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Methionine sulfoxide reductase A deficiency exacerbates acute liver injury induced by acetaminophen

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ABSTRACT

Acetaminophen (APAP) overdose induces acute liver injury via enhanced oxidative stress and glutathione (GSH) depletion. Methionine sulfoxide reductase A (MsrA) acts as a reactive oxygen species scavenger by catalyzing the cyclic reduction of methionine-*S*-sulfoxide. Herein, we investigated the protective role of MsrA against APAP-induced liver damage using MsrA gene-deleted mice ($MsrA^{-/-}$). We found that $MsrA^{-/-}$ mice were more susceptible to APAP-induced acute liver injury than wild-type mice ($MsrA^{+/+}$). The central lobule area of the $MsrA^{-/-}$ liver was more impaired with necrotic lesions. Serum alanine transaminase, aspartate transaminase, and lactate dehydrogenase levels were significantly higher in $MsrA^{-/-}$ than in $MsrA^{+/+}$ mice after APAP challenge. Deletion of MsrA enhanced APAP-induced hepatic GSH depletion and oxidative stress, leading to increased susceptibility to APAP-induced liver injury in MsrA-deficient mice. APAP challenge increased Nrf2 activation more profoundly in $MsrA^{-/-}$ than in $MsrA^{+/+}$ livers. Expression and nuclear accumulation of Nrf2 and its target gene expression were significantly elevated in $MsrA^{-/-}$ than in $MsrA^{+/+}$ livers after APAP challenge. Taken together, our results demonstrate that MsrA protects the liver from APAP-induced toxicity. The data provided herein constitute the first *in vivo* evidence of the involvement of MsrA in hepatic function under APAP challenge.

1. Introduction

Acetaminophen (*N*-acetyl-*p*-aminophenol, APAP) is commonly used as an analgesic and antipyretic agent worldwide. Although APAP is considered safe at therapeutic doses, its accidental overdose can cause severe liver injury, including total failure and death [1]. APAP overdose is thought to be a major cause of drug-induced liver damage in developed countries such as the United Kingdom and the United States [2]. APAP is metabolized to the highly reactive electrophile, *N*-acetyl-*p*-benzoquinone imine (NAPQI), through cytochrome p450-mediated biotransformation in the liver [3]. Most NAPQI is detoxified by conjugation with glutathione (GSH) and excreted without causing toxicity [4]. However, excess NAPQI first depletes the GSH level, and then covalently binds to thiol groups of intracellular proteins, thus generating reactive oxygen species (ROS) that triggers hepatocellular necrosis [5–7]. Enhancement of hepatic GSH levels concomitant with scavenging ROS by treatment with *N*-acetylcysteine (or GSH) is an effective therapeutic strategy for APAP overdose patients [8,9]. However, despite extensive studies in the last few decades, the pathophysiological responses to APAP overdose have yet to be fully elucidated and the therapeutic options for APAP-induced acute liver injury are currently limited.

Methionine sulfoxide reductases (Msrs) are ubiquitous and conserved oxidoreductases that catalyze the reduction of methionine sulfoxide to methionine in proteins [10]. These enzymes consist of two stereospecific families that completely reduce mixtures of methionine-(*R,S*)-sulfoxide residues. MsrA is specific for the *S*-form of methionine sulfoxide, whereas MsrB only reduces the *R*-form [11–13]. There are one MsrA and three MsrB (MsrB1–3) genes in mammalian cells [14]. Msrs are thought to be important enzymes that repair oxidatively damaged proteins and scavenge cellular ROS. A number of studies have shown that MsrA functions as an antioxidant enzyme from bacteria and mammals [15]. We recently reported the protective role of MsrA against ischemia/reperfusion injury in the mouse kidney [16].

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a master transcription factor that enhances transcription of cytoprotective

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genes, including heme oxygenase-1 (HO-1) and GSH synthesizing and conjugating enzymes, in response to oxidative, electrophilic, and xenobiotic stresses [17]. It is well known that hepatic Nrf2 is activated by APAP treatment [18,19].

Considering that oxidative stress is strongly associated with APAP-induced acute liver injury, we hypothesized that MsrA, an abundant antioxidant enzyme in the liver [20], might play a protective role against acute liver injury induced by APAP. To test this hypothesis, we examined wild-type ($MsrA^{+/+}$) and MsrA gene-deleted ($MsrA^{-/-}$) mice. We found that $MsrA^{-/-}$ mice were more susceptible to APAP-induced liver injury than $MsrA^{+/+}$ mice. MsrA deficiency enhanced GSH depletion and oxidative stress in the livers after APAP challenge. In addition, MsrA deficiency led to increased hepatic Nrf2 activation in response to APAP.

2. Materials and methods

2.1. Animal preparation

The generation of MsrA knockout mice has been described elsewhere [21]. $MsrA^{-/-}$ and $MsrA^{+/+}$ mice used in this study were generated by breeding heterozygous knockout ($MsrA^{+/-}$) mice that were obtained by backcrossing into the C57BL/6N genetic background for >8 generations. All experiments were conducted using 8- to 10-week-old $MsrA^{-/-}$ or $MsrA^{+/+}$ mice. This study was conducted according to the guidelines of the Institutional Animal Care and Use Committee of Yeungnam University. Mice were intraperitoneally administered with sterile saline control (10 μl/g body weight) or APAP (300 mg/kg body weight; Sigma–Aldrich) dissolved in warm saline solution after overnight food deprivation. Food was returned to the mice immediately following dosing. After 6 h of treatment, mice were sacrificed to obtain liver tissues and blood from the heart.

