

The Significance of Urinary RBP and KIM-1 Determination in Cisplatin – Induced Acute Kidney Injury

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Abstract: *Objective:* Cisplatin is one of the most commonly used and most effective chemotherapy drugs in clinical treatment of solid tumors, because of its strong anti-cancer effect, no cross resistance with other cancer drugs, but cisplatin is one of the significant renal toxicity drugs either, which greatly limits its clinical application. The traditional diagnostic methods for AKI are to measure Scr and changes in urine volume, but these indicators are affected by non-renal factors and cannot reflect renal function in an early and timely manner, which delays the optimal early treatment time for AKI. To discuss the clinical value of detecting urinary retinol binding protein (RBP) and kidney injury molecule 1 (KIM-1) in early diagnosis of cisplatin-induced acute kidney injury. *Methods:* 48 rats were randomly divided into the cisplatin group (CP group, 42 rats) and the control group (NS group, 6 rats). The urinary RBP and KIM-1, renal pathological changes were observed at each injection time points. *Results:* Urinary KIM-1 it significantly increased at 12h point ($P < 0.05$), which was higher obviously than NS group. Urinary RBP it significantly elevated at 6h point ($P < 0.05$). It has correlation with Scr. *Conclusion:* In cisplatin-induced acute kidney injury, urinary RBP and KIM-1 increased obviously earlier than serum creatinine, they were early sensitive markers.

Keywords: Cisplatin, Acute Kidney Injury, Urinary Retinol Binding Protein, Kidney Injury Molecule 1

1. Introduction

With the rapid development of the pharmaceutical industry in recent years, new drugs have been applied in clinical practice, and drug-induced AKI has been increasing year by year, which has become an unavoidable problem for clinical medicine workers. Cisplatin is one of the most commonly used and most effective chemotherapy drugs in clinical treatment of solid tumors, because of its strong anti-cancer effect, no cross resistance with other cancer drugs, as well as the most commonly used antitumor drug combination chemotherapy. Its antitumor effect is positively correlated with dose, but cisplatin is one of the significant renal toxicity drugs either, and renal toxicity was positively correlated with its dosage. In other words, the higher the dose of cisplatin, the better the anti-tumor effect, at the same time, the stronger the nephrotoxicity, the greater the probability of renal injury. Clinical investigation shows that the incidence of cisplatin renal damage as much as 25% - 35% [1-5]. Therefore, its

clinical application is greatly limited. How to effectively ensure the quality of chemotherapy for cancer patients, reduce kidney injury, and better play the anti-tumor effect of cisplatin has become an urgent problem to be solved. The traditional diagnosis of AKI is mainly based on SCR or changes in urine volume, but there are many factors affecting these indicators [6]. Studies have shown that SCR increases only when glomerular filtration rate drops by more than 50%, which cannot achieve the purpose of early diagnosis and delays the optimal treatment time for AKI. Therefore, it is an urgent requirement for new drug development and clinical treatment to find early, sensitive and quantifiable AKI markers.

2. Materials and Methods

2.1. Animals

Healthy adult male SD rats, weighing 200-220g, SPF grade, were purchased from Experimental Animal Center of Guangxi

Medical University. Rats were kept in the Animal Experiment Center of the Fourth Affiliated Hospital of Guangxi Medical University. The temperature of the animal center was set at 20°C-28°C, and the air humidity was controlled at 50%-60%. The light and darkness were alternated for 12h, and the rats were separately caged and fed freely within 24h. A total of 48 adult SD rats were included, including 42 in CP group and 6 in NS group.

2.2. Treatment

The experiment was divided into CP group and NS group. The CP group was divided into CP3h group, CP6h group, CP12h group, CP24h group, CP48h group, CP72h group and CP96h group according to different time after injection, with 6 rats in each group; After adaptive feeding for 3 days, SD rats were divided into 8 groups according to random number method, and randomly assigned to CP group and NS group.

2.3. Material

6ml/30mg cisplatin injection from Jiangsu Hausen Pharmaceutical Co., Ltd. Batch number: 120318.

