Selonsertib, a Potential Drug for Liver Failure Therapy by Rescuing the Mitochondrial Dysfunction of Macrophage via ASK1-JNK-DRP1 Pathway

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Research

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Abstract

Background

Acute liver failure (ALF) is associated with high mortality rate and there are still no effective treatments expect liver transplantation and artificial liver therapies. This study was aimed to determine the effects, therapeutic window and mechanisms of selonsertib, a selective inhibitor of ASK1, for ALF therapy.

Results

Lipopolysaccharide and D-galactosamine (LPS/GalN) was used to simulate ALF. We found that selonsertib pretreatment significantly ameliorated ALF as determined by reduced hepatic necrosis and serum alanine aminotransferase, aspartate aminotransferase and inflammatory cytokine levels. However, selonsertib is only effective early after LPS/GalN administration and the limited therapeutic window was related to the activation and mitochondrial translocation of JNK and DRP1. Further experiments revealed that selonsertib could alleviate LPS-induced mitochondrial damage in macrophages by evaluating the mitochondrial membrane potential and mitochondrial permeability transition pore opening in macrophages. Selonsertib also suppressed the release of inflammatory cytokines from macrophages by reducing DRP1-mediated mitochondrial dysfunction, which was confirmed by using mdivi, the specific DRP1 inhibitor.

Conclusions

Selonsertib protected against LPS/GalN-induced ALF by attenuating JNK-mediated DRP1 mitochondrial translocation and then rescuing mitochondrial damage in macrophages and may have therapeutic potential for early ALF patients.

Introduction

Acute liver failure (ALF) is a severe consequence of abrupt hepatocyte injury manifested as rapid-onset elevation of aminotransferases, altered mentation, and disturbed coagulation. ALF can be caused by a variety of factors including paracetamol toxicity, virus, hepatic ischaemia and autoimmune hepatitis, and drug-induced liver injury from prescription drugs and herbal supplements, and it can evolve over days or weeks to a lethal outcome [1]. Liver transplantation and artificial liver therapies are presented as main treatments for ALF in clinical. However, the shortage of available donor livers limit the widespread clinical application of liver transplantation and the multiple postoperative complications also limited the efficacy of artificial liver therapy against ALF [2]. Thus, there is an urgent medical need to identify novel therapies for ALF.

Apoptosis signal-regulating kinase 1 (ASK1) is a ubiquitously expressed apical mitogen-activated kinase kinase kinase (MAP3K) that is activated by various types of pathological stimuli, including neurodegenerative disorders, inflammatory diseases and cancer [3-5]. Recently, several investigations

have showed that ASK1 is over-activated in ALF and liver injury, such as APAP-induced ALF, CCl_4-induced liver injury, and hepatic ischemia/reperfusion injury, suggesting inactivation of ASK1 might be a potential strategy for ALF therapy [6, 7]. Selonsertib, a selective inhibitor of ASK1, has been reported as an effective treatment for non-alcoholic steatohepatitis (NASH) and multidrug resistance (MDR) in various types of cancer in human patients by reducing hepatic steatosis, inflammation and fibrosis or reversing ATP-binding cassette transporters-mediated MDR [8, 9]. Its phase III clinical trial on NASH has been initiated by the U.S. Food and Drug Administration [10].

Lipopolysaccharide and D-galactosamine (LPS/GalN) is widely used to simulate ALF [11]. The present study aimed to investigate the effects, underlying mechanisms, and therapeutic window of selonsertib in LPS/GalN-induced ALF.

Methods

Animals

C57BL/6 mice (aged 5–6 weeks) were purchased from Nanjing BioMedical Research Institute of Nanjing University (Nanjing, China). Animals were housed in a controlled environment with a 12 hour light/dark cycle and free access to food and water. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the First Affiliated Hospital of Zhejiang University.

ALF mouse model and treatment

C57BL/6J mice were intraperitoneally injected with LPS (10 μg/kg, Sigma-Aldrich, St Louis, MO, USA) and D-GalN (400 mg/kg, Sigma-Aldrich) to establish a mouse model of LPS/GalN-induced ALF. Selonsertib (15, 30, 60 mg/kg, MCE) were administered *via* i.p. injection 30 min prior to LPS/GalN injection or at 0.5, 1, 2, and 4 hours after LPS/GalN injection. For another therapy, mdivi (30 mg/kg, MCE) were administered via *i.p.* injection 30 min prior to LPS/GalN injection. The control group was administered with vehicle (n=6). At 0.5, 1, 2, 4, 6 h after LPS/GalN injection, the mice were sacrificed, and serum and liver samples were collected for assessing the extent of liver injury. Serum was evaluated for biochemical parameters. The liver samples were evaluated for histochemistry and Western blot analysis.

Liver histological and serum biochemical analysis

Liver tissues were processed for paraffin embedding and were sectioned into 4 μm sections. The sections were then routinely stained with hematoxylin and eosin (H&E staining). The serum levels of alanine aminotransferase aspartate (ALT) and aspartate aminotransferase (AST) were measured by using FUJI DRI-CHEM Slide GFP/ALT-PIII and GOT/AST-PIII, respectively, according to the manufacturer's instructions with DRI-CHEM 4000ie (FUJIFILM). Total bilirubin (TBiL) was measured using standard clinical chemistry techniques (Integra II; Roche, UK).

Cell culture and treatment

Mouse macrophage cell line, RAW264.7 were cultured in 1640 (Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (Thermo Fisher Scientific, Inc.), 100 U/ml penicillin, and 100 µg/ml streptomycin (Thermo Fisher Scientific, Inc.) and maintained at 37°C in a humidified 5% CO_2 atmosphere. The cells were pre-incubated with selonsertib (5 µM) or mdivi (10 µM) for 6 h and then incubated with LPS (500 ng/ml) for 4 h. The supernatant and cell samples were then collected for further analysis.

Cytokine detection by ELISA

The cytokine levels in supernatants from cell cultures were quantified with an enzyme-linked immunosorbent assay (ELISA) with a commercial mouse Tumor Necrosis Factor-α (TNF-α) and Interleukin-1α (IL-1α, MultiSciences, China) ELISA kit according to the manufacturer's instruction.

