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

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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 CCl_4-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 interlukein-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 : CCl_4-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 (CCl_4) 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 CCl_4-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 CCl_4-induced ALF.

Materials and Methods

MAS preparation

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

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silicon, potassium, and sodium were determined by inductively coupled plasma optical emission spectroscopy (ICP/OES) (PerkinElmer, USA).

Cisplatin-induced ARF and CCl_4-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 CCl_4-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 CCl_4 administration. CCl_4 (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% CCl_4 or vehicle (olive oil) i.p. Livers were isolated and blood samples were collected *via* cardiac puncture on day 1 after CCl_4 administration.

Serum analysis

Serum concentrations of blood urea nitrogen (BUN), creatine, 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 gene-specific 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 GGT 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).

Table 1. The concentrations of essential and possible essential trace elements in the mineral aqueous solution (MAS) were measured by inductively coupled plasma optical emission spectroscopy (ICP/OES)

Chemical elements	Concentration (%)
Si	26.00
K	2.90
Na	5.36

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).

MAS protects mice from ARF and ALF

We investigated the protective effects of the MAS on cisplatin-induced ARF and CCl_4-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 CCl_4 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 creatine 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 (40.9 ± 18.67 mg/L *vs.* 163.5 ± 45.18 mg/L *vs.* 363.6 ± 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 CCl_4 administration for

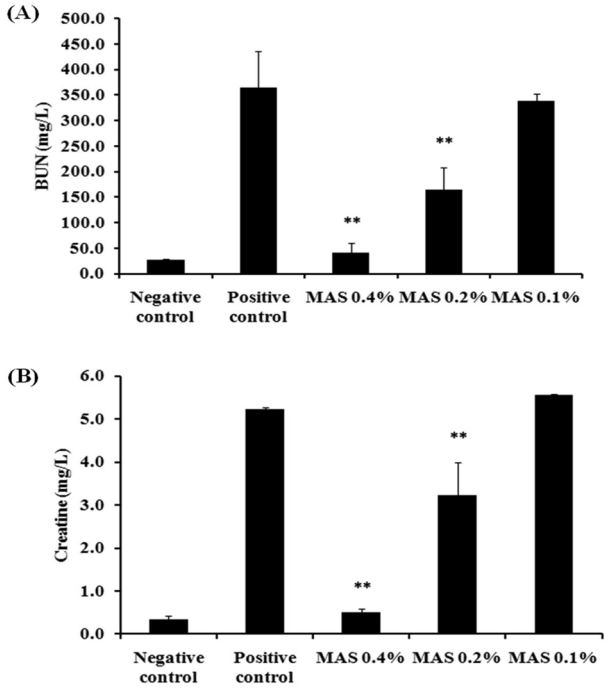

Fig. 1. Effect of the MAS on renal function in cisplatin-induced acute renal failure (ARF). Mice received drinking water or water containing the MAS (0.4, 0.2, and 0.1%) for 2 weeks before cisplatin administration. Blood urea nitrogen (BUN) (A) and plasma creatine (B) were measured 72 h after cisplatin injection. Cisplatin caused severe renal dysfunction, which was partially prevented by the MAS. $^{*}p < 0.05$, $^{**}p < 0.005$; n = 5.

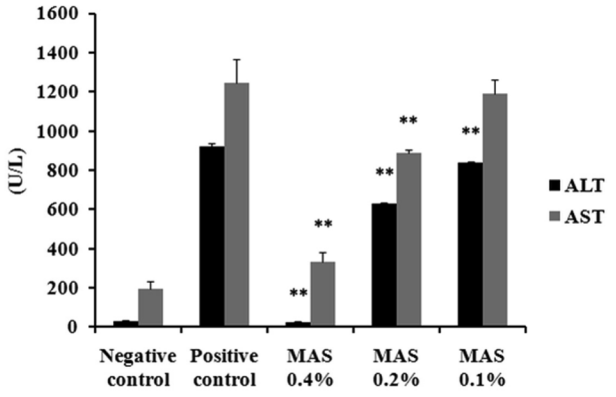

Fig. 2. Effect of the MAS on liver function in CCl_4-induced acute liver failure (ALF). Mice received drinking water or water containing the MAS (0.4, 0.2, and 0.1%) for 2 weeks before CCl_4 administration. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured 24 h after CCl_4 injection. CCl_4 caused severe liver dysfunction, which was partially prevented by the MAS. *P < 0.05, **P < 0.005; n = 5.

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).

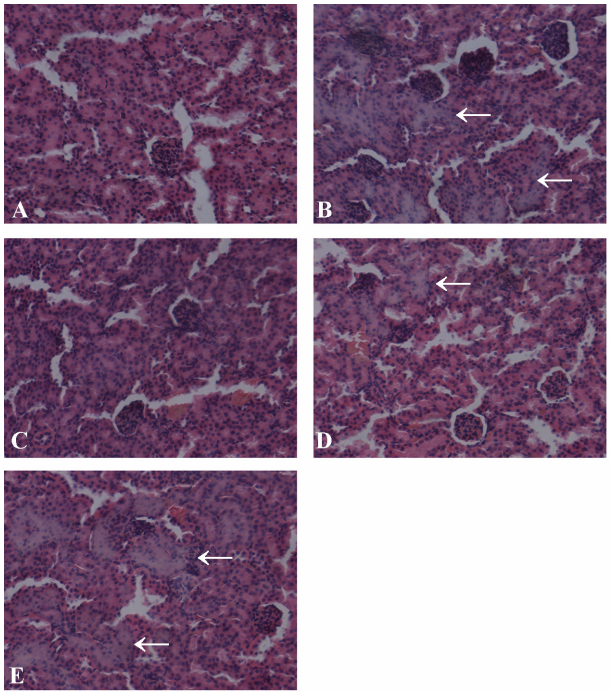

Fig. 3. Effect of the MAS on renal histology in mice. Kidney tissue was fixed in 4% paraformaldehyde at 72 h after cisplatin injection embedded in paraffin, cut into 4 μm sections, and stained with H&E. (A; Negative control, B; Positive control, C; 0.4% MAS, D; 0.2% MAS, E; 0.1% MAS). Kidney sections 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.

