Original Research

Berberine protects steatotic donor undergoing liver transplantation via inhibiting endoplasmic reticulum stress-mediated reticulophagy

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Impact statement

Berberine is isolated from traditional Chinese medicine plants and has dramatically therapeutic potential against inflammation, diarrhea, and diabetes. But the benefits of BBR on steatotic grafts after liver transplantation remain poorly understood. Our findings might help explain the mechanism of berberine in protecting steatotic livers undergoing transplantation and give advantageous insights that berberine has potential as a suitable candidate for preventing hepatic injury after steatotic liver transplantation by inhibiting ER stress-mediated reticulophagy.

Abstract

Steatotic livers are more susceptible to ischemia/reperfusion injury, and increase the risk of primary graft non-function after liver transplantation. The protective effects of berberine have been described in various liver pathological models. However, it is unknown if berberine exerts its beneficial action in steatotic donors undergoing liver transplantation. In the present study, male Wistar rats were fed with high-fat diet (HFD) for 12 weeks to induce moderate steatotic liver. Then orthotropic liver transplantation was constructed. Berberine (200 mg/kg/d) was given intragastrically one week before liver transplantation. Thapsigargin (TG) (0.2 mg/kg) was administrated intravenously 24 h before liver transplantation. Liver function, oxidative stress, and inflammatory cytokine were detected by biochemical or histopathological analysis. The morphology of autophagosomes and endoplasmic reticulum (ER) was observed by transmission electron microscopy. The expression of CHOP, BIP, the phosphorylation of PERK, LC3-II/I, Beclin-1, and p62 were determined by Western blot assay. The co-localization of endoplasmic reticulum marker (KDEL) and autophagic protein (LC3B) was analyzed by immunofluorescence microscopy. The level of reticulophagy hallmark (FAM134B) was determined by immunohistochemistry. Compared with HFD + LT group, berberine ameliorated hepatocellular damage, decreased the oxidative stress level and inflammatory cytokine release. Simultaneously, berberine inhibited the expression of both endoplasmic reticulum stress parameters and autophagy-related proteins. Additionally, the co-localization of endoplasmic reticulum marker and LC3B was also reduced in HFD + BBR + LT group. berberine down-regulated the level of FAM134B. TG reversed the beneficial effects of berberine. Our study revealed that berberine exerts protective effects on steatotic livers undergoing transplantation by inhibiting endoplasmic reticulum stress-mediated reticulophagy.

Keywords: Berberine, fatty liver, liver transplantation, endoplasmic reticulum, reticulophagy, ischemia–reperfusion

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Introduction

Owing to the mismatch between organ donation and the demand for liver transplantation, expanding the liver donor pool is of vital importance. Among the common types of organs from extended-criteria donors, the potential use of steatotic livers has become a major focus of investigation for transplantation.[1,2] Unfortunately, more than half of livers are considered as not suitable for transplantation and will be discarded due to the presence of severe fatty infiltration. Steatotic livers are more susceptible to ischemia/reperfusion (I/R) injury, which may lead to the high

risk of primary graft non-function after liver transplantation.[3] Some medicine has been proven effective in animal studies but none of them has been applicated in clinical liver transplantation.[4,5]

Endoplasmic reticulum (ER) is an important organelle responsible for protein synthesis and assemblage. Under pathological conditions, unfolded protein response (UPR) would be activated by sustained accumulation of misfolded or unfolded proteins in the lumen of ER, that is, ER stress. Numerous reports have suggested that ER stress is critical in the pathophysiological development of various diseases.[6–9] In steatotic livers, ER stress has been implicated as a major contributor of post-transplant injury.[10] But the exact down-stream mechanism involved in the steatotic allograft injury remains unknown and need to be further investigated.

Autophagy is a critical process responsible for maintaining intracellular homeostasis by disassembling the detrimental cytoplasmic parts.[11] However, uncontrolled activation of autophagy may lead to irreversible cell damage.[12] Recent data indicate the crosstalk between ER stress and autophagy.[13] The distention of membrane from ER or Glogi complex is the beginning of autophagy initiation, which, in turn, is helpful for degrading misfolded proteins in ER. In aging adipose tissue, compromised autophagy was linked to the increase of ER stress and subsequent inflammation.[14] Guo *et al*.[15] found that ER stress-triggered autophagy was involved in cocaine-related neuroinflammation. However, the relationship between ER stress and autophagy has not been fully clarified.

Berberine (BBR) is a compound derived from the traditional Chinese medicine plants and has dramatically therapeutic potential against inflammation, diarrhea and so on.[16,17] Clinical evidence suggested that BBR was a fantastic cholesterol-lowering drug with its unique mechanism different from statins.[18] A large body of experimental data has supported the notion that BBR preconditioning attenuates I/R injury of various organs including kidney, heart, and intestine.[19–21] Our previous study found that BBR mediated the protection of non-steatotic donors undergoing liver transplantation by activating Sirt1/FoxO3α signaling pathway.[22] But there is limited information available in identifying the action of BBR in steatotic livers undergoing liver transplantation.

Taken together, our present study was aimed to evaluate the beneficial effect of BBR on steatotic donor of rats after liver transplantation and explore the underlying molecular mechanisms in BBR-offered protection.

Materials and methods

Animals

All animal experiments were approved by the Committee on the Ethics of Animal Experiments of Tianjin Medical University. Male Wistar rats (six weeks) were obtained from the Military Medical Science Academy Laboratory, Beijing, China. Animals were kept four each cage and raised in a temperature-controlled environment with 12-h light/dark cycle. Rats were fed with high-fat diet (HFD) or standard diet (SD) for 12 weeks to induce steatotic or non-steatotic liver. HFD rats would show moderate fatty infiltration in hepatocytes (40–60% steatosis). The ingredients of HFD were documented in Supplementary Table 1.

Experimental design

Rats were randomly assigned into the following nine groups ($n = 6$):

Group 1. SD. Rats with non-steatotic livers were subjected to transverse laparotomy.

Group 2. SD + BBR. Rats with non-steatotic livers were administrated with BBR (200 mg/kg/day) intragastrically for one week before the transverse laparotomy. The optimization of BBR treatment dosage was seen in Supplementary Figure 1.

Group 3. SD + LT. Rats underwent liver transplantation using non-steatotic donors.

Group 4. SD + BBR + LT. Rats with non-steatotic livers were administrated with BBR (200 mg/kg/day) intragastrically for one week before liver transplantation as donors.

Group 5. HFD. Rats with steatotic livers were subjected to transverse laparotomy.

Group 6. HFD + BBR. Rats with steatotic livers were administrated with BBR (200 mg/kg/day) intragastrically for one week before the transverse laparotomy.

Group 7. HFD + LT. Rats underwent liver transplantation using steatotic donors.

Group 8. HFD + BBR + LT. Rats with steatotic livers were administrated with BBR (200 mg/kg/day) intragastrically for one week before liver transplantation as donors.

Group 9. HFD + BBR + TG + LT. Rats with steatotic livers were administrated with BBR intragastrically (200 mg/kg/day, one week) and thapsigargin (TG, 0.2 mg/kg, intravenously 24 h before operation) before liver transplantation as donors. A flow-chart of experimental design is shown in Figure 1.

