hyperhomocysteinemia on cisplatin-induced acute kidney damage, as well as the mechanism involved.*
Methods: *Forty-eight healthy mice were assigned to control and model groups, having 16 and 32 mice, respectively. Cisplatin was intraperitoneally given to model mice at a level of 20 mg/kg. Serum levels of homocysteine (Hcy), BUN and creatinine (Scr) were measured in each group, and changes in kidney coefficient were calculated. Changes in levels of glucose regulatory protein 78 (GRP78) and cysteine-dependent aspartate-directed protease-12 (Caspase-12) were determined with immunohistochemistry and Western blot assay.*
Results: *Serum Hcy, BUN, Scr, renal coefficient, and the expression levels of GRP78 and Caspase-12 in kidney of model mice were markedly elevated, relative to control values ($p < 0.05$). However, relative to model mice, serum Hcy, BUN, Scr, renal coefficient, apoptosis level of renal tubular epithelial cells, and GRP78, Caspase-12 expression levels in renal tissue were significantly increased in the high-methionine intervention group ($p < 0.05$).*
Conclusion: *Cisplatin induces acute renal injury in mice. Hyperhomocysteinemia may aggravate cisplatin-induced acute renal injury by upregulating the expression of endoplasmic reticulum stress protein.*

Keywords: *Hyperhomocysteinemia, Endoplasmic reticulum stress protein, Cisplatin, Acute kidney injury*

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INTRODUCTION

Due to aggravation of environmental pollution and increased economic pressure on the human population, the incidence of tumor is on the increase. Chemotherapy is one of the major ways of tumor treatment, and cisplatin is one of the chemotherapeutic agents popularly used in clinics. However, although cisplatin is popularly applied for treating many solid cancers, it often produces some adverse side effects in cancer patients [1]. For instance, long-term use of high-dose cisplatin produces serious adverse reactions such as bone marrow suppression,

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nephrotoxicity and ototoxicity. Nephrotoxicity is the most serious and common adverse reaction, and it may result in acute renal injury [2].

Acute renal injury is a severe clinical syndrome characterized by rapid deterioration of renal function. It has become a global public health problem with high incidence and mortality. Some important causes of acute renal injury are renal ischemia-reperfusion injury, use of nephrotoxic drugs, and sepsis caused by infection.

At present, apart from dialysis, there is no effective treatment for improving the survival of acute renal injury patients and reducing kidney injury [3]. Without timely and effective treatment, acute kidney injury often develops into chronic kidney disease and gradually degenerates into end-stage nephropathy which has a serious effect on lives of patients. Therefore, it is important to find ways of preventing or alleviating the side effects of cisplatin without affecting its antitumor effects. Homocysteine is an intermediate metabolic product of methionine. High blood level of homocysteine is referred to as hyperhomocysteinemia [4]. Hyperhomocysteinemia is closely related to the pathogenesis of many diseases [5]. However, the mechanism underlying the nephrotoxicity of cisplatin is not yet clearly understood. In the present investigation, the influence of hyperhomocysteinemia on cisplatin-mediated nephrotoxicity was investigated.

EXPERIMENTAL

Materials

A total of 48 healthy male SPF C57/BL6 mice weighing 22 ± 3 g and aged 8 weeks, were obtained from Guangdong Medical Laboratory Animal Center, SCXK (Guangdong 2018-0002).

This study received approval from the Ethical Committee of Guizhou University School of Medicine, Guizhou University, Guiyang, PR China (approval no. 201946799), and was conducted according to Principles of Laboratory Animal Care [6].

Major reagents and equipment

The instruments and reagents used, and their suppliers (in brackets) were: cisplatin (Qilu Pharmaceutical Co. Ltd, batch no. 37171258, specification:10mg); GRP78 antibody (Shanghai Shifeng Biotechnology Co. Ltd.); anhydrous ethanol (Jianxing Reagent Factory, Panyu District, Guangzhou); phosphate buffer (Jiangsu Enmoasai Biotechnology Co. Ltd.); paraffin slicing machine (Beijing Shengke Xinde Technology Co. Ltd., Model RM2245); cryogenic high-speed centrifuge (Shanghai Luxiangyi Centrifuge Instrument Co. Ltd., model TGL-17M); -80 ℃ ultra-low temperature refrigerator (Nanjing Baden Medical Co. Ltd., model MDF-C8V); electronic balance (Shanghai Youzhen Electronic Technology Co. Ltd., model BSM); real-time fluorescence quantitative PCR analyzer (Xi 'an Tianlong Technology Co. Ltd., model TL988-IV); and biological microscope (Shanghai Yuguang Instrument Co. Ltd., model WMS-1033).

Animal grouping and treatments

All mice were kept at laboratory temperature of 20 ± 2 ℃ and humidity of 60 ± 5 % in an environment with 12-h light/12-h dark cycle for 1 week. Thereafter, the mice were assigned to control group (16 mice) and model group (32 mice). The mice in the model group were split equally into 2 sub-groups, one of which was given standard feed (model group) while the other group was given 2 % methionine feed (high methionine intervention group) and standard feed. Mice in the control group were fed with standard diet. After 2 weeks of feeding, mice in model and high methionine intervention groups received intraperitoneal injection of 20 mg of cisplatin, while mice in the control group were given intraperitoneal injection of the same dose of normal saline.