2.2. Histology

A slice of liver was fixed in freshly prepared 4% paraformaldehyde solution, embedded in paraffin, and then cut into 5 μm thick sections. After deparaffinization and dehydration, liver sections were stained with haematoxylin and eosin (H&E) for microscopic examinations.

2.3. Measurements of serum ALT, AST, and LDH levels

Serum was obtained from the whole blood by centrifugation after coagulation. Serum alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH) levels were measured using assay kits (IVD Lab, Korea) according to the manufacturer's instructions and by a Shimadzu UV visible spectrophotometer.

2.4. Western blot analysis

Western blot analysis was performed as described previously [16]. Antibodies against MsrA, Nrf2, HO-1, γ-glutamylcysteine ligase (γGCL), and glyceraldehyde 3-phosphate dehydrogenase (Gapdh) were used as described previously [22,23]. Antibodies against lamin B and α-tubulin were purchased from Santa Cruz Biotechnology. Densities of blots were measured using the ImageJ program (National Institutes of Health, USA).

2.5. Measurement of GSH contents

GSH content was measured using a GSH fluorescent detection kit (Arbor Assays) according to the manufacturer's instructions. Briefly, liver tissue homogenates were prepared in 5% sulfosalicylic acid to remove the proteins. Hepatic GSH contents were analyzed and calculated as μmole/g of tissue.

2.6. Measurements of hydrogen peroxide, protein-carbonyl, and 4-hydroxynonenal (HNE) levels

H_2O_2 levels in liver samples were determined using the ferric-sensitive dye, xylenol orange (Sigma–Aldrich), as previously described [24]. Protein-carbonyl levels were measured using an OxyBlot protein oxidation detection kit (Millipore) according to the manufacturer's recommendations. HNE levels were determined by measuring HNE-protein adducts using anti-HNE antibodies (Abcam). The blot signals were quantitatively analyzed using the ImageJ software.

2.7. Quantitative polymerase chain reaction (qPCR) analysis

Total RNA from sliced liver samples was isolated using a TRI solution (Bioscience), and then reverse-transcribed to cDNA using a reverse transcription kit (Applied Biosystems). Next, samples were subjected to qPCR using a Real-Time PCR 7500 System and Power SYBR Green PCR Master Mix (Applied Biosystems) according to the manufacturer's instructions. Gapdh was used to normalize the amounts of RNA in each sample. The primers used were: 5′- CGAAGAGGCGATTTCTTGTCA-3′ and 5′- CAGTTCCAGTGATAAGTGAGCC-3′ for Nrf2; 5′-AGGTACACATCCAAGCCGAGA-3′ and 5′-ATCACCAGCTTAAAGCCTTCT-3′ for HO-1; 5′-GGACAAACCCCAACCATCC-3′ and 5′-GTTGAACTCAGACATCGTTCCT-3′ for γGCL; 5′-GGTCCTCAGTGTAGCCCAAG-3′ and 5′-AATGTGTCCGTCGTGGATCT-3′ for Gapdh.

2.8. Nuclear protein fractionation

Nuclear and cytoplasmic proteins from livers were fractionated using an NE-PER Nuclear and Cytoplasmic Extraction kit (Thermo Scientific) according to the manufacturer's instructions. Lamin B and α-tubulin were used as nuclear and cytoplasmic marker proteins, respectively.

2.9. Statistical analysis

Statistical analysis was conducted using a Student's *t*-test or one-way analysis of variance (ANOVA) where appropriate. A p value < 0.05 was considered significant.

3. Results

3.1. MsrA-deleted mice are more susceptible to APAP-induced acute liver injury

$MsrA^{+/+}$ and $MsrA^{-/-}$ male mice were treated with APAP (300 mg/kg body weight) for 6 h, after which liver damage was analyzed based on histological alterations and serum ALT, AST, and LDH levels. Livers from control $MsrA^{-/-}$ mice were histologically normal, similar to those from control $MsrA^{+/+}$ mice (Fig. 1A). APAP treatment induced liver damage in $MsrA^{+/+}$ and $MsrA^{-/-}$ mice, with more profound damage being observed in the $MsrA^{-/-}$ than the $MsrA^{+/+}$ mice (Fig. 1A). The central lobule area of the $MsrA^{-/-}$ liver was more impaired with necrotic lesions. The increased susceptibility to APAP-induced hepatotoxicity in $MsrA^{-/-}$ mice was confirmed by the significantly elevated ALT and AST levels in APAP-treated $MsrA^{-/-}$ mice compared to APAP-treated $MsrA^{+/+}$ mice (Fig. 1B and C). In addition, $MsrA^{-/-}$ mice treated with APAP showed significantly increased LDH enzyme levels compared to $MsrA^{+/+}$ mice treated with APAP (Fig. 1D). Female mice were also tested.

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