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Curcumin Attenuates D-Galactosamine/Lipopolysaccharide–Induced Liver Injury and Mitochondrial Dysfunction in Mice[1–3]

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Abstract

Curcumin, a naturally occurring antioxidant, has various beneficial effects in the treatment of human diseases. However, little information regarding the protection it provides against acute liver injury is available. The present study investigated the protective effects of curcumin against D-galactosamine/lipopolysaccharide (D-GalN/LPS)-induced acute liver injury in mice. A total of 40 male Kunming mice were randomly assigned into 5 groups: *1*) mice administered saline vehicle injection (control), *2*) mice administered 200 mg/kg body weight (BW) curcumin by i.p. injection (CUR), *3*) mice administered D-GalN/LPS (700 mg and 5 μg/kg BW) via i.p. injection (GL), *4*) mice administered 200 mg/kg BW curcumin i.p. 1 h before D-GalN/LPS injection (CUR-GL), and *5*) mice administered 200 mg/kg BW curcumin i.p. 1 h after D-GalN/LPS injection (GL-CUR). Twenty h after D-GalN/LPS injection, serum alanine aminotransferase activities were 18.5% and 13.5% lower ($P < 0.05$) and aspartate aminotransferase (AST) activities were 26.6% and 9.6% lower ($P < 0.05$) in the CUR-GL and GL-CUR groups, respectively, than in the GL group. The CUR-GL and GL-CUR groups had 64.4% and 15.0% higher ($P < 0.05$) mitochondrial membrane potentials, respectively, and the CUR-GL group had a 44.7% lower reactive oxygen species concentration than the GL group ($P < 0.05$). Mitochondrial manganese superoxide dismutase activities were 111% and 77.9% higher ($P < 0.05$) and the percentages of necrotic cells were 47.0% and 32.4% lower ($P < 0.05$) in the CUR-GL and GL-CUR groups, respectively, than in the GL group. Liver mRNA levels of sirtuin 1 (*Sirt1*) were 56.4% lower ($P < 0.05$) in the CUR-GL group than in the GL group. Moreover, compared with the GL-CUR group, the CUR-GL group had an 18.7% lower serum AST activity, a 31.7% lower mitochondrial malondialdehyde concentration, a 36.0% lower hepatic reactive oxygen species concentration, and a 43.0% higher mitochondrial membrane potential. These results suggested that curcumin protects against D-GalN/LPS–induced liver damage by the enhancing antioxidant defense system, attenuating mitochondrial dysfunction and inhibiting apoptosis. This was especially true for curcumin pretreatment, which highlighted its promise as a preventive treatment for acute liver injury in clinical settings. J. Nutr. doi: 10.3945/jn.114.193573.

Introduction

Acute hepatic failure is clinically characterized by massive hepatocyte apoptosis and very high patient mortality (1). Multiple factors contribute to hepatic failure, including excessive use of alcohol, viral infection, liver transplant failure, the side effects of chemical drugs, and the metabolism of xenobiotics (2). In fact, an experimental model of liver failure induced by D-galactosamine (D-GalN)[5]/LPS in rodents was shown to mimic acute hepatic failure in clinical settings (3–5). In this model, D-GalN/LPS induces liver damage through the elevation of reactive oxygen species (ROS), especially in Kupffer cells (6). The excessive ROS are not only toxic to the cells but also trigger a number of pathological events, such as oxidant stress.

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[3] Supplemental Tables 1 and 2 and Supplemental Figures 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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[5] Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BW, body weight; CON, mice that were i.p. injected with isotonic saline solution; CUR, mice administered 200 mg/kg body weight curcumin by i.p. injection; CUR-GL, mice administered 200 mg/kg body weight curcumin i.p. 1 h before D-galactosamine/LPS injection; DCFH-DA, dichlorofluorescein-diacetate; D-GalN, D-galactosamine; *Foxo3*, Forkhead box O 3; GL, mice administered D-galactosamine/LPS (700 mg and 5 μg/kg body weight) via i.p. injection; GL-CUR, mice administered 200 mg/kg body weight curcumin i.p. 1 h after D-galactosamine/LPS injection; GPx, glutathione peroxidase; GR, glutathione reductase; *Ho1*, heme oxygenase 1; MMP, mitochondrial membrane potential; MnSOD, manganese superoxide dismutase; *Nrf2*, nuclear factor erythroid 2–related factor 2; PC, protein carbonyl; ROS, reactive oxygen species; *Sirt1*, sirtuin 1.

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Mitochondria have been described as "the powerhouse of the cell" because of the efficient electron transport chain system. However, mitochondria are the major source of ROS within mammalian cells, and they are susceptible to oxidant stress (7). The ROS boost that occurs during oxidant stress leads to an imbalance within the antioxidant defense system, a reduction in mitochondrial membrane potential (MMP), and finally, mitochondrial dysfunction (8). Recently, apart from an inflammatory response, both ROS overgeneration and mitochondrial dysfunction were considered possible mechanisms of D-GalN/LPS–induced acute liver injury in rodents. Lee et al. (9) verified the induction of synergistic ROS production and the loss of MMP via D-GalN/LPS during hepatic cell death.

