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Original Research Article

Aloperine attenuates carbon tetrachloride-induced mouse hepatic injury via Nrf2/HO-1 pathway

Rui Xiong[1], Shuzhong Shan[2]*, Xiaoming Wang[1], Xiaowen Zhang[1], Haixia Yu[1], Haomin Shi[1], Xuejiao Wang[1]
[1]Department of Clinical Nutrition, [2]Department of Orthopaedics, Qinhai Provincial People's Hospital, Xining City, Qinghai Province 810007, China

For correspondence:* ***Email:* *ShuzhongShandjk@163.com;* ***Tel:*** *+86-971-8066178*

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Abstract

Purpose: *To investigate whether aloperine pretreatment ameliorates acute liver injury in carbon tetrachloride (CCl_4)-treated mice.*
Methods: *Mice were injected with CCl_4 and orally administered aloperine. Blood samples and liver tissues were used for histopathological and biochemical analyses, respectively. Protein expression levels were determined by western blotting.*
Results: *Histopathological analysis indicate that aloperine pretreatment significantly alleviated CCl_4-induced mouse hepatic injury. CCl_4 treatment induced the upregulation of aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine amino transferase (ALT), and total bilirubin ($p < 0.05$). However, these alterations were significantly inhibited by aloperine treatment. Moreover, aloperine pretreatment markedly decreased ($p < 0.05$) the CCl_4-induced expression of oxidative stress biomarkers, including malondrialdeline (MDA), glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD). Compared to the control group, the protein levels of Nrf2, HO-1, iNOS, and COX-2 were significantly increased in the CCl_4 group, while Nrf2 and HO-1 were upregulated. Furthermore, iNOS and COX-2 were downregulated in mouse liver in CCl_4 + aloperine group compared to CCl_4 group in a concentration-dependent manner ($p < 0.05$).*
Conclusion: *Aloperine pretreatment appears to markedly upregulate Nrf2 and HO-1 and downregulate iNOS and COX-2 to suppress hepatic injury in mice. Thus, aloperine is a promising treatment for acute liver injury.*

Keywords: *Hepatic injury, Aloperine, Oxidative stress, Nrf2/HO-1 pathway*

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INTRODUCTION

Environmental toxins often disturb hepatic metabolic function and increase the expression of liver enzymes, leading to liver fibrosis, cirrhosis, and even cancer [1]. Liver injury can be caused by a combination of oxidative stress, necrosis, inflammation, and apoptosis [2]. Carbon tetrachloride (CCl_4) is frequently used to induce liver injury and to study the effects of chemical compounds on the liver [3]. Existing hepatoprotective drugs are used sparingly

because of their side effects [4]. Therefore, there is an urgent need to develop safer hepatoprotective drugs.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is an important leucine zipper-containing, redox-sensitive transcription factor that can induce the production of antioxidant enzymes via the antioxidant response element [5]. By activating heme oxygenase-1 (HO-1) expression, Nrf2 can suppress the nuclear translocation of NF-κB and subsequent inflammatory responses [6]. Thus, interventions that target the Nrf2/HO-1 signaling pathway have recently been suggested to be important therapeutic approaches for the treatment of alcoholic liver injury [7]. Antioxidants not only remove free radicals, but are also cytoprotective [8]. For example, the combination of metformin and luteolin has been reported to reduce liver injury induced by CCl_4 by activating the Nrf2/HO-1 pathway [9]. The Nrf2/HO-1 pathway thus appears vital for treating liver injury.

Aloperine, a compound that can be extracted from bitter beans, plays an important role in certain inflammatory disorders, such as allergic contact dermatitis and experimental colitis [10,11]. A recent study showed that aloperine protects mice from ischemia-reperfusion-induced renal injury by reducing levels of oxidative stress [12]. Furthermore, aloperine was found to attenuate allergic airway inflammation by regulating the Nrf2/HO-1 signaling pathway [13]. Therefore, whether aloperine could alleviate liver injury remains to be investigated. The present study was undertaken to explore the influence of aloperine pretreatment on acute liver injury caused by CCl_4 in mice and determine the possible involvement of the Nrf2/HO-1 pathway in this process.

EXPERIMENTAL

Chemical and reagents

Aloperine and CCl_4 were purchased from Abcam (Cambridge, MA, USA) and Sigma-Aldrich (St. Louis, MO, USA), respectively.

Mice and treatments

The animal study was approved the Medical Ethics Committee of Qinhai Provincial People's Hospital (approval no. 20191016) and conducted according to the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health [14]. Twenty-four male C57BL/6 mice (6–8 weeks of age, 16–18 g; Animal Research Center of Nanjing University (Nanjing, China) were randomly divided into four groups (six mice/group): Control, CCl_4, CCl_4 + 50 mg/kg aloperine, and CCl_4 + 100 mg/kg aloperine. In the CCl_4 group, mice were intraperitoneally (i.p.) injected with CCl_4 [10 mL/kg, 0.5% (v/v), dissolved in olive oil]. In the CCl_4 + 50 mg/kg aloperine and CCl_4 + 100 aloperine groups, mice were orally administered aloperine (50 and 100 mg/kg, respectively, resuspended in ethanol) for 7 days. Two hours after the last dose of aloperine, mice were injected with CCl_4. Mice in the control and CCl_4 groups were given the same dose of solvent. Mice were sacrificed 24 h post-CCl_4 injection. Blood samples were taken from the orbital vein, while liver tissues were obtained after dissection and used for the following analyses.

