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Original Research Article

Chloroquine prevents acute kidney injury induced by lipopolysaccharide in rats via inhibition of inflammatory factors

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Abstract

Purpose: *To investigate the role of chloroquine (CQ) in lipopolysaccharide (LPS)-induced renal injury in rats.*
Methods: *Rats were assigned to one of four groups (n = 10). Control group was only given saline solution, whereas the model control, LPS + CQ, and LPS + yohimbine (YOH) + CQ groups were administered LPS intraperitoneally. At the end of the study, blood urea nitrogen (BUN) and creatinine (Cr) levels were determined.*
Results: *CQ treatment significantly decreased the blood concentrations of tissue necrosis factor alpha (TNF-α), interleukin-6 (IL-6), IL-18, BUN, and Cr in the model control rats. There were also significant decreases in the levels of high mobility group protein 1 and kidney injury molecule-1 in the renal injury rats compared to the model control group. However, the inhibitory effects of CQ in the LPS-treated rats were blocked by treatment with YOH, an α-2-adrenergic receptor antagonist.*
Conclusions: *Treatment with CQ attenuates LPS-induced renal injury by inhibiting inflammatory response.*

Keywords: *Creatinine, Chloroquine, Inflammatory reactions, Kidney injury, Lipopolysaccharide*

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INTRODUCTION

Various factors can trigger a systemic inflammatory response, which is characterized by a reduction in oxygen uptake and myocardial contractility such as pathogen administration, trauma, hypoxia, reperfusion injury, and postoperative sepsis [1]. Sepsis has a mortality rate of 40 – 80 % and most commonly targets the kidneys [2]. Acute kidney injury (AKI) induced by sepsis has a 70 % mortality rate [3,4]. Factors related to the onset of inflammation include tissue necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and IL-18, which are expressed at higher concentrations under pathological conditions. Of these, IL-18 is a biomarker of AKI [5]. Injuries to organs are induced by the IL-18-mediated expression of TNF-α and IL-6, which initiate an inflammatory reaction [6]. It is believed that inhibiting this inflammatory reaction is a promising strategy for preventing organ injury.

Chloroquine (CQ, Figure 1) and its analogs exhibit a wide spectrum of biological activities including anti-cancer [7] and anti-hepatitis C virus [8,9] effects. CQ also readily crosses the blood-brain barrier [10]. In addition, CQ analogs such as hydroxychloroquine have shown promise for the treatment of systemic lupus erythematosus

[11] and rheumatoid arthritis [12]. Experiments using a number of CQ analogs in a human diploid embryonic lung cell line (MRC-5) revealed their potential biological properties and lack of cytotoxic effects [13]. Therefore, this study evaluated the nephroprotective effects of CQ in LPS-induced sepsis.

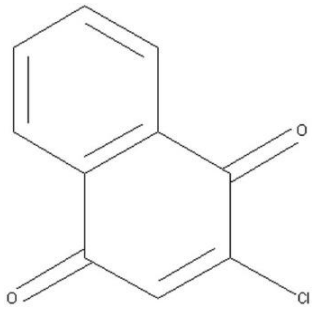

Figure 1: Chemical structure of chloroquine (CQ)

EXPERIMENTAL

Animals

Male 18-week-old Sprague–Dawley rats were obtained from Beijing Vital River Experimental Animal Technology (Beijing, China). The study was performed according to the European guidelines for animal use [14]. Study approval was obtained from the animal research ethics committee of The Provincial Hospital Affiliated with Shandong University (Shandong Sheng, China)

Drugs

CQ was dissolved in dimethyl sulfoxide and stored at -20 °C. Yohimbine (YOH) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Treatment protocols

The rats were separated into four groups: untreated normal control group, model control group that was administered LPS intraperitoneally (i.p.), LPS + CQ treatment group that was treated with CQ after LPS administration, and LPS + YOH + CQ treatment group that was pretreated with YOH followed by CQ treatment after LPS administration. LPS, CQ, and YOH were administered at doses of 4, 5, and 1 mg/kg body weight, respectively after 12 h.

Kidney damage analysis

The rats were sacrificed by pentobarbital overdose (70 mg/kg, i.p.) after 12 h and the blood Cr, BUN, IL-6, IL-18, and TNF-α levels were determined using blood collected from the abdominal artery. An automated biochemistry analyzer (Hitachi 7600-020/7170A; Hitachi High-Technologies, Tokyo, Japan) was used to measure the Cr and BUN levels. Immediately after sacrifice, the left kidney was isolated from all of the rats under liquid nitrogen, fixed in formalin, and stored at -78 °C. In addition, two biomarker molecules, high mobility group protein 1 (HMGB-1) and kidney injury molecule-1 (KIM-1), were measured in the renal tissue sections. To examine renal histology, renal tissues were fixed in formalin, embedded in paraffin, and examined under a microscope at 400 × magnification after hematoxylin and eosin staining.