Cytometric Bead Array (CBA) analysis

Cytokine levels in the serum samples were detected by BD LSR Fortessa cytometer (Becton Dickinson Holdings Pte Ltd, Singapore) using commercially available kit BD™ Cytometric Bead Array (CBA) Mouse Inflammation Kit (BD Biosciences, USA) following manufacturer's instructions. In brief, cytokine standards were prepared using a vial of lyophilized mouse inflammatory cytokines, including IL-6, IL-10, Monocyte Chemoattractant Protein-1 (MCP-1), Interferon-γ (IFN-γ), TNF-α, and IL-12p70, and assay diluent by the method of serial dilutions. Capture bead was added into each tube that is samples, standards, and negative control and was incubated for 30 min at room temperature in the absence of light. The flow cytometer was calibrated using cytometer setup beads and the assay was performed.

Mitochondrial Permeability Transition Pore (mPTP) opening detection and mitochondrial membrane potential observation

The mitochondrial membrane potential (Δψm) was observed by staining with the JC-1 probe (Beyotime Biotechnology, Jiangsu, China) according to the manufacturer's instruction. After JC-1 incubation for 20 min at 37 °C, monomeric green fluorescence emissions and aggregate red fluorescence intensities in cells were observed by two-photon confocal laser scanning microscopy (Lei TCS SPB-MaiTai MP). The mPTP opening was evaluated through the mPTP Assay Kit (Beyotime Biotechnology) according to the manufacturer's instruction and observed with the above microscopy. A decrease in green fluorescence indicated that the increased opening of mPTP.

Determination of Mitochondrial oxidative stress

Cells were pelleted by centrifugation and resuspended and incubated in pre-warmed growth medium containing 250 nM MitoSOX™ Red Mitochondrial Superoxide Indicator (Thermo Fisher Scientific, Inc.) for 30 min at 37 °C in a 5% CO_2 atmosphere. After incubation, the cells were washed with PBS and the fluorescence was then assessed by fluorescence microscopy.

Mitochondria isolation

The mitochondria from macrophages and liver tissues were isolated by using Cell Mitochondria Isolation Kit (Beyotime Biotechnology) and Tissue Mitochondria Isolation Kit (Beyotime Biotechnology), respectively, according to the manufacturer's instruction. The protein expression levels in the isolated mitochondria were then examined by Western blot analysis.

Western blot analysis

To determine protein expression levels, whole-cell, cell mitochondrial cytoplasmic fractions, tissue extracts, and tissue mitochondrial fractions were lysed with RIPA peptide lysis buffer (Beyotime Biotechnology) containing 1% protease inhibitors (Pierce). Samples were quantified using a Pierce BCA Protein Assay Kit according to the manufacturer's instructions, and 30 μg protein was resolved by SDS-PAGE and transferred to PVDF membranes. Membranes were blocked in 5% nonfat milk and incubated overnight at 4°C with primary antibodies, including anti-ASK1 (1:1000; Cell Signaling Technology), anti-p-ASK1 (1:1000; Cell Signaling Technology), anti-JNK (1:1000; Cell Signaling Technology), anti-p-JNK (1:1000; Cell Signaling Technology), anti-p38 (1:1000; Cell Signaling Technology), anti-p-p38 (1:1000; Cell Signaling Technology), anti-dynamin-related protein 1 (DRP1, 1:1000; Abcam), anti-p-DRP1 (1:1000; Cell Signaling Technology), anti-OPA1 (1:1000; Abcam), anti-mitofusin 1 (MFN1, 1:1000; Abcam), anti-mitofusin 2 (MFN2, 1:1000; Abcam), anti-mitochondrial fission protein (MFF, 1:1000; Abcam), anti-mitochondrial fission protein 1 (FIS1, Proteintech), anti-voltage-dependent anion channel 1 (VDAC1, 1:1000; Abcam), and anti-GAPDH (1:3000; Cell Signaling Technology). The membranes were then probed with HRP-tagged secondary antibodies at room temperature for 1 hour. The protein bands were visualized using Enhanced Chemiluminescence System and detected in ChemiScope Western Blot Imaging System (Clinx Science Instruments Co., Ltd.). Image J software was used for gray-scale value assay.

Statistics

All data are presented as mean ± SD. Comparisons were made between two groups using independent samples t-test, or between three or more groups using one-way analysis of variance (ANOVA) with Tukey's post-hoc test. $P < 0.05$ was considered significant.

Results

Selonsertib protects against LPS/GalN-induced ALF by inhibiting the activation of JNK pathway

To investigate the effect of selonsertib on LPS/GalN-induced ALF, mice were treated with 15, 30, or 60 mg/kg of selonsertib, 30 min prior to administration of LPS/GalN and then examined 6 h later. As shown in Figure 1A and B, LPS/GalN induced significant liver injury as indicated by dramatically elevated serum ALT, AST, and TBiL levels accompanied with massive hepatic necrosis, while pretreatment with all of these three dosages of selonsertib resulted in remarkable reduction in serum ALT, AST, and TBiL levels (Figure 1A) and less hepatic necrosis (Figure 1B). CBA cytokine analysis also showed that selonsertib pretreatment could significantly reduce the elevated serum levels of inflammatory cytokines and chemokines, such as TNF-α, IFN-γ, IL-12p70, IL-6, and MCP-1, whereas enhance the serum level of IL-10,

an anti-inflammatory cytokine, in LPS/GalN-administered mice, especially at the dosage of 30 and 60 mg/kg (Figure 1C). These data indicate that selonsertib can effectively prevent LPS/GalN-induced ALF.

As selonsertib is a specific ASK1 inhibitor, we then determined the protective mechanism of selonsertib in this model by focusing ASK1-mediated signaling pathway. JNK activation has been implicated in variety of liver injuries and ASK1 is suggested to function upstream of JNK pathway [3, 12]. As we expected, LPS/GalN administration induced remarkable elevation of phosphorylated JNK (p-JNK) levels in liver tissues, while selonsertib pretreatment markedly reduced the phosphorylation of JNK (Figure 1D). Quantitative densitometric analysis also confirmed the changes of p-JNK/total JNK levels by pretreatment with all of these three dosages of selonsertib. These results confirmed that inhibition of ASK1-mediated JNK activation by selonsertib may contribute to the prevention of LPS/GalN-induced ALF.

