Linoleic Acid Alleviates Lipopolysaccharide Induced Acute Liver Injury via Activation of Nrf2

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Summary

Linoleic acid (LA) not only functions as an essential nutrient, but also profoundly modulates oxidative stress and inflammatory response. However, the potential mechanisms have not been adequately researched. Hence, this study examined the potential pharmacological roles of LA and the underlying mechanisms in mice with lipopolysaccharide (LPS)-associated acute liver injury (ALI). The results indicated that treatment with LA alleviated the histopathological abnormalities in the hepatic and plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glutathione-S-transferase (GST) in mice with LPS exposure. In addition, LA inhibited the LPS-associated generation of proinflammatory factors, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), and downregulated the hepatic myeloperoxidase (MPO) level. In addition, the administration of LA resulted in a reduction in hepatic malondialdehyde (MDA) levels and an elevation in liver superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), and glutathione peroxidase (GSH-PX) levels. Further investigations revealed that LA promoted the expression of nuclear factor E2-related factor (Nrf2) and NAD(P)H: quinone oxidoreductase 1 (NQO1). In addition, the beneficial outcomes of LA on LPS-induced acute liver failure were revered when Nrf2 was pharmacologically suppressed by ML385. These experimental results demonstrated that LA supplementation attenuated LPS-associated acute hepatic impairment in mice *via* the activation of Nrf2.

Key words

Acute liver injury • Lipopolysaccharide • Linoleic acid • Nrf2

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Introduction

The liver is involved in many physiological processes, for instance, detoxification, and immunity. Hence, the organ is prone to damage [1]. Acute liver injury (ALI) is usually caused by toxins, infection, hypoxia, drugs, or alcohol [2]. It is a complicated clinical syndrome is usually closely correlated with increased mortality [3]. Several studies have suggested that uncontrolled inflammatory response and oxidative stress might be the major reasons underlying the development of ALI [4,5]. Lipopolysaccharide (LPS) produced by gram-negative bacteria, is a representative pathological factor that induces strong inflammatory response and oxidative stress in both experimental studies and under certain clinical situations [6-8]. LPS-associated hepatic damage in mice has been widely used for the investigation of hepatoprotective reagents [9,10].

Linoleic acid (LA), is the most widely consumed polyunsaturated fatty acid in the diet of human beings,

and is not synthesized by the body [11]. Previous studies have indicated that LA effectively alleviates oxidation reaction and inflammatory response in a mouse model of spontaneous multiple sclerosis [12], serum-starved hepatocytes [13], palmitic acid-induced microglia inflammation [14], and ethidium bromide-induced demyelination [15]. Moreover, several studies have stablished that the deficiency of LA is linked with the exposures of hepatic disorders, such as nonalcoholic fatty liver disease [16] and liver fat deposition [17]. Thus, LA might function as a bioactive nutrient under pathological conditions. However, whether LA is effective in LPS-induced ALI remains to be determined.

In this study, ALI was induced *via* the intraperitoneal administration of lipopolysaccharide (LPS) to C57BL/6 mice, and its possible effects on liver damage, inflammatory response, and oxidative stress were evaluated. As previous studies have observed that the antioxidant and anti-inflammatory effects of LA in aluminum-induced Alzheimer's disease model are associated with nuclear factor E2-related factor (Nrf2) activation [18], this research determined the potential effects of LA on the Nrf2 pathway. ML385, an Nrf2 inhibitor [19-21], was administered to clarify the hepatic protection of LA in relation to the Nrf2 pathway.

Materials and Methods

Animals

Experimental animals used in this research were C57BL/6 wild-type mice (age: 7-8 weeks weight: 20-22 g), which were received from the Laboratory Animal Center at Chongqing Medical University (Chongqing, China). All experimental animals were housed in a specific pathogen-free (SPF) animal care room and provided with regulated environments at 25±2 ℃, and under a light/dark cycle of 12 h each. The animals were fed with standard lab diets and liquids ad libitum. Before use, all mice were subjected to a minimum of 1 week of acclimation.

