Role of dihydroceramides in the progression of acute-on-chronic liver failure in rats

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Abstract

Background: Previously, dihydroceramide (d18:0/24:0) (dhCer (d18:0/24:0)) was reported to be a potential biomarker for acute-on-chronic liver failure (ACLF) prognosis. In this study, we further explored the role of dhCer (d18:0/24:0) in the progression of ACLF to validate the biomarker using ACLF rat model.

Methods: ACLF rats were sacrificed at 4 and 8 h post-D-galactosamine (D-gal)/lipopolysaccharide (LPS) administration to investigate the liver biochemical markers, prothrombin time and liver histopathology. Change in dhCer and other sphingolipids levels were investigated by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). Rats were treated with N-(4-hydroxyphenyl) retinamide (4-HPR) to examine the mortality rate and its role in improving ACLF.

Results: LPS/D-gal administration resulted in significant elevation in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Prothrombin time was prolonged and histopathological examination showed abnormality. HPLC-MS/MS results showed total dhCer levels in ACLF group (64.10 ± 8.90 pmol/100 μL, 64.22 ± 6.78 pmol/100 μL for 4 and 8 h, respectively) were decreased significantly compared with control group (121.61 ± 23.09 pmol/100 μL) ($P < 0.05$). In particular, dhCer (d18:0/24:0), dhCer (d18:0/20:0), and dhCer (d18:0/22:0) levels were decreased. Treatment with 4-HPR significantly increased the levels of dhCers, including dhCer (d18:0/24:0) compared with ACLF group, for the level of dhCer (d18:0/24:0) in 4-HPR group was 20.10 ± 8.60 pmol/100 μL and the level of dhCer (d18:0/24:0) in ACLF group was 9.74 ± 2.99 pmol/100 μL ($P < 0.05$). This was associated with reduced mortality rate and prolonged survival time. The ALT and AST in 4-HPR group were significantly decreased compared with ACLF group. The prothrombin time of 4-HPR group (41.49 s) was significantly lower than the prothrombin time of ACLF group (57.96 s) ($P < 0.05$). 4-HPR also decreased plasma ammonia levels slightly, as the plasma ammonia levels in 4-HPR group and ACLF group were 207.37 ± 60.43, 209.15 ± 60.43 μmol/L, respectively. Further, 4-HPR treatment improved histopathological parameters.

Conclusions: DhCer, especially dhCer (d18:0/24:0), is involved in the progression of ACLF. Increasing the levels of dhCer can reduce the mortality rate of ACLF rats and alleviate liver injury.

Keywords: N-(4-hydroxyphenyl) retinamide; Acute-on-chronic liver failure; Ceramides; Dihydroceramides

Introduction

Acute-on-chronic liver failure (ACLF) is a clinical syndrome characterized by acute decompensation of chronic liver disease associated with organ failures, and short-term mortality is very high, which is distinct from chronic liver failure or decompensated cirrhosis.[1,2] The 28-day mortality rate among ACLF patients was at least 15%.[3] Liver transplantation is the only efficient therapeutic strategy for ACLF, the 1-year survival rate of which exceeds 80% in China.[4,5] However, patients with infection do not meet the criteria for liver transplant, which rules out almost half of the patients. Due to the shortage of liver donors, 50% to 70% of patients die while on the organ transplant waiting list. The pathogenesis of ACLF remains unclear. It is necessary to study the pathophysiological mechanism of ACLF and find an effective therapeutic approach.

Sphingolipids are a class of lipids that play a vital role in many cellular processes, including apoptosis, autophagy, and cell growth.[6] Sphingolipids such as ceramide and sphingosine-1-phosphate (Sph-1-P), have been widely studied. Dihydroceramide (dhCer) is considered as an inactive precursor of ceramide.[7] However, recent studies have suggested that dhCers are involved in cell cycle, apoptosis, and autophagy.[8] Increased dhCer results in impairment of autophagic flux and promotes fibrosis in hepatic steatosis model.[9] Previously, our group demonstrated that dhCer (d18:0/24:0) was suggested to be a

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useful prognostic biomarker for ACLF.[10] The aim of this study was to determine serum sphingolipid composition in ACLF rat. We particularly focused on dhCer to clarify its role in ACLF and suggest a potential therapy for ACLF. In this study, we used 4-HPR, an inhibitor of desaturase (DES) which could increase the levels of dhCer, to help us explore the role of dhCer in ACLF.

Methods

Animals and reagents

Male Wistar rats (120–150 g) were purchased from the Vital River Laboratories (Beijing, China). All rats were raised in an environment with a 12-h light/dark cycle at a temperature of 22 ± 1°C with free access to food and water. Animal care and all experiments were performed according to the guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Peking Union Medical College & Chinese Academy of Medical Sciences (PUMC&CAMS). Porcine serum was purchased from Solarbio Science & Technology (Beijing, China). Lipopolysaccharide (LPS), D-galactosamine (D-gal), and N-(4-hydroxyphenyl) retinamide (4-HPR) were purchased from Sigma-Aldrich (St Louis, MO, USA). Cytochrome C primary antibody was purchased from Santa Cruz (Dallas, Texas, USA). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) kits were purchased from Biosino (Beijing, China). Prothrombin times (PTs) determination kit and plasma ammonia kit were purchased from the Nanjing Institute of Jiancheng Bioengineering (Nanjing, China).

Experimental procedures

Experimental methods were performed using a protocol we established previously.[11] Rats were randomly assigned to two groups, control group ($n = 8$) and ACLF group ($n = 16$). Rats in ACLF group were intraperitoneally injected with 0.5 mL porcine serum twice a week for 11 consecutive weeks. After 11 weeks, the rats were divided into two groups, ACLF model group ($n = 8$) and 4-HPR treatment group. In 4-HPR group, 4-HPR was injected intraperitoneally at a dose of 20 mg/kg 12 h before LPS treatment. Then rats in ACLF model group and 4-HPR treatment group were injected intravenously with LPS (50 μg/kg). Thirty minutes later, D-gal (600 mg/kg) was injected intraperitoneally to induce ACLF. The control rats were administrated with equal volume of normal saline.

Serum biochemical parameters

Serum ALT and AST were measured with TBA-40FR automatic biochemistry analyzer (Toshiba, Tokyo, Japan) using commercial kits following the manufacturer's instructions.

PTs determination

Based on the method described previously,[11] the PT was measured using PT kits following the manufacturer's instructions.

Liver histopathologic examinations

Liver samples were fixed in 10% formalin for 48 h and embedded in paraffin. The sample was sectioned (5 μm per section). The sections were then deparaffinization in xylene and dehydrated in alcohol. Sections were stained with hematoxylin-eosin (H&E). Sections were then analyzed by light microscopy (Olympus, Tokyo, Japan).

Measurement of sphingolipids

High-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) was used to analyze the level of sphingolipids in serum as previously described.[12]

Measurement of plasma ammonia levels

The plasma ammonia level was measured using kits following the manufacturer's instructions as previously