Measuring TNF-A, IL-6, and IL-18 levels in the rat plasma

To determine the TNF-α, IL-18, and IL-6 levels in the rat plasma, blood samples were taken from the rats and centrifuged at 12000 × *g* for 10 min. Then the serum was isolated and stored at -78 °C. IL-6, IL-18, and TNF-α concentrations were measured using ELISA kits (Nanjing KeyGen Biotech, Jiangsu, China).

Western blot analysis

The renal tissue samples were washed twice with phosphate-buffered saline (PBS) and subsequently lysed in lysis buffer containing Tris-HCl pH 7.4 (50 µM), sodium chloride (137 µM), 10 % glycerol, 100 µM Na_2 (VO_3), 1 µM PMSF, aprotinin (10 mg/mL), leupeptin (10 mg/mL), 1 % NP-40, and cocktail (5 µM). The bicinchoninic acid assay was used to determine the protein concentration. The protein samples were loaded on 10 % polyacrylamide gels, resolved by electrophoresis, and electro transferred to poly (vinylidene fluoride) membranes using the semi-dry method. The membranes were washed with Tris-buffered saline Tween (TBST) and blocked in 5 % non-fat dry milk for 12 h. Subsequently, the membranes were incubated overnight with primary antibodies against KIM-1 and HMGB-1 (both purchased from Santa Cruz Biotechnology, Dallas, TX, USA), washed again with TBST, and incubated with secondary antibodies for 2 h. A chemiluminescent detection system was used to detect the protein bands, which were quantified using the Quantity One software package (Bio-Rad, Hercules, CA, USA) using GAPDH (Thermo Fisher Scientific, Fremont, CA, USA) as an internal control.

Statistical analysis

All of the experiments were conducted three times and the results are reported as the mean ±

SD. The results were compared using the Student's *t*-test and $p < 0.05$ was considered statistically significant. SPSS software version 16.0 was used for the data analysis.

RESULTS

Effects of CQ on BUN and creatinine levels in rats with LPS-induced AKI

The BUN and creatinine (Cr) levels were markedly higher in the LPS-treated group than in the controls (Figure 2), and CQ treatment caused significant decreases in serum BUN and Cr levels in the LPS-induced sepsis rat model. However, the decrease in Cr and BUN caused by CQ was inhibited in rats pretreated with the α-2-adrenergic receptor antagonist YOH, and Cr and BUN levels were similar to those of rats injected with LPS.

The renal histology micrographs of LPS-treated and untreated control groups showed luminal swelling and flattened glomerular and renal tubule cells in LPS-treated rats. LPS treatment also induced degeneration of the tubular epithelium, the appearance of small tubular structures, and an inflammatory cell infiltrate. However, CQ treatment inhibited these changes, and the findings were similar to those of the non-LPS control group. Pretreating rats with YOH followed by CQ treatment prevented the effects of CQ treatment (Figure 3).

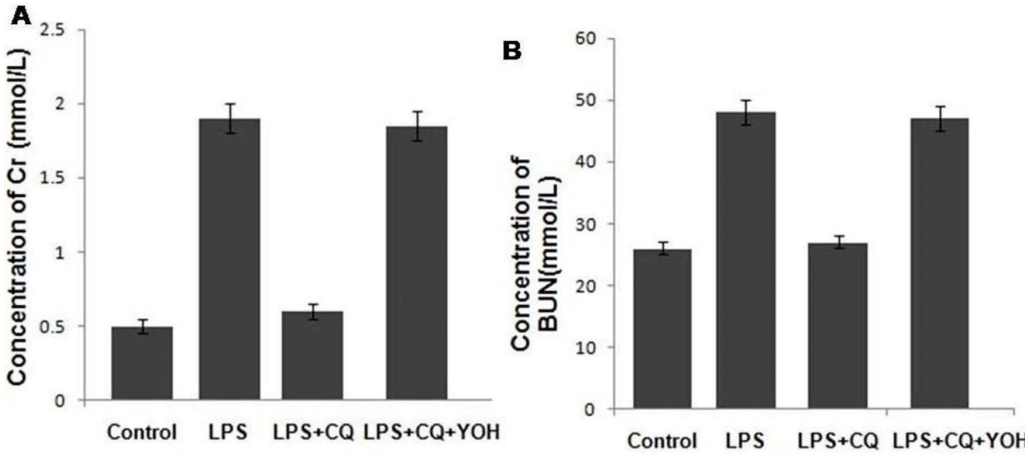

Figure 2: Effects of CQ and YOH on Cr and BUN levels in rats with LPS-induced renal injury. CQ treatment caused a significant decrease in serum levels of Cr and BUN in the LPS-induced kidney injury rat model