Groups	Treatments	Berberine 200mg/kg	Thapsigargin 0.2mg/kg	Liver transplantation
SD+ (12 weeks)	Sham			
	BBR	√		
	LT			√
	BBR+LT	√		√
HFD+ (12 weeks)	Sham			
	BBR	√		
	LT			√
	BBR+LT	√		√
	BBR+TG+LT	√	√	√

√: apply this treatment; n=6

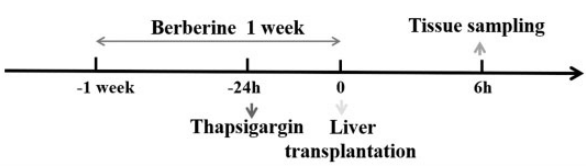

Figure 1. Illustration of the experimental design. Animals were randomly assigned into the following nine groups ($n = 6$). The rats were fed with standard diet (SD) or high-fat diet (HFD) for 12 weeks. BBR was intragastrically given at a dose of 200 mg/kg/day for one week. Liver transplantation was undergone after given BBR. Thapsigargin (TG) was intravenously given at a dose of 0.2 mg/kg for 24 h before operation.

Establishment of rat liver transplantation model

The rat liver transplantation model was conducted without hepatic artery reconstruction according to the report from Kamada *et al*.[23] The rats were anesthetized by intraperitoneal injection of phenobarbitone. Donor liver was harvested in a standardized process including freeing ligaments, ligating blood vessels, cannulating common bile duct and perfusing with heparinized normal saline. Then liver donors were excised and placed in 4°C normal saline before transplantation. Recipient hepatectomy was performed and donor liver was implanted by suturing supra-hepatic and sub-hepatic vena cava, connecting cuffs with the related vessels and inserting the stent of bile duct. During the surgery, the average time for operation was 132 ± 27 min and anhepatic phase (from vena porta clamping to graft re-perfusion) was 15 ± 3 min.[24] Tissue collections and blood were collected 6 h after reperfusion.

Determination of hepatic function

The blood (3 mL) was collected from hepatic inferior cava vena and centrifugated at 3000 r/min for 20 min. Serum was measured using an automated Biochemical Analyzer (AU5400, Beckman Coulter) was used to determine the level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Assessing oxidative stress and inflammation parameters

Liver tissues were homogenized in phosphate buffer saline (PBS) and centrifugated at 14,000 r/min for 20 min (4°C). The supernatants were collected for detecting the concentrations of malondialdehyde (MDA), superoxide dismutase (SOD), TNF-α, and IL-β by commercially assay kits.

Histopathologic examination

Liver tissues were fixed with 10% formalin dehydrated and embedded in paraffin. The sections were dewaxed, hydrated, and stained with hematoxylin-eosin (HE) according to standard protocols. Then the slides were observed under optical light microscope. Histopathologic change of each sample was evaluated by the same pathologist in a blinded study. Hepatic damage was scored as described by Suzuki *et al*.[25] (Table 1).

Transmission electron microscopy

Liver samples were fixed in 2.5% glutaraldehyde overnight and washed with PBS for three times. Then tissues were dehydrated, embedded, fixed in 10% buffered glutaraldehyde and 1% osmic acid, and finally sliced. The ultrastructure of autophagosome and ER was observed under a transmission electron microscope.

Table 1. Suzuki scores for the assessment of hepatic damage.

Score	Congestion	Vacuolization	Necrosis
0	None	None	Nonnecrotic cell
1	Minimal	Minimal	Single cell
2	Mild	Mild	0%–30%
3	Moderate	Moderate	30%–60%
4	Severe	Severe	>60%

Immunofluorescence

Liver samples were incubated with anti-LC3B (1:1000, Abcam) and anti-KDEL (1:100, Abcam) overnight at 4°C followed by fluorescence secondary antibodies, anti-rabbit Alexa 568 (1:300, CST), and anti-mouse Alexa 488 (1:300, CST). The slides were counterstained with nuclear dye 4′,6-diamidino-2-phenylindole (DAPI) 10 min before examined under the fluorescence microscopy.

Immunohistochemistry

Liver specimens were fixed and embedded in paraffin. After dewaxing and hydration, endogenous peroxidase activity was quenched by 3% hydrogen peroxide for 15 min. Then sections were blocked with 5% normal goat serum for 20 min. The slices were incubated with primary antibody (anti-FAM134B, 1:1000) overnight. After washing, the slices were incubated with secondary antibody at room temperature for 15 min. Sections were stained with DAB kits. The FAM134B-positive cells with brown particles were counted in at least 10 different images.

Western blotting

Equal amounts of protein lysates (50 μg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to PVDF membranes. Membranes were blocked in 5% skim milk for 1.5 h at room temperature and incubated at 4°C overnight with primary antibodies against PERK, p-PERK, CHOP, LC3II/LC3I, Beclin-1, p62, and GAPDH. Then membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:2000, Abcam) for 1 h and visualized with enhanced chemiluminescence reagents (Millipore, MA, USA).

Statistical analysis

The SPSS 22.0 software was used for descriptive and comparative statistical analysis. Histologic damage scores were presented as median ±interquartile range and Kruskal–Wallis H test was used to calculate the significance of the data. Other values were expressed as means ± SD and analyzed by one-way ANOVA. Significant differences were defined as P value less than 0.05 (two tailed).

Results

Effect of BBR pretreatment on hepatic injury after steatotic liver transplantation

To investigate the effect of BBR on hepatic injury after steatotic liver transplantation, we initially evaluated the level of serum ALT and AST. Compared with HFD group, liver transplantation dramatically enhanced ALT and AST levels in steatotic donors, which were reduced by BBR pretreatment ($P < 0.05$) (Figure 2(a) and (b)). Histological

examination revealed that HFD group displayed micro- and macro-vesicular steatosis scattered in the liver lobule ($P < 0.05$). After liver transplantation, the steatotic donors displayed severe sinusoidal congestion, necrosis, inflammatory infiltration with a dramatically higher Suzuki score ($P < 0.05$). And a comparatively improved lobular structure and the reduced damage score were observed in steatotic grafts preconditioned with BBR ($P < 0.05$) (Figure 3).

Effects of BBR on hepatic oxidative stress and inflammation after steatotic liver transplantation

The activation of oxidative stress and inflammation is considered to be secondary to direct cellular damage resulting from ischemic insult. Compared with HFD group, liver transplantation elevated the expression of MDA with the reduced SOD activity in steatotic grafts ($P < 0.05$). Pretreatment with BBR drastically suppressed the oxidative stress level in steatotic grafts after liver transplantation ($P < 0.05$) (Figure 2(c) and (d)). Similarly, compared with HFD group, the production of inflammatory factors (TNF-α, IL-1β) was greatly increased in steatotic grafts after liver transplantation. Pretreatment of BBR inhibited the hepatic inflammatory reaction after steatotic liver transplantation ($P < 0.05$) (Figure 2(e) and (f)).