Selonsertib efficacy is related to JNK mitochondrial translocation

Since sustained JNK activation and translocation to mitochondria has been implicated in liver injury [12, 13], we then assessed the effect of selonsertib on the activation and mitochondrial translocation of JNK. As shown in Figure 2A and B, the LPS/GalN-induced elevation in p-JNK (Figure 2A) was accompanied by increases in total JNK and p-JNK in mitochondria (Figure 2B) and this was prevented by administration of selonsertib.

We then analyzed the therapeutic window of selonsertib in this model. Mice treated with selonsertib 0.5 h or 1 h after LPS/GalN also showed significant protection against liver injury when compared to vehicle-treated mice but when selonsertib administration was delayed to 2 h or 4 h post LPS/GalN, no protection against liver injury were evident as determined by serum ALT, AST and TBiL levels (Figure 2C), HE staining (Figure 2D), and CBA cytokine analysis (Figure 2E). We further investigated whether the limited therapeutic window of selonsertib on ALF was related to the aberrant JNK activation. Limited LPS/GalN-induced JNK activation was observed as early as 0.5 h and 1 h with almost no JNK and p-JNK translocation to the mitochondria. However, by 2 h, massive JNK activation and extensive mitochondrial translocation had occurred (Figure 2F and G). The above data suggested that the protective effect of selonsertib on LPS/GalN-induced ALF was depended on suppression of early JNK activation and mitochondrial translocation.

Selonsertib suppresses DRP1 mitochondrial translocation and rectifies abnormal mitochondrial dynamics

As DRP1 translocation to mitochondria has been suggested to be downstream of JNK activation and mediate mitochondrial fission [14, 15], we then investigated the effect of selonsertib on JNK-mediated DRP1 activation and mitochondria translocation in the whole and mitochondrial fraction of liver tissues from the mice following 0.5, 1, 2, 4 h LPS/GalN exposure. As shown in Figure 3A, LPS/GalN administration induced gradually elevation of DRP1 phosphorylation at Ser616 (p-DRP1) in the whole tissue samples and there was a dramatic increase of p-DRP1 was detected at 2 h after LPS/GalN injection. Further analyses of the levels of DRP1 and p-DRP1 proteins in mitochondrial fraction showed

that LPS/GalN injection promoted the translocation of both DRP1 and p-DRP1 to mitochondria, especially from 2 h after LPS/GalN injection (Fig. 3B). Interestingly, selonsertib pretreatment could effectively suppress the elevation of DRP1 phosphorylation and its translocation to mitochondria by LPS/GalN induction (Figure 3C and D).

As altered DRP1 activity was known to result in abnormal mitochondrial dynamics [16], we then investigate the effect of selonsertib on mitochondrial dynamics by determining the expression of mitochondrial fission- and fusion-related proteins. Compared with those in the normal control (NC) group, the expression of fission-related proteins FIS1, and MFF were significantly upregulated whereas the fusion-related proteins MFN1, MFN2, and OPA1 were concurrently significantly decreased in the liver tissue from LPS/GalN injected mice (Figure 3E). While, the dysregulated expressions of mitochondrial dynamics-related proteins were markedly rescued by selonsertib pretreatment. These data suggest that selonsertib may prevent ALF through modulating mitochondrial dynamics by suppressing DRP1 mitochondrial translocation.

Selonsertib prevents mitochondrial damage of macrophages in ALF through suppressing DRP1 activation

Macrophages play a pivotal role in modulating hepatic immune microenvironment and inflammatory response in ALF [17]. As previous studies showed that LPS exposure usually induced mitochondrial dysfunction in macrophages [18, 19], we further determine the role of selonsertib in protecting mitochondrial function in the LPS-primed mouse macrophage cell line, RAW264.7. As we expected, LPS exposure induced a marked increase in the mitochondrial oxidative stress in macrophages as indicating by MitoSox, while selonsertib treatment significantly weakened the red fluorescence intensity in the LPS-primed macrophages (Figure 4A-left). In addition, LPS priming caused progressive opening of the mPTP, as inferred from the apparent decrease of the fluorescent probe, calcein, which had been preloaded into mitochondria (Figure 4A-right). LPS priming also decreased mitochondrial membrane potential (Δψm), as indicated by the increased conversion of red fluorescence to green fluorescence of JC-1 dye (Figure 4A-middle). While, selonsertib pretreatment effectively prevented the LPS-induced the mPTP opening and Δψm dissipation of macrophage as shown by mitochondrial accumulation of calcein fluorescence and reduced the ratio of red to green fluorescence of JC-1 dye in macrophages, respectively (Figure 4A). Moreover, LPS exposure also induced the activation and mitochondrial translocation of JNK and DRP1 in macrophages just as we observed in the liver tissue of LPS/GalN-induced ALF, while, selonsertib pretreatment effectively suppressed those activation and mitochondrial translocation by LPS priming (Figure 4B and C). Meanwhile, LPS stimulation induced significant release of inflammatory cytokines, such as TNF-α and IL-1α, whereas selonsertib treatment alleviated the release of these cytokines (Figure 4D).

By using mdivi, a DRP1 inhibitor, we further found that inhibition of DRP1 activation could effectively inhibit its mitochondrial translocation and then alleviated the LPS-primed abnormal mitochondrial dynamics and mitochondrial dysfunction, which were indicated by the alleviation of the abnormal

expressions of mitochondrial dynamics-related proteins and the reduction of mPT pore opening and Δψm dissipation in the LPS-primed macrophages (Figure 5A and B). We further confirmed the protective effects of mdivi on LPS/GalN-induced ALF by the observation of reduced serum ALT, AST, and TBiL levels and less hepatic necrosis in those mice by mdivi pretreatment (Figure 5C and D). Thus, JNK-mediated DRP1 mitochondrial translocation might be an important link that mediated the protective effect of selonsertib on ALF and mitochondrial damage.

Discussion

ALF, also known as fulminant hepatic failure, is a life-threatening clinical syndrome induced by massive hepatic necrosis. Its medical management is associated with high rates of morbidity and mortality and there are still no effective treatments expect liver transplantation and artificial liver therapies [1]. Thus, it is important to identify a potential drug and further clarify its treatment mechanism and determine its therapeutic window for ALF. In this study, we showed that selonsertib, a selective inhibitor of ASK1 and currently in a phase III clinical trial on NASH [20], could effectively protect against LPS/GalN-induced ALF by inhibiting the activation of hepatic ASK1-JNK-DRP1 pathway and alleviating mitochondrial damage of macrophages. Unfortunately, selonsertib is only effective early after LPS/GalN administration and the limited therapeutic window may be related to the time point of JNK and DRP1 mitochondrial translocation.

