European Review for Medical and Pharmacological Sciences 2017; 21: 5813-5820

Carvedilol alleviates the biliary cirrhosis through inhibiting the endoplasmic reticulum stress

P. GAO[1], B. YANG[1], H.-Y. YU[1], R.-R. MENG[1], J.-Y. SI[2]

[1]Department of Vascular Surgery, No. 1 People's Hospital of Jining City, Shandong Province, China
[2]Department of Gastroenterology, No. 1 People's Hospital of Jining City, Shandong Province, China

Abstract. – OBJECTIVE: To investigate the effects of carvedilol on inflammation, apoptosis, and hepatic fibrosis caused by biliary cirrhosis and its mechanisms in mice.

MATERIALS AND METHODS: 60 male C57/BL6 mice were randomly divided into sham-operation group (Sham group, n=20), biliary cirrhosis group (BDL group, n=20) and carvedilol group (CAR group, n=20). The CAR group was treated with gavage using 12.5 mg/kg carvedilol, once a day for 14 consecutive days, while the Sham group and BDL group were treated with gavage using the equivalent normal saline. After that, the mice in Sham group received the laparotomy under chloral hydrate anesthesia, followed by direct abdominal closure. The mice in BDL group and CAR group received the common bile duct ligation after anesthesia for modeling. After modeling, the survival rate of mice in each group was detected, and the blood and liver tissues were taken for detection. The morphological changes in liver tissues and apoptosis in mice in each group were detected and compared. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), superoxide dismutase (SOD), malondialdehyde (MDA), hydroxyproline, and α-smooth muscle actin (α-SMA) were also detected. The mRNA expression levels of pro-inflammatory factors, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), transforming growth factor β-1 (TGF-β1), α-SMA and collagen-1 were detected via reverse transcription-polymerase chain reaction (RT-PCR). The protein expression levels of CHOP (CCAAT-enhancer-binding protein homologous protein), activating transcription factor 4 (ATF4), ATF6, inositol-requiring enzyme 1 (IRE1), phosphorylated Jun N-terminal kinase (pJNK), α-SMA, and collagen-1, were detected via Western-blotting.

RESULTS: Our study showed that carvedilol could significantly alleviate the biliary cirrhosis in mice, and improve the survival rate of mice. The ALT, AST and TBIL levels, severity of cirrhosis, and number of apoptotic cells in CAR group were significantly lower than those in BDL group. The levels of α-SMA and hydroxyproline in CAR group were also significantly lower than those in BDL group. The activity of SOD in CAR group was significantly higher than that in BDL group; the above differences were statistically significant ($p<0.05$). In addition, it was also found that carvedilol could down-regulate the mRNA expression levels of iNOS, COX-2 and TGF-β1, down-regulate the mRNA and protein expression levels of α-SMA and collagen-1, and negatively regulate the ATF4-CHOP, ATF6-CHOP and IRE1-pJNK signaling pathways.

CONCLUSIONS: Carvedilol has a significant effect on alleviating the biliary cirrhosis in mice, and its relevant mechanism may be that carvedilol inhibits the endoplasmic reticulum stress through the negative regulation of ATF4-CHOP, ATF6-CHOP and IRE1-pJNK signaling pathways, which needs to be confirmed by further *in vitro* experiments.

Key Words:
Carvedilol, Biliary cirrhosis, Endoplasmic reticulum stress (ERS).

Introduction

Liver cirrhosis is pathologically characterized by the formation of pseudo-lobe, which brings great pain to patients and easily induces liver cancer. The main causes of liver cirrhosis are the drinking, hepatitis B virus, portal hypertension and bile duct obstruction. In addition, chronic cholestasis caused by gene defects, poisoning and mechanical obstruction can ultimately lead to the occurrence of biliary cirrhosis[1]. Biliary cirrhosis is characterized by the impairment of bile production and bile excretion in hepatic duct[2]. It has been reported[3] that with the aggravation of cholestasis, the cell oxidative stress response and mitochondrial dysfunction can be induced, ultimately leading to the degeneration and apoptosis of liver cells