BBR pretreatment inhibited the ER stress induced autophagy in steatotic grafts after liver transplantation

To analyze the role of ER stress-mediated autophagy in the salutary effects of BBR, the expression of ER stress parameters (p-PERK/PERK, CHOP, Bip) and autophagy-related proteins was detected using Western blotting. Figure 4 showed that the level of CHOP, Bip, and the phosphorylation of PERK was significantly increased in steatotic grafts after liver transplantation compared with HFD group, which could be down-regulated after BBR pretreatment ($P < 0.05$). Figure 5 showed the expression of autophagy-related proteins in different groups. Compared with HFD group, the ratio of LC3II/I, Beclin-1, and p62 expression was concurrently increased in steatotic grafts after liver transplantation. And BBR treatment could significantly inhibit the level of autophagy, which was counteracted by ER stress inducer TG ($P < 0.05$).

BBR treatment inhibited the selective autophagy of ER in steatotic grafts after liver transplantation

Transmission electron microscopy revealed the accumulation of autophagosomes and the reduction of rough ER were induced in steatotic grafts after liver transplantation, which could be reversed by BBR (Figure 6). Accordingly, we hypothesized that autophagy mediated the degradation of ER in steatotic grafts. To confirm the hypothesis, the co-localization of ER (KDEL) and autophagy (LC3B) was analyzed by double-immunofluorescence microscopy. In Figure 7, the co-localization of ER and autophagy appeared weak in SD group but was gradually stronger in HFD group. Liver transplantation further enhanced the co-localization of ER and autophagy. However, BBR pretreatment reduced the ER localization with LC3B, which could be abolished by TG.

FAM134B is a kind of ER-resident receptor which specifically binds to autophagy and facilitates ER degradation. In our study, LT enhanced the expression of FAM134B in steatotic livers compared with HFD group. Also, BBR preconditioning significantly impedes the level of FAM134B, which was reversed by TG ($P < 0.05$) (Figure 8). These results indicated that BBR could inhibit reticulophagy in steatotic grafts after liver transplantation.

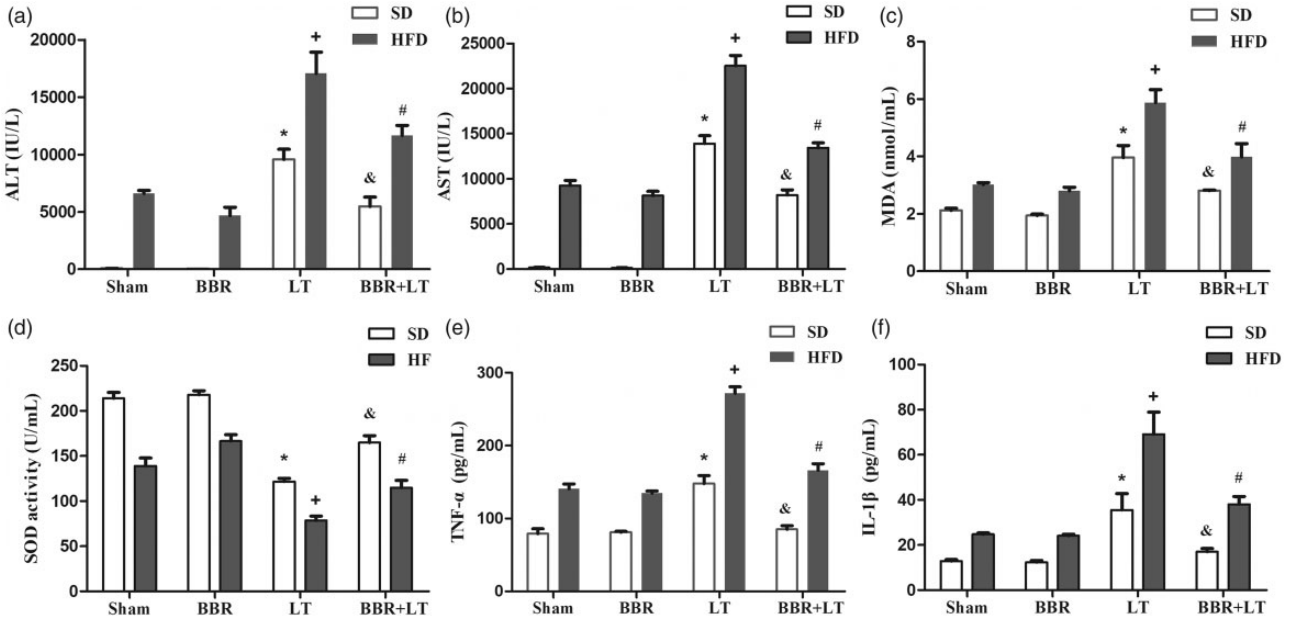

Figure 2. BBR ameliorated the hepatic injury of steatotic donors undergoing liver transplantation. (a) ALT level. (b) AST level. (c) MDA concentration. (d) SOD activity. (e) TNF-α level. (f) IL-1β level. The data were means $\pm$ standard deviation. $^{*}P < 0.05$ vs. SD group; $^{+}P < 0.05$ vs. HFD group; $^{\&}P < 0.05$ vs. SD+LT group; $^{\#}P < 0.05$ vs. HFD+LT group.

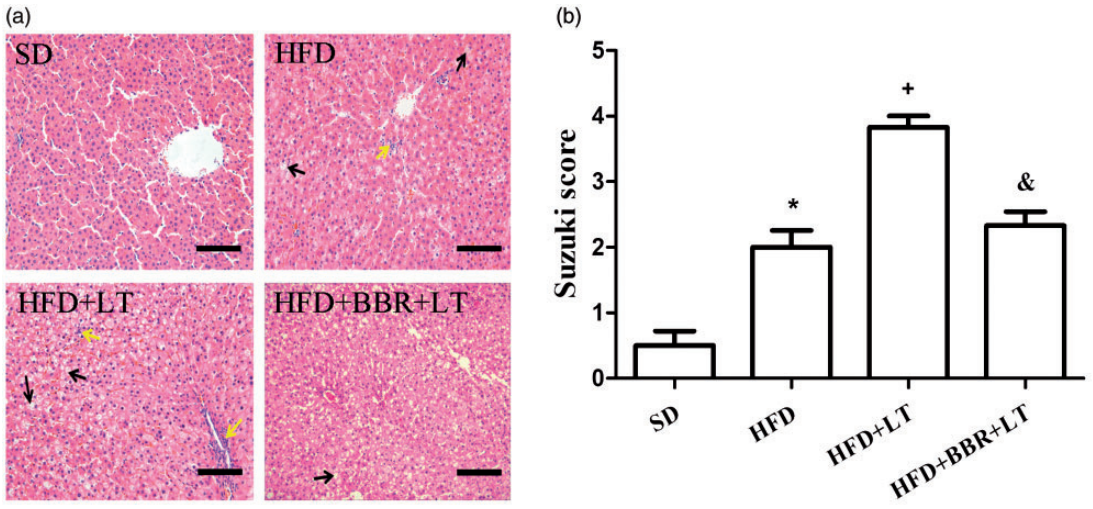

Figure 3. BBR alleviated the pathological injury of steatotic donors undergoing liver transplantation. (a) Representative photomicrographs of liver samples with hematoxylin–eosin staining (200×magnification). Yellow arrows indicated necrotic cells and inflammatory infiltration. Black arrows indicated steatotic hepatocytes. (b) Suzuki scores of liver samples. The data were means ± standard deviation. $^{*}P < 0.05$ vs. SD group; $^{+}P < 0.05$ vs. HFD group; $^{\&}P < 0.05$ vs. HFD+LT group. (A color version of this figure is available in the online journal.)