Recently, hepatoprotective agents of natural origins have drawn much attention because they show promise for use in the treatment of experimental and clinical liver diseases. Curcumin, a polyphenol derived from the natural herb turmeric (*Curcuma longa*), has been described as having various beneficial effects, including anti-inflammatory (10), antioxidant (11), antimicrobial (12), and antimutagenic and anticancer (13) activities. Over the past decades, growing experimental data have demonstrated that curcumin is effective in protecting against the following: carbon tetrachloride–induced hepatic fibrosis in rats (14), heat stress–induced oxidant stress in quail (15), hexavalent chromium–induced renal oxidant damage (16), cisplation-induced hepatotoxicity (17), aluminum-induced mitochondrial dysfunction in the brain (18), arsenic-induced hepatic damage in mice (19), and perfluoro-octane sulfonate-induced genotoxicity (20). It was reported that the outstanding free radical scavenging and antioxidant activities of curcumin are mainly responsible for the above-mentioned protection found in animal studies (21). In addition, curcumin is a lipophilic substance, which allows it to become incorporated into biologic membranes and directly protecting cells from ROS attack (22). However, there is little research addressing the protective effects of curcumin against D-GalN/LPS–induced acute liver injury in mice.

In the present study, we aimed to investigate the protective effects of curcumin on D-GalN/LPS–induced liver injury in mice, focusing on the role of mitochondrial function and apoptosis. The discrepancy regarding the protective effects of curcumin against liver damage in the pre- and post-treatment groups was further investigated in our study.

Materials and Methods

Chemicals and reagents. LPS (*Escherichia coli* 0111:B4), D-GalN, and curcumin were obtained from Sigma-Aldrich. All other chemicals were commercially available and of reagent grade.

Animals. Male Kunming mice with a body weight (BW) of 20–25 g were obtained from the Animal Multiplication Centre of Qinglong Mountain and used throughout the present study. The mice were allowed water and standard granulated diet (AIN-93 diet; see **Supplemental Table 1**) (23) ad libitum and were maintained under standard light (i.e., 12/12-h light/dark), temperature (22 ± 2°C), and relative humidity (50 ± 10%) conditions. All of the procedures involving the mice were carried out in accordance with the Guiding Principles on the Care and Use of Laboratory Animals, China. The experiment performed in the present study was approved by the Animal Care Committee of Nanjing Agricultural University.

Experimental design. Five groups of mice were studied (n = 8 per group): *1*) mice that were i.p. injected with isotonic saline solution [D-GalN/LPS and curcumin vehicle; control (CON) group], *2*) mice administered curcumin injection alone [for which curcumin was dissolved in olive oil and injected i.p. at a single dose of 200 mg/kg BW (CUR)] (19), *3*) mice administered D-GalN/LPS injection [in which D-GalN (700 mg/kg BW) was administered i.p. along with LPS (5 μg/kg BW) (GL) to induce nonlethal fulminant hepatic failure], *4*) mice administered curcumin 1 h before D-GalN/LPS injection [in which curcumin (200 mg/kg BW) was injected i.p. 1 h before D-GalN/LPS injection (CUR-GL)], and *5*) mice administered curcumin 1 h after D-GalN/LPS injection [in which curcumin (200 mg/kg BW) was injected i.p 1 h after D-GalN/LPS injection (GL-CUR)]. All mice were anesthetized 20 h after D-GalN/LPS or D-GalN/LPS–vehicle injection. Serum was obtained from the blood via centrifugation at 3000 × g for 20 min. Hepatic samples of the right lobe were collected immediately for morphologic examination, and then the left lobe of the liver was snap-frozen and stored at −80°C for other assays. The nonlethal dose of D-GalN/LPS and sampling time adopted in the present study were determined on the basis of our preliminary study and previous reports (9,24–26).

Measurement of serum aminotransferase activities. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured via the enzymatic kinetic method by using an automatic biochemistry analyzer (SELECTA XL; Vital Scientific) according to the manufacturer's protocol.

Histologic examination. Liver tissue specimens from the right lobe were fixed in pH 7.4, 10% buffered neutral formalin, embedded in paraffin, and sliced into 5-mm-sections. After hematoxylin and eosin staining, the morphologic evaluation was carried out by using a light microscope (Nikon ECLIPSE 80i).

Isolation of hepatic mitochondria. Hepatic mitochondria were prepared via the method previously described (27). Protein concentration was determined by using Coomassie brilliant blue (27).

Assay of lipid peroxidation and protein oxidation. Lipid peroxidation was expressed as malondialdehyde concentration, which is a byproduct of lipid peroxidation and can react with thiobarbituric acid to generate a colored product with an absorption maximum at 532 nm. A malondialdehyde assay kit (no. A003; Nanjing Jiancheng Bioengineering Institute) was used. Malondialdehyde concentrations were expressed in nmol/100 mg protein. Protein oxidation in the liver mitochondria was estimated via the concentration of protein carbonyls (PCs). The PC concentration was measured spectrophotometrically by using the method of Wei et al. (28) and expressed in nmol/mg protein.

Determination of mitochondria antioxidant system. Activities of manganese superoxide dismutase (MnSOD), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione in the hepatic mitochondria were determined via enzymatic colorimetric methods by using commercial kits for MnSOD (no. A001; Nanjing Jiancheng Bioengineering Institute), GPx (no. A005; Nanjing Jiancheng Bioengineering Institute), GR (no. A062; Nanjing Jiancheng Bioengineering Institute), and glutathione (no. A006; Nanjing Jiancheng Bioengineering Institute). Concentrations of protein in the mitochondria were determined by using a commercial kit (no. A045; Nanjing Jiancheng Bioengineering Institute).

Transmission electron microscopy. For the ultrastructural examination, fresh hepatic fragments of the right lobe were fixed in 2.5% glutaraldehyde (pH 7.4, 0.1 mol/L sodium cacodylate buffer) and 1% osmium tetroxide (v:v). After dehydration by using alcohol and acetone, the changes in the ultrastructure were detected via a transmission electron microscope (H-7650; Hitachi).