Corresponding Author: Jiyuan Si, MD; e-mail: sitou483073@163.com

and bile duct cells[4]. A series of inducible factors produced by the apoptosis of liver cells can activate hepatic stellate cells, which is a key link in promoting the occurrence of hepatic fibrosis[5,6]. Studies[7,8] have proved that cholestasis can lead to endoplasmic reticulum stress (ERS), which has a protective effect on cells under normal conditions. Activating transcription factor 4 (ATF4), ATF6 and inositol-requiring enzyme 1 (IRE1) are three classical signaling pathways that have been proved to be associated with ERS regulation[9]. Excessive ERS can induce the CHOP-mediated apoptosis response, oxidative stress response and inflammatory response[10]. Besides, transforming growth factor β-1 (TGF-β1) is an inducible factor that has been proved to induce the activation of hepatic stellate cells. Researches have confirmed that tauro ursodesoxy cholic acid (TUDCA) can effectively inhibit the unfolded protein response caused by cholestasis, and alleviate the severity of liver cirrhosis and apoptosis[7]. Hamdy et al[11] reported that carvedilol can alleviate the CCl_4-induced hepatic fibrosis in mice, but its mechanism was not clear. It was found in clinical observation that the liver function and ascites of patients with cholestasis-induced liver cirrhosis can be improved significantly after carvedilol treatment. Moreover, It has been proved that carvedilol can inhibit the activation of hepatic stellate cells, but its related mechanism remains unclear[12]. Carvedilol, as the third generation of non-selective β receptor inhibitor, has been widely applied in patients with cirrhotic portal hypertension, because it can effectively reduce the portal pressure[13]. ERS can be induced by oxidative stress, acute liver injury and poisoning[8]. Therefore, it is speculated that the occurrence of biliary cirrhosis may be related to the excessive ERS caused by it. The primary purpose of this study was to investigate the effects of carvedilol on inflammation, apoptosis, and hepatic fibrosis caused by biliary cirrhosis and its mechanisms in mice.

Materials and Methods

Animals and Treatments

60 male C57/BL6 mice (purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) were randomly divided into three groups: sham-operation group (Sham group, n=20), biliary cirrhosis group (BDL group, n=20) and carvedilol group (CAR group, n=20). The CAR group was treated with gavage using 12.5 mg/kg carvedilol, once a day for 14 consecutive days, while the Sham group and BDL group were treated with gavage using the equivalent normal saline. After that, the mice in Sham group received the laparotomy under chloral hydrate anesthesia, followed by direct abdominal closure; the mice in BDL group and CAR group received the common bile duct ligation after anesthesia for modeling. After modeling, the survival rate of mice in each group was detected, and the blood and liver tissues were taken for detection. This investigation was approved by the Animal Ethics Committee of No.1 People's Hospital of Jining City.

Detection of Serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Total Bilirubin (TBIL)

The levels of serum transaminase and TBIL are important indexes of measuring the severity of liver damage[15]. ALT, AST and TBIL in the serum sample collected from each experimental group above were detected using the fully automatic biochemical analyzer (Philips, Eindhoven, The Netherlands).

Detection of Levels of α-smooth Muscle Actin (α-SMA) and Hydroxyproline in Liver Tissues

The α-SMA content in liver tissues in each experimental group was detected and compared via immunohistochemical measured according to a previous work[16]. The same amount of liver tissues was taken from each experimental group, and colorimetric method was used to detect the hydroxyproline content[17].

Detection of Contents of Serum Superoxide Dismutase (SOD) and Malondialdehyde (MDA)

SOD and MDA are important products in oxidative stress response. The level of SOD and MDA in each experimental group was measured. The equivalent serum was taken from each experimental group, and SOD and MDA contents were detected according to the literature report[18].

Detection of Morphological Changes in Liver Tissues and Apoptosis

After fixation using 10% formaldehyde solution, the liver tissues collected from each experimental group were prepared into paraffin sections according to the method shown in a previous study[19]. The pathological changes in liver tissues

were observed under optical microscope (400×) (Olympus Optical Co., Ltd, Tokyo, Japan), and the number of pseudo-lobules in the same visual field was counted and compared. After TUNEL staining for paraffin sections, the severity of liver cell apoptosis in each experimental group was observed under optical microscope (400×), and the apoptotic index was calculated[20].

Detection of mRNA Expression Via Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

The liver tissues were cleaved and the RNA kit (Eurofins MWG Operon, Ebersberg, Germany) was used to extract the total RNA. The relevant primer sequences were searched in Pubmed database (Table I). The extracted RNA was reversely transcribed into cDNA, followed by amplification via RT-PCR to detect its expression. The mRNA expression in each experimental group was calculated using $2^{-\Delta\Delta CT}$ method[21].

Detection of the Protein Expression Level Via Western-blotting

The liver tissues in each experimental group were cleaved, and the expressions of relevant proteins (α-SMA, collagen-1, ATF4, ATF6, CHOP, IRE1 and pJNK) were detected via Western-blotting[21]. GAPDH was used as the internal reference in the experiment. Antibodies used in each experiment were purchased from Abcam (Cambridge, MA, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis of relevant experimental data. All data were presented as mean ± standard deviation. The difference between the two experimental groups was compared via *t*-test. $p<0.05$ suggested that the difference was statistically significant.

Table I. Primer sequences used in this study.