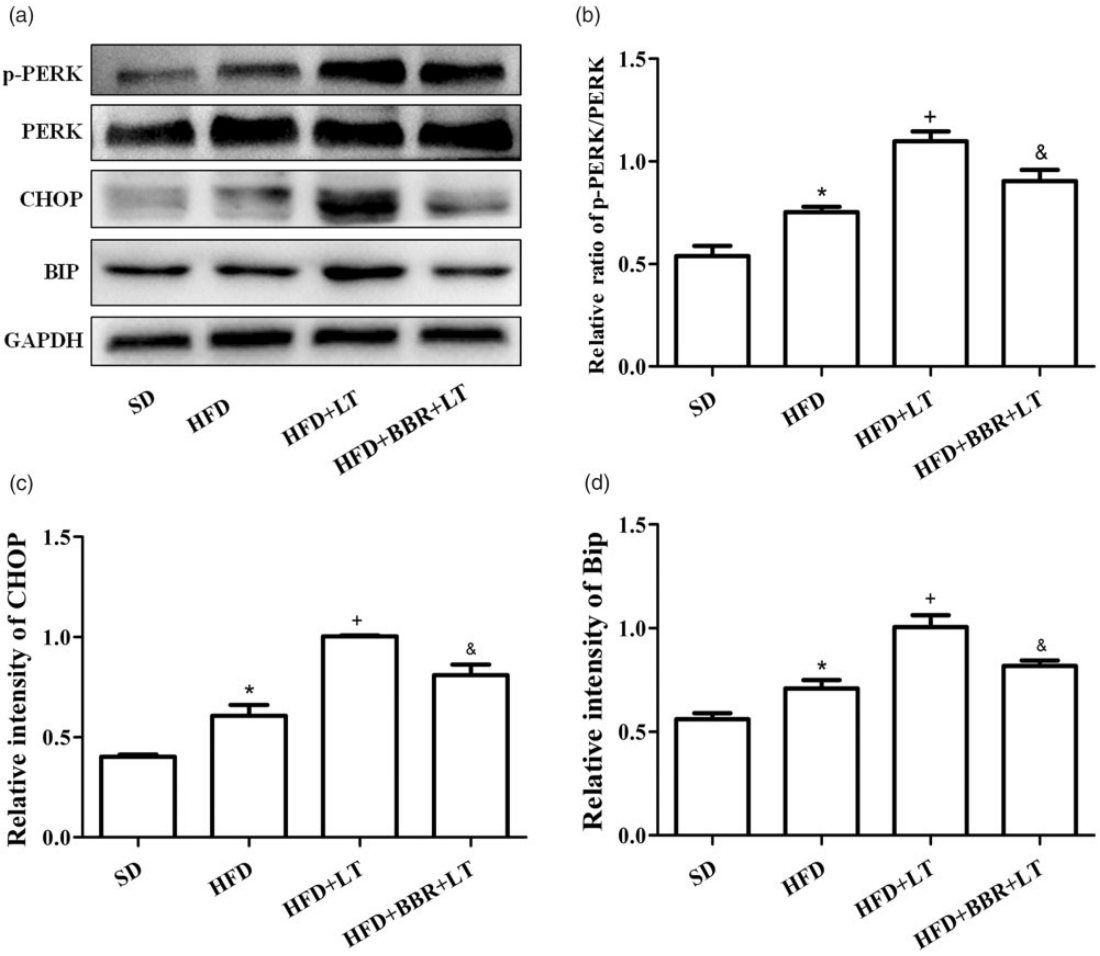

Figure 4. Preconditioning with BBR rescued the ER stress in steatotic donors undergoing liver transplantation. (a) Western blotting images of PERK phosphorylation, CHOP and Bip. (b) Relative ratio of p-PERK/PERK. (c) Relative intensity of CHOP. (d) Relative intensity of Bip. The data were means ± standard deviation. $^{*}P < 0.05$ vs. SD group; $^{+}P < 0.05$ vs. HFD group; $^{\&}P < 0.05$ vs. HFD+LT group.

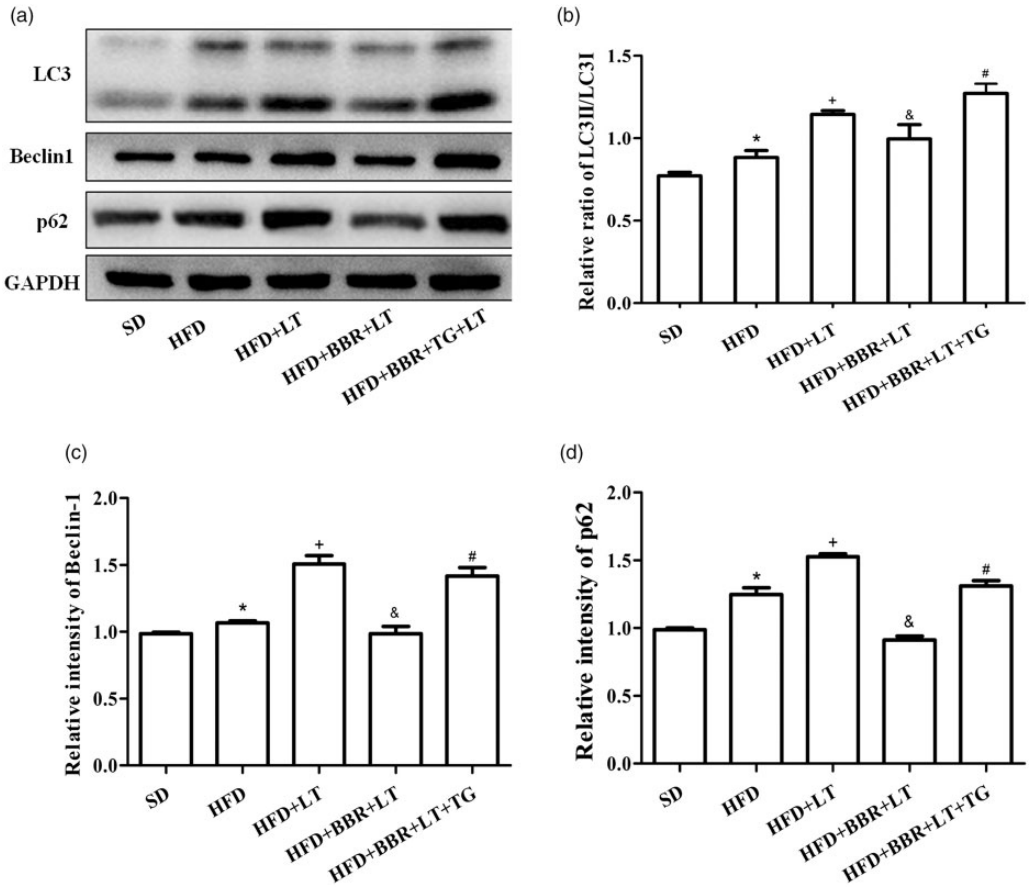

Figure 5. BBR attenuated the ER stress-induced autophagy in steatotic donors after liver transplantation. (a) Western blotting images of Beclin-1, LC3, and p62. (b) Relative ratio of LC3-II/I. (c) Relative intensity of Beclin-1. (d) Relative intensity of p62. The data were means ± standard deviation. $^{*}P < 0.05$ vs. SD group; $^{+}P < 0.05$ vs. HFD group; $^{\&}P < 0.05$ vs. HFD+LT group; $^{\#}P < 0.05$ vs. HFD+BBR+LT group.