ASK1, a mammalian MAP3K, is activated in response to various cytotoxic stresses, including serum withdrawal, ROS, tumor necrosis factor, microtubule interfering agents, and cancer chemotherapeutic agents and it has been implicated in a variety of cellular functions, including cell survival, and inflammatory response [5, 21, 22]. Activated ASK1 then activates downstream kinases such as JNK and p38 pathways, resulting cell apoptosis and inflammatory cytokine expression [3, 23]. Recently, several reports showed that ASK1 is over-activated in variety of liver diseases including drug- and toxin-induced ALF [6, 24, 25]. In this study, we found that ASK1 was significantly activated in the liver tissues of LPS/GalN-induced ALF (Supplementary Figure S1A). Thus, as a specific inhibitor of ASK1, selonsertib might be a potential drug for ALF therapy. As we expected, pretreatment with selonsertib could remarkably lessen hepatic necrosis and reduce serum levels of ALT, AST, TBiL, and inflammatory cytokines and chemokines in a dose-dependent manner. In addition, selonsertib pretreatment also suppressed the activation of JNK (Figure 1) and p38 (Supplementary Figure S1A) in the live tissue from the ALF mouse model. Other studies also supported the above findings as ASK1 inhibition protected against acetaminophen-induced liver injury and high-fat diet-induced hepatic steatosis by attenuating JNK and p38 activation [6, 26]. Unfortunately, further experiments showed that selonsertib is only effective early after LPS/GalN administration (within 1h in the model) and loses its efficacy relatively rapidly after that time. The limited therapeutic window of selonsertib on ALF may be due to the time point of JNK activation and mitochondrial translocation as limited LPS/GalN-induced JNK activation and mitochondria translocation was observed as early as 1 h, whereas a massive JNK activation and an extensive mitochondrial translocation were occurred from 2 h later. While, there is no obvious regularity of the activation of hepatic p38 after LPS/GalN exposure (Supplementary Figure S1B), suggesting that in

this model ASK1-midiated JNK activation and mitochondrial translocation might be the key factor determining selonsertib therapeutic efficiency. A previous study also showed that JNK, but not p38, contributed to ASK1-mediated cellular damage in direct APAP-induced hepatocyte injury [24].

The primary pathophysiological event in LPS/GalN-induced ALF is the release of pro-inflammatory cytokines derived from macrophages [11, 27]. Emerging evidence now indicates that mitochondrial dysfunction is a possible mechanism of LPS/GalN-induced ALF, which causes abnormal mitochondrial dynamics, ROS over-production, and the release of pro-apoptotic proteins and other factors, resulting in cell death [28]. Mitochondrial quality control and dynamics are essential for regulating mitochondrial homeostasis. Excessive mitochondrial fragmentation with increased fission or impaired fusion is a hallmark of many degenerative diseases and contributes to their pathophysiology [29, 30]. It was also reported that hepatitis B virus induces mitochondrial fission, which contributes to mitochondrial dysfunction and cell injury [31]. The processes of mitochondrial dynamics are regulated by conserved dynamin-related GTPases, including the fission proteins DRP1, FIS1, and MFF and the fusion proteins MFN1, MFN2, and OPA1 [32]. Phosphorylation of DRP1 at Ser616 and translocation from cytoplasm into mitochondria seem to be the key upstream event of mitochondrial fragmentation and dysfunction [33]. In the present study, we also found that selonsertib could effectively suppress DRP1 activation and mitochondrial translocation and then rescue the mitochondrial dysfunction and suppress the mitochondrial oxidative stress in the liver tissue of ALF. Since JNK activation is thought to be the critical controlled in the processes of DRP1-mediated fission, the protective role of selonsertib on ALF may through modulating mitochondrial dynamics by JNK-DRP1 pathway.

Macrophages play a pivotal role in the pathophysiology of inflammatory diseases [34]. Several studies proved that the danger of ALF was dependent on the liver immune homeostasis and inflammation microenvironment mainly modulated by macrophages [17, 35]. As LPS exposure was known to induce macrophage mitochondrial dysfunction in acute tissue injury [19], we then used LPS-primed macrophages as *in vitro* model to determine whether selonsertib could protect the mitochondrial function of macrophages in ALF. We found that selonsertib pretreatment effectively prevented the LPS-induced mitochondrial dysfunction of macrophage and the release of pro-inflammatory cytokines TNF-α and IL-1α, which were known as major cytokines causing liver injury and inflammation [36, 37]. By using mdivi-1, a selective inhibitor of DRP1, we further confirmed the role of DRP1 in mediating LPS-induced mitochondrial dysfunction in macrophages and LPS/GalN-induced fulminant liver injury and inflammation. These data indicate that DRP1 may be the key molecule contributing to the protective effect of selonsertib on ALF by affecting the mitochondrial function of macrophage. Although previous studies mainly focused on the role of ASK1 in modulating cytotoxic stress-induced hepatocellular necrosis and apoptosis [14, 25, 38], our study showed that the therapeutic effect of ASK1 inhibition on ALF may also through protecting macrophage mitochondrial function.

Conclusion

In summary, our data identified the specific ASK1 inhibitor selonsertib as a potential therapeutic drug for the early treatment of ALF by inhibiting JNK-mediated DRP1 mitochondrial translocation and then rescuing mitochondrial damage. In addition, our study further confirmed the important role of mitochondrial function in the pathogenesis of ALF by determining the interaction between ASK1-JNK-DRP1 axis-mediated mitochondrial shape and the abnormal secretion of inflammatory cytokines in macrophages, suggesting restoring mitochondrial homeostasis may also offer a novel strategy for ALF therapy development.

Abbreviations

ALF: acute liver failure; ASK1: apoptosis signal-regulating kinase 1; MAP3K: mitogen-activated kinase kinase kinase; NASH: non-alcoholic steatohepatitis; MDR: multidrug resistance; LPS/GalN: lipopolysaccharide and D-galactosamine; ALT: alanine aminotransferase aspartate; AST: aspartate aminotransferase; TBiL: total bilirubin; ELISA: enzyme-linked immunosorbent assay; TNF-α: tumor necrosis factor-α; IL-1α: Interleukin-1α; CBA: Cytometric Bead Array; MCP-1: Monocyte Chemoattractant Protein-1; IFN-γ: Interferon-γ; mPTP: mitochondrial permeability transition pore; Δψm: mitochondrial membrane potential; DRP1: dynamin-related protein 1; FIS1: mitochondrial fission protein 1; MFF: mitochondrial fission protein; MFN1: mitofusin 1; MFN2: mitofusin 2; OPA1: optic atrophy 1; VDAC1: voltage-dependent anion channel 1.