Gene	Sequence
iNOS	F: 5′-GGAATCTTGGAGCGAGTTG-3′
	R: 5′-GTCCAGGAAGTAGGTGAGGG-3′
COX-2	F: 5′-GAACCGCATTGCCTCTGA-3′
	R: 5′-GCCTTTGCCACTGCTTGTA-3′
TGF-β1	F: 5′- GCGGACTACTATGCTAAAGAGG-3′
	R: 5′- GTAGAGTTCCACATGTTGCTCC-3′
α-SMA	F: 5′-TGACCCAGATTATGTTTGAGACC-3′
	R: 5′-CCAGAGTCCAGCACAATACCA-3′
Collagen-1	F: 5′-CAAGGTCCTTCTGGATCAAGTG-3′
	R: 5′-CCTTTATGCCTCTGTCACCTTG-3′

Results

Carvedilol Could Reduce the Serum ALT/AST and TBIL Levels and Improve the Liver Function of Patients with Liver Cirrhosis

In clinical work, 8 patients with cirrhosis were randomly selected. The blood was drawn, the ALT/AST and TBIL levels were detected, and the abdominal girth was measured. After the oral administration of carvedilol (12.5 mg, q.d.) for 14 consecutive days, the blood was drawn from patients again to detect the ALT/AST and TBIL levels, and the abdominal girth was measured; the results were compared with those on admission. It was found that the levels of ALT/AST (Figure 1A) and TBIL (Figure 1B) in patients were decreased after carvedilol treatment, and the differences were statistically significant ($p<0.05$). To study its mechanism, the animal experiments were performed.

Carvedilol Alleviated the Liver Function Impairment, Liver Morphological Changes and Apoptosis in mice Caused by biliary Cirrhosis, and Improved the Survival Rate

ALT/AST and TBIL are important indexes of liver function impairment in cirrhosis[15]. The results showed that the levels of ALT/AST (Figure 2A) and TBIL (Figure 2B) in CAR group were significantly lower than those in BDL group, and the differences were statistically significant ($p<0.05$), indicating that carvedilol can effectively reduce the liver enzyme and bilirubin levels in mice with biliary cirrhosis. The survival rate of mice in CAR group was significantly higher than that in BDL group (Figure 2C), and the difference was statistically significant ($p<0.05$), suggesting that carvedilol can effectively prolong the survival of mice with biliary cirrhosis. The formation of pseudo lobule in cirrhosis is a landmark morphological change in cirrhosis[22]. The morphological changes in liver tissues of mice in each experimental group were compared. The number of pseudo lobule in CAR group was significantly lower than that in BDL group (Figure 2D), and the difference was statistically significant ($p<0.05$), indicating that carvedilol can alleviate the severity of hepatic fibrosis in mice with bili-

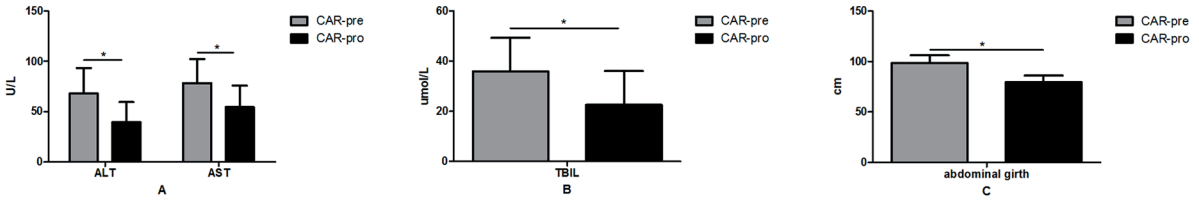

Figure 1. Comparisons of ALT/AST ***(A)***, TBIL ***(B)***, and abdominal girth ***(C)*** in the clinical patients (*p<0.05).

ary cirrhosis. The number of apoptotic liver cells was also compared. The number of apoptotic cells in CAR group was significantly lower than that in BDL group (Figure 2E), and the difference was statistically significant (p<0.05), suggesting that carvedilol can significantly reduce the cell apoptosis in mice with biliary cirrhosis. In summary, carvedilol can effectively alleviate the damage in mice with biliary cirrhosis and improve the survival rate.

Carvedilol Reduced the Contents of α-SMA and Hydroxyproline in Liver Tissues

α-SMA is an important marker of activation of hepatic stellate cells[23], and the activated hepatic stellate cells play important roles in the occurrence of cirrhosis[24]. Hydroxyproline is a marker of collagen production[7], and its rise indicates the deposition of collagen in cells, thus lading to liver cell fibrosis. The contents of α-SMA and hydroxyproline in hepatic tissues in each experimental group were measured, and it was found that α-SMA (Figure 3) and hydroxyproline in CAR group were significantly lower than those in BDL group, and the differences were statistically significant (p<0.05), indicating that they can reduce the severity of hepatic fibrosis in biliary cirrhosis.