Biochemical characterization

Blood (4 mL) was taken from the tail vein of each mouse from each group. The blood samples were allowed to clot at room temperature, followed by centrifugation at 3000 rpm. The supernatants (sera) were preserved in a cryogenic refrigerator at -80 °C. Serum Hcy, BUN and Scr levels of each group were measured with automatic biochemical analyzer, and changes in kidney coefficients were calculated as shown in Eq 1.

$$K (\%) = (B/M)100 \quad \ldots\ldots\ldots\ldots (1)$$

where B = sum of bilateral kidney weight and M = mouse weight.

Following sacrifice, the kidneys were excised and processed for light microscopy via preparation of paraffin sections. Histopathological changes in each group were observed using periodic acid Schiff staining.

Apoptosis of mice in each group was analyzed using TUNEL technique. Under the microscope, normal renal tissue nucleus was blue in color,

while apoptotic cells were green. Superimposition of blue and green nuclei indicated TUNEL-positive cells. Five fields of vision were taken from each section for calculation of percentage apoptosis of renal tubular epithelial cells, relative to entire cell population in the field.

Changes in kidney expression levels of Caspase-12 and GRP78 were determined with immunohistochemistry and western blot.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical analysis was done with SPSS 23.0 software package. Measurement data for BUN, Scr and renal coefficient were compared between two groups using independent sample *t*-test, while multiple groups were compared using ANOVA. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Changes in BUN, Scr and renal coefficient

Serum Hcy, BUN, Scr and renal coefficient were markedly elevated in mice in model group, relative to control ($p < 0.05$). Compared with the model group, serum Hcy, BUN, Scr and renal coefficients in the high-methionine intervention group were significantly increased ($p < 0.05$). These results are shown in Table 1.

Histopathological changes in mouse kidney

In the control group, the renal tissue structure was clear without nuclear fragmentation or abscission. In the model group, the epithelial cells of renal tubules were swollen and necrotic, with nuclear fragmentation and abscess, and a large number of tubules were observed. The swelling, necrosis, nuclear fragmentation and abscission in the high-methionine intervention group were significantly higher than those in the model group. These results are shown in Figure 1.

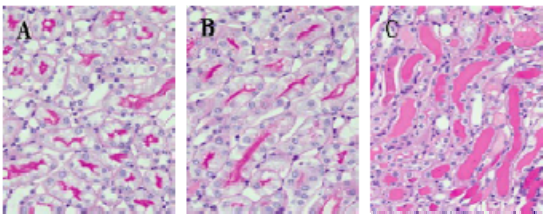

Figure 1: Histopathological changes in kidney of mice in the 3 groups. (A) Control, (B) model and (C) high-methionine

Effect of hyperhomocysteinemia on apoptosis

Compared with the control group, there was marked increase in apoptosis of model mice kidney tubule cells. Moreover, apoptosis level of these cells in the high-methionine intervention group was significantly higher than that of model group ($p < 0.05$). These results are shown in Figure 1 and Table 2.

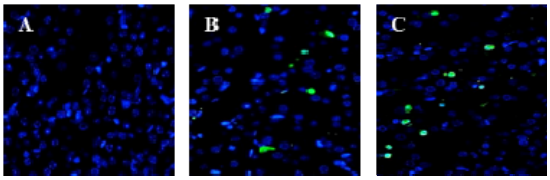

Figure 2: Apoptosis of renal tubular epithelial cells in the 3 mice groups. A: control, B: model, and C: high methionine intervention

Table 2: Apoptosis of cells

Group	TUNEL positive cell (%)
Control	0.01 ± 0.01
Model	9.74 ± 0.83
High-methionine	21.58 ± 1.53
F	1386.43
P-value	< 0.001

Data are mean ± SD (n = 12).

Changes in concentrations of GRP78 and Caspase-12 in renal tissues

Figures 3 and 4, and Table 3 show that GRP78 and Caspase-12 expression levels in the kidney tissues of model mice were markedly enhanced, relative to control values ($p < 0.05$).

Table 1: Changes in BUN, Scr and renal coefficient

Group	Hcy (μmol/L)	BUN (mmol/L)	Scr (μmol/L)	Kidney coefficient
Control	2.11 ± 1.01	5.21 ± 0.98	28.44 ± 6.56	1.46 ± 0.21
Model	5.97 ± 1.29	15.10 ± 0.98	63.72 ± 6.03	1.68 ± 0.09
High methionine	23.26 ± 3.17	36.17 ± 2.73	150.15 ± 17.54	1.83 ± 0.09
F	358.67	960.31	364.73	20.68
P-value	< 0.001	< 0.001	< 0.001	< 0.001

Values are mean ± SD (n = 12)

However, the expressions of GRP78 and Caspase-12 in the kidney tissues of mice in the high-methionine intervention group were markedly unregulated, relative to model mice ($p < 0.05$).