Design of the experiments

A common model of ALI induced by LPS was utilized [22-24]. The mice were categorized randomly into four groups of 6 each, as follows: (a) control group (solvent control without linoleic acid and saline), (b) LA group (50 mg/kg of LA and saline), (c) LPS group (solvent control without linoleic acid and 20 mg/kg of LPS), and (d) LA+LPS group (50 mg/kg of LA and 20 mg/kg of LPS). LPS (L2880, Sigma-Aldrich, USA) was soluble in normal saline, while LA (L1376, Sigma-Aldrich, USA) was dissolved in dimethyl sulfoxide (A503039, Sangon Biotech, Shanghai) and further diluted in olive oil. In this test, LA or solvent control without linoleic acid was administered intraperitoneally to each mouse 1 h before the LPS injection into the abdomen. After 24 h, all mice were alive, they were anesthetized and then sacrificed *via* cervical dislocation. The blood and hepatic tissues were collected for further investigations. To evidence that the Nrf2 signaling was involved in the protective effect of LA against LPS-associated ALI, ML385 (SML1833, Sigma-Aldrich) was used to inhibit Nrf2 activation. The inhibitor group (ML385+LA+LPS, n=6 per group) was added, and this group was treated with an intraperitoneal injection of ML385 2 h earlier, with the other details being the same as mentioned above.

Histopathology

The mice left lobe liver tissues were removed and immediately placed in pre-prepared tissue fixative at room temperature overnight. Subsequently, the tissues were sequentially embedded, sectioned, and stained with hematoxylin and eosin (H&E). Then, the morphologically examination was analyzed by an imaging system (Leica, Wetzlar, Germany). The histological changes were observed under 10× eyepiece and objective lenses at magnifications of 10× and 20×.

Determination of liver enzymes and oxidative stress-related markers

In this research, we used commercially available kits (Nanjing Jiancheng Bioengineering Institute, China) to survey the plasma activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glutathione-S-transferase (GST) in the mice as per the manufacturer's instructions. The liver tissues were blended with a normal saline for the further detection of superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA), myeloperoxidase (MPO), catalase (CAT), and glutathione peroxidase (GSH-PX). All laboratory steps were conducted following the instructions of the corresponding assay kits (Nanjing Jiancheng Bioengineering Institute).

Enzyme-linked immunosorbent assay (ELISA)

The liver tissue samples were meticulously collected and rinsed in ice-cold saline solution to remove

any residual blood. After blotting dry with a filter paper, the tissues were accurately weighed and transferred into a tissue grinding tube. An appropriate volume of ice-cold normal saline was added to the tube based on the weight measurement, followed by thorough homogenization using a tissue homogenizer to obtain a 10 % tissue homogenate. Subsequently, the prepared homogenate was centrifuged at 3000 rpm for 15 min in a cryogenic centrifuge. The supernatant was collected, diluted to the appropriate concentration, and then the levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in each sample were analyzed according to the instruction of the ELISA test box (Neobioscience, Shenzhen, China).

Real-time qPCR

The extraction of total RNA from the mouse liver tissues was performed by the Trizol (Sangong Biotech, China) method. Total RNA was reverse transcribed into cDNA by using the PrimeScript RT two-step assay kit (Takara Biotech). The gene expression levels of β-actin, TNF-α, IL-6, and NQO1 were calculated *via* real-time fluorescence quantitative PCR (qPCR) using TB GreenVR Premix Ex TaqTMII (Takara Biotech, China). β-actin was employed for reference. The relative transcripts standards of the target genes were analyzed by using the $2^{-\Delta\Delta CT}$ method. The primers were synthesized by Tsingke Biotechnology (Beijing, China); the primer primers sequences are shown in Table 1.

Table 1. Primer sequences used for real-time qPCR.

Gene	**Forward primer (5'-3')**	**Reverse primer (5'-3')**
NQO1	CTGAAAGGCTGGTTTGAGAGAGT	TGGACACCCTGAAGAGAGTACAT
IL-6	AACCGCTATGAAGTTCCTCTCTG	TGGTATCCTCTGTGAAGTCTCCT
TNF-α	GTGATCGGTCCCCAAAGG	GGTGGTTTGCTACGACGTG
β-Actin	ACTGTCGAGTCGCGTCC	GTGACCCATTCCCACCATCA

Western blotting

The liver tissues were removed from -80 °C refrigerator and appropriate weights of liver tissues were immediately weighed and their total protein contents were extracted with RIPA buffer (Beyotime, Shanghai). The amount of protein was calculated using the BCA Protein Detection Reagent Kits (Beyotime, Shanghai). Equal mass of protein was denatured by boiling for 10 min and separated by 7.5 % or 12.5 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis and then transferred onto polyvinylidene difluoride (PVDF) membranes (HVLP02500, Millipore, USA). The membranes were blocked with 5 % skim milk powder diluted in TBS-T (Sangong Biotech, China) for 1 h at indoor temperature. After obstructing, the membrane was treated by appropriate primary antibodies, Nrf2 (1:500, Proteintech, China), NQO1(1:2000, Abcam, England), GAPDH (1:20000, ABclonal, China) and β-actin (1:6000, Bioss, China) at 4 °C for overnight. The next day, the primary antibody was removed, and the membrane was washed five times with TBS-T. The PVDF membrane was then incubated for 1 h at room temperature with goat antirabbit secondary antibody (1:6000, Bioss, China). Finally, the proteins were detected using a chemiluminescence reagent (VIBER, France). The binding was analyzed by the VIBER software (VIBER, France).