2.4. Definition and Diagnostic Criteria of AKI

2.4.1. Definition of AKI

The Acute Kidney Injury Network (AKIN) Panel defines AKI as abnormalities in renal function or structure not exceeding 3 months, including abnormalities in blood, urine, and tissue testing or imaging markers of renal injury.

2.4.2. Diagnostic Criteria for AKI

The AKIN consensus determined the diagnostic criteria for AKI as follows: sudden renal function decrease within 48 hours, and Scr increase with absolute value $>26.5\mu\text{mol/L}$ (0.3mg/dL); Or Scr increased by $>50\%$ compared with the original level; And/or decreased urine volume $<0.5\text{ml}/(\text{kg}\cdot\text{h})$ for more than 6 hours, obstructive nephropathy or dehydration were excluded [4].

2.5. Methods

- 1) The rat model of cisplatin AKI was established by single intraperitoneal injection of 10mg/Kg cisplatin to SD rats

in CP group [15], NS group SD rats were intraperitoneally injected with 2ml/Kg normal saline as control group.

- 2) At each time point after drug injection, SD rats were subjected to ventral median incision after ether inhalation anesthesia, and 5ml of blood was collected by portal venipuncture and put into EP tube and sent to the laboratory of our hospital for Scr and BUN determination; 0.8-1ml of urine was taken by bladder puncture and sent to the laboratory of our hospital for urine RBP and KIM-1 determination; bilateral kidneys were taken and sent to the pathology department of our hospital for HE pathological section.

2.6. Investigated Rats Parameters

- 1) General situation of rats: the body weight of rats in CP group and NS group, death during the experiment, mental condition, hair condition, activity and feeding condition of rats in each group and their changes over time were observed;
- 2) Blood and urine biochemical indexes: The detection values of Scr, BUN, urinary RBP, and urinary KIM-1 were observed at each time point, and the change trend of Scr, urinary RBP, KIM-1 and whether there was correlation between urinary RBP, KIM-1 and Scr;
- 3) HE staining of renal tissue pathology: observe the structure of glomerulus and renal tubule in renal HE section, especially observe whether the epithelial cells of renal tubule shed like brush edge, cytoplasmic vacuolar degeneration, lumen dilation, renal tubule blockage, interstitial inflammatory cell infiltration and apoptotic cells.

2.7. Statistical

All measurement data were expressed as mean plus or minus standard deviation ($\bar{x} \pm s$), and analyzed by SPSS17.0 statistical software. Block design analysis of variance was used for multivariate comparison of group design, LSD test or SNK test was used for pairwise comparison of multiple mean within the group, and Spearman correlation analysis was used for correlation analysis of measurement data. The test level was set as 0.05, and $P < 0.05$ was statistically significant.

Table 1. Scr, BUN, KIM-1 and RBP.

Group	N	Scr (mol/l)	BUN (mmol/l)	KIM-1 (ng/ml)	RBP (mg/l)
NS	6	45.733 \pm 4.541	5.129 \pm 0.913	1.375 \pm 0.432	4.367 \pm 0.659
3h	6	45.133 \pm 4.301	5.076 \pm 1.075	1.361 \pm 0.408	4.467 \pm 0.596
6h	6	45.083 \pm 4.354	5.147 \pm 1.149	1.933 \pm 0.501	6.050 \pm 0.909*
12h	6	45.400 \pm 4.392	5.139 \pm 1.342	2.769 \pm 0.532*	7.317 \pm 1.408*
CP	24 h	47.400 \pm 5.653	5.253 \pm 1.491	3.633 \pm 0.585*	8.800 \pm 1.820*
48h	6	59.083 \pm 6.956* ∇	6.644 \pm 1.647* ∇	6.667 \pm 1.256* ∇	10.783 \pm 1.992*
72h	6	83.233 \pm 11.165* ∇	8.343 \pm 2.193* ∇	3.417 \pm 0.655*	11.433 \pm 2.192*
96h	6	96.950 \pm 11.265* ∇	10.217 \pm 2.638* ∇	2.183 \pm 0.560*	12.917 \pm 2.395*
F values		56.353	21.647	20.726	45.693
P values		0.000	0.000	0.000	0.000

* comparison between CP group and NS $P < 0.05$,

∇ comparison within CP group, $P < 0.05$.