Hepatic histopathological examination

Liver tissues were fixed, embedded, and stained with hematoxylin and eosin (H&E). The extent of injury was scored from 0 to 4, based on the severity of the vacuolization of hepatocyte cytoplasm, sinusoidal congestion, and parenchymal necrosis [15]. Ten different views of an image were randomly chosen and scored by two pathologists independently.

Evaluation of biomarkers of liver function

Serum AST, ALP ALT, and total bilirubin levels were measured utilizing commercial assay kits (Jiancheng Biological Technology, China).

Antioxidants in hepatic tissues

Hepatic tissues were homogenized and centrifuged. The supernatants of the liver tissue homogenates were used for the determination of malondrialdeline (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) levels. Commercial assay kits (Jiancheng Biological Technology) were used to examine the effects of the antioxidants on hepatic tissues.

Protein extraction and western blotting analysis

Western blotting was conducted, as described previously [16,17]. Total protein was extracted, subjected to SDS-PAGE, and then transferred onto PVDF membranes. The membranes were then blocked with 5% nonfat milk and incubated with the following antibodies: anti-Nrf2 (Abcam); anti-HO-1(Cell Signaling Technology, Danvers, MA, USA), anti-iNOS (Abcam); and anti-COX-2 (Abcam). The level of β-actin was used as an internal control. Proteins were visualized and quantified.

Statistical analysis

The results are expressed as mean ± SD and analyzed by one-way ANOVA among the four groups using SPSS software. $P < 0.05$ was considered statistically significant.

RESULTS

Aloperine attenuates CCl_4-induced liver injury in mice

To verify the role of aloperine in reducing hepatic damage, liver tissues were collected from mice in the different groups and examined by histological analysis and scored. Compared to control mice, CCl_4 treatment resulted in severe liver damage (Figure 1). The hepatic damage was alleviated to a certain extent by treatment with 50 mg/kg aloperine, but was ameliorated to a much greater extent by treatment with 100 mg/kg aloperine. These findings demonstrate that aloperine reduces CCl_4-induced liver damage in mice.

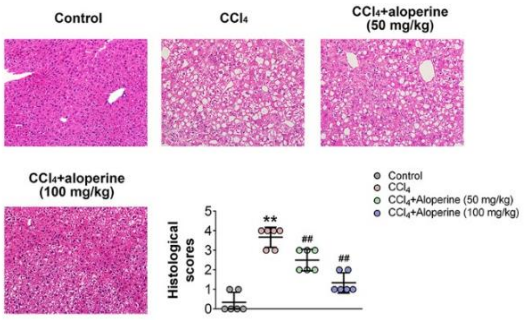

Figure 1: Morphological and histological features of liver tissues from mice in the different groups. H&E staining of liver sections from the various mouse groups was performed. Scores were assigned, according to the severity of liver injury, and are shown as a scatter diagram. Original magnification, 200×. **/##, $P < 0.01$

Aloperine treatment decreases CCl_4-induced serum levels of hepatic functional markers in mice

The effects of aloperine on the serum levels of hepatic functional markers were determined. Compared to the control group, the serum levels of AST, ALT, ALP, and total bilirubin were significantly increased in mice by CCl_4 treatment. However, aloperine treatment (50 mg/kg) markedly reduced the levels of these hepatic functional markers compared to mice with CCl_4 treatment. Moreover, the serum concentrations of these markers were lower in the CCl_4 + 100 mg/kg aloperine group than in the CCl_4 + 50 mg/kg aloperine group (Figure 2).

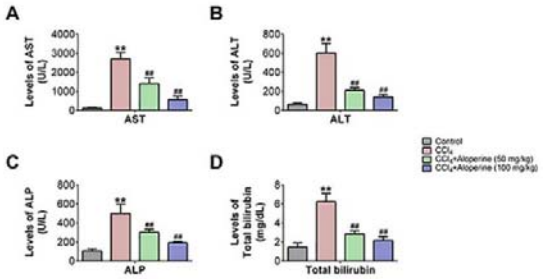

Figure 2: Effect of aloperine on the serum levels of hepatic functional markers in mice. **/##, $P < 0.01$

Aloperine treatment reduces CCl_4-induced oxidative stress in mouse liver

CCl_4 treatment for 24 h markedly upregulated the levels of MDA and downregulated the activities of GSH, CAT, and SOD. Aloperine pretreatment reversed these effects in a dose-dependent manner (Figure 3).