Carvedilol Increased the Serum SOD Level and Decreased the Serum MDA Content

SOD and MDA are two important indexes of detecting oxidative stress response[25]. We found

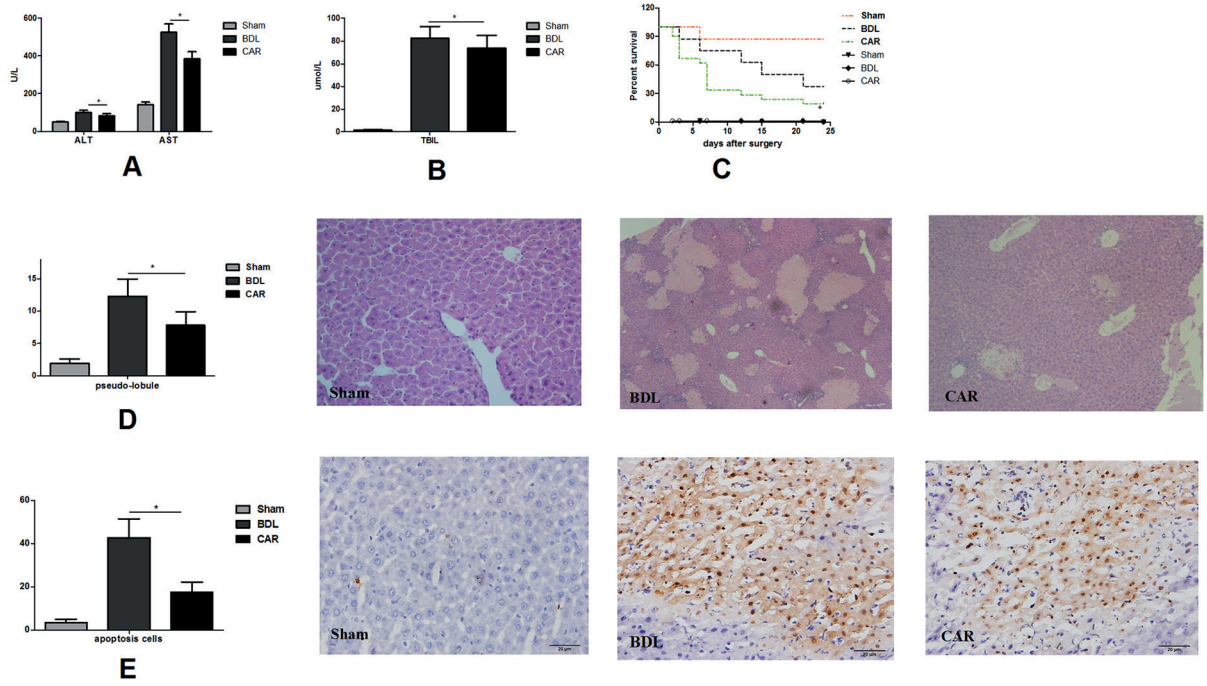

Figure 2. Comparisons of ALT/AST ***(A)***, TBIL ***(B)*** in the serum and the survival rate ***(C)*** in each experimental group (*p<0.05); Pseudo-lobules ***(D)*** were counted in tissues (HE staining, 400×) in each experimental group; the TUNEL staining ***(E)*** was performed and 100 cells per field were counted in three fields in each experimental group (400×); (*p<0.05).