Declarations

Ethics approval and consent to participate

Animal experimental protocols were approved by the Institutional Animal Care and Use Committee of the First Affiliated Hospital of Zhejiang University.

Consent for publication

Not applicable.

Availability of data and materials

All data and materials are available upon request.

Competing Interests

The authors have declared that no competing interest exists.

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Author Contributions

Guohua Lou and Aichun Li performed the *in vivo* and *in vitro* experiments and drafted the manuscript. Tianbo Zhang and Jinjin Qi participated in the in vitro studies and performed the statistical analysis. Zhi Chen participated in the design of this study. Yanning Liu conceived of the study and participated in its design and coordination. All the authors have read and approved the final manuscript.

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Figures

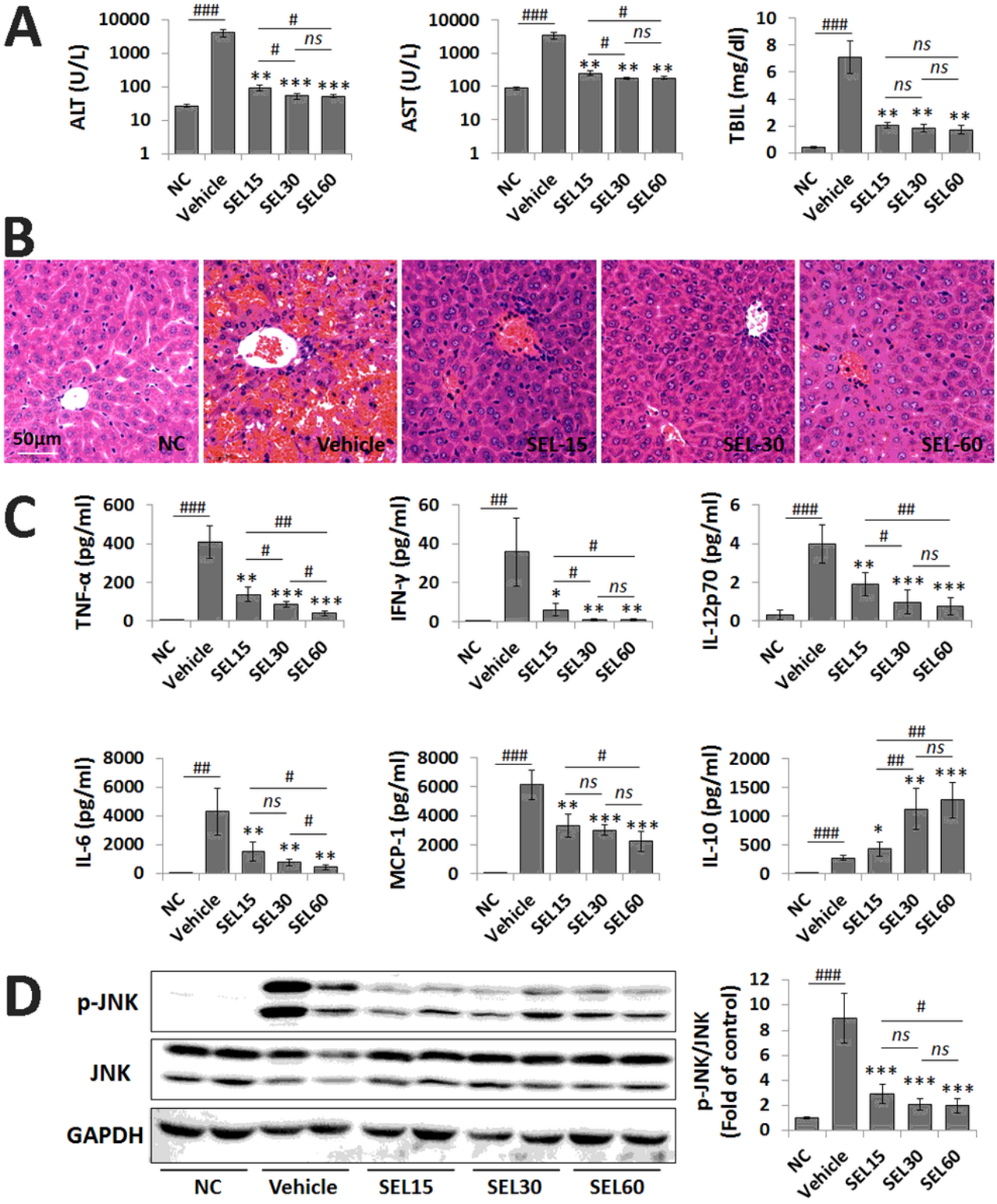

Figure 1

Selonsertib administration ameliorates LPS/GalN-induced liver failure (A) Serum levels of ALT, AST, and TBIL were elevated by LPS/GalN injection (Vehicle group) and decreased by selonsertib treatment in a dose-dependent manner. (B) Pathological changes in the liver tissue were shown using H&E staining. (C) The serum levels of inflammatory cytokines were determined by CBA cytokine analysis. (D) Western blot analysis and gray value assay on p-JNK and JNK expression levels in murine liver samples (2 from 6

samples were shown in the blots). Data are presented as mean ± SD (n=6). *P < 0.05 vs. Vehicle; **P < 0.01 vs. Vehicle; ***P < 0.001 vs. Vehicle; #P < 0.05; ##P < 0.01; ###P < 0.001; ns, none significant; NC, normal control; SEL15, selonsertib (15 mg/kg); SEL30, selonsertib (30 mg/kg); SEL60, selonsertib (60 mg/kg).