Detection of ROS. The ROS assay kit (no. S0033; Beyotime Biotech) was used to detect the intracellular ROS concentrations via a sensitive fluorescent dichlorofluorescein-diacetate (DCFH-DA) probe, as previously described in detail (29). ROS production was presented as the mean DCFH-DA fluorescence intensity over that of the control.

Measurement of MMP. An MMP assay kit (no. C2006; Beyotime Biotech) was used to monitor the change in MMP, as previously

described in detail (30). The MMP of hepatocytes was calculated as the fluorescence ratio of aggregates (red) to monomers (green).

Assay of cell apoptosis. The Annexin V-FITC/PI Apoptosis Detection Kit (no. V13245; Invitrogen) was used to measure phosphatidylserine exposure on the outer leaflet of the plasma membrane, as previously described in detail (22).

Real-time qPCR analyses. The total RNA was isolated from the frozen liver tissue by using Trizol reagent [Takara Biotechnology (Dalian)] and then reverse-transcribed by using a commercial kit (Perfect Real Time, SYBR PrimeScriP; Takara Biotechnology) according to the manufacturer's instructions. The mRNA expression levels of specific genes were quantified via real-time PCR by using SYBR Premix Ex Taq II (Tli RNaseH Plus; Takara Biotechnology) and an ABI 7300 Fast Real-Time PCR detection system (Applied Biosystems). The SYBR Green PCR reaction system was 20 μL in total, which consisted of 10 μL SYBR Premix Ex Taq (2×), 0.4 μL of the forward and reverse primers, 0.4 μL of ROX reference dye (50×), 6.8 μL of double-distilled H_2O_2, and 2 μL of cDNA template. The reaction conditions were as follows: 30 s at 95°C, 40 cycles of 5 s at 95°C, and 30 s at 60°C. Each sample was run in triplicate. The fold expression of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method (31), in which $\Delta Ct = Ct_{target\ gene} - Ct_{\beta\text{-actin}}$ and $\Delta\Delta Ct = \Delta Ct_{samples\ in\ all\ groups} - \Delta Ct_{the\ mean\ of\ the\ control\ group}$. The β-actin gene was amplified as an internal standard in the present study. The primer sequences are given in **Supplemental Table 2**.

Statistical analysis. Data were analyzed by using SPSS 17.0. All of the data were tested for homogeneity of variance, and the AST and ALT data were logarithmically transformed to a normal distribution. When the *F* test was significant, means were compared by using 1-factor ANOVA and Bonferroni's multiple-comparisons test. The differences between the compared groups were considered significant at $P < 0.05$. Results are presented as means ± SEMs.

Results

Serum AST and ALT activities. Compared with the CON group, serum ALT and AST activities in the GL group were decreased ($P < 0.05$) due to D-GalN/LPS exposure (**Fig. 1**). The CUR-GL and GL-CUR groups had 18.5% and 13.5% lower ($P < 0.05$) serum ALT activities, respectively, than those of the GL group, and they had 26.6% and 9.6% lower ($P < 0.05$) serum AST activities, respectively, than those of the GL group. Compared with the GL-CUR group, the CUR-GL group had an 18.7% lower serum AST activity ($P < 0.05$).

Histopathological analysis. A normal lobular liver architecture and cell structure were observed in the CON and CUR groups (**Fig. 2**). The D-GalN/LPS treatment resulted in pathological alternations including apparent hepatocyte vacuolation, the dissolution of the hepatocyte architecture, and the disorganization of the parenchyma and the nuclei. The D-GalN/LPS–induced morphologic changes, especially the vacuolation and necrosis of hepatocytes, were alleviated in the CUR-GL and GL-CUR groups.

Lipid peroxidation and PC. Compared with the CON group, the hepatic mitochondrial malondialdehyde concentration in the GL group was higher ($P < 0.05$) due to the D-GalN/LPS injection (**Fig. 3**). Curcumin treatment decreased ($P < 0.05$) mitochondrial malondialdehyde concentrations by 68.5% and 53.9% in the CUR-GL and GL-CUR groups, respectively, in comparison with those in the GL group. Moreover, the CUR-GL group had a 31.7% lower mitochondrial malondialdehyde concentration compared with the GL-CUR group ($P < 0.05$). The hepatic PC concentration in the isolated mitochondria was also 85.6% higher ($P < 0.05$) in the GL group than in the CON group (Fig. 3). However, there were no differences when the mitochondrial PC concentrations in the CUR-GL and GL-CUR groups were compared with those in the GL group.

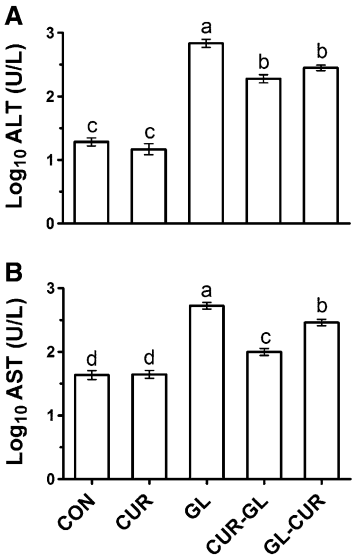

FIGURE 1 Effect of curcumin on serum ALT (*A*) and AST (*B*) activities in D-GalN/LPS–injected mice. Values are means ± SEMs ($n = 8$). Means for a variable without a common letter differ, $P < 0.05$. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CON, mice that were i.p. injected with isotonic saline solution; CUR, mice administered 200 mg/kg body weight curcumin by i.p. injection; CUR-GL, mice administered 200 mg/kg body weight curcumin i.p. 1 h before D-galactosamine/LPS injection; D-GalN, D-galactosamine; GL, mice administered D-galactosamine/LPS (700 mg and 5 μg/kg body weight) via i.p. injection; GL-CUR, mice administered 200 mg/kg body weight curcumin i.p. 1 h after D-galactosamine/LPS injection.