Immunohistochemical analysis

The mice left lobe liver tissues were removed and immediately placed in pre-prepared tissue fixative at room temperature overnight. Subsequently, the tissues were embedded in paraffin, sliced into 4-um sections, and dried for 2 h at 65 °C. The sections were then immersed in xylene, and deparaffinized and hydrated in a gradient series of ethanol. Antigen repair was performed by heating the samples in EDTA (PH 9.0, Solarbio, China) for 15 min at 95 °C. Endogenous peroxidase was blocked using an endogenous peroxidase blocker for 15 min at room temperature and then incubated with anti-Nrf2 (1:100, Proteintech, China) primary antibody at 37 °C for 1 h. After washing thrice with PBS for 3 min each, the reaction enhancement solution was incubated sequentially for 15 min at room temperature and then washed. Biotin-labeled goat anti-rabbit IgG polymer was added, incubated for 30 min at room temperature, and then washed. The DAB (ZLI-9018, ZSGB-BIO, China) staining solution was prepared, stained and observed under the microscope, followed by counterstaining, dehydrating, and sealing the slides, per the manufacturer's

instructions (PV-9001, ZSGB-BIO, China). The number of nuclei densely stained for Nrf2 was counted in 150 cells in five fields at ×40 magnification, and the cells from each group were compared [25]. All the images were viewed by using imaging system (Leica, Wetzlar, Germany).

Statement of ethics

All animals involved in this study were handled following the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. The experiment received approval from the Ethics Committee at University-town Hospital, Chongqing Medical University (LL. 202239).

Analysis of statistical data

The data was expressed as the mean ± standard deviation (SD), as appropriate following Shapiro-Wilk test for normality. To evaluate the differences between the groups, a one-way analysis of variance (ANOVA) was performed to evaluate the group differences, subsequently, *post hoc* comparisons were made using Tukey's test for multiple comparisons. All statistical analyses and graphical representations were performed using GraphPad Prism 8.0 software. In this study, a threshold of $P<0.05$ was used to determine statistical significance.

Results

LA alleviated LPS-induced hepatic damage

To evaluate the potential hepatoprotective properties of LA against liver damage induced by LPS, a murine model of ALI was created through intraperitoneal administration of LPS. We examined the histological changes in the liver using H&E staining. As shown in Figure 1, the control and single LA groups revealed normal hepatic architecture, with a central vein and radiating cords of hepatocytes. When compared with the control group, single LPS treatment induced liver injury, which was manifested as edema or congestion, irregularly dilated central vein with inflammatory reactions, and macrophage infiltration around the central vein. On the contrary, LA treatment of LPS-induced ALI mitigated the hepatic damage (Fig. 1A). Past studies have shown that liver enzyme indexes (AST, ALT and GST) in the plasma are significantly increased during liver injury. Therefore, the trend of elevated liver enzyme indicators is often employed to assess the liver injury and abnormal liver functions in clinical practice [26]. Furthermore, the plasma ALT, AST, and GST levels were calculated, indicating that the levels were higher in comparison with the controls. However, treatment with LA reduced the levels of these enzymes in mice with LPS-induced ALI (Fig. 1B-D). These findings indicate that LA has beneficial impact in safeguarding the liver from damage induced by LPS.

LA dampened the inflammatory response caused by LPS

We used RT-qPCR and enzyme-linked immunosorbent assay to examine the levels of proinflammatory cytokines in the liver tissues so as to clearly define the expressions of inflammatory factors in mice. It has been showed that the gene levels of TNF-α and IL-6 were greatly increased by stimulation with LPS, but significantly decreased by the administration of LA (Fig. 1F-G). Similarly, the hepatic IL-6, TNF-α, and MPO levels were tested using enzyme linked immunosorbent assay, as shown in the Figure 1H, I, E, the expressions of TNF-α, IL-6, and MPO in the hepatic tissues were increased in the LPS groups when compared with that in the control group. Treatment with LA partially blocked LPS-induced IL-6 and TNF-α expression when compared with the LPS group. In summary, LPS stimulation induces inflammation reaction within mice liver, which could be weakened through LA administration.