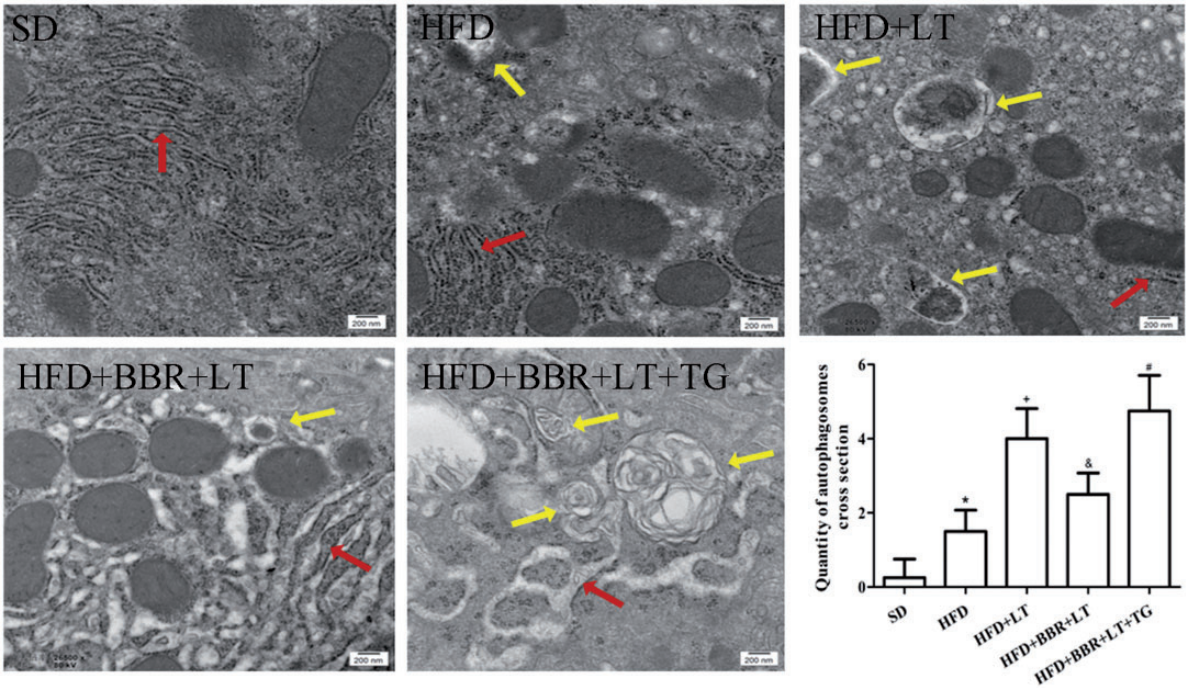

Figure 6. Effects of BBR on the ultrastructure of autophagosomes and ER under transmission electron microscopy (15,000×magnification). Yellow arrows indicated autophagic vacuoles. Red arrows indicated ER. The data were means ± standard deviation. $^{*}P < 0.05$ vs. SD group; $^{+}P < 0.05$ vs. HFD group; $^{\&}P < 0.05$ vs. HFD+LT group; $^{\#}P < 0.05$ vs. HFD+BBR+LT group. (A color version of this figure is available in the online journal.)

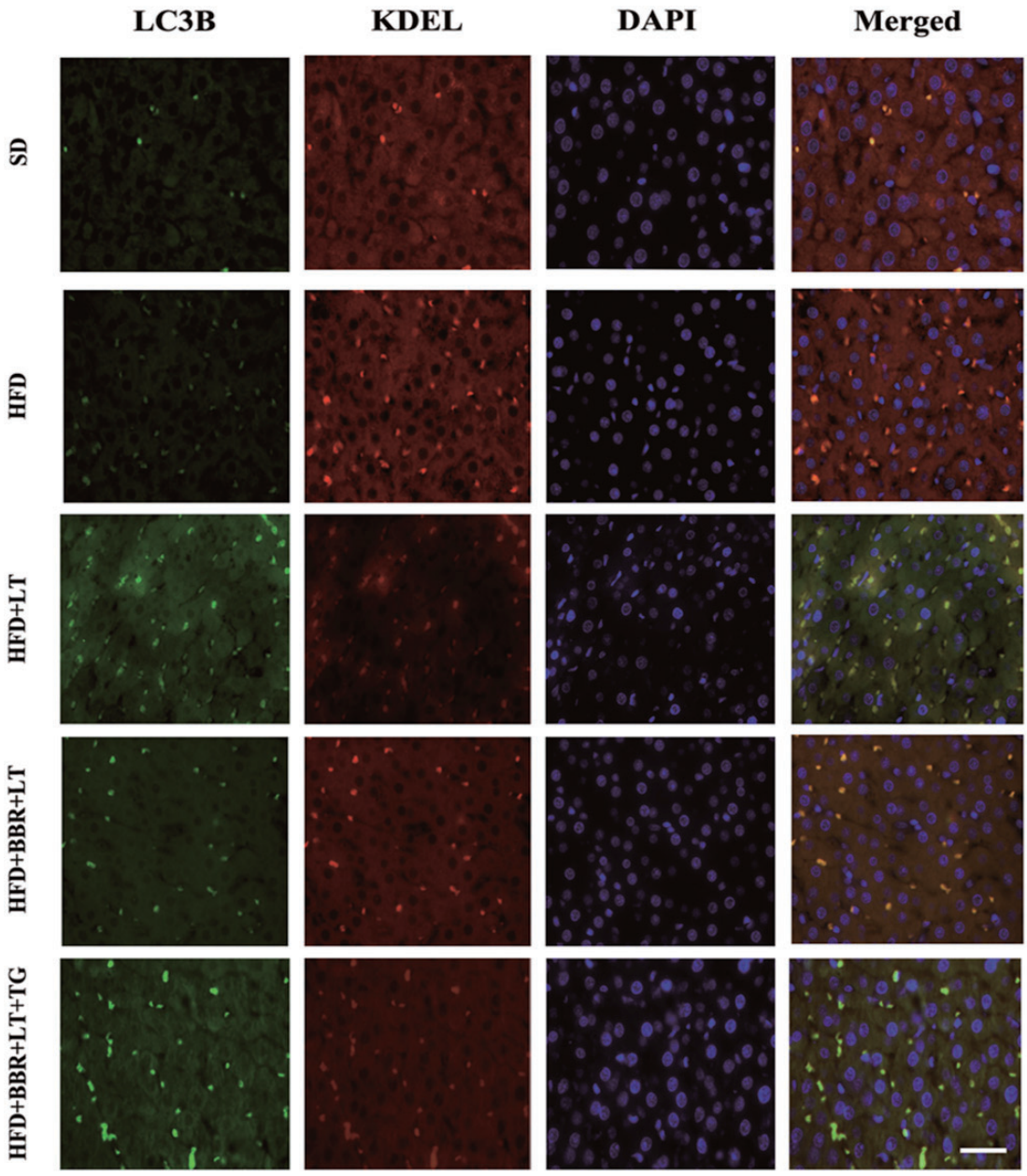

Figure 7. BBR reduced the co-localization of ER (KDEL) and autophagy (LC3B) by double-immunofluorescence microscopy. Hepatic samples were labeled with autophagy marker LC3B (green) and ER marker KDEL (red). Nuclei were stained with DAPI (blue). Then the co-localization of ER and autophagy was detected under fluorescence microscope (400×magnification). (A color version of this figure is available in the online journal.)