Mitochondrial antioxidant defense system. The effects of curcumin on the mitochondrial antioxidant defense systems, namely MnSOD, glutathione, GPx, and GR, against D-GalN/LPS–induced oxidant stress are represented in **Table 1**. The GL group had 62.8%, 53.1%, and 78.0% lower ($P < 0.05$) mitochondrial activities for MnSOD, GPx, and GR, respectively, than those of the CON group. MnSOD activities in the mitochondrial fraction were 111% and 77.9% higher ($P < 0.05$) in the CUR-GL and GL-CUR groups, respectively, than those in the GL group. Curcumin treatment also increased mitochondrial GPx activities by 76.2% and 48.0% ($P < 0.05$) in the CUR-GL and GL-CUR groups, respectively, in comparison with the GL group. Compared with the GL group, mitochondrial GR activities were increased ($P < 0.05$) by curcumin post-treatment in the GL-CUR group. However, no differences in mitochondrial glutathione content were observed in the CUR-GL and GL-CUR groups compared with the GL group.

Transmission electron microscope. There were many well-developed mitochondria accompanied with neatly arranged endoplasmic reticulums in the CON group (**Fig. 4**). There were no obvious changes in hepatic ultrastructure in the CUR group compared with the CON group. Compared with the CON group, the mitochondria in the GL group were characterized as markedly swollen with dilated cristae, decreased electron density of the intramitochondrial matrix, and uneven or deformed membranes (Fig. 4). The abnormality or degeneration of hepatic mitochondria was dramatically alleviated in both the CUR-GL