3. Results

3.1. General Situation of Rats

After injection, the body weight of rats in CP group and NS group showed no significant change, and no death occurred. However, SD rats in CP group showed listlessness, sparse yellow and dull hair color, lazy eye closure, reduced food intake and other phenomena, and the above phenomena increased with time.

3.2. Morphological Structure

HE staining of kidney tissue in NS group showed clear renal tissue structure without obvious abnormality, normal glomeruli and renal tubule structure with less interstitium; No obvious abnormalities were observed in glomeruli and renal tubules in the CP3H, CP6H and 12H groups. In CP24h group, no obvious glomeruli abnormality was observed, but some renal tubules showed vacuolar degeneration. No obvious glomeruli abnormality was observed in the CP48h group, but tubular vacuolar degeneration was significantly worse than that in the CP24h group. There was no obvious glomeruli abnormality in the CP72h group, but the renal tubular degeneration was worse than that in the CP48h group, and inflammatory cell infiltration was observed in the renal interstitium. In the CP96h group, there was erythrocyte stasis in the glomeruli, but no obvious abnormality was observed. However, the epithelial cells of the renal tubules showed obvious brush edge abscission, and the vacuolar degeneration of the cytoplasm was significantly worse than before. In addition, the lumen dilated significantly, with the appearance of tubules and even the aggregation of apoptotic cells, as shown in Figure 1.

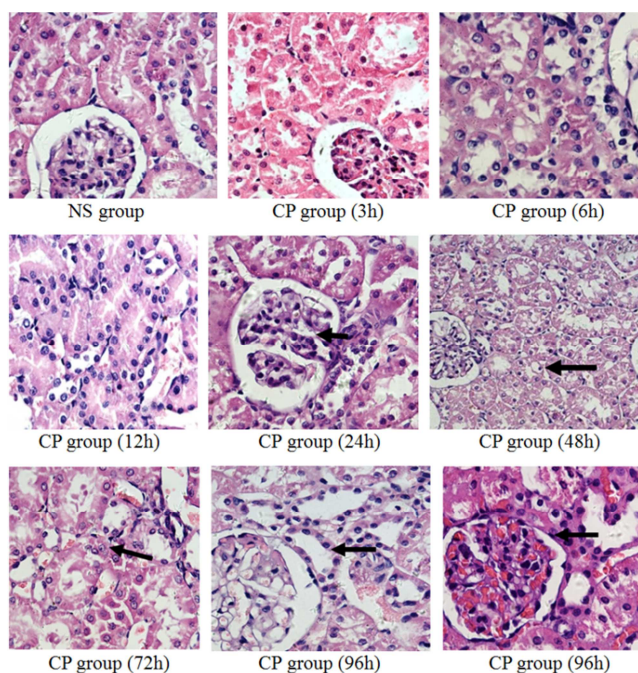


Figure 1. HE staining Results (HE×400).

4. Discussion

Cisplatin is a widely used chemotherapeutic agent for periodic nonspecific heavy metal tumors, and it is also the most commonly used anti-tumor drug in combination chemotherapy because it has no cross resistance with other tumor drugs [7-8]. Cisplatin has high accumulation, high metabolism and high excretion in the kidney, and its nephrotoxicity is particularly prominent, which is positively correlated with the drug dosage. Clinical statistics show that the incidence of AKI during cisplatin chemotherapy is as high as 35%, which greatly limits its clinical application. The traditional diagnostic methods for AKI are to measure Scr and changes in urine volume, but these indicators are affected by non-renal factors and cannot reflect renal function in an early and timely manner, which delays the optimal early treatment time for AKI. Therefore, it is an urgent requirement for the development of The Times to find early, sensitive and quantifiable AKI markers.