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 administration, whereas kidney sections from one of the groups receiving MAS (0.4%) showed no differences with the negative-control group histology. The 0.2% MAS group showed only mild renal necrosis (Fig. 3).

In CCl_4-induced ALF, liver sections from the positive-control group showed liver necrosis at day 1 after CCl_4 administration, 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. 4). 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

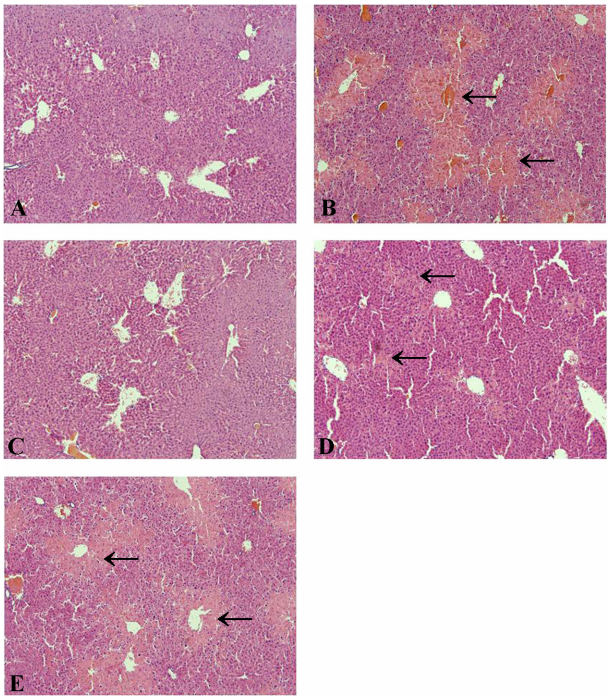

Fig. 4. Effect of the MAS on liver histology in mice. Liver tissue was fixed in 4% paraformaldehyde at 24 h after CCl_4 injection, embedded in paraffin, cut into 4 μm sections, and stained with H&E. (A; Negative control, B; Positive control, C; 0.4% MAS, D; 0.2% MAS, E; 0.1% MAS). Liver sections from the 0.4% MAS group (C) were not different from those in the negative control (A), and the 0.2% MAS group (D) showed only mild liver necrosis. arrows: necrotic areas, ×40.

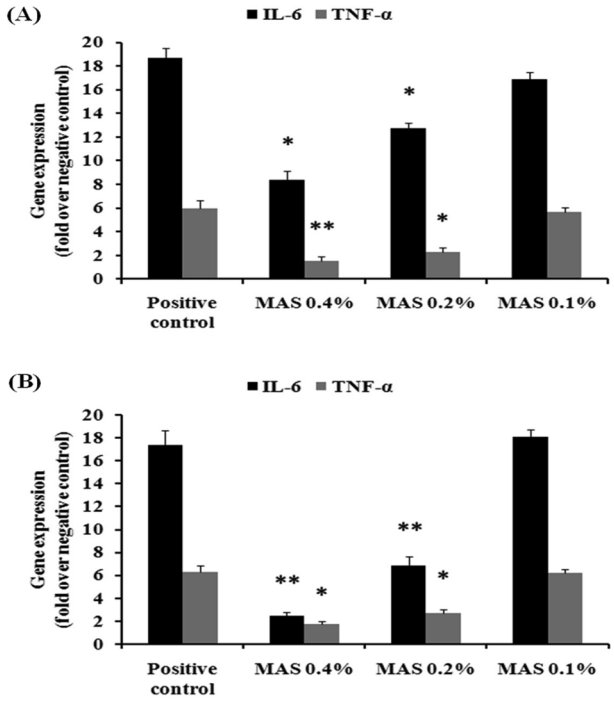

Fig. 5. Effect of the MAS on proinflammatory cytokine expression. Proinflammatory cytokine expression was determined by real-time reverse transcription polymerase chain reaction. (A; cisplatin-induced ARF, B; CCl_4-induced ALF). Compared with the negative control, mice that received the MAS had reduced tumor necrosis factor (TNF)-α and interleukin (IL)-6 expression. $^{*}p < 0.05$, $^{**}p < 0.005$.

after treatment with cisplatin in the negative-control, positive-control, and the MAS (0.4, 0.2 and 0.1%) groups. In CCl_4-induced ALF, livers were harvested at 24 h after treatment with CCl_4 in the negative-control, positive-control, and the MAS (0.4, 0.2 and 0.1%) groups. Cytokine gene expression was determined by real-time RT-PCR. IL-6 and TNF-α expression in kidneys and livers of the groups receiving MAS (0.4 and 0.2%) were significantly lower than those in the positive-control group (Fig. 5).

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 CCl_4 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 CCl_4-induced liver injury [9, 17].

In our previous examination, we tested the protective effect of 2% MAS on cisplatin-induced ARF and CCl_4-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 CCl_4-induced ALF.

In the cisplatin-induced ARF group, serum BUN and creatine 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 creatine. In the CCl_4-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 CCl_4 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 CCl_4-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 CCl_4-induced ALF.

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