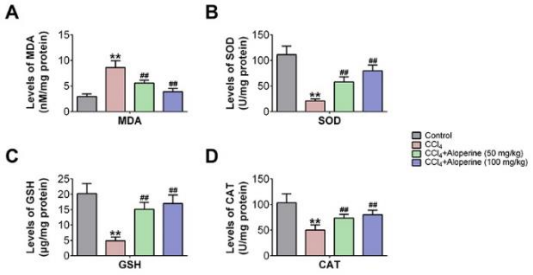

Figure 3: Effects of aloperine on CCl_4-induced oxidative stress in mouse liver. **/##, $P < 0.01$.

Aloperine reverses the effects of CCl_4 treatment on the regulation of the Nrf2/HO-1 axis in mouse liver

The results shown in Figure 4 showed that, compared to the control group, the protein levels of Nrf2, HO-1, iNOS, and COX-2 were significantly increased in the CCl_4 group. On the other hand, Nrf2 and HO-1 were upregulated, whereas iNOS and COX-2 were downregulated in mouse liver in CCl_4 + aloperine group compared to the CCl_4 group in a concentration-dependent manner.

DISCUSSION

The liver has numerous important functions that are essential for life, including protein synthesis, glucose homeostasis, and detoxification. Although the liver has a strong regenerative capacity, it can still become damaged due to exposure environmental toxins, resulting in organ

dysfunction and metabolic abnormalities. The underlying molecular mechanism of acute liver injury has been found to be associated with oxidative stress, apoptosis, and inflammation [1]. CCl_4 treatment is known to stimulate lipid peroxidation, reactive oxygen species production, and centrilobular necrosis and steatosis; it has been extensively used to induce acute hepatic damage in a mouse model of liver toxicity [18].

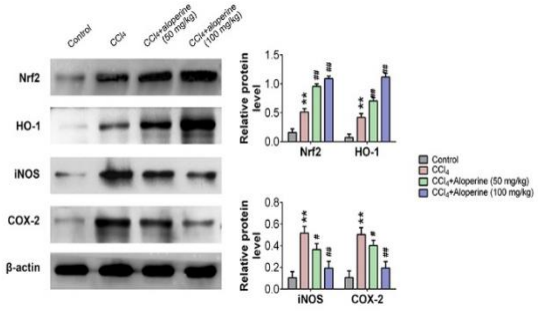

Figure 4: Effects of aloperine treatment on the expression of Nrf2/HO-1 pathway components in mouse liver. Relative protein levels are shown in the western blot. #, $P < 0.05$, **/##, $P < 0.01$

Some natural products have been shown to relieve CCl_4-induced liver damage. For example, curcumin, an extract from turmeric rhizomes, can inhibit oxidative stress and inflammation, ameliorating CCl_4-induced liver injury [19]. In addition, sesamin has been suggested to regulate the JNK pathway and inhibit hepatic oxidative stress induced by CCl_4 [20]. In the present study, aloperine treatment effectually relieved hepatic dysfunction and histopathologic damage by downregulating serum AST, ALT, and ALP activities, and total levels of bilirubin. The combination of two or more agents may result in synergistic effects to alleviate hepatic injury, which could potentially lessen the dose of a single drug and reduce untoward side effects. For example, the combination of metformin and luteolin has been shown to exhibit a potential synergy for the treatment of hepatic injury mediated by CCl_4 [9]. Whether these medications can be used in combination with aloperine to prevent liver damage deserves further study.

Aloperine has been found to possess some beneficial medicinal functions. It protects mice against DSS-induced colitis via the suppression of the PI3K/Akt signaling pathway [21]. Aloperine pretreatment has also been shown to reduce inflammation, the apoptosis of tubular cells, and renal damage caused by ischemia-reperfusion [12]. Furthermore, aloperine was shown to inhibit the proliferation and differentiation of fibroblasts, alleviating lung fibrosis induced by bleomycin [22]. The present study demonstrated that aloperine could inhibit oxidative stress responses and elicit hepatoprotective effects. Based on these findings, aloperine may have other medicinal benefits that should be explored in the future.

Nrf2 has been shown to regulate antioxidant genes; its activation might provide effective cellular protection by regulating intracellular redox status [8]. Previous studies have suggested that natural antioxidants may activate Nrf2 and HO-1. For example, curcumin, an activator of the Nrf2 pathway, was shown to stimulate the activity of antioxidants and suppress the oxidative stress caused by CCl_4 [19]. In addition, morin [23], anwulignan [24], and andrographolide [25] have been shown to upregulate Nrf2/HO-1, exert antioxidative functions, and protect against mouse liver damage. Therefore, the stimulation of the Nrf2/HO-1 pathway is a promising strategy for the clinical treatment of liver injury.

CONCLUSION

The findings of this study indicate that aloperine pretreatment suppresses mouse hepatic injury, partly via the enhancement of Nrf2/HO-1 pathway and stimulation of its antioxidant activity. These findings may thus lead to the development of novel drug candidates for the management of acute liver injury.

DECLARATIONS

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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 authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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