Protective effects of a mineral aqueous solution on toxicity in mouse liver and kidney

In-Jae Park, Se-Yeoun Cha, Min Kang, Yang-Sub So, Ji-Yun Bahng, Hyung-Kwan Jang*

Department of Veterinary Infectious Diseases and Avian Diseases, College of Veterinary Medicine & Korea Zoonosis Research Institute, Chonbuk National University, Jeonju 561-756, Korea

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Abstract : We demonstrated that a mineral aqueous solution (MAS) administered to mice functionally and histologically protected against cisplatin-induced acute renal failure (ARF) and CCl₄-induced acute liver failure (ALF). In ARF model, 0.4 and 0.2% MAS decreased mortality and the serum concentrations of blood urea nitrogen (BUN) and creatine in mice. Additionally, 0.4 and 0.2% MAS reduced contraction of distal convoluted tubules and suppressed expression of the proinflammatory cytokines interleukine-6 (IL-6) and tumor necrosis factor (TNF-α) in the kidney. In ALF model, 0.4 and 0.2% MAS decreased serum concentrations of alanine aminotransferase and aspartate aminotransferase in mice. Additionally, 0.4 and 0.2% MAS reduced necrotic areas and suppressed expression of IL-6 and TNF-α in the liver. These results indicate that a MAS might have protective effects against ARF and ALF.

Keywords : CCl₄-induced acute liver failure, cisplatin-induced acute renal failure, mineral aqueous solution, silicon

Introduction

Cisplatin and other platinum derivatives are important chemotherapeutic agents used to treat solid tumors, including ovarian, head and neck, and testicular germ cell tumors [26]. A known complication of cisplatin administration is acute renal failure (ARF). The nephrotoxic effect of cisplatin is cumulative and dose-dependent and often necessitates dose reduction or withdrawal. Approximately 25~35% of patients develop evidence of nephrotoxicity following a single dose of cisplatin [24]. Despite this toxicity, cisplatin remains one of the most commonly used chemotherapeutic drugs due to its efficacy [26].

Much attention has been focused on the direct toxic effects of cisplatin to renal tubular cells in vitro [24]. In this setting, cisplatin induces DNA damage [13, 24], mitochondrial dysfunction [31], formation of reactive oxygen species [15], caspase activation [12], and either necrotic or apoptotic cell death depending on the cisplatin concentration [14, 19]. Inflammatory mechanisms appear to play an important role in the pathogenesis of ischemic acute renal injury [4, 28].

Acute liver failure (ALF) is a rare condition consisting of rapid-onset severe liver injury accompanied by coagulopathy and encephalopathy, and a patient mortality rate of 90% [5, 18]. Approximately 2,000 cases per year occur in the USA resulting in liver transplantation or death in >35% of these cases, frequently due to multi-organ failure. Nevertheless, the etiology of ALF is mysterious in approximately 20% of adult patients in the USA [20].

Acute administration of carbon tetrachloride (CCl₄) has been used to establish an experimental model of severe hepatocellular damage involving generation of oxidative stress and recruitment of inflammatory cells [10, 21, 29], which induces liver architectural and functional damage [11, 16, 25]. Liver regeneration involves a complex regulated response to CCl₄-induced ALF [17, 30].

Silicon is the second most abundant element in the lithosphere after oxygen and is an essential element. However, silicon has no known biochemistry to describe its requirement by biota. Although silicon is not widely regarded as an essential element in mammals, it has some beneficial actions in chicks and rats through effects on growth and skeletal development [6, 27]: abnormalities involving articular cartilage and connective tissue are produced in chicks fed a silicon-deficient diet.

The objective of this study was to evaluate the protective effects of a silicon-rich mineral aqueous solution (MAS) on cisplatin-induced ARF and CCl₄-induced ALF.