Discussion

Although pharmacological studies have demonstrated the therapeutic actions of BBR on hepatic steatosis or I/R injury,[22,26] the benefits of BBR on steatotic grafts after liver transplantation remain poorly understood. Several findings in the present study help to elucidate the protective mechanism of BBR against I/R injury after steatotic liver transplantation. Firstly, BBR could ameliorate hepatic injury in steatotic livers undergoing transplantation by inhibiting the level of oxidative stress and inflammation. Secondly, this protective action might be attributed to the inhibition of ER stress-mediated reticulophagy.

A large body of studies have proved the multitude biological effects of BBR including anti-hyperglycemic, anti-tumorigenesis, and anti-hyperlipidemia activities.[18,27,28] Clinical evidence demonstrated that BBR treatment markedly decreased serum levels of lipid metabolites in patients with nonalcoholic fatty liver disease.[29] Our previous data documented that BBR could ameliorate I/R injury in non-steatotic grafts undergoing liver transplantation via activating Sirt1/FoxO3α pathway.[22] In the present study, BBR attenuated the pathological damage and reduced the expression of plasma liver enzymes compared with HFD + LT group. The oxidative stress and inflammation were also inhibited at the same time. These data

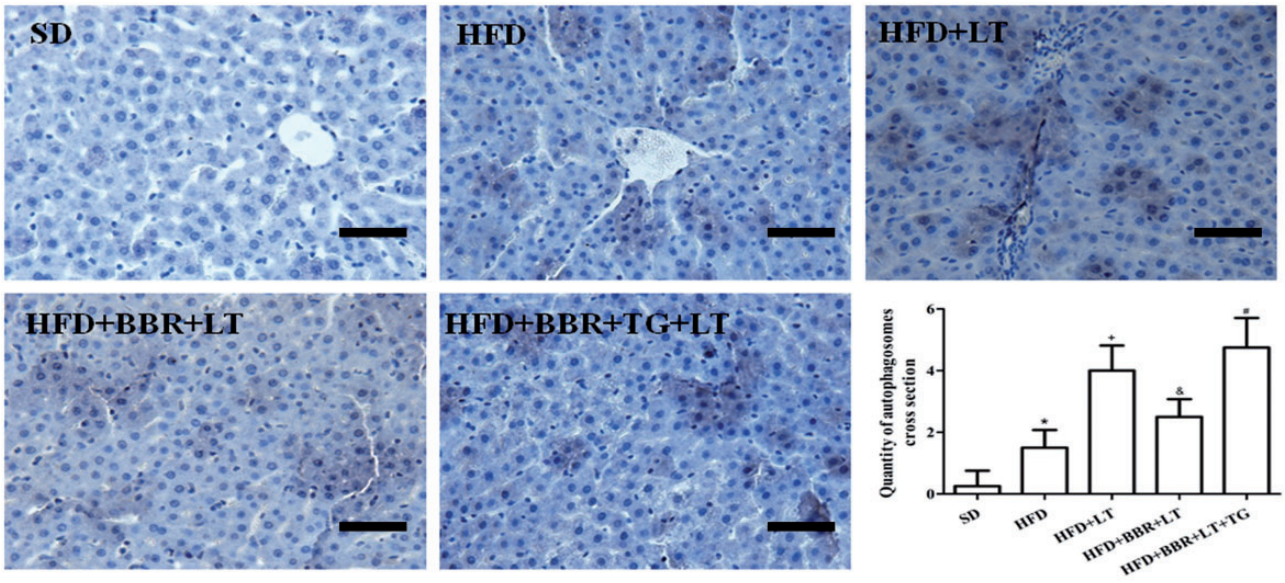

Figure 8. Effects of BBR on the expression of FAM134B in liver samples by immunohistochemistry (200×magnification). The data were means ± standard deviation. $^{*}P < 0.05$ vs. the SD group, $^{+}P < 0.05$ vs. the HFD group, $^{\&}P < 0.05$ vs. the HFD+LT group, $^{\#}P < 0.05$ vs. the HFD+BBR+LT group. (A color version of this figure is available in the online journal.)

confirmed the protective effects of BBR against steatotic liver transplantation.

ER is essential for maintaining the homeostasis of intracellular calcium and lipid metabolism. Under stress conditions, the UPR would be induced or exacerbated, which augments I/R injury of multiple organs.[30,31] In the model of myocardial I/R injury of rats, BBR inhibited ER stress via activating JAK2/STAT3 pathway.[6] ER stress also occurs in both non-steatotic and steatotic donors after liver transplantation and its magnitude is more prolonged and severe in steatotic grafts.[10] Consistent with previous literatures, liver transplantation was sufficient to activate the expression of ER stress parameters (p-PERK, CHOP, Bip) in steatotic donors, which could be inhibited by BBR pretreatment.

Under ER stress, several adaptive mechanisms including autophagy can be initiated to restore the metabolic balance. Accumulated evidences show that controlled autophagy is seen as a compensatory mechanism following ER stress,[32,33] whereas severe or prolonged autophagy may deteriorate cellular functions and end in irreversibly damage.[34,35] In our previous work, up-regulation of autophagy exhibited a pro-survival function after liver transplantation using non-steatotic donors.[22] Interestingly, steatotic grafts undergoing liver transplantation showed the aggravated hepatic injury along with a notable increase of autophagy. Furthermore, BBR exhibited protective effect on steatotic grafts by down-regulating the expression of LC3II/LC3I, p62, and Beclin1, which could be abolished by ER stress inducer TG. Herein, our results implicated that BBR mediated hepatic protection by inhibiting ER stress-triggered autophagy.

Among all the autophagic proteins, p62 is the first protein to recognize ubiquitinylated proteins and connect ubiquitin noncovalently during autophagy.[36] Although a large body of evidences demonstrate that p62 can be eliminated under autophagy initiation.[37,38] However, in special cases, the activation of selective autophagy is dependent on p62 induction. Selective autophagy involves in the generation of autophagosomes targeting special organelles including mitochondria (mitophagy), ER (reticulophagy), or ribosomes (ribophagy).[39] Chi *et al*.[40] implicated that thyroid hormone promoted selective autophagy via enhancing p62 expression, which in turn defended against hepatotoxicity and carcinogenesis. In Parkinson's disease model, the transcription of genes encoding for p62 was increased following mitophagy induction.[41] Our current study also detected the up-regulation of p62 in steatotic grafts undergoing liver transplantation. In addition, under transmission electron microscopy, we noticed the more autophagosomes with the more severe fragmentation of ER in steatotic livers subjected to liver transplantation compared with HFD group. Accordingly, it was reasonable to postulate that reticulophagy occurred in steatotic grafts after liver transplantation.