LA activated the Nrf2 signaling pathway and mitigated LPS-induced oxidative stress

To detect the conservation mechanism of LA in LPS-induced ALI, the Nrf2 and NAD(P)H: quinone oxidoreductase 1 (NQO1) protein levels were detected. When compared with the control group, the standards of Nrf2 and NQO1 were reduced by LPS. However, treatment with LA increased the expressions of Nrf2 and NQO1 (Fig. 2A-D). In order to further investigate the nuclear translocation of Nrf2, immunohistochemical results indicated that no obvious nuclear translocation in the control group, LA group, and LPS group, while deep nuclear staining was observed in the LA+LPS group (Fig. 2E, F). In addition, the NQO1 gene expression was markedly reduced in the LPS group but greatly elevated in the LA+LPS group (Fig. 2G). As we all know, the Nrf2 signals are closely connected with oxidative stress, hence the levels of oxidative stress markers were assessed to evaluate the influence of LA on oxidative stress in the liver tissues. The liver tissues exhibited a notable increase in MDA levels among the LPS groups, while

LA intervention partially mitigated the enhancement of oxidative stress (Fig. 2H). On the contrary, the liver tissues exhibited a significant decrease in the levels of SOD, CAT, GSH and GSH-PX expression. However, this decline was effectively reversed by LA therapy (Fig. 2I-L). This phenomenon demonstrated that, LA can alleviate LPS-induced oxidative stress, which may be related to Nrf2 pathway activation.

ML385 suppressed Nrf2 activity and attenuated the protective effect of LA against LPS-induced oxidative damage in ALI

To explore the potential molecular mechanism in more depth, we administered ML385, an Nrf2 inhibitor, *via* intraperitoneal injection to suppress Nrf2 activity. Subsequently, we monitored the expression of NQO1, an antioxidant downstream of Nrf2. As shown in Figure 3A-C, the use of ML385 decreased the NQO1 gene expression and protein levels in the ML385+LA+LPS group when compared that to those in the LA+LPS group. Similarly, the immunohistochemistry findings demonstrated that in the LA+LPS group, there was a presence of nuclear NRF2 staining. However, when ML385 intervention was applied, it effectively hindered the translocation of NRF2 into the nucleus (Fig. 2D, E). The presence of ML385 partially attenuated the protective effect of L against oxidative stress induced by LPS-associated hepatic injury. As depicted in Figure 3F-J, the hepatic tissue of mice in the LA+LPS group exhibited a significant reduction in MDA levels compared to those in the LPS group. Conversely, there was a noticeable increase observed in the levels of SOD, CAT, GSH, and GSH-PX. Compared to the LA+LPS group, there was a significant increase in MDA levels observed in the liver tissues of mice in the ML385+LA+LPS group. Conversely, a significant decrease was noted in the activities of SOD, CAT, GSH, and GSH-PX. This finding provides additional evidence that the Nrf2 pathway is responsible for the hepatoprotective effect of LA in mitigating oxidative stress induced by LPS.

ML385 suppressed the remission effects of LA on LPS-induced liver injury and inflammatory

We confirmed whether the LA treatment reduced the hepatic damage and inflammation induced by LPS. Moreover, the protective role of LA on the LPS-associated liver damage and inflammatory were partly abolished by ML385. As shown in Figure 4A, pathological examinations revealed improved liver injury, with the treatment of LA in LPS-associated hepatic injury. However, the use of ML385 suppressed the remission effects of LA on LPS-induced liver injury. Comparatively, the hepatic injury biomarkers ALT, AST, and GST in the plasma were also increased in the ML385+LA+LPS group than in the LA+LPS group (Fig. 4B-D). Meanwhile, we assessed the inflammatory expressions in the liver. Our findings indicated that LA intervention led to a decrease in the TNF-α, IL-6, and MPO levels, which were subsequently enhanced after treatment with ML385 (Fig. 4E-G). These results indicated that LA failed to exert liver protection and attenuate inflammation in the presence of ML385. It was further demonstrated that LA alleviated LPS-induced liver injury and inflammatory response by activating the Nrf2 pathway.