Materials and Methods

MAS preparation

The MAS was provided by Green Nanobiotech (Korea). Concentrations of nutrient constituents in the MAS such as

*Corresponding author
Tel: +82-63-270-3885, Fax: +82-63-270-2135
E-mail: hkjang@chonbuk.ac.kr
Cisplatin-induced ARF and CCl₄-induced ALF

Four-week-old female Balb/c mice were randomly divided into five groups (n = 10) for the cisplatin-induced ARF study. The negative and positive control groups did not receive MAS. Three experimental groups received drinking water containing MAS (0.4, 0.2, and 0.1%; 6.24, 3.12 and 1.56 mg/kg body weight as silicon concentration in MAS) for 2 weeks before cisplatin administration. Cisplatin (Sigma-Aldrich, USA) was freshly prepared in sterile normal saline at a concentration of 0.5 mg/mL the day of administration. Mice were given either 20 mg/kg body weight of cisplatin or vehicle (saline) intraperitoneally (i.p.). Kidneys were isolated and blood samples were collected via cardiac puncture on day 3 after cisplatin administration.

Four-week-old female Balb/c mice were randomly divided into five groups (n = 10) for the CCl₄-induced ALF study. The negative and positive control groups did not receive the MAS. Three experimental groups received drinking water containing MAS (0.4, 0.2, and 0.1%; 6.24, 3.12 and 1.56 mg/kg body weight as silicon concentration in MAS) for 2 weeks before CCl₄ administration. CCl₄ (Sigma-Aldrich) was freshly prepared in olive oil at a concentration of 0.5% (v/v) the day of administration. Mice were given either 25 µL of 0.5% CCl₄ or vehicle (olive oil) i.p. Livers were isolated and blood samples were collected via cardiac puncture on day 1 after CCl₄ administration.

Serum analysis

Serum concentrations of blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in mice were measured using a COBAS INTEGRA 400 plus system (Roche, Germany).

Histological examination

Kidney and liver tissues were fixed in 10% formaldehyde and embedded in paraffin, and 4-µm sections were stained with hematoxylin and eosin. Histological analyses of kidney and the liver injury were performed using an Eclipse Ti-U inverted microscope (Nikon, Japan).

Quantitation of mRNA by real-time reverse-transcription polymerase chain reaction (RT-PCR)

Total RNA in kidney and liver tissues was isolated using an Easy-BLUE Total RNA Extraction kit (Intron Biotech, Korea). Real-time RT-PCR was performed with a Stratagene Mx3000P Real-Time PCR system (Stratagene, USA). Three micrograms of total RNA was reverse transcribed in a reaction volume of 20 µL using GoScript Reverse Transcriptase (Promega, USA) and random primers. The product was diluted to 80 µL, and a 3 µL aliquot was used as a template for amplification using Brilliant III Ultra-Fast SYBR Green QPCR Master Mix (Agilent Technologies, USA) and genespecific primers. The primer sets used were: mouse interleukin (IL)-6 (forward: CTA TAC CAC TTC ACA AGT CGG AGG C TT; reverse: TAG GAG AGC ATT GGA AAT TGG GTG AGG), tumor necrosis factor (TNF)-α (forward: CAT CAG TTC TAT GGC CCA GAC CCT C; reverse: CGG CAG AGA GGA GGT TGA CTT TCT C). The amount of DNA was normalized to the GAPDH signal amplified in a separate reaction (forward: CCC CTT CAT TGA CCT CAA CTA CAT GGT; reverse: GTT GT C ATA TTT CTC GTG GTT CAC ACC C).

Statistical analysis

All data were analyzed with SPSS 12.0 statistical software (SPSS, USA). Data are expressed as mean ± standard deviation. Statistical differences were examined independently using the Student’s t test and Pearson’s correlation test. A p value < 0.05 was considered significant.

Results

The constituents of the MAS by ICP/OES

The concentration of essential trace elements in the MAS are listed in Table 1. Silicon was found at a high concentration (26.0%), whereas potassium and sodium were at low concentrations (2.9 and 5.36%, respectively).

<table>
<thead>
<tr>
<th>Chemical elements</th>
<th>Concentration (%)</th>
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<tbody>
<tr>
<td>Si</td>
<td>26.00</td>
</tr>
<tr>
<td>K</td>
<td>2.90</td>
</tr>
<tr>
<td>Na</td>
<td>5.36</td>
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</tbody>
</table>

MAS protects mice from ARF and ALF

We investigated the protective effects of the MAS on cisplatin-induced ARF and CCl₄-induced ALF. The mice received drinking water or water containing the MAS (0.4, 0.2 and 0.1%) for 2 weeks before cisplatin and CCl₄ administration.