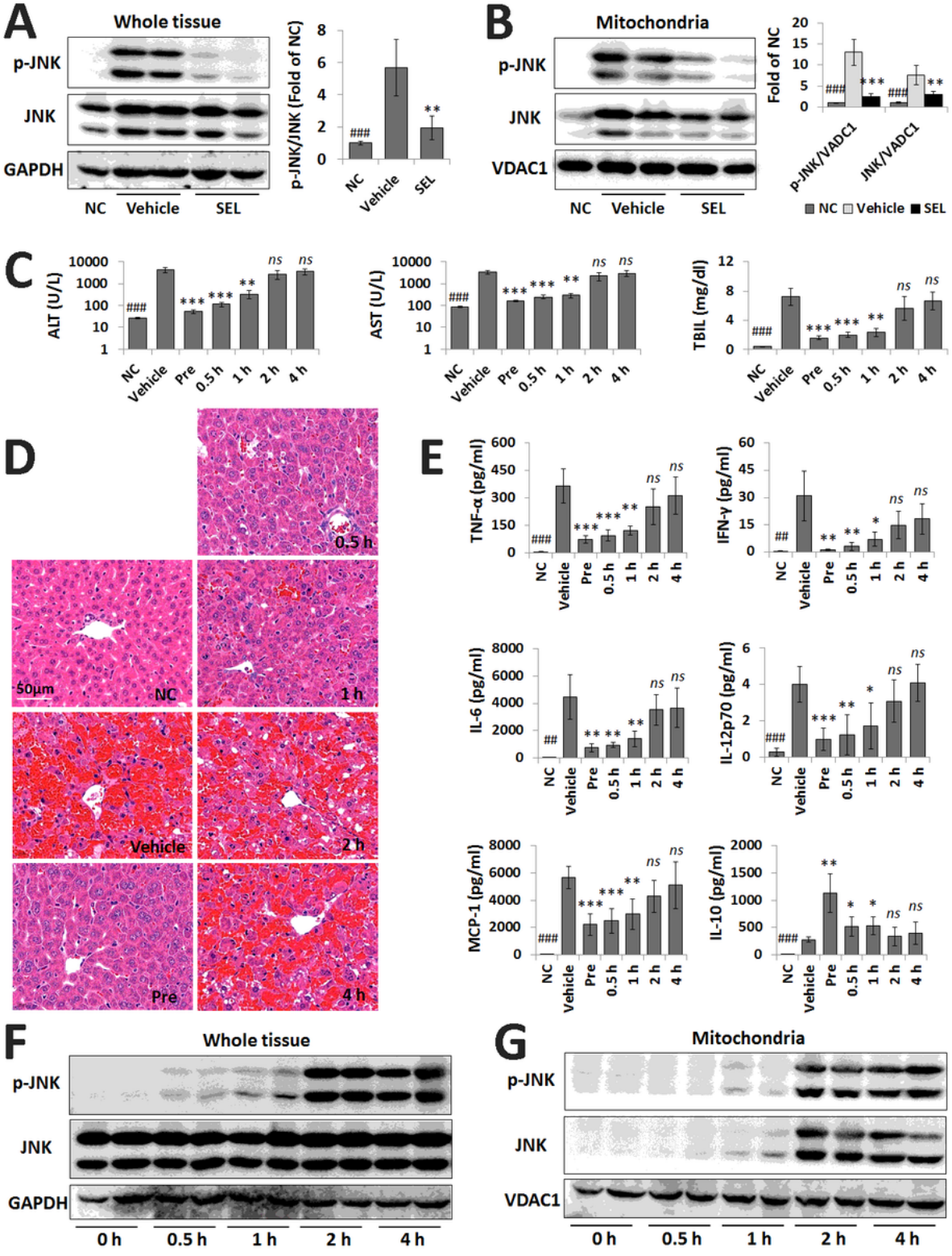

Figure 2

The therapeutic efficacy of selonsertib is related to JNK mitochondrial translocation (A, B) Western blot analysis and gray value assay on p-JNK and JNK levels in murine whole liver samples (A) and the mitochondria fraction of liver samples (B), 1 or 2 from 6 samples were shown in the blots. (C) Serum levels of ALT, AST, and TBIL in different groups with selonsertib pretreatment (Pre) or treatment at 0.5 h, 1 h, 2 h, and 4 h after LPS/GalN injection. (D) Pathological changes in the liver tissue were shown by H&E staining. (E) The serum levels of inflammatory cytokines and chemokines were determined by CBA cytokine analysis. (F, G) Western blot analysis on p-JNK and JNK levels in murine whole liver samples (F) and the mitochondria fraction of liver samples (G) at initial (0 h) and 0.5 h, 1 h, 2 h, and 4 h after LPS/GalN injection (2 from 6 samples were shown in the blots). Data are presented as mean ± SD (n=6). *P < 0.05 vs. Vehicle; **P < 0.01 vs. Vehicle; ***P < 0.001 vs. Vehicle; ##P < 0.01 vs. Vehicle; ###P < 0.001 vs. Vehicle; ns, none significant vs. Vehicle; NC, normal control; SEL, selonsertib (30 mg/kg).