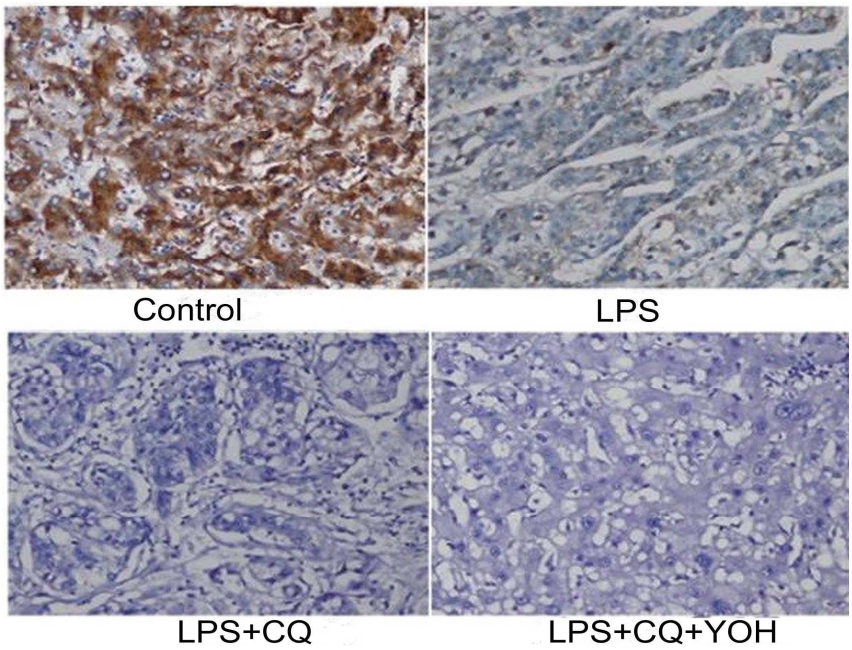

Figure 3: Effects of CQ and YOH on renal histology in LPS-induced renal injury in rats. The changes in kidney tissue histopathology were examined with hematoxylin and eosin staining. CQ, chloroquine; YOH, yohimbine; LPS, lipopolysaccharide; magnification 200×

Effects of CQ on HMGB-1 and KIM-1 expression in the kidneys of LPS-treated rats

The analysis of KIM-1 and HMGB-1, biomarkers of kidney damage, revealed significantly greater expression in rats treated with LPS (Figure 4). However, CQ treatment resulted in marked decreases in KIM-1 and HMGB-1 expression in LPS-treated rats. Treatment of rats given LPS with YOH followed by CQ prevented the CQ-mediated decreases in KIM-1 and HMGB-1.

Effects of CQ on the inflammatory reaction in the kidney

There was increased expression of the inflammatory factors IL-6, IL-18, and TNF-α in the LPS-treated rats (Figure 5). CQ treatment induced a marked decrease in the expression of all three inflammatory factors, whereas pretreatment with YOH inhibited the effects of CQ.

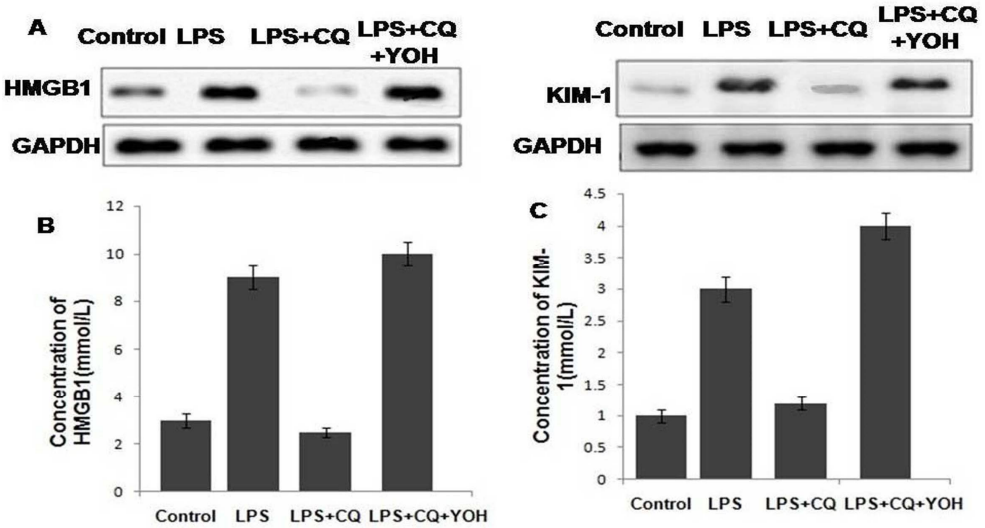

Figure 4: Effects of CQ and YOH on HMGB-1 and KIM-1 expression in the renal tissues. YOH, yohimbine; LPS, lipopolysaccharide