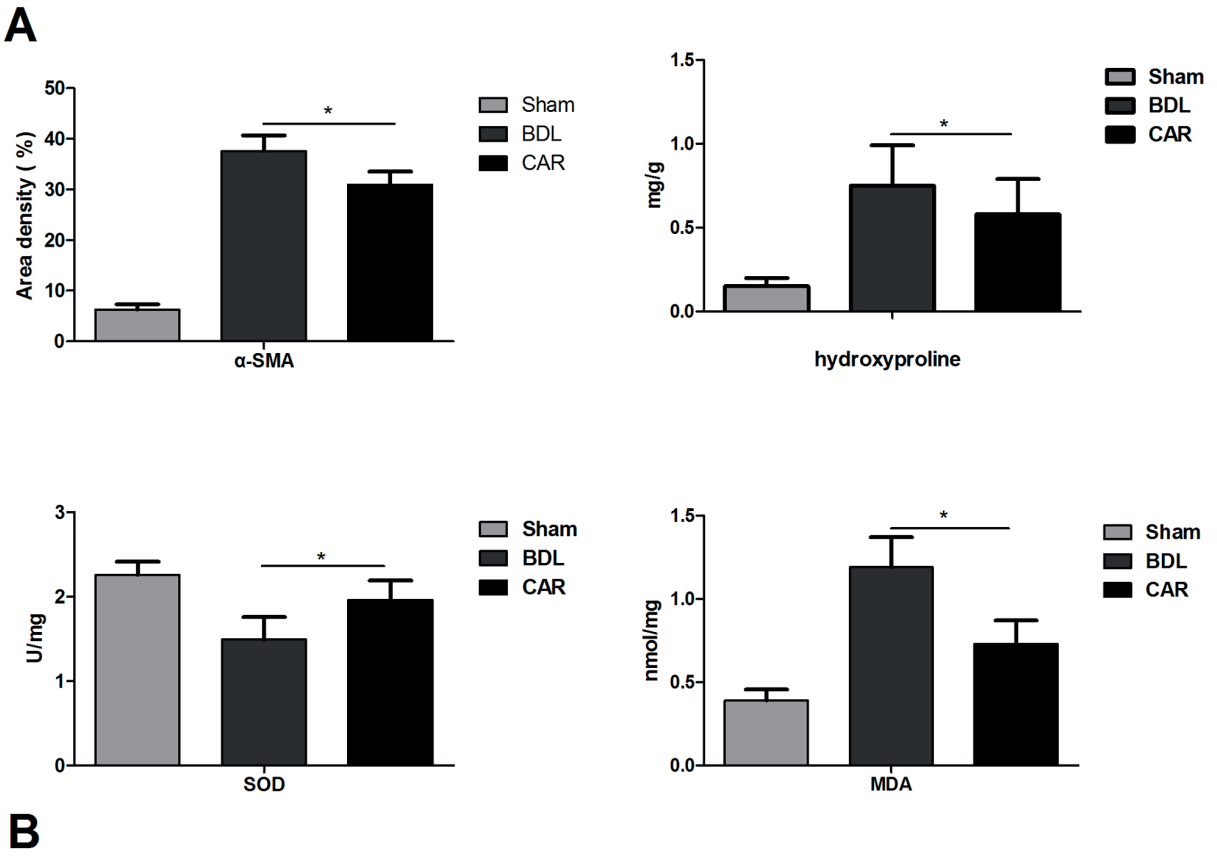

Figure 3. Comparisons of α-SMA ***(A)*** and hydroxyproline ***(A)*** content in liver tissues of each experimental group (*p<0.05); Comparisons of SOD ***(B)*** activity and MDA ***(B)*** content in serum of each experimental group (*p<0.05).

that the activity of SOD (Figure 3B) in CAR group was significantly increased, but the MDA content (Figure 3B) was significantly decreased compared with those in BDL group; the differences were statistically significant (p<0.05). The experimental results showed that carvedilol can effectively inhibit the oxidative stress.

Carvedilol Inhibited the mRNA Expressions of iNOS, COX-2 and TGF-β1

It has been found that the levels of pro-inflammatory factors in biliary cirrhosis were significantly increased, and TGF-β1 could activate the hepatic stellate cells, thereby aggravating hepatic fibrosis[26]. We found that the expressions of pro-inflammatory cytokines, iNOS and COX-2 (Figure 4A), in CAR group were significantly lower than those in BDL group, and the differences were statistically significant (p<0.05). Therefore, it is speculated that carvedilol can alleviate the injury in mice with biliary cirrhosis through inhibiting the pro-inflammatory cytokines and reducing TGF-β1.

Carvedilol Could Down-regulate the mRNA and Protein Expressions of α-SMA and Collagen-1, and Negatively Regulate the ATF4-CHOP, ATF6-CHOP and IRE1-pJNK Signaling Pathways

Our results showed that the mRNA (Figure 4C, p<0.05) and protein (Figure 5) expressions of α-SMA and collagen-1 in CAR group were significantly decreased compared with throe in BDL group, indicating that carvedilol can down-regulate the α-SMA and collagen-1 expressions and alleviate the severity of hepatic fibrosis. CHOP is a marker protein produced by ERS, which can be regulated via ATF4 and ATF6 signaling pathways; another important signaling pathway of ERS, IRE1-pJNK, is associated with inflammation and apoptosis[8]. In our study, the expression of ERS marker protein, CHOP, in CAR group was

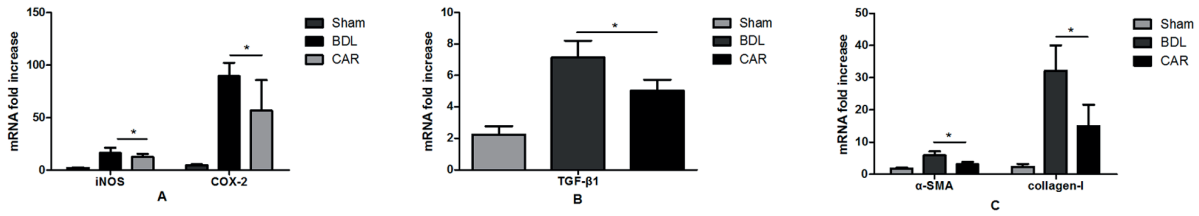

Figure 4. Comparisons of mRNA expression levels of iNOS and COX-2, TGF-β1, α-SMA, and collagen-1in liver tissues of each experimental group (*p<0.05).

significantly lower than that in BDL group, indicating that carvedilol can inhibit ERS response. The expressions of ATF4, ATF6, IRE1 and pJNK in CAR group were decreased, suggesting that carvedilol may inhibit ERS through the negative regulation of ATF4-CHOP, ATF6-CHOP and IRE1-pJNK signaling pathways, thus alleviating biliary cirrhosis (Figure 5).