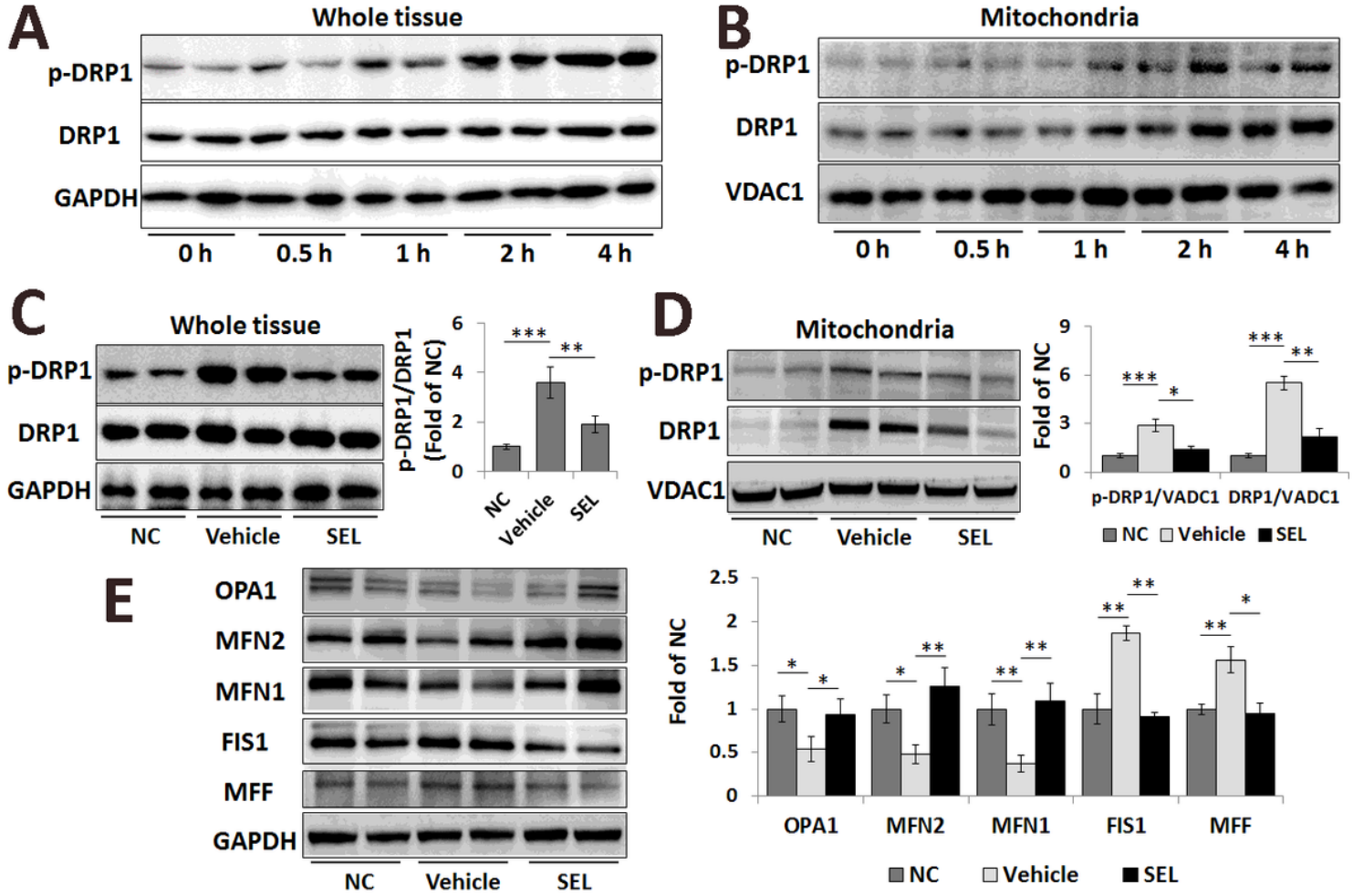

Figure 3

Selonsertib reverses Drp1 mitochondrial translocation and abnormal mitochondrial dynamics (A, B) Western blot analysis on p-DRP1 and DRP1 levels in murine whole liver samples (A) and the mitochondria fraction of liver samples (B) at initial (0 h) and 0.5 h, 1 h, 2 h, and 4 h after LPS/GalN injection. (C) Western blot analysis and gray value assay on p-DRP1 and DRP1 levels in murine whole liver samples (C) and the mitochondria fraction of liver samples (D) from different groups. (E) Western blot analysis and gray value assay on the expression levels of mitochondrial dynamics-related proteins in murine liver

samples. Two from 6 samples were shown in the blots. Data are presented as mean ± SD (n=6). *P < 0.05; **P < 0.01; ***P < 0.001; NC, normal control; SEL, selonsertib (30 mg/kg).

