Original Article

Intravenous injection of Xuebijing attenuates acute kidney injury in rats with paraquat intoxication

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BACKGROUND: The study aimed to investigate the therapeutic benefits of intravenous Xuebijing on acute kidney injury (AKI) in rats with paraquat intoxication.

METHODS: Male Sprague-Dawley rats were randomly divided equally into three groups: sham group ($n=8$), paraquat group ($n=8$) and Xuebijing-treated group ($n=8$) using a random number table. The rats were intraperitoneally injected with 50 mg/kg of paraquat. One hour after paraquat administration, the rats were treated intravenously with Xuebijing (8 mL/kg). At 12 hours after paraquat administration, serum was collected to evaluate kidney function, then the rats were sacrificed and kidney samples were immediately harvested. AKI scores were evaluated by renal histopathology and pro-inflammatory cytokines mRNA levels in kidney were assayed using real-time RT-PCR.

RESULTS: Serum urea nitrogen, creatinine and AKI scores were significantly higher in the paraquat group, compared with the sham group ($P<0.05$, respectively). Moreover, interleukin (IL)-1 β , IL-6 and TNF- α mRNA levels were significantly higher in the paraquat group ($P<0.01$, respectively). However, intravenous Xuebijing significantly decreased serum urea nitrogen, creatinine, AKI scores and IL-1 β , IL-6 and TNF- α mRNA levels, compared with the paraquat group ($P<0.05$, respectively).

CONCLUSION: Intravenous Xuebijing attenuates AKI following paraquat poisoning by suppressing inflammatory response.

KEY WORDS: Paraquat; Xuebijing; Kidney injury; Inflammation

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INTRODUCTION

Paraquat intoxication is a serious health concern in the developing countries. In China, paraquat is commonly implicated in acute poisonings. The patients with paraquat intoxication have a high mortality rate ranging from 50% to 90%.^[1] Paraquat has been known to injure the various organ systems including the lung, kidney, liver and heart. Acute lung injury is the usual cause of death in most cases of paraquat toxicity.^[2] Additionally, acute kidney injury (AKI) is also common death of patients with paraquat intoxication. The main pathogenesis of paraquat intoxication includes redox

cycling and intracellular oxidative stress generation; however, inflammatory reaction plays a vital role in paraquat toxicity.^[3] Therefore, suppressing the inflammation may help to reduce acute kidney injury.

Xuebijing injection is a traditional Chinese medicine preparation that has been proved to exert strong anti-inflammatory effects.^[4] Many studies had shown that Xuebijing treatment could prevent inflammatory response-induced diseases including systemic inflammatory response syndrome and pyemia.^[5–6] Therefore, the research mainly investigated the protective effects of Xuebijing injection on paraquat-induced acute kidney injury in rats.

METHODS

Experimental animals

Eight-week-old male healthy Sprague-Dawley (SD) rats weighing 200–250 g were purchased from the Laboratory Animal Center of Fujian Medical University (Fuzhou, China). All rats were housed on a 12-hour light-dark cycle with access to standard laboratory chow and water was provided *ad libitum*. The experiments were approved by the Institutional Animal Care and Use Committee of Fujian Medical University.

Experimental groups and drug treatment

After an overnight fast except for free access to water, the animals were randomly divided into three groups: sham group ($n=8$), paraquat group ($n=8$) and paraquat Xuebijing-treated group ($n=8$). According to previous reports,^[3] the rats were intraperitoneally administered with 50 mg/kg of paraquat (Sigma, USA). One hour after administration of paraquat, the rats were treated with Xuebijing (8mL/kg, HongRi Pharmaceutical Company, Tianjin) by injecting into the tail vein.^[4] The rats from the sham group received 2 mL/kg of normal saline intravenously. At 12 hours after administration of paraquat, the rats were deeply anesthetized and sacrificed for renal function assessment (blood urea nitrogen and creatinine), mRNA level measurements, and pathological examination.