Discussion

In clinical work, it was found that carvedilol could decrease the levels of ALT/AST and TBIL in serum of patients with biliary cirrhosis and reduce the severity of ascites; the differences before and after medication were statistically significant. This experiment was performed to further study its relevant mechanism. We found that carvedilol could reduce the levels of serum aminotransferase and TBILI, and improve the survival rate, indicating that carvedilol can alleviate the injury caused by biliary cirrhosis. The pathological result and TUNEL staining result of liver tissues showed that the number of pseudo lobules and apoptotic cells in CAR group were lower than those in BDL group, indicating that carvedilol can alleviate the severity of hepatic fibrosis in biliary cirrhosis, and reduce the apoptosis of hepatic cells and bile duct cells. In summary, carvedilol has a definite effect on alleviating the biliary cirrhosis. Then, the research on mechanism of action was conducted. It is generally believed that[24] the activation of hepatic stellate cells plays an important role in the course of liver cirrhosis. It is reported that carvedilol can significantly improve the heart and kidney fibrosis of experimental animals[27], and its mechanism may be related to its inhibition of hepatic stellate cell activation, antioxidant effect[28], and proliferation of vascular smooth muscle cells[2], but the further mechanism is still not clear. α-SMA is an important marker of the activation of hepatic stellate cells[4]. In this experiment, the content of α-SMA in CAR group was decreased compared with that in BDL group, suggesting that carvedilol can inhibit the activation of hepatic stellate cells. The content of hydroxyproline is a marker of collagen production in cells[7], and it was also decreased in CAR group compared with that in BDL group, indicating that carvedilol can reduce the collagen deposition in cells. Further molecular research showed that carvedilol could down-regulate the mRNA and protein expressions of α-SMA and collagen-1. The above studies proved that carvedilol can inhibit the activation of hepatic stellate cells in biliary cirrhosis, reduce the accumulation of collagen, and alleviate the severity of hepatic fibrosis. Its regulatory mechanism was further studied. Researches have proved that the toxic deoxycholic acid and glycochenodeoxycholic acid induce ERS and unfolded protein response[29,30]. In this investigation, CHOP in BDL group was significantly increased compared with that in Sham group, indicating that the ERS response is activated. CHOP is the effector molecule of downstream regulation of ERS, which can mediate the liver apoptosis[31]. The severity of liver cirrhosis in CHOP gene-deleted mice is significantly alleviated[6]. It has been reported that TUDCA can

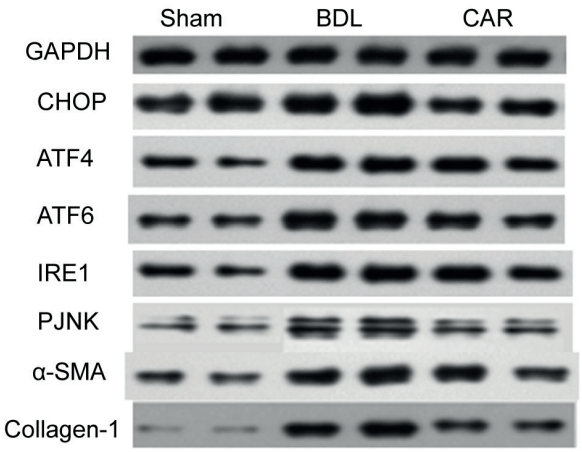

Figure 5. Comparisons of protein expression levels of α-SMA, collagen-1, ATF4, CHOP, ATF6, IRE1 and PJNK.

reduce the severity of hepatic fibrosis and apoptosis in biliary cirrhosis by inhibiting UPR, whereas TUDCA has been proved to be the ERS inhibitory drug[33]. It has also been reported[34] that the expression of CHOP is increased during the development of biliary cirrhosis, so we believe that its effect on relieving cirrhosis is associated with the inhibition of ERS. It was found that the expression of CHOP in CAR group was significantly lower than that in BDL group. Besides, ATF4 and ATF6 are two signaling pathways that have been confirmed to regulate CHOP[8]; the expressions of ATF4 and ATF6 in CAR group were significantly decreased compared with those in BDL Group. Therefore, it is speculated that carvedilol can down-regulate the expression of CHOP by negatively regulating the ATF4-CHOP and ATF6-CHOP signaling pathways. Moreover, IRE1-pJNK signaling pathway is another signaling pathway associated with inflammation that regulates ERS response. Compared with those in BDL group, the mRNA expressions of iNOS and COX-2 in CAR group were decreased, suggesting that carvedilol can alleviate the inflammatory response in mice with biliary cirrhosis. In this study, the protein expressions of IRE1 and pJNK were decreased in CAR group, so it is speculated that carvedilol can alleviate the inflammatory response by negatively regulating IRE1-pJNK signaling pathway. What's more, oxidative stress response is another key link in ERS response, which can not only activate the hepatic stellate cells and induce apoptosis[35], but also up-regulate the mRNA expression of TGF-β1[36]. Studies have shown that the activation of TGF-β1 can, on one hand, activate hepatic stellate cells[26], and, on the other hand, up-regulate the collagen-1 protein expression[37] and promote the development of hepatic fibrosis. It was found that MDA content and TGF-β1 expression in CAR group were decreased, but the level of SOD was increased, indicating that carvedilol can inhibit the oxidative stress, thus inhibiting TGF-β1 production and activation of hepatic stellate cells, and alleviating the severity of cirrhosis. In conclusion, carvedilol alleviates biliary cirrhosis, whose relevant mechanism is at least partially related to the inhibition of ERS.