In the cisplatin-induced ARF group, 50% of the positive-control group died within 72 h, whereas only 20% of the groups receiving the MAS (0.4 and 0.2%) died (data not shown). The mice were sacrificed 72 h after cisplatin administration for BUN and creatinine determinations. At the time of killing, BUN levels in the groups receiving MAS (0.4 and 0.2%) were significantly lower than those in the positive-control group (45.18 mg/L vs. 163.5 ± 45.18 mg/L, 18.67 mg/L vs. 5.36 ± 71.77 mg/L) (Fig. 1A). Creatine levels in the groups receiving MAS (0.4 and 0.2%) were significantly lower than those in the positive-control group (0.5 ± 0.07 mg/L vs. 3.2 ± 0.75 mg/L vs. 5.2 ± 0.04 mg/L) (Fig. 1B).

The mice were sacrificed 24 h after CCl₄ administration for quantification of mRNA by real-time reverse-transcription polymerase chain reaction (RT-PCR). Total RNA in kidney and liver tissues was isolated using an Easy-BLUE Total RNA Extraction kit (Intron Biotech, Korea). Real-time RT-PCR was performed with a Stratagene Mx3000P Real-Time PCR system (Stratagene, USA). Three micrograms of total RNA was reverse transcribed in a reaction volume of 20 µL using GoScript Reverse Transcriptase (Promega, USA) and random primers. The product was diluted to 80 µL, and a 3 µL aliquot was used as a template for amplification using Brilliant III Ultra-Fast SYBR Green QPCR Master Mix (Agilent Technologies, USA) and genespecific primers. The primer sets used were: mouse interleukin (IL)-6 (forward: CTA TAC CAC TTC ACA AGT CGG AGG C TT; reverse: TAG GAG AGC ATT GGA AAT TGG GTG AGG), tumor necrosis factor (TNF)-α (forward: CAT CAG TTC TAT GGC CCA GAC CCT C; reverse: CGG CAG AGA GGA GGT TGA CTT TCT C). The amount of DNA was normalized to the GAPDH signal amplified in a separate reaction (forward: CCC CTT CAT TGA CCT CAA CTA CAT GGT; reverse: GTT GT C ATA TTT CTC GTG GTT CAC ACC C).

Statistical analysis

All data were analyzed with SPSS 12.0 statistical software (SPSS, USA). Data are expressed as mean ± standard deviation. Statistical differences were examined independently using the Student’s t test and Pearson’s correlation test. A p value < 0.05 was considered significant.
Protection against liver and kidney injury by mineral aqueous solution

ALT and AST determination in the CCl$_4$-induced ARF group. At the time of killing, ALT levels in the groups receiving MAS (0.4, 0.2, and 0.1%) were significantly lower than those in the positive-control group (24.7 ± 6.22 U/L vs. 627.4 ± 7.57 U/L vs. 836.8 ± 10.15 U/L vs. 923.5 ± 15.08) (Fig. 2). AST levels in the groups receiving MAS (0.4 and 0.2%) were significantly lower than those in the positive-control group (331.4 ± 48.68 U/L vs. 887.4 ± 19.31 U/L vs. 1246.9 ± 121.44 U/L) (Fig. 2).

MAS reduces kidney and liver injury from ARF and ALF

In cisplatin-induced ARF, kidney sections from the positive-control group showed renal necrosis at 72 h after cisplatin injection embedded in paraffin, cut into 4 µm sections, and stained with H&E. (A; Negative control, B; Positive control, C; 0.4% MAS, D; 0.2% MAS, E; 0.1% MAS). Kidney sections from the 0.4% MAS group (C) were not different with those in the negative-control (A), and the 0.2% MAS (D) showed only mild renal necrosis. arrows: necrotic areas, ×200.

In CCl$_4$-induced ALF, liver sections from the positive-control group showed liver necrosis at day 1 after CCl$_4$ injection, whereas liver sections from the 0.4% MAS group showed no differences with the negative control group histology. The 0.2% MAS group showed only mild renal necrosis (Fig. 3).

We found that the necrotic areas diminished significantly around the central vein in the MAS groups at 24 h.