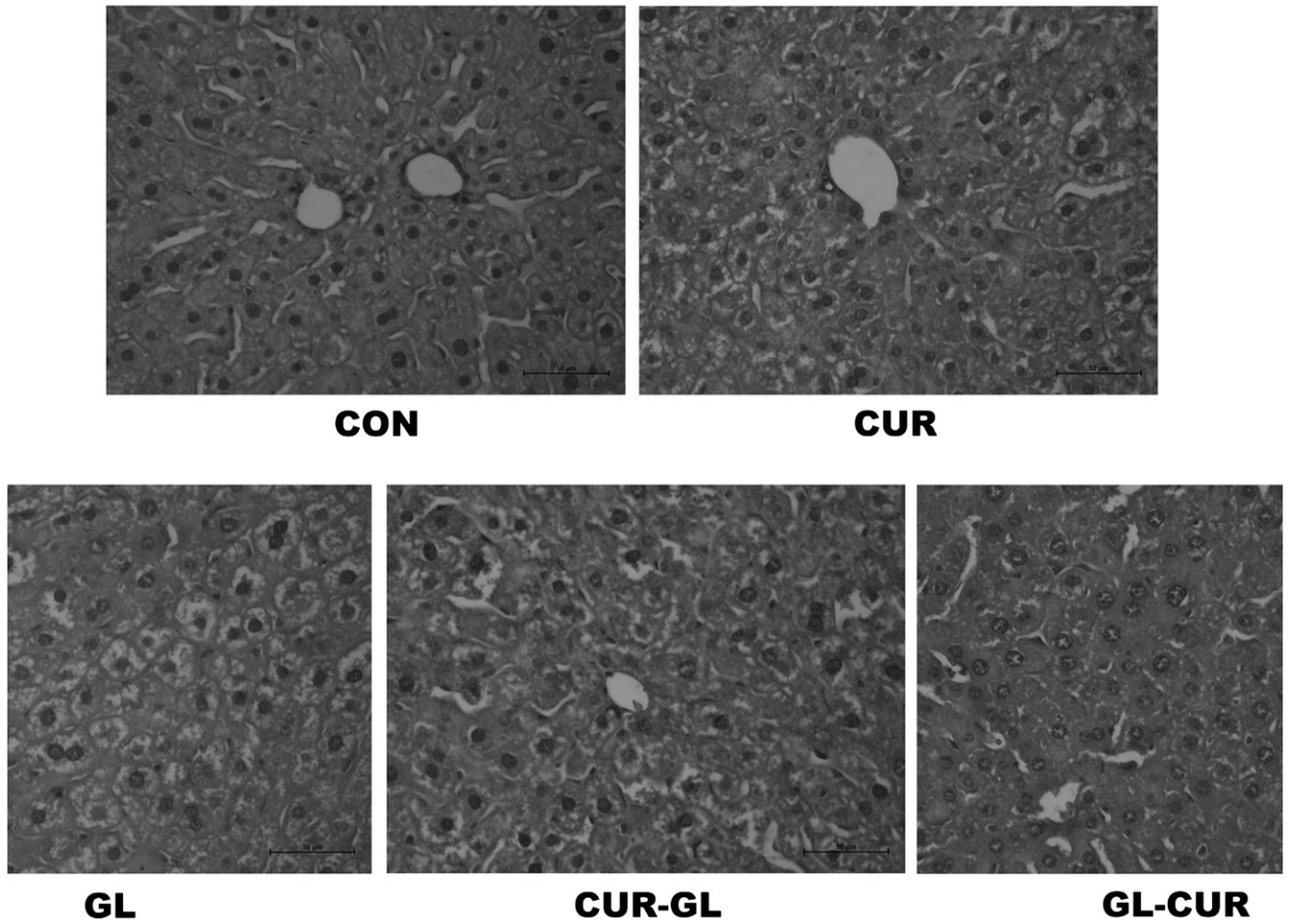

FIGURE 2 Effect of curcumin on hepatic histology in D-GalN/LPS–injected mice. Liver sections from the different groups were stained with hematoxylin and eosin (original magnification: 400×). CON, mice that were i.p. injected with isotonic saline solution; CUR, mice administered 200 mg/kg body weight curcumin by i.p. injection; CUR-GL, mice administered 200 mg/kg body weight curcumin i.p. 1 h before D-galactosamine/LPS injection; D-GalN, D-galactosamine; GL, mice administered D-galactosamine/LPS (700 mg and 5 μg/kg body weight) via i.p. injection; GL-CUR, mice administered 200 mg/kg body weight curcumin i.p. 1 h after D-galactosamine/LPS injection.

and GL-CUR groups, although mitochondrial swelling and damaged membranes were still observed.

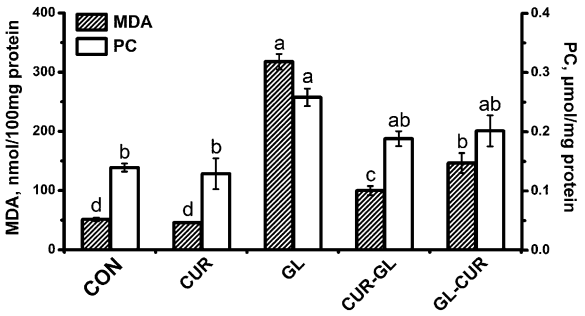

FIGURE 3 Effect of curcumin on hepatic mitochondrial MDA and PC concentrations in D-GalN/LPS–injected mice. Values are means ± SEMs (n = 8). Means for a variable without a common letter differ, $P <$ 0.05. CON, mice that were i.p. injected with isotonic saline solution; CUR, mice administered 200 mg/kg body weight curcumin by i.p. injection; CUR-GL, mice administered 200 mg/kg body weight curcumin i.p. 1 h before D-galactosamine/LPS injection; D-GalN, D-galactosamine; GL, mice administered D-galactosamine/LPS (700 mg and 5 μg/kg body weight) via i.p. injection; GL-CUR, mice administered 200 mg/kg body weight curcumin i.p. 1 h after D-galactosamine/LPS injection; MDA, malondialdehyde; PC, protein carbonyl.

ROS and MMP. Mice in the GL group had a 165% increase (P < 0.05) in intracellular ROS concentration (**Fig. 5** and **Supplemental Fig. 1**) and a 48.2% decrease (P < 0.05) in MMP (Fig. 5) when compared with the CON group. The mean DCFH-DA fluorescence intensity was lower (P < 0.05) by 44.7% in the CUR-GL group when compared with the CON group. MMPs were actually 64.4% and 15.0% greater (P < 0.05) in the CUR-GL and GL-CUR groups, respectively, than in the GL group. Interestingly, the CUR-GL group had a 36.0% lower hepatic ROS concentration and a 43.0% higher MMP when compared with the GL-CUR group (P < 0.05).

Apoptosis. Compared with the CON group, mice in the GL group had more (P < 0.05) apoptotic and necrotic liver cells (Fig. 5 and **Supplemental Fig. 2**). Curcumin treatment decreased the percentage of necrotic cells by 47.0% and 32.4% (P < 0.05) in the CUR-GL and GL-CUR groups, respectively, in comparison with the GL group. The percentage of apoptotic cells was 24.7% greater (P < 0.05) in the GL-CUR group than in the GL group.

Gene expression. Compared with the CON group, D-GalN/LPS administration increased (P < 0.05) the liver mRNA levels of nuclear factor erythroid 2–related factor 2 (*Nrf2*), heme oxygenase 1 (*Ho1*), *Mnsod*, *Gpx*, and sirtuin 1 (*Sirt1*) in the GL

TABLE 1 Effect of curcumin on the mitochondrial activities of MnSOD, GSH, GPx, and GR in the livers of D-GalN/LPS–injected mice[1]

	Treatment group				
Antioxidant capacity	CON	CUR	GL	CUR-GL	GL-CUR
MnSOD, *U/mg protein*	97.5 ± 13.2[a]	101 ± 6.18[a]	36.3 ± 12.8[c]	76.4 ± 5.84[b]	64.5 ± 5.73[b]
GSH, *mg/g protein*	0.414 ± 0.0656[a,b]	0.612 ± 0.0356[a]	0.295 ± 0.0318[b]	0.358 ± 0.0502[b]	0.318 ± 0.0700[b]
GPx, *U/mg protein*	52.3 ± 1.79[a]	52.7 ± 1.82[a]	24.5 ± 2.48[c]	43.2 ± 2.91[a,b]	36.3 ± 2.28[b]
GR, *U/mg protein*	2.86 ± 0.278[a]	3.06 ± 0.143[a]	0.634 ± 0.111[c]	1.44 ± 0.140[b,c]	1.78 ± 0.317[b]

[1] Values are means ± SEMs, $n = 8$. Means in a row without a common letter differ, $P < 0.05$. CON, mice that were i.p. injected with isotonic saline solution; CUR, mice administered 200 mg/kg body weight curcumin by i.p. injection; CUR-GL, mice administered 200 mg/kg body weight curcumin i.p. 1 h before D-galactosamine/LPS injection; D-GalN, D-galactosamine; GL, mice administered D-galactosamine/LPS (700 mg and 5 μg/kg body weight) via i.p. injection; GL-CUR, mice administered 200 mg/kg body weight curcumin i.p. 1 h after D-galactosamine/LPS injection; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; MnSOD, manganese superoxide dismutase.

group (**Table 2**). The liver mRNA levels of *Sirt1* were 56.4% lower ($P < 0.05$) in the CUR-GL group than in the GL group. In contrast, no differences in the liver mRNA levels of *Nrf2*, *Ho1*, *Mnsod*, *Gpx*, or *Sirt1* were observed in the CUR-GL and GL-CUR groups when compared with the GL group.