Acute kidney injury scores

The left kidney was fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 4- μ m thick sections. Then the sections were stained with hematoxylin and eosin. According to the histopathologic damage scoring system established previously,^[7] each section for each animal was evaluated by an investigator blinded to the groups. Briefly, each section was evaluated separately with a microscope for swelling, vacuolar degeneration, necrosis and interstitial hyperemia, edema in renal tubular epithelial cell. The lesion scores of each category were evaluated on a four-point scale (normal=0 point, minimal changes=1 point, moderate changes=2 points, severe changes=3 points). The score of each animal was calculated by dividing the total scores for the number of examined sections.

Measurement of IL-1 β , IL-6 and TNF- α mRNA expression

Total RNA was extracted from the right kidney samples using TRIzol Reagent (Invitrogen, USA) according to the instructions. cDNA was synthesized by reverse transcription for RT-PCR according to the

manufacturer's directions (PrimeScript RT Master Mix; TaKaRa, China). All primers for RT-PCR in this experiments were listed as follows: interleukin (IL)-1 β forward, 5'-TCCTCTGTGACTCGTGGGAT-3'; reverse, 5'-TCAGACAGCACGAGGCATTT-3'; IL-6 forward, 5'-AGAGACTTCCAGCCAGTTGC-3'; reverse, 5'-AGCCTCCGACTTGTGAAGTG-3'; TNF- α forward, 5'-TCGTCTACTCCTCAGAGCCC-3'; reverse, 5'-ACTTCAGCGTCTCGTGTGTT-3'; GAPDH forward, 5'-CAAGGTCATCCATGACAACTTTG-3'; reverse, 5'-GTCC ACCACCCTGTTGCTGTAG-3'.

The cDNA was amplified by GoTaq qPCR Master Mix (Promega, USA). Real-time PCR reactions were performed with the following program: initial denaturation at 95 °C for 2 minutes, followed by 40 cycles of 95 °C for 3 seconds, and 60 °C for 30 seconds. Target gene mRNA expression was calculated based on the $2^{-\Delta\Delta C_t}$ methods normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

Statistical analysis

Data were presented as mean $\pm$ standard deviation. Multiple comparisons were made using ANOVA followed by Bonferroni's post hoc test (SPSS, Inc., Chicago, IL). A P value <0.05 was considered statistically significant.

RESULTS

Evaluation of renal function and AKI scores

Blood urea nitrogen (BUN), creatinine levels and AKI scores in the paraquat group were significantly higher than those in the sham group (all $P<0.01$; Table 1). However, intravenous Xuebijing treatment significantly decreased BUN, creatinine levels and AKI scores, compared with the paraquat group (all $P<0.05$; Table 1).

Evaluation of renal pathology

Normal renal tissues under a light microscope were clear and there was no obvious hyperemia, edema and degeneration of pathology (Figure 1A). However, paraquat administration caused obvious swelling, vacuolar

Table 1. Effects of Xuebijing on blood urea nitrogen (BUN), creatinine (Cr) levels and acute kidney injury (AKI) scores in paraquat poisoning rats

Groups	<i>n</i>	BUN (mmol/L)	Cr (μ mol/L)	AKI scores
Sham	8	6.11 $\pm$ 0.53	58.42 $\pm$ 5.67	0.26 $\pm$ 0.09
Paraquat	8	20.81 $\pm$ 3.14*	165.75 $\pm$ 16.29*	8.57 $\pm$ 0.42*
Paraquat Xuebijing-treated	8	15.28 $\pm$ 4.36 [#]	129.16 $\pm$ 10.44 [#]	5.64 $\pm$ 0.36 [#]

Compared with the sham group, * $P<0.01$; compared with the paraquat group, [#] $P<0.05$.

degeneration, necrosis and part of the lumen atresia, interstitial hyperemia, edema and nuclear pyknosis. Cell structure disappeared in proximal tubular epithelial cell and there exists a few glomerular structural disorder and extravasation of erythrocytes (Figure 1B). Xuebijing treatment significantly attenuated the above pathological injury (Figure 1C).

IL-1 β , IL-6 and TNF- α mRNA levels in kidney tissues

The expression of IL-1 β , IL-6 and TNF- α mRNA in kidney tissues in the paraquat group was significantly increased, compared with the sham group ($P<0.01$, respectively). However, intravenous Xuebijing treatment significantly reduced IL-1 β , IL-6 and TNF- α mRNA levels, compared with the paraquat group ($P<0.01$, respectively; Figure 2).