CP group of Scr in this study results suggest that CP group of SD rats abdominal cavity injection of cisplatin, within 24 h when Scr no obvious change, began to rise within 24 h - 48 h, 48 h was significantly higher compared with the NS control group, the difference was statistically significant. During 48h (24h-72h) SCR increased > by 50% relative to baseline, and no death in rats, conform to the Scr rise within 48 h > 50% AKI diagnosis standard, indicates that under the condition of this experiment, cisplatin, 10 mg/kg to create a single intraperitoneal injection drug AKI cisplatin SD rats model was established, at the same time 72 h can be diagnosed AKI class I, AKI grade II can be diagnosed at 96h. The results of this study are consistent with the results of Vikash Sinha *et al.* that Scr of rat AKI model was significantly increased within 3 days after 5.5mg/Kg cisplatin was injected into the rat tail vein [9-10].

The results of BUN in CP experimental group showed that no significant change of BUN was observed within 24h, but it began to rise within 24h-48h and was significantly higher than that in NS control group at 48h, and the difference was statistically significant. Scr and BUN are both evaluation indexes of glomerular filtration rate impairment of renal function. The results of this study indicated that no significant changes were observed in both of them within 24h, but showed a continuous upward trend within 24h to 96h, indicating that renal function of rats in this study was continuously impaired and gradually aggravated.

Large Numbers of clinical studies and basic studies show that cisplatin acute kidney injury kidney disease mainly for renal tubular epithelial cells dysfunction, a main mechanism is to cause kidney toxicity: cisplatin can cause renal tubular epithelial cells in ischemia and directly lead to the lack of oxygen, if damage is severe and no intervention will eventually lead to renal tubular epithelial cell necrosis, which in turn causes kidney structure change; The other mechanism is to cause renal ischemia: cisplatin can cause renal vasoconstriction, resulting in decreased renal blood flow and glomerular filtration rate, thereby causing renal injury. The

results of HE staining of kidney pathology in this experiment indicated that, compared with the NS group, no obvious abnormalities were observed in the glomeruli of the CP group except erythrocyte deposition. Renal tubular of NS group not seen obvious abnormality, and CP group rat renal tubule degeneration obviously. In the early stage, the main manifestation was renal tubular vacuolar degeneration, further development of renal tubular epithelial cells, loss of brush border, malpighian tube cavity expansion, interstitial inflammatory cell infiltration, in the absence of interference will gradually develop to control cavity exposed basement membrane, red and white cell or protein appeared on renal tubular plug pipe type, eventually lead to renal tubular epithelial cell apoptosis; The change trend of renal tubules in this study was consistent with the change of Scr and BUN, that is, the results of this study confirmed that the renal toxicity target of cisplatin was mainly on renal tubules rather than glomeruli. The results of this study were consistent with the results of Gravedi P, Gaspari F and Mori K, and Mishra J et al.'s study on renal tubules as the main renal lesions in acute renal injury with cisplatin [11-12].

RBP is a low molecular weight lipophile carrier protein synthesized and secreted by the rough endoplasmic reticulum of the liver, with a molecular weight of 21KD, a pH of 4.4-4.8 and a half-life of 3-12h. The main function of RBP is to transport retinol from the liver to epithelial cells, and can specifically bind to retinal epithelial cells to provide retinol to the retina. RBP is widely distributed in human serum, cerebrospinal fluid, urine and other body fluids, since it has the characteristics of small molecular weight and short half-life, in the liver, it has important clinical significance in early diagnosis and curative effect observation of kidney disease. And because RBP can reflect the body's nutritional status in specificity, it is also a diagnosis early sensitive indicator of malnutrition. In the blood, RBP and retinol, prealbumin In the form of a 1:1:1 (mol) polymer complex and transports 90% of retinol in the body, it can't be filtered by the glomerular membrane. After retinol is transported to the target cell and binds to the RBP receptor on the surface of the target cell, complex dissociation, retinol into cells, RBP was free in the plasma, was quickly glomerular filtration, almost all in renal proximal convoluted tubules heavy absorption, and broken down into amino acids for organizations to use, only a small amount is excreted in the urine. Under normal circumstances, RBP has strong stability in urine, is not easily decomposed, is not affected by pH and blood pressure, and the excretion of RBP is very little <0.7mg/L. When the function of the near convoluted tubule is impaired, its reabsorption function is decreased, and the urinary RBP can be increased, so it is also recognized as one of the early biomarkers of AKI. Due to the numerous etiology of AKI, the types and changes of biological markers of AKI caused by different etiology are different. In AKI caused by nephrotoxic drug cisplatin, no urine RBP has been reported.