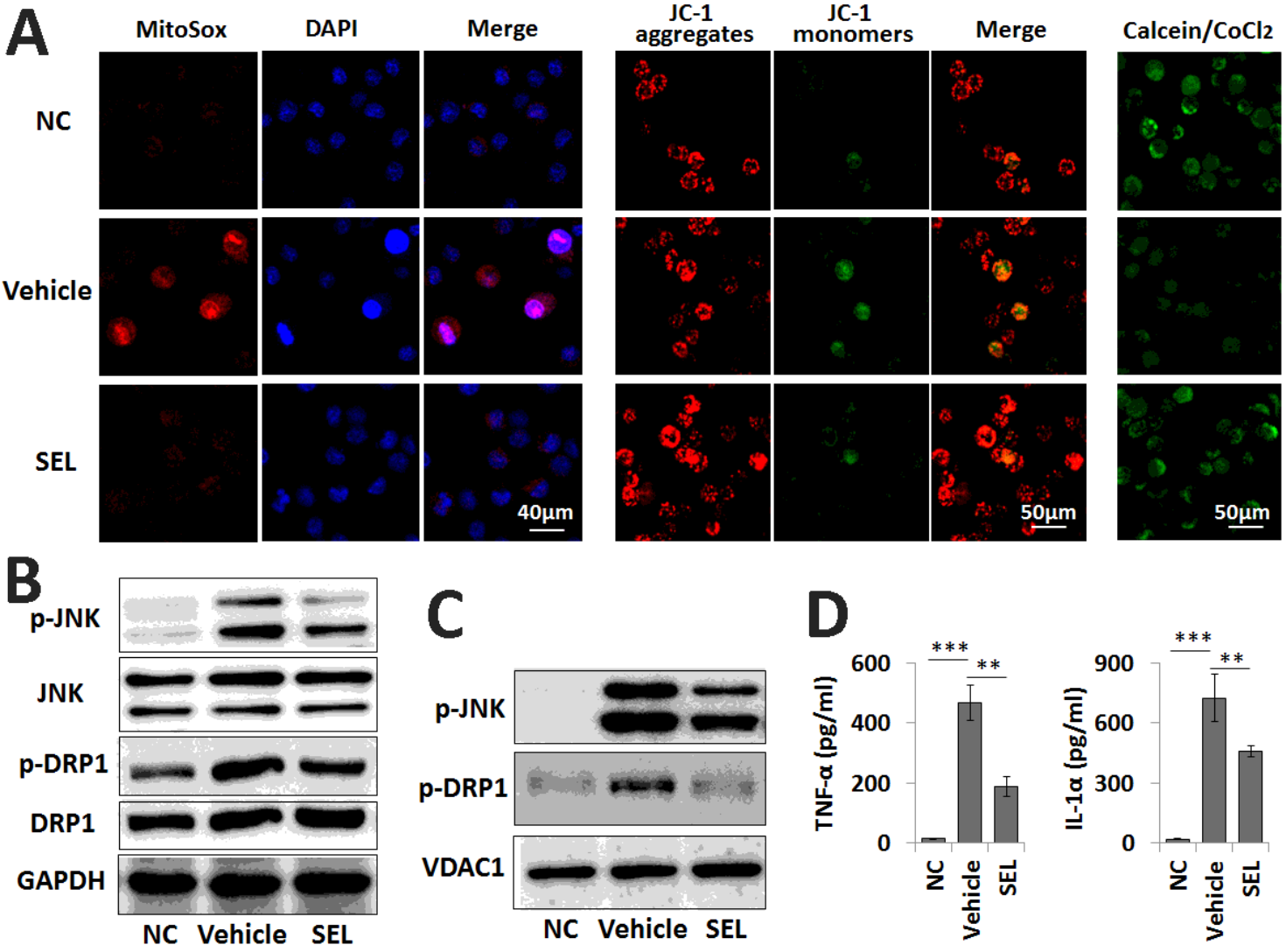

Figure 4

Selonsertib prevents LPS-primed JNK and Drp1 mitochondrial translocation and mitochondrial damage of macrophages (A) The mitochondrial oxidative stress, mitochondrial membrane potential (Δψm), and mitochondrial permeability transition pore (mPTP) opening in normal cultured (NC) and LPS-primed macrophages were shown by MitoSox (left), JC-1 (middle) and calcein staining (right), respectively. (B, C) Western blot analysis on p-JNK, JNK, p-DRP1 and DRP1 levels in the whole cell (B) and the mitochondria fraction (C) of macrophages. (D) TNF-α and IL-1α secretion levels from macrophages by LPS exposure were measured by ELISA. Data are presented as mean ± SD (n=3). **P < 0.01; ***P < 0.001; SEL, selonsertib (5 μM).

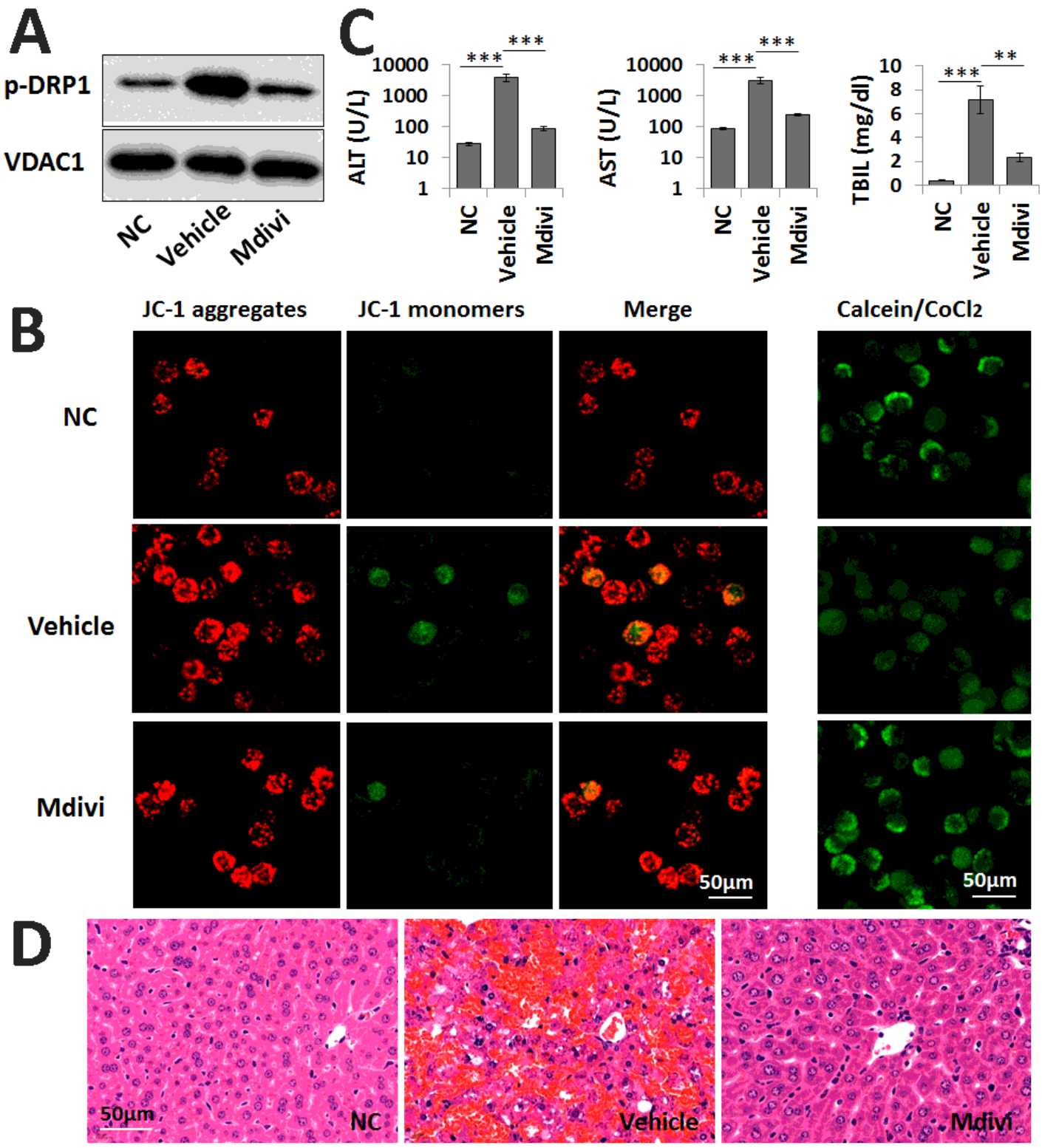

Figure 5

Drp1 mediates the mitochondrial damage of macrophages in LPS/GalN-induced liver failure (A) Western blot analysis on p-DRP1 levels in the mitochondria fraction of normal cultured (NC) and LPS-primed macrophages. Mdivi (10 μM) was used to inhibit DRP1 activity. (B) The mitochondrial membrane potential (Δψm) and mitochondrial permeability transition pore (mPTP) opening in normal cultured (NC) and LPS-primed macrophages were shown by JC-1 (left) and calcein staining (right), respectively. (C)

Serum levels of ALT, AST and TBIL in normal control group (NC), vehicle-treated ALF group (Vehicle), and mdivi-treated group (Mdivi, 30 mg/kg). Data are presented as mean ± SD (n=6). **P < 0.01; ***P < 0.001. (D) Pathological changes in the liver tissue were shown by H&E staining.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials.pdf