Discussion

Apart from being an important dietary and cellular component, mounting evidence suggests that LA functions as a bioactive regulator under various pathological conditions. For instance, in a model of long-chain fatty acid-induced mitochondrial dysfunction in CD8+ T cells (CTL), LA augmented mitochondrial function and CTL antitumor immunity [27]. In LPS-stimulated HTR-8/SVneo trophoblast cells, LA maintained cell viability and significantly increased the total antioxidant capacity and the heme oxygenase-1 (HO1) level [28]. A recent study using the nematode Caenorhabditis elegans as a model found that supplementation with 1 μM LA enhanced stress-associated genes, such as sod-1, sod-3, mtl-1, and so on, thereby reducing oxidative stress and extending the lifespan of the worm [29]. ω-6 polyunsaturated fatty acids (LA) can activate a synergistic feedback loop between autophagy and antioxidant systems to exert antioxidant effects [13]. Moreover, previous studies have demonstrated that LA may have a protective effect on the liver. For example, linoleic acid supplementation can significantly reduce the level of MDA in liver tissues [30]; the higher the proportion of LA and unsaturated fatty acids in fatty acids, the lower the risk of liver fibrosis [31]. In the present study, treatment with LA ameliorated LPS-induced hepatic damage, as indicated by improvement in histopathological damages and decreased plasma AST and ALT levels. Therefore, LA may have potential value in pharmacological intervention of acute liver injury.

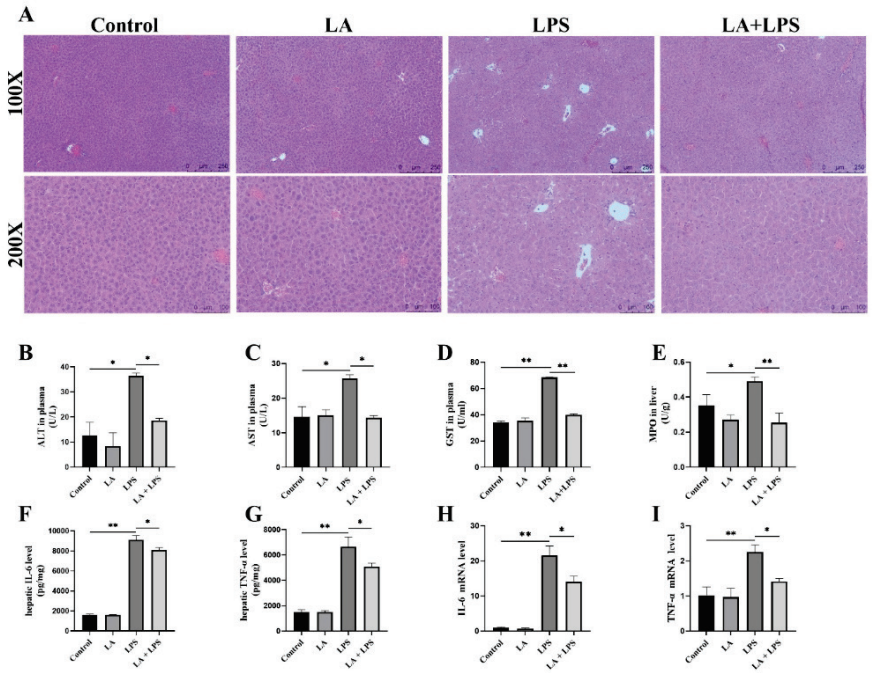

Fig. 1. Linoleic acid alleviated LPS-induced hepatic damage and inflammatory response. The experimental mice were sacrificed 24 h after LPS intervention, and their liver tissues were collected, while the plasma was separated. (**A**) HE staining of the liver tissues (magnification, 100× or 200×); (**B**) The ALT levels in the plasma; (**C**) The AST levels in the plasma; (**D**) The GST levels in the plasma; (**E**) The relative MPO levels. (**F**) The relative IL-6 mRNA levels; (**G**) The relative TNF-α mRNA levels; (**H**) ELISA showing IL-6 levels; (**I**) ELISA showing TNF-α levels; * P<0.05, ** P<0.01. LPS, lipopolysaccharide, LA, linoleic acid, ALT, alanine transaminase, AST, aspartate transaminase, GST, glutathione-S-transferase, IL-6, interleukin-6, TNF-α, tumor necrosis factors-α, MPO, myeloperoxidase, ELISA, enzyme-linked immunosorbent assay.