DISCUSSION

This study showed that intravenous Xuebijing could reduce inflammatory responses in renal tissue and prevent acute kidney injury induced by paraquat. The main component of Xuebijing injection is composed of the safflower, radix paeoniae rubra, Rhizoma Chuanxiong, radix salviae miltiorrhizae, and Radix Angelicae Sinensis, etc.^[8] Xuebijing injection is developed into an intravenous

preparation based on Xuefu Zhuyu decoction, which has many beneficial effects against bacterial toxins, reducing endotoxin,^[9] suppressing the generation and release of inflammatory mediators,^[10] regulating the immune system,^[11] and protecting microcirculation and vascular endothelial cells.^[12] In line with our study,^[4] Xuebijing had therapeutic benefits regulating lung inflammation and lung function.

Studies from toxicokinetics of paraquat have found that paraquat is eliminated mainly through the kidney. Within 12 to 24 hours of ingestion, more than 90% of paraquat is excreted unchanged by the kidney if the renal function remains normal.^[13] In addition, the concentration of paraquat is the highest in the kidney except the lung.^[14] Therefore, acute kidney injury was a recognized complication of paraquat intoxication. The main lesions are located in renal proximal tubule including swelling, degeneration and partial necrosis of epithelial cells, interstitial congestion and edema. However, the lesions in the glomerular, distal tubule and collecting duct are not obviously found.^[15] After Xuebijing intervention, proximal tubular lesions and acute kidney injury were significantly reduced. The results of this study showed that Xuebijing intervention had a protective effect on renal function.

Research showed that pro-inflammatory cytokines were involved in acute kidney injury induced by paraquat.^[16] Removal of inflammatory mediators such as continuous blood purification could reduce renal damage and protect

Figure 1. The kidney histopathology in the sham group (A), paraquat group (B) and paraquat Xuebijing-treated group (C) at 12 hours after administration of paraquat (H&E, original magnification $\times 200$).

Figure 2. IL-1 β , IL-6 and TNF- α mRNA expression in kidney tissues from the sham group, paraquat group and paraquat Xuebijing-treated group at 12 hours after administration of paraquat. Administration of paraquat significantly increased pro-inflammatory factors in kidney tissues. Intravenous Xuebijing significantly decreased these pro-inflammatory factors. Mean $\pm$ SD, $n=8$. * $P<0.01$ versus the sham group; # $P<0.01$ versus the paraquat group.

renal function.^[17] On the other hand, the study also found that Xuebijing treatment could reduce the levels of pro-inflammatory cytokines and suppress inflammatory responses.^[18] In this study, Xuebijing intervention significantly reduced the levels of pro-inflammatory cytokines (IL-1 β , IL-6 and TNF- α), demonstrating that Xuebijing had anti-inflammatory effects on paraquat intoxication.

Of course, the study had the following limitations. Firstly, this study did not directly evaluate renal inflammatory changes pathologically. Because inflammatory scores in renal pathology reflect inflammation levels, the marker would be involved in the further study. Secondly, this study did not observe the effects of Xuebijing injection on oxidative stress. In fact, oxidative stress also plays an important role in renal injuries induced by paraquat. The further experiments would be considered to explore the mechanism. Finally, in order to exclude other interference factors, a single treatment in this research only was adopted. In fact, at present there is no specific antidote of paraquat intoxication. Symptomatic and supportive treatment in clinical practice mainly includes emetic, gastric lavage, adsorption, catharsis, fluid infusion, diuresis, blood purification, glucocorticoid and immunosuppressants, etc. Therefore, this treatment may be used as an adjunctive therapy.

CONCLUSION

The study demonstrates that intravenous injection of Xuebijing attenuates AKI following paraquat intoxication via suppressing inflammatory responses.

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Ethical approval: The experiments were approved by the Institutional Animal Care and Use Committee of Fujian Medical University.

Conflicts of interest: The authors declare there is no competing interest related to the study, authors, other individuals or organizations.

Contributors: Xu JJ proposed the study and wrote the first draft. All authors read and approved the final version of the paper.

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