Nonetheless, reticulophagy, the autophagic sequestration of ER fragments into autophagosomes, can occur in both yeast and mammalian cells.[42] A large proportion of proteins newly synthesized in ER fail to mature properly and need to be degraded by autophagy. The observation of ER-membrane in autophagic vacuoles under electron micrography provides the strong evidence supporting to the view. The study from Lennemann *et al*.[43] reported the protective effects of reticulophagy against Dengue and Zika viruses, which provided a promising molecular target for the development of anti-viral drug. However, in kidney cells exposed to quantum dots, UPR-mediated reticulophagy triggered the renal toxicity.[44] In line with the previous reports, our immunofluorescence staining assays found that the co-localization between ER tracker (KDEL) and autophagy marker (LC3B) was increased in steatotic grafts undergoing liver transplantation. Moreover, the

pretreatment with BBR reduced the ER localization with LC3B, which was countered by TG. FAM134B has been proven to be the hallmark of ER-phagy which serves as the cargo receptor binding to autophagy-related proteins to facilitate ER turnover. Our initial analysis showed that FAM134B expression was markedly provoked in steatotic donors after liver transplantation. Pretreatment with BBR impeded the level of FAM134B, which could be reversed by TG. Altogether, the above results supported the therapeutic potential of BBR on steatotic grafts after liver transplantation via inhibiting ER stress-mediated reticulophagy.

Although it is the first time to report the protective mechanism of BBR against steatotic liver transplantation, there are still a number of important questions remaining unresolved and warranting further research. The regulation of reticulophagy is not simple but involved in many players. Apart from FAM134B, SEC62 and reticulon 3 have been identified as the key receptors connecting the ER with autophagosomal membrane. Our finding preliminarily confirmed the presence of reticulophagy in steatotic donors after liver transplantation; the precise functions and the interplay of these receptors remain unclear and should be elucidated by further analyses. Additionally, while we have observed the inhibition of ER stress-mediated reticulophagy by BBR in steatotic grafts after liver transplantation, the deeper evaluation of molecular mechanisms using genetic engineering mice by disrupting the critical gene expression is in urgent need.

In conclusion, our findings identify that steatotic liver transplantation-mediated induction of ER stress is the upstream of reticulophagy response which results in the aggravation of hepatic injury. BBR exerts the protective effects on steatotic livers undergoing transplantation by inhibiting ER stress-mediated reticulophagy, which provides a new insight into the mechanisms underlying BBR's hepatoprotection.

Authors' contributions: ZN and SMW participated in research design, performance of all the experiments and writing of the manuscript; WM performed the biochemical analysis and part of the histological study; ZXY managed the protein detecting and analyzed the results; DYJ was responsible for establishing the animal model; LYB acquired data and analyzed them; YWL participated in research design; WSS participated in manuscript preparation; and DHY was the overall advisors, helped to revise the design of the experiment and sponsored the project.

DECLARATION OF CONFLICTING INTERESTS

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REFERENCES

1. Wong TC, Fung JY, Chok KS, Cheung TT, Chan AC, Sharr WW, Dai WC, Chan SC, Lo CM. Excellent outcomes of liver transplantation using severely steatotic grafts from brain-dead donors. *Liver Transpl* 2016;**22**:226–36
2. Gabrielli M, Moisan F, Vidal M, Duarte I, Jiménez M, Izquierdo G, Domínguez P, Méndez J, Soza A, Benitez C, Pérez R, Arrese M, Guerra J, Jarufe N, Martínez J. Steatotic livers.Can we use them in PLTX?outcome data from a prospective baseline liver biopsy study. *Ann Hepatol* 2012;**11**:891–8
3. Todo S, Demetris AJ, Makowka L, Teperman L, Podesta L, Shaver T, Tzakis A, Starzl TE. Primary nonfunction of hepatic allografts with preexisting fatty infiltration. *Transplantation* 1989;**47**:903–5
4. Luo XY, Takahara T, Hou J, Kawai K, Sugiyama T, Tsukada K, Takemoto M, Takeuchi M, Zhong L, Li XK. Theaflavin attenuates ischemia-reperfusion injury in a mouse fatty liver model. *Biochem Biophys Res Commun* 2012;**417**:287–93
5. Ramachandran S, Liaw JM, Jia J, Glasgow SC, Liu W, Csontos K, Upadhya GA, Mohanakumar T, Chapman WC. Ischemia-reperfusion injury in rat steatotic liver is dependent on NFkappaB P65 activation. *Transpl Immunol* 2012;**26**:201–6
6. Zhao GL, Yu LM, Gao WL, Duan WX, Jiang B, Liu XD, Zhang B, Liu ZH, Zhai ME, Jin ZX, Yu SQ, Wang Y. Berberine protects rat heart from ischemia/reperfusion injury via activating JAK2/STAT3 signaling and attenuating endoplasmic reticulum stress. *Acta Pharmacol Sin* 2016;**37**:354–67
7. Puri P, Mirshahi F, Cheung O, Natarajan R, Maher JW, Kellum JM, Sanyal AJ. Activation and dysregulation of the unfolded protein response in nonalcoholic fatty liver disease. *Gastroenterology* 2008;**134**:568–76
8. Tajiri S, Oyadomari S, Yano S, Morioka M, Gotoh T, Hamada JI, Ushio Y, Mori M. Ischemia-induced neuronal cell death is mediated by the endoplasmic reticulum stress pathway involving CHOP. *Cell Death Differ* 2004;**11**:403–15
9. Cheng X, Liu H, Jiang CC, Fang L, Chen C, Zhang XD, Jiang ZW. Connecting endoplasmic reticulum stress to autophagy through IRE1/JNK/beclin-1 in breast cancer cells. *Int J Mol Med* 2014;**34**:772–81
10. Anderson CD, Upadhya G, Conzen KD, Jia J, Brunt EM, Tiriveedhi V, Xie Y, Ramachandran S, Mohanakumar T, Davidson NO, Chapman WC. Endoplasmic reticulum stress is a mediator of posttransplant injury in severely steatotic liver allografts. *Liver Transpl* 2011;**17**:189–200
11. Guan X, Qian Y, Shen Y, Zhang L, Du Y, Dai H, Qian J, Yan Y. Autophagy protects renal tubular cells against ischemia/reperfusion injury in a time-dependent manner. *Cell Physiol Biochem* 2015;**36**:285–98
12. Huang Z, Han Z, Ye B, Dai Z, Shan P, Lu Z, Dai K, Wang C, Huang W. Berberine alleviates cardiac ischemia/reperfusion injury by inhibiting excessive autophagy in cardiomyocytes. *Eur J Pharmacol* 2015;**762**:1–10
13. Kania E, Pajak B, Orzechowski A. Calcium homeostasis and ER stress in control of autophagy in cancer cells. *Biomed Res Int* 2015;**2015**:352794
14. Ghosh AK, Mau T, O'Brien M, Garg S, Yung R. Impaired autophagy activity is linked to elevated ER-stress and inflammation in aging adipose tissue. *Aging* 2016;**8**:2525–37
15. Guo ML, Liao K, Periyasamy P, Yang L, Cai Y, Callen SE, Buch S. Cocaine-mediated microglial activation involves the ER stress-autophagy axis. *Autophagy* 2015;**11**:995–1009
16. Yu L, Li Q, Yu B, Yang Y, Jin Z, Duan W, Zhao G, Zhai M, Liu L, Yi D, Chen M, Yu S. Berberine attenuates myocardial ischemia/reperfusion