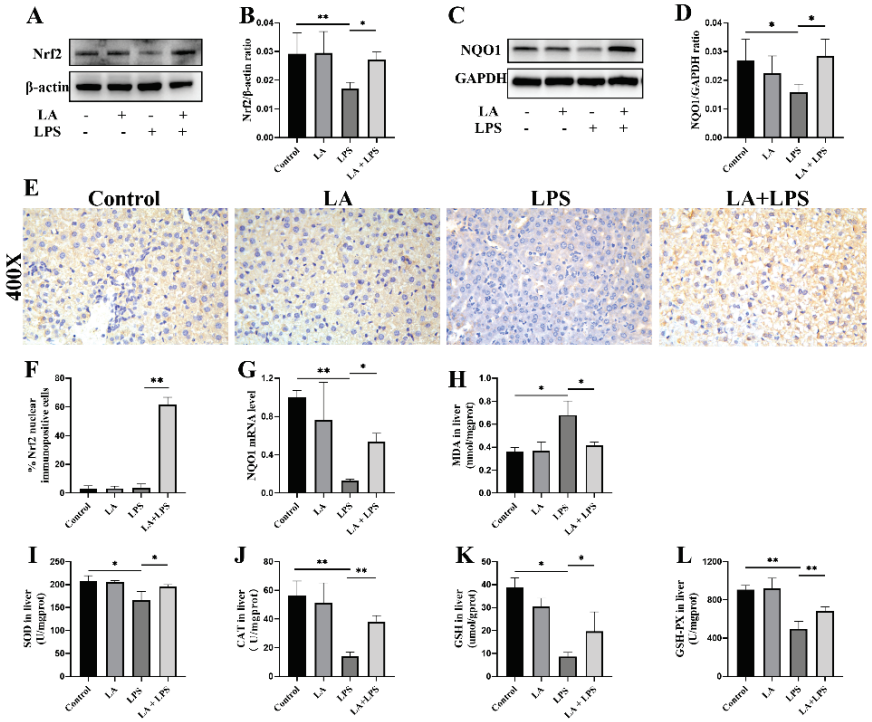

Fig. 2. Linoleic acid activated the Nrf2 signaling pathway and mitigated LPS-induced oxidative stress. (**A, B, C, D**) Western blotting displaying the protein levels of Nrf2 and NQO1; (**E**) Nrf2 protein was analyzed by immunohistochemical analysis (magnification, 400×); (**F**) Charts illustrate the % Nrf2 nuclear immunopositive cells; (**G**)The relative NQO1 mRNA levels; (**H**) The relative lipid peroxidation protein (MDA) levels; (**I**) The relative SOD levels. (**J**) The relative CAT levels; (**K**) The relative GSH levels; (**L**) The relative GSH-PX levels. * P<0.05, ** P<0.01. LPS, lipopolysaccharide, LA, linoleic acid, Nrf2, nuclear factor E2-related factor 2, NQO1, NAD(P)H: quinone oxidoreductase 1, MDA, malondialdehyde, SOD, superoxide dismutase, CAT, catalase, GSH, glutathione, GSH-PX, glutathione peroxidase.