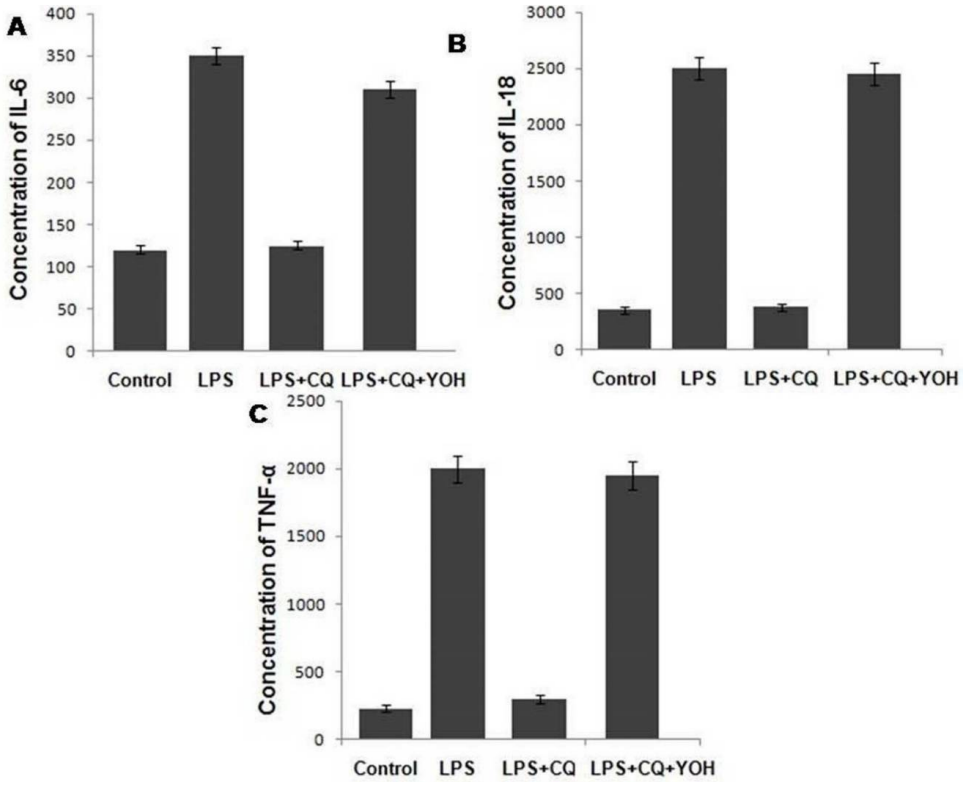

Figure 5: Effects of CQ and YOH on TNF-α, IL-18, and IL-6 expression in rats with LPS-induced renal injury. IL, interleukin; TNF-α, tumor necrosis factor-α; YOH, yohimbine; LPS, lipopolysaccharide

DISCUSSION

Renal tubule injury is the cause of renal failure in more than 80 % of affected patients [15, 16]. This study demonstrated the effects of CQ on AKI in rats administered LPS. CQ treatment inhibited the effects of LPS and prevented renal damage, as evidenced by the decrease in KIM-1 and HMGB-1 expression. Pre-treatment of LPS-treated animals with YOH blocked the effects of CQ, suggesting that CQ elicits its effects via the α-2 adrenoceptor. HMGB-1 binds to inflammatory mediators and induces the release of pro-inflammatory cytokines [17]. KIM-1, a transmembrane tubular protein, is expressed in the kidneys after renal injury and serves as a marker of renal injury [17]. Kidney injury involves an inflammatory reaction [18]. The reduction in the expression of factors responsible for inflammatory reactions caused by CQ, which was enhanced by LPS treatment, demonstrated the inhibitory effects of CQ on IL-6, IL-18, and TNF-α expression. The inflammatory reactions promoted by TNF-α and IL-6 factors are induced by IL-18 and cause organ injury [19].

This study showed that CQ treatment in LPS-treated rats inhibited kidney injury via the suppression of factors involved in inflammation such as IL-6, IL-18, and TNF-α. However, pretreatment with the α-2-adrenergic receptor antagonist YOH blocked the effects of CQ in the renal tubules, collecting ducts, and microvascular system.

CONCLUSION

The nephroprotective effects of CQ in LPS-induced renal injury can be attributed to the inhibition of the inflammatory response. CQ may play a role in the management renal injury but further studies are required confirm this.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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