When the kidney is damaged by cisplatin, renal tubule function is impaired, leading to RBP reabsorption disorder, which leads to the increase of urinary RBP, and the more

severe the renal tubule injury is, the higher the urinary RBP is. In this study, the RBP results in the CP group indicated that there was no obvious abnormality in urine RBP within 3h, but it began to rise within 3h-6h, and the difference at 6h was statistically significant compared with that in the NS group, showing a continuous upward trend within 6h-96h. In this study, the results of RBP in CP group indicated that the abnormal increase of RBP could be detected after 6h, which was significantly earlier than the increase of SCR, indicating that urinary RBP was as sensitive as KIM-1 in cisplatin acute renal injury as SCR. The results of this study are consistent with Zhong Fangcai et al.'s study that urinary RBP is a sensitive marker for acute kidney injury and Wang Yongqing et al.'s study that urinary RBP is a sensitive marker for acute kidney injury in tumor chemotherapy.

According to RBP and Scr related statistical analysis indicate that the urine RBP and Scr were positively correlated, the correlation coefficient of $r = 0.964$ ($P = 0.000 < 0.05$), indicated that the higher the degree of renal injury, the higher the SCR and the more serious the renal tubule lesion, the higher the urine RBP. It means the urine RBP can reflect cisplatin in rats with acute kidney injury degree of kidney damage, consistent with the experimental Scr and pathology results, and Huang Yunfang wen-li Chen et al. Study of RBP in AKI significance in early diagnosis and classification research results: the urine RBP can be used as evaluation index AKI illness severity.

KIM-1 is a type I transmembrane protein found in proximal renal tubular epithelial cells, belonging to the immunoglobulin gene superfamily. It is not expressed in fetal liver and kidney, slightly expressed in adult liver, kidney and spleen, but significantly enhanced expressed in kidney after ischemia injury. It is the adhesion molecule of damaged proximal confluent tubular epithelial cells, and may be involved in the process of kidney injury and repair, the development and metastasis of renal tumor, and immune response. Vaidya et al. used rat renal ischemia/reperfusion model to study and showed that KIM-1 can be used as a sensitive, specific and quantitative biological marker for early detection of renal injury [13-15].

The monitoring results of urine KIM-1 in CP group in this experiment showed that there was no obvious abnormality in the urine KIM-1 in CP group within 6h, but it began to rise within 6h to 12h, and then increased significantly at 12h, which was significantly higher than that in NS control group. The difference was statistically significant, reaching a peak value within 24h to 72h and presenting a decreasing trend within 72h to 96h. These results indicated that in the AKI model of rats induced by cisplatin, the expression of KIM-1 was significantly up-regulated within 12h, indicating that the increase of KIM-1 was much earlier than that of SCR, that is, KIM-1 compared with SCR was an early sensitive marker of acute kidney injury induced by cisplatin.

5. Conclusion

In conclusion: Renal tubule is the main target site of acute renal injury induced by cisplatin in rats, abnormal urine RBP,

KIM - 1 rise significantly higher before the Scr, urine RBP and KIM - 1 are early sensitive markers of acute kidney injury induced by cisplatin in rats, and the urine RBP level changes can also reflect the cisplatin in rats with acute kidney injury degree of kidney damage. However, the urinary RBP and KIM-1 data obtained in this study were not corrected by urinary creatinine, and there may be deviation in the data. In addition, the interval of grouping time points was long and the total recording time was short, so the changes of SCR, urinary RBP and KIM-1 could not be recorded in detail. In future studies, we will gradually improve the experimental design, increase the number of rats and the consistency of grouping time points, make detailed records of urinary RBP and KIM-1, increase the diagnostic specificity and sensitivity and the correlation with other biomarkers, so as to obtain a more comprehensive and in-depth study of acute renal injury with cisplatin.

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