Discussion

Curcumin, derived from the rhizome *Curcuma longa*, is 1 of the primary ingredients in the ginger and curry powders that are widely consumed in Middle Eastern and Asian countries,

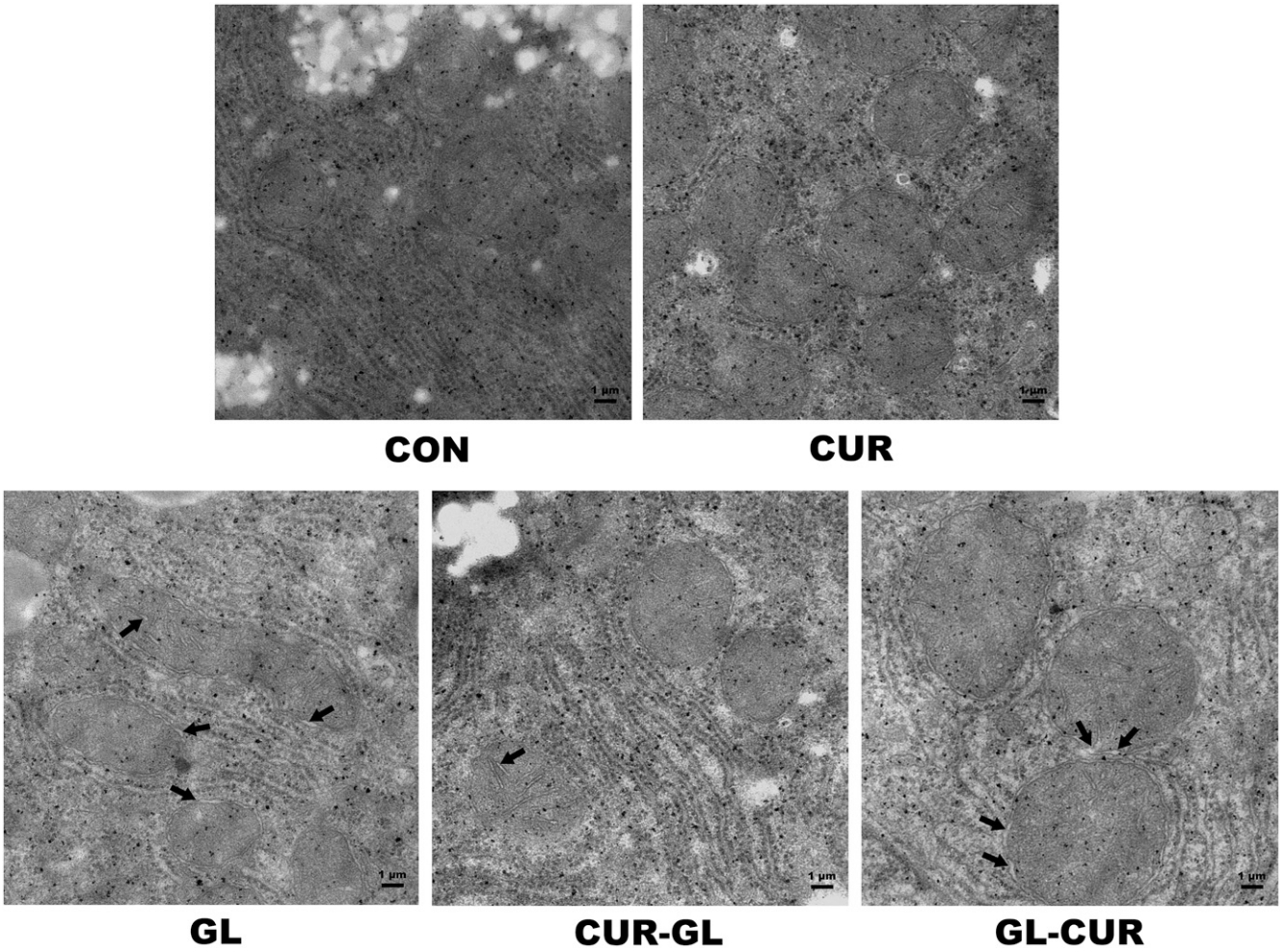

FIGURE 4 Effects of curcumin on liver ultrastructure in D-GalN/LPS–injected mice under transmission electron microscopy. Arrows indicate the morphologic alternations of hepatic mitochondria in the transmission electron microscopy images (original magnification: 8000×), including swollen mitochondrial, dilated cristae, deformed membrane, or decreased electron density of the intramitochondrial matrix. CON, mice that were i.p. injected with isotonic saline solution; CUR, mice administered 200 mg/kg body weight curcumin by i.p. injection; CUR-GL, mice administered 200 mg/kg body weight curcumin i.p. 1 h before D-galactosamine/LPS injection; D-GalN, D-galactosamine; GL, mice administered D-galactosamine/LPS (700 mg and 5 μg/kg body weight) via i.p. injection; GL-CUR, mice administered 200 mg/kg body weight curcumin i.p. 1 h after D-galactosamine/LPS injection .