injury by reducing oxidative stress and inflammation response: role of silent information regulator 1. *Oxid Med Cell Longev* 2016;**2016**:1689602
17. Xia X, Wang H, Niu X, Wang H, Liu Z, Liu Y, Qi Z, Wang S, Liu S, Liu S. Assessment of the anti-diarrhea function of compound chinese herbal medicine cangpo oral liquid. *Afr J Tradi Complement Altern Med* 2013;**11**:140–7
18. Derosa G, D'Angelo A, Bonaventura A, Bianchi L, Romano D, Maffioli P. Effects of berberine on lipid profile in subjects with low cardiovascular risk. *Expert Opin Biol Ther* 2013;**13**:475–82
19. Visnagri A, Kandhare AD, Bodhankar SL. Renoprotective effect of berberine via intonation on apoptosis and mitochondrial-dependent pathway in renal ischemia reperfusion-induced mutilation. *Ren Fail* 2015;**37**:482–93
20. Chen K, Li G, Geng F, Zhang Z, Li J, Yang M, Dong L, Gao F. Berberine reduces ischemia/reperfusion-induced myocardial apoptosis via activating AMPK and PI3K-Akt signaling in diabetic rats. *Apoptosis* 2014;**19**:946–57
21. Gu L, Li N, Yu W, Gong J, Li Q, Zhu W, Li J. Berberine reduces rat intestinal tight junction injury induced by ischemia-reperfusion associated with the suppression of inducible nitric oxide synthesis. *Am J Chin Med* 2013;**41**:1297–312
22. Lin Y, Sheng M, Weng Y, Xu R, Lu N, Du H, Yu W. Berberine protects against ischemia/reperfusion injury after orthotopic liver transplantation via activating Sirt1/FoxO3alpha induced autophagy. *Biochem Biophys Res Commun* 2017;**483**:885–91
23. Kamada N, Calne RY. Orthotopic liver transplantation in the rat. Technique using cuff for portal vein anastomosis and biliary drainage. *Transplantation* 1979;**28**:47–50
24. Ishii E, Shimizu A, Takahashi M, Terasaki M, Kiunugi S, Nagasaka S. Surgical technique of orthotopic liver transplantation in rats: the kamada technique and a new splint technique for hepatic artery reconstruction. *J Nippon Med Sch* 2013;**80**:4–15
25. Suzuki S, Toledo-Pereyra LH, Rodriguez FJ, Cejalvo D. Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Modulating effects of FK506 and cyclosporine. *Transplantation* 1993;**55**:1265–72
26. Chang X, Yan H, Fei J, Jiang M, Zhu H, Lu D, Gao X. Berberine reduces methylation of the MTTP promoter and alleviates fatty liver induced by a high-fat diet in rats. *J Lipid Res* 2010;**51**:2504–15
27. Chang W, Chen L, Hatch GM. Berberine as a therapy for type 2 diabetes and its complications: from mechanism of action to clinical studies. *Biochem Cell Biol* 2015;**93**:479–86
28. Chu SC, Yu CC, Hsu LS, Chen KS, Su MY, Chen PN. Berberine reverses epithelial-to-mesenchymal transition and inhibits metastasis and tumor-induced angiogenesis in human cervical cancer cells. *Mol Pharmacol* 2014;**86**:609–23
29. Yan HM, Xia MF, Wang Y, Chang XX, Yao XZ, Rao SX, Zeng MS, Tu YF, Feng R, Jia WP, Liu J, Deng W, Jiang JD, Gao X. Efficacy of berberine in patients with non-alcoholic fatty liver disease. *PLoS One* 2015;**10**:e0134172
30. Le Pape S, Dimitrova E, Hannaert P, Konovalov A, Volmer R, Ron D, Thuillier R, Hauet T. Polynomial algebra reveals diverging roles of the unfolded protein response in endothelial cells during ischemia-reperfusion injury. *FEBS Lett* 2014;**588**:3062–7
31. Barreto-Torres G, Parodi-Rullan R, Javadov S. The role of PPARalpha in metformin-induced attenuation of mitochondrial dysfunction in acute cardiac ischemia/reperfusion in rats. *Int J Mol Sci* 2012;**13**:7694–709
32. Lv SX, Qiao X. Isovitexin (IV) induces apoptosis and autophagy in liver cancer cells through endoplasmic reticulum stress. *Biochem Biophys Res Commun* 2018;**496**:1047–54
33. Yan WJ, Liu RB, Wang LK, Ma YB, Ding SL, Deng F, Hu ZY, Wang D. Sirt3-mediated autophagy contributes to resveratrol-induced protection against ER stress in HT22 cells. *Front Neurosci* 2018;**12**:116
34. Xu D, Zhu J, Jeong S, Li D, Hua X, Huang L, Zhang J, Luo Y, Xia Q. Rictor deficiency aggravates hepatic ischemia/reperfusion injury in mice by suppressing autophagy and regulating MAPK signaling. *Cell Physiol Biochem* 2018;**45**:2199–212
35. Liu Y, Levine B. Autosis and autophagic cell death: the dark side of autophagy. *Cell Death Differ* 2015;**22**:367–76
36. Katsuragi Y, Ichimura Y, Komatsu M. p62/SQSTM1 functions as a signaling hub and an autophagy adaptor. *FEBS J* 2015;**282**:4672–8
37. Bartlett BJ, Isakson P, Lewerenz J, Sanchez H, Kotzebue RW, Cumming RC, Harris GL, Nezis IP, Schubert DR, Simonsen A, Finley KD. p62, ref (2)P and ubiquitinated proteins are conserved markers of neuronal aging, aggregate formation and progressive autophagic defects. *Autophagy* 2014;**7**:572–83
38. Xie Y, Xiao J, Fu C, Zhang Z, Ye Z, Zhang X. Ischemic preconditioning promotes autophagy and alleviates renal ischemia/reperfusion injury. *Biomed Res Int* 2018;**2018**:8353987
39. Knaevelsrud H, Simonsen A. Fighting disease by selective autophagy of aggregate-prone proteins. *FEBS Lett* 2010;**584**:2635–45
40. Chi HC, Chen SL, Tsai CY, Chuang WY, Huang YH, Tsai MM, Wu SM, Sun CP, Yeh CT, Lin KH. Thyroid hormone suppresses hepatocarcinogenesis via DAPK2 and SQSTM1-dependent selective autophagy. *Autophagy* 2016;**12**:2271–85
41. Ivankovic D, Chau KY, Schapira AH, Gegg ME. Mitochondrial and lysosomal biogenesis are activated following PINK1/parkin-mediated mitophagy. *J Neurochem* 2016;**136**:388–402
42. Nakatogawa H, Mochida K. Reticulophagy and nucleophagy: new findings and unsolved issues. *Autophagy* 2015;**11**:2377–8
43. Lennemann NJ, Coyne CB. Dengue and zika viruses subvert reticulophagy by NS2B3-mediated cleavage of FAM134B. *Autophagy* 2017;**13**:322–32
44. Jiang S, Lin Y, Yao H, Yang C, Zhang L, Luo B, Lei Z, Cao L, Lin N, Liu X, Lin Z, He C. The role of unfolded protein response and ER-phagy in quantum dots-induced nephrotoxicity: an in vitro and in vivo study. *Arch Toxicol* 2018;**92**:1421–34

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