Conclusions

Carvedilol has a significant effect on alleviating the inflammatory response, apoptosis and hepatic fibrosis caused by biliary cirrhosis in mice. Its relevant mechanism may be that carvedilol inhibits ERS through the negative regulation of ATF4-CHOP, ATF6- CHOP and IRE1-pJNK signaling pathways. There were some shortcomings in this experiment, and further *in vitro* experimental studies are needed.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Tian X, Zhao C, Guo J, Xie S, Yin F, Huo X, Zhang X. Carvedilol attenuates the progression of hepatic fibrosis induced by bile duct ligation. Biomed Res Int 2017; 2017: 4612769.
2) Lotze U, Heinke S, Fritzenwanger M, Krack A, Muller S, Figulla HR. Carvedilol inhibits platelet-derived growth factor-induced signal transduction in human cardiac fibroblasts. J Cardiovasc Pharmacol 2002; 39: 576-589.
3) Hirschfield GM, Heathcote EJ, Gershwin ME. Pathogenesis of cholestatic liver disease and therapeutic approaches. Gastroenterology 2010; 139: 1481-1496.
4) Siddiqui MA, Ahmad J, Farshori NN, Saquib Q, Jahan S, Kashyap MP, Ahamed M, Musarrat J, Al-Khedhairy AA. Rotenone-induced oxidative stress and apoptosis in human liver HepG2 cells. Mol Cell Biochem 2013; 384: 59-69.
5) Zhang QD, Xu MY, Cai XB, Qu Y, Li ZH, Lu LG. Myofibroblastic transformation of rat hepatic stellate cells: the role of Notch signaling and epithelial-mesenchymal transition regulation. Eur Rev Med Pharmacol Sci 2015; 19: 4130-4138.
6) Schattenberg JM, Zimmermann T, Worns M, Sprinzl MF, Kreft A, Kohl T, Nagel M, Siebler J, Schulze BH, He YW, Galle PR, Schuchmann M. Ablation of c-FLIP in hepatocytes enhances death-receptor mediated apoptosis and toxic liver injury in vivo. J Hepatol 2011; 55: 1272-1280.
7) Paridaens A, Raevens S, Devisscher L, Bogaerts E, Verhelst X, Hoorens A, Van Vlierberghe H, van Grunsven LA, Geerts A, Colle I. Modulation of the unfolded protein response by tauroursodeoxycholic acid counteracts apoptotic cell death and fibrosis in a mouse model for secondary biliary liver fibrosis. Int J Mol Sci 2017; 18: pii: E214.
8) Dara L, Ji C, Kaplowitz N. The contribution of endoplasmic reticulum stress to liver diseases. Hepatology 2011; 53: 1752-1763.
9) Liu J, Ren F, Cheng Q, Bai L, Shen X, Gao F, Busuttil RW, Kupiec-Weglinski JW, Zhai Y. Endoplasmic reticulum stress modulates liver inflammatory immune response in the pathogenesis of liver ischemia and reperfusion injury. Transplantation 2012; 94: 211-217.
10) Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. Embo Rep 2006; 7: 880-885.

11) Hamdy N, El-Demerdash E. New therapeutic aspect for carvedilol: antifibrotic effects of carvedilol in chronic carbon tetrachloride-induced liver damage. Toxicol Appl Pharmacol 2012; 261: 292-299.

12) Li T, Ke W, Sun P, Chen X, Belgaumkar A, Huang Y, Xian W, Li J, Zheng Q. Carvedilol for portal hypertension in cirrhosis: systematic review with meta-analysis. BMJ Open 2016; 6: e10902.

13) Tsochatzis EA, Triantos CK, Burroughs AK. Gastrointestinal bleeding: carvedilol-the best beta-blocker for primary prophylaxis? Nat Rev Gastroenterol Hepatol 2009; 6: 692-694.

14) Gani AR, Uppala JK, Ramaiah KV. Tauroursodeoxycholic acid prevents stress induced aggregation of proteins in vitro and promotes PERK activation in HepG2 cells. Arch Biochem Biophys 2015; 568: 8-15.

15) Rej R. Aspartate aminotransferase activity and isoenzyme proportions in human liver tissues. Clin Chem 1978; 24: 1971-1979.

16) Guesdon JL, Ternynck T, Avrameas S. The use of avidin-biotin interaction in immunoenzymatic techniques. J Histochem Cytochem 1979; 27: 1131-1139.

17) Woessner JJ. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys 1961; 93: 440-447.