MAS suppresses proinflammatory cytokine expression

In cisplatin-induced ARF, kidneys were harvested at 72 h...
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In cisplatin-induced ARF, serum BUN and creatinine levels in the positive-control group increased dramatically compared with those in the negative control group, indicating renal dysfunction. In contrast, the 0.4 and 0.2% MAS groups showed markedly attenuated release of BUN and creatinine. In the CCl₄-induced ALF group, serum ALT and AST levels in the positive-control group increased dramatically compared with those in the negative-control group, indicating severe hepatocellular damage. In contrast, the 0.4 and 0.2% MAS groups showed markedly attenuated ALT and AST release.

The kidney and the liver histological observations strongly support the protective effect of the MAS on ARF and ALF (Figs. 3 and 4). Cisplatin and CCl₄ caused histological changes.

**Discussion**

Cisplatin is an important antitumor agent used for treating various solid tumors. The key limitation of this drug is its nephrotoxicity. Studies on the pathogenesis of cisplatin nephrotoxicity have mainly focused on the direct toxicity of cisplatin *in vitro*, including the role of oxidative stress [1, 7]. However, recent studies have demonstrated the important role of inflammation and cytokine activity in the pathogenesis of cisplatin nephrotoxicity [8, 23].

The CCl₄ model of acute intoxication has been used for decades to investigate the response to acute and chronic liver injury, because the elementary lesions caused by this hepatotoxin replicate those seen in most cases of human liver diseases. Proinflammatory cytokines are believed to play a key role in the pathogenesis of CCl₄-induced liver injury [9, 17].

In our previous examination, we tested the protective effect of 2% MAS on cisplatin-induced ARF and CCl₄-induced ALF. Serum BUN, creatine, ALT, and AST levels in mice that received 2% MAS were similar to those in mice that received 0.4% MAS. Additionally, the kidney and the liver histological observations in the 2% MAS mice were similar to those in the 0.4% MAS mice. Therefore, the protective effects of 0.4, 0.2, and 0.1% MAS were examined using models of cisplatin-induced ARF and CCl₄-induced ALF.

In the cisplatin-induced ARF group, serum BUN and creatinine levels in the positive-control group increased dramatically compared with those in the negative control group, indicating renal dysfunction. In contrast, the 0.4 and 0.2% MAS groups showed markedly attenuated release of BUN and creatinine. In the CCl₄-induced ALF group, serum ALT and AST levels in the positive-control group increased dramatically compared with those in the negative-control group, indicating severe hepatocellular damage. In contrast, the 0.4 and 0.2% MAS groups showed markedly attenuated ALT and AST release.

The kidney and the liver histological observations strongly support the protective effect of the MAS on ARF and ALF (Figs. 3 and 4). Cisplatin and CCl₄ caused histological changes.
in the kidney and liver, including necrosis. These alterations were significantly attenuated by the MAS, as the kidneys and the livers showed only minor necrotic areas. These results suggest that the MAS may have potential clinical applications for preventing kidney and liver disorders.

TNF-α and IL-6 are pleiotropic proinflammatory cytokines that are rapidly produced by macrophages in response to tissue damage [2, 32]. While low levels of TNF-α may play a role in cell protection, excessive amounts cause cell impairment. An increase in TNF-α level has been directly correlated with histological evidence of hepatic necrosis and an increase in serum AST levels [3]. Recent studies have shown that proinflammatory cytokines such as TNF-α and IL-6 are associated with cisplatin-induced acute renal failure [22]. Real-time RT-PCR was performed to analyze proinflammatory cytokine levels in kidney and liver tissue. As a result, TNF-α and IL-6 expression decreased in the kidneys and livers of the 0.4 and 0.2% MAS groups. These results indicate that the MAS may suppress expression of proinflammatory cytokines such as TNF-α and IL-6. Additional studies are required to examine this effect in further detail.

Our results provide evidence for the protective effect of the MAS in cisplatin-induced ARF and CCl4-induced ALF. Overall, the MAS not only suppressed the inflammatory response but also prevented functional damage in ARF and ALF models. Further studies will be needed to fully understand the association between the mode of action of silicon and the inflammatory responses in the protective effect of the MAS against cisplatin-induced ARF and CCl4-induced ALF.

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