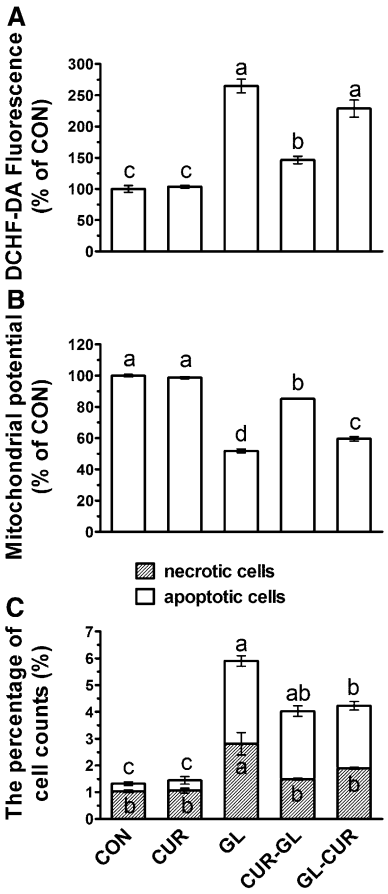

FIGURE 5 Effects of curcumin on hepatic ROS concentrations (*A*), mitochondrial membrane potential (*B*), and cell apoptosis (*C*) in D-GalN/LPS–injected mice. In panels *A* and *B*, the value of control group was set to 100%. In panel *C*, the values were the percentages of the apoptotic and necrotic cell counts. Values are means ± SEMs (n = 6). Means for a variable without a common letter differ, $P < 0.05$. CON, mice that were i.p. injected with isotonic saline solution; CUR, mice administered 200 mg/kg body weight curcumin by i.p. injection; CUR-GL, mice administered 200 mg/kg body weight curcumin i.p. 1 h before D-galactosamine/LPS injection; DCFH-DA, dichlorofluorescein-diacetate; D-GalN, D-galactosamine; GL, mice administered D-galactosamine/LPS (700 mg and 5 μg/kg body weight) via i.p. injection; GL-CUR, mice administered 200 mg/kg body weight curcumin i.p. 1 h after D-galactosamine/LPS injection; ROS, reactive oxygen species.

especially on the Indian subcontinent. The daily intake of curcumin has been reported to be as much as 100 mg/d [~2 mg/(kg × d) for a 50-kg adult] (32). However, efficient curcumin amounts that exhibited potential health-promoting effects have been reported to range from 50 to 400 mg/(kg × d) in various rodent models (16–18). This dose was considerably higher than that seen in the human diet. Phase I clinical trials demonstrated that curcumin, when taken at doses up to 12 g/d, is well tolerated in humans (33). Hence, a 200-mg/kg dose of curcumin may be nontoxic in humans if administered in clinical trials. In the present study, the dose of curcumin was acceptable and proved to be efficient in terms of hepatoprotection without any adverse effects.

The assessment of serum aminotransferase activities and histopathological changes suggested that liver damage induced via D-GalN/LPS was alleviated by curcumin intervention. In the liver, ALT is normally located in the cytoplasm, and AST is distributed in both the cytosolic and mitochondrial fractions. However, when the hepatic structure is acutely damaged, ALT and AST are released into the circulatory system, leading to increased activities in the serum (34,35). In the current study, the fact that curcumin treatment reduced the release of hepatic ALT and AST suggested attenuated liver injury, which was also directly evidenced by histopathological observation (Fig. 2). A similar protective role of curcumin against hepatotoxicity was also reported in carbon tetrachloride–treated rats (14).

Mitochondrial function was involved in D-GalN/LPS–induced oxidant damage and the antioxidant capacity of curcumin. It has been accepted that high amounts of D-GalN/LPS–mediated ROS generation and the enhancement of lipid peroxidation and protein oxidation play a pathological role in oxidant damage (9,36,37). The mitochondria, due to the enrichment of PUFAs in their membranes, are among the most vulnerable targets of free radicals. Under oxidant stress, excessive free radicals interact with the hepatic mitochondrial membranes, which causes the elevation of malondialdehyde concentrations and the subsequent disintegration of the mitochondrial membrane (38). Consistent with other research (5), we also showed that acute exposure to D-GalN/LPS markedly increased intracellular ROS, malondialdehyde, and PC concentrations in the hepatic mitochondria of mice. Interestingly, pre- and post-treatment with curcumin remarkably decreased mitochondrial malondialdehyde concentrations and attenuated ROS overgeneration in D-GalN/LPS–treated mice. Curcumin, as a chain-breaking antioxidant, could efficiently scavenge excessive free radicals against oxidant stress. Moreover, studies have found that because of its high hydropho-

TABLE 2 Effect of curcumin on hepatic mRNA expression of antioxidant genes in D-GalN/LPS–injected mice[1]