18) Ulusoy S, Ozkan G, Yucesan FB, Ersoz S, Orem A, Alkanat M, Yulug E, Kaynar K, Al S. Anti-apoptotic and anti-oxidant effects of grape seed proanthocyanidin extract in preventing cyclosporine A-induced nephropathy. Nephrology (Carlton) 2012; 17: 372-379.

19) Kozutsumi Y, Segal M, Normington K, Gething MJ, Sambrook J. The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. Nature 1988; 332: 462-464.

20) Shi J, Shao W, Yang D, Zhao L, Deng L, Wang X, Sun B. Hydrodynamics-based transfection of plasmid encoding receptor activator for nuclear factor kappa B-Fc protects against hepatic ischemia/reperfusion injury in mice. Liver Transpl 2010; 16: 611-620.

21) Zhang X, Wang Z, Li J, Gu D, Li S, Shen C, Song Z. Increased 4-hydroxynonenal formation contributes to obesity-related lipolytic activation in adipocytes. PLoS One 2013; 8: e70663.

22) Lee YA, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: a translational success story. Gut 2015; 64: 830-841.

23) Li J, Li J, Li S, He B, Mi Y, Cao H, Zhang C, Li L. Ameliorative effect of grape seed proanthocyanidin extract on thioacetamide-induced mouse hepatic fibrosis. Toxicol Lett 2012; 213: 353-360.

24) Jiao J, Friedman SL, Aloman C. Hepatic fibrosis. Curr Opin Gastroenterol 2009; 25: 223-229.

25) Xu ZC, Yin J, Zhou B, Liu YT, Yu Y, Li GQ. Grape seed proanthocyanidin protects liver against ischemia/reperfusion injury by attenuating endoplasmic reticulum stress. World J Gastroenterol 2015; 21: 7468-7477.

26) Kawada N. Evolution of hepatic fibrosis research. Hepatol Res 2011; 41: 199-208.

27) Zhu JN, Chen R, Fu YH, Lin QX, Huang S, Guo LL, Zhang MZ, Deng CY, Zou X, Zhong SL, Yang M, Zhuang J, Yu XY, Shan ZX. Smad3 inactivation and MiR-29b upregulation mediate the effect of carvedilol on attenuating the acute myocardium infarction-induced myocardial fibrosis in rat. PLoS One 2013; 8: e75557.

28) Shen XH, Cheng WF, Li XH, Sun JQ, Li F, Ma L, Xie LM. Effects of dietary supplementation with vitamin E and selenium on rat hepatic stellate cell apoptosis. World J Gastroenterol 2005; 11: 4957-4961.

29) Bochkis IM, Rubins NE, White P, Furth EE, Friedman JR, Kaestner KH. Hepatocyte-specific ablation of Foxa2 alters bile acid homeostasis and results in endoplasmic reticulum stress. Nat Med 2008; 14: 828-836.

30) Sasaki M, Yoshimura-Miyakoshi M, Sato Y, Nakanuma Y. A possible involvement of endoplasmic reticulum stress in biliary epithelial autophagy and senescence in primary biliary cirrhosis. J Gastroenterol 2015; 50: 984-995.

31) Uzi D, Barda L, Scaiewicz V, Mills M, Mueller T, Gonzalez-Rodriguez A, Valverde AM, Iwawaki T, Nahmias Y, Xavier R, Chung RT, Tirosh B, Shibolet O. CHOP is a critical regulator of acetaminophen-induced hepatotoxicity. J Hepatol 2013; 59: 495-503.

32) Tamaki N, Hatano E, Taura K, Tada M, Kodama Y, Nitta T, Iwaisako K, Seo S, Nakajima A, Ikai I, Uemoto S. CHOP deficiency attenuates cholestasis-induced liver fibrosis by reduction of hepatocyte injury. Am J Physiol Gastrointest Liver Physiol 2008; 294: G498-G505.

33) Zhang L, Xin Z, Yu X, Ma C, Liang W, Zhu M, Cheng Q, Li Z, Niu Y, Ren Y, Wang Z, Lin T. Osmotic stress induced cell death in wheat is alleviated by tauroursodeoxycholic acid and involves endoplasmic reticulum stress-related gene expression. Front Plant Sci 2017; 8: 667.

34) Lebeaupin C, Proics E, de Bieville CH, Rousseau D, Bonnafous S, Patouraux S, Adam G, Lavallard VJ, Rovere C, Le Thuc O, Saint-Paul MC, Anty R, Schneck AS, Iannelli A, Gugenheim J, Tran A, Gual P, Bailly-Maitre B. ER stress induces NLRP3 inflammasome activation and hepatocyte death. Cell Death Dis 2015; 6: e1879.

35) Richter K, Kietzmann T. Reactive oxygen species and fibrosis: further evidence of a significant liaison. Cell Tissue Res 2016; 365: 591-605.

36) Hakucho A, Liu J, Liu X, Fujimiya T. Carvedilol improves ethanol-induced liver injury via modifying the interaction between oxidative stress and sympathetic hyperactivity in rats. Hepatol Res 2014; 44: 560-570.

37) Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008; 134: 1655-1669.