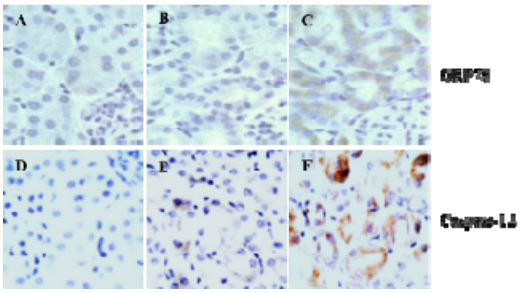

Figure 3: Changes in GRP78 and Caspase-12 levels in kidney tissues in the 3 mice groups. A and D: control, B and E: model, C and F: high-methionine intervention.

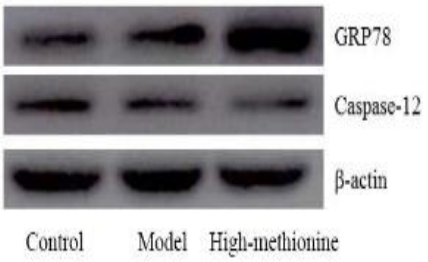

Figure 4: Changes in kidney GRP78 and caspase-12 levels

Table 3: Renal GRP78 and caspase-12 levels

Group	GRP78	Caspase-12
Control	1.01 ± 0.01	1.00 ± 0.01
Model	7.18 ± 0.43	3.87 ± 0.47
High-methionine	11.23 ± 1.28	8.52 ± 0.66
F	522.93	789.61
P-value	< 0.001	< 0.001

Values are mean ± SD (n = 12)

DISCUSSION

Cisplatin, a broad-spectrum anticancer drug, is widely used in chemotherapy for different solid cancers e.g. non-small cell lung cancer, ovarian cancer and cervical cancer [7]. Studies have found that the anticancer effect of cisplatin is significantly correlated with its dose: the higher the dose of cisplatin, the stronger the anticancer effect [8]. However, cisplatin not only kills tumors, it also damages normal body tissue cells due to its nephrotoxicity, neurotoxicity and ototoxicity. Nephrotoxicity is the most common toxic side effect of cisplatin, and this may be due to the fact that cisplatin is excreted mainly by the kidneys. Thus, non-toxic blood levels of cisplatin may even reach toxic levels in the kidneys due to concentration of the drug [9].

It has been reported that cisplatin toxicity occurs mainly in renal tubular epithelial cells of proximal tubules [10]. Oxidative stress, inflammation and cell necrosis or apoptosis are the mechanisms that underlie renal tubular epithelial cell injury. So far, attempts have been made to use hydration, diuresis and other measures to prevent cisplatin-induced nephrotoxicity, but their therapeutic effects are still poor. Some scholars believe that kidney tubular epithelial cell apoptosis is one of the important mechanisms involved in cisplatin-induced renal damage [11]. The pathways involved in this apoptosis (i.e. exogenous apoptosis mediated by death receptors, endogenous mitochondrial apoptosis, and endoplasmic reticulum stress) play important roles in the cisplatin-induced apoptosis of renal tubular epithelial cells.

Endoplasmic reticulum (ER) is the largest organelle in cells. It participates in protein synthesis and maintenance of intracellular calcium homeostasis. Endoplasmic reticulum stress is induced by stimulating factors such as ischemia-reperfusion, hypoxia, and increased levels of unfolded or misfolded proteins. Glucose regulatory protein 78 (GRP78), a chaperone of ER, prevents excessive accumulation of misfolded proteins in ER. When the ER environment is disordered, and there are increased levels of misfolded proteins, the expression level of GRP78 significantly increases [12]. This is in agreement with the results obtained in this study. Caspase-12 is distributed in the cytoplasmic surface of the ER, and it is most expressed in the proximal renal tubular epithelial cells. It has been reported that Caspase-12, a specific molecule in ER stress apoptotic pathway, is involved in renal tubular epithelial cell apoptosis [13]. The results of this study showed that cisplatin induced ER stress response in cells during renal toxicity.

Homocysteine is a sulfur-containing amino acid which is an intermediate metabolic product of methionine. Studies have reported that hyperhomocysteinemia directly led to pathological changes in the glomeruli [14]. The results of this study showed that hyperhomocysteinemia significantly aggravated acute renal injury and increased the expression levels of GRP78 and caspase-12. It is likely that hyperhomocysteinemia induced ER stress and unfolded protein response, and further mediated amplification of inflammatory response cascade. It has been reported that hyperhomocysteinemia directly inhibited methyltransferase, resulting in

DNA damage and cell apoptosis [15,16]. Thus, hyperhomocysteinemia may be an independent risk factor for endothelial cell injury.

CONCLUSION

Cisplatin induces acute kidney injury in mice. Hyperhomocysteinemia may aggravate cisplatin-induced acute kidney injury in mice by upregulating the expression of endoplasmic reticulum stress protein.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors, all authors read and approved the manuscript for publication. Yanjun Long conceived and designed the study, Mei Zhang, Yanjun Long, Yan Zha, Jing Yuan, Yan Ran collected and analyzed the data, while Mei Zhang wrote the manuscript.

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