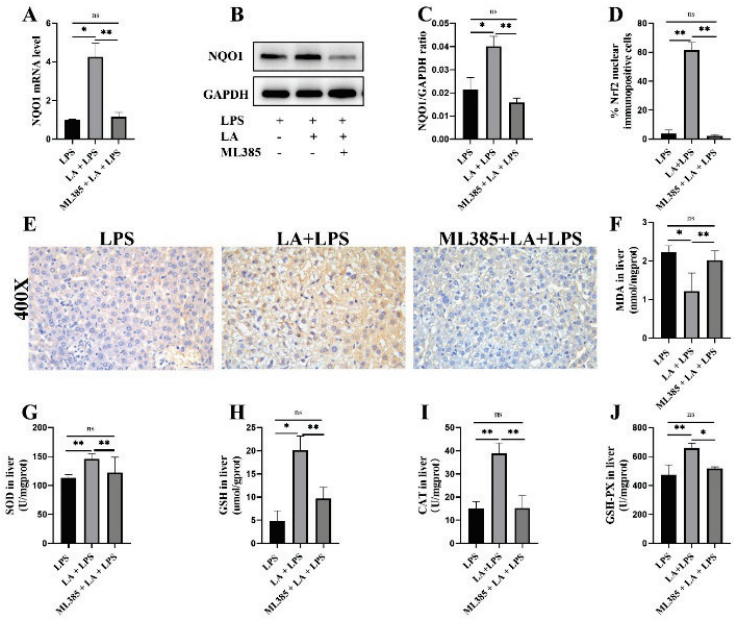

Fig. 3. ML385 suppressed Nrf2 activity and attenuated linoleic acid's protective effect against LPS-induced oxidative damage in acute liver injury. (**A, B**) Western blotting exhibits the protein levels of NQO1; (**C**) The relative NQO1 mRNA levels; (**D**) Charts illustrate the % Nrf2 nuclear immunopositive cells; (**E**) Nrf2 protein was analyzed by immunohistochemical analysis (magnification, 400×); (**F**) MDA activity in the liver; (**G**) SOD activity in the liver; (H) GSH activity in the liver; (**I**) CAT activity in the liver; (**J**) GSH-PX activity in the liver. * P<0.05. LPS, lipopolysaccharide, LA, linoleic acid, NQO1, NAD(P)H: quinone oxidoreductase 1, MDA, malondialdehyde, GSH, increased glutathione, SOD, superoxide dismutase, CAT, catalase, GSH-PX, glutathione peroxidase, ML385, an Nrf2 inhibitor.

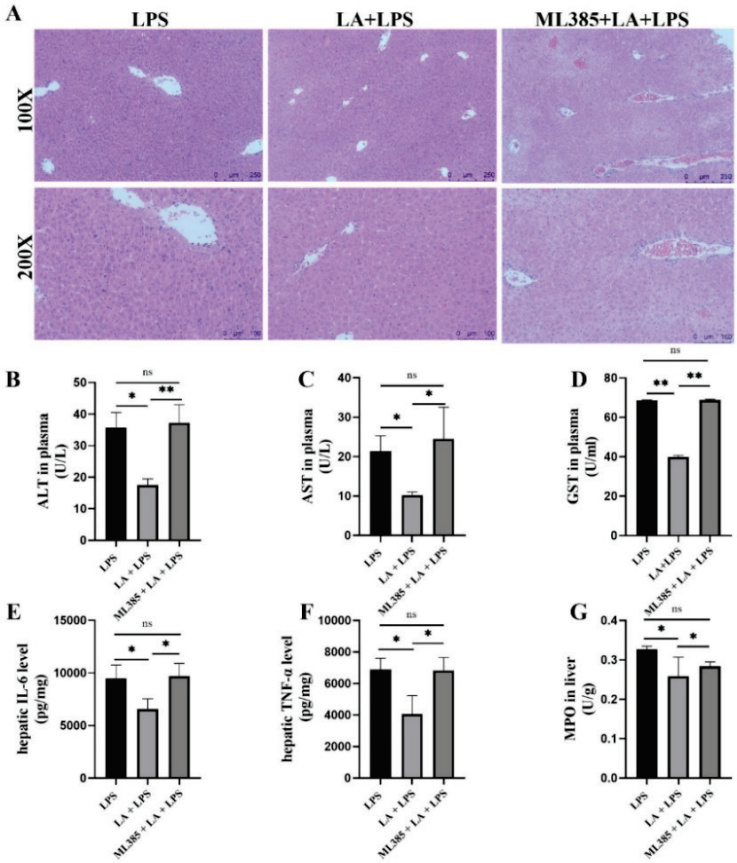

Fig. 4. ML385 suppressed the remission effects of linoleic acid on LPS-induced liver injury and inflammatory. (**A**) HE staining of the liver tissues (magnification, 100× or 200×). (**B**) The ALT levels in the plasma of mice; (**C**) The AST levels in the plasma of mice; (**D**) The GST levels in the plasma of mice; (**E**) ELISA showing the IL-6 levels; (**F**) ELISA showing the TNF-α levels; (**G**) The relative MPO levels; * P<0.05. LPS, lipopolysaccharide, LA, linoleic acid, ML385, an Nrf2 inhibitor, ALT, alanine transaminase, AST, aspartate transaminase, IL-6, ininterleukin-6, TNF-α, tumor necrosis factors-α, MPO, myeloperoxidase, ELISA, enzyme-linked immunosorbent assay.

Liver damage is strongly associated with an unregulated inflammatory reaction. An overactive inflammatory response facilitates the onset and progression of liver injury, and numerous investigations have verified that restraining excessive inflammation plays a pivotal role in managing liver damage. For instance, the inhibition of the inflammatory response ameliorated liver injury in a carbon tetrachloride- induced ALI mouse model [32], In LPS/D-galactosamine-induced ALI, oxyberberine exerted hepatoprotective effects by activating anti-inflammatory and antioxidant pathways [33]. In present study, LA intervention alleviated the inflammatory response in the liver, as indicated by a reduction in hepatic the TNF-α, and IL-6 levels. In agreement with our study, another investigation reported that treatment with LA improved hyperuricemia and renal inflammation induced by high fructose in rats [34]. These results suggested that LA alleviates liver injury by inhibiting the inflammatory response.