	Treatment group				
Gene expression:β-actin	CON	CUR	GL	CUR-GL	GL-CUR
Nrf2	1.03 ± 0.114^{b}	1.23 ± 0.277^{b}	2.28 ± 0.351^{a}	1.75 ± 0.264^{a,b}	2.15 ± 0.245^{a}
Ho1	1.01 ± 0.0831^{b}	0.950 ± 0.179^{b}	1.76 ± 0.153^{a}	1.46 ± 0.287^{a,b}	1.68 ± 0.443^{a,b}
Mnsod	1.00 ± 0.0450^{b}	1.19 ± 0.191^{b}	3.13 ± 0.735^{a}	1.93 ± 0.349^{a,b}	2.15 ± 0.559^{a,b}
Gpx1	1.00 ± 0.0253^{b}	2.42 ± 0.938^{b}	5.86 ± 1.76^{a}	3.67 ± 1.06^{a,b}	3.12 ± 0.877^{a,b}
Nqo1	1.03 ± 0.126	0.902 ± 0.0862	0.872 ± 0.214	1.10 ± 0.177	0.981 ± 0.159
Sirt1	1.03 ± 0.118^{c}	1.00 ± 0.228^{c}	3.90 ± 1.15^{a}	1.70 ± 0.418^{b,c}	3.02 ± 0.761^{a,b}

[1] Values are means ± SEMs, n = 8. Means in a row without a common letter differ, $P < 0.05$. CON, mice that were i.p. injected with isotonic saline solution; CUR, mice administered 200 mg/kg body weight curcumin by i.p. injection; CUR-GL, mice administered 200 mg/kg body weight curcumin i.p. 1 h before D-galactosamine/LPS injection; D-GalN, D-galactosamine; GL, mice administered D-galactosamine/LPS (700 mg and 5 μg/kg body weight) via i.p. injection; GL-CUR, mice administered 200 mg/kg body weight curcumin i.p. 1 h after D-galactosamine/LPS injection; *Gpx1*, glutathione peroxidase 1; *Ho1*, heme oxygenase 1; *Mnsod*, manganese superoxide dismutase; *Nqo1*, NAD(P)H quinone oxidoreductase 1; *Nrf2*, nuclear factor erythroid 2–related factor 2; *Sirt1*, sirtuin 1.

bicity, curcumin easily incorporated itself into the hydrophobic regions of the cell membrane, where it could directly prevent lipid peroxidation via its strong free radical scavenging effect (39).

In mice, mitochondrial antioxidant defense was enhanced by curcumin when challenged with D-GalN/LPS. One possible mechanism involved in D-GalN/LPS–mediated mitochondrial dysfunction is the disruption of redox status (40). The ROS defense network in the mitochondrial fraction consists of the enzymatic (e.g., MnSOD, GPx, and GR) and nonenzymatic (mainly glutathione) antioxidants. In the current study, the activities of MnSOD, GPx, and GR were weakened and most of the glutathione was depleted after D-GalN/LPS injection. We found that pre- and postadministration of curcumin strongly elevated the activities of MnSOD, GPx, and GR, as reported previously (15,17). One explanation for the above results is that curcumin enhanced the antioxidant defense systems and was able to efficiently detoxify the excessive ROS via this enhanced enzymatic reaction.

Numerous studies indicated that apoptosis is an important step in the development of liver injury. The apoptosis of hepatocytes was previously reported in acute liver injury induced by D-GalN/LPS (41). In the present study, the flow cytometric assay performed via annexin/propidium iodide staining confirmed apoptosis in D-GalN/LPS–treated mice. The induction of apoptosis via D-GalN/LPS was attenuated to a significant extent upon curcumin pre- and post-treatment, which was consistent with the results of others (42). Two main pathways leading to cell apoptosis have been studied in detail, namely mitochondrial-dependent and -independent apoptotic pathways (43). Alterations in MMP are thought to be a central regulatory mechanism of cell death (44,45). The collapse of the MMP after D-GalN/LPS addition suggested the role of mitochondrial dysfunction in D-GalN/LPS–induced liver injury. As previously described, curcumin partially prevented the processes of the mitochondrial apoptotic pathway by modulating the initial step of apoptosis (42). The similar antiapoptotic effects of curcumin were reconfirmed by elevating the MMP in our studies, which might be closely related to its free radical scavenging activity (46). Chen et al. (47) also indicated that curcumin attenuated MPP^+-induced apoptosis in pheochromocytoma cells through the mitochondria-ROS pathway.

Curcumin intervention may further regulate the gene expression of transcription factors involved in several key antioxidant signals. In the present study, the abnormal changes in mRNA expression were concomitant with observed liver injury and mitochondrial dysfunction in this study. Curcumin pretreatment significantly alleviated the liver mRNA levels of *Sirt1*. *Sirt1*, a member of the deacetylase family, was shown to regulate oxidative stress in cells through the induction of Forkhead box O 3 *(Foxo 3)*, which directly affects the expression of *Mnsod* and catalase (48). A recent study verified the protective effects of curcumin against ischemia reperfusion–induced ROS boost via the regulation of *Sirt1* (49). Our results indicated that the modulatory effects of curcumin on the expression of some antioxidant genes may reduce oxidant damage and maintain mitochondrial function in D-GalN/LPS–treated mice.

In conclusion, the present work showed that curcumin is a potential antioxidant and effective against D-GalN/LPS–related liver injury and mitochondrial dysfunction in mice. Curcurmin supplementation significantly prevented D-GalN/LPS–induced ROS overproduction, mitochondrial dysfunction, and cell apoptosis in mice, possibly via directly scavenging free radicals and indirectly enhancing the endogenous antioxidant defense system, and blocking the oxidant stress–mediated induction of gene expression. Our data also highlighted the fact that curcumin-attenuated cell apoptosis was partially dependent on the mitochondrial pathway. Interestingly, when compared with post-treatment with curcumin, we found that pretreatment with curcumin was much more effective in terms of protection against D-GalN/LPS–induced oxidant damage, which highlights its potential role in preventing clinical liver failure. In the future, we hope that more attention will be given to finding the molecular mechanism that explains the discrepancy between curcumin pre- and post-treatment in the D-GalN/LPS model.

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