Several research studies have indicated a potential association between the antioxidant properties of LA and its ability to reduce inflammation. In an *in vitro* model of Parkinson's induced by 6-OHDA in SH-SY5Y cells, LA stimulated lipid droplet biogenesis, improved autophagic/lipophilic flux, and exerted antioxidant effects, thereby alleviating an inflammatory response [35]. In addition to direct oxidative damage, oxidative stress is profoundly involved in the regulation of inflammation by modulating the redox-sensitive proinflammatory signaling pathways [36,37]. The present study noted that LA alleviated oxidative stress in the liver, as evidenced by a decrease in the MDA level and an increase in the GSH and SOD levels. The attenuated oxidative stress might have contributed to the suppressed inflammatory response in this study.

Nrf2 is a classical antioxidant pathway that is activated by oxidative stress. It is then transferred from the cytoplasm to the nucleus and promotes the transcription of antioxidant enzymes, such as NQO1 and HO1 [38]. This study identified that treatment with LA upregulated the expressions of Nrf2 and NQO1, which suggests that the Nrf2 pathway was activated. Interestingly, coadministration of the Nrf2 inhibitor ML385 abolished the antioxidant and anti-inflammatory effects of LA. In line with our findings, dietary supplementation of conjugated LA was reported to alleviate depression markers in mice *via* the modulation of the Nrf2 pathway [39]. Thus, treatment with LA might protect the liver from LPS-induced oxidative and inflammatory injury in an Nrf2-dependent manner.

In summary, as illustrated in Figure 5, our findings signify that LA exhibits hepatoprotective roles by inhibiting oxidative stress and inflammation on ALI. The antioxidant and anti-inflammatory activities of LA might result from the activation of the Nrf2 pathway. Although the direct molecular mechanism by which LA activates Nrf2 needs to be further examined, the results from this study allude that LA has potential value in the pharmacological control of ALI resulting from oxidative stress and inflammatory response.

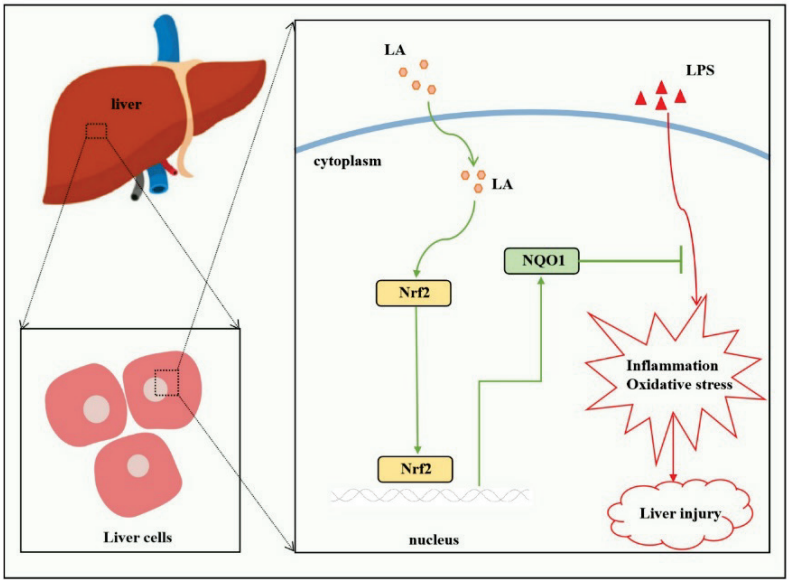

Fig. 5. Graphical abstract of linoleic acid ameliorated LPS-induced liver injury. In mice, LA activates the Nrf2 signaling pathway and promotes the transcription of NQO1 that contributes to the inhibition of inflammation and oxidative stress induced by LPS, as well as the mitigation of LPS-induced acute liver injury.

Conclusions

These experimental results demonstrate that LA supplementation attenuates LPS-associated acute liver impairment in mice *via* the activation of the Nrf2 pathway. Nonetheless, the specific action mechanism main to be elucidated, warranting further studies.

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Conflict of Interest

There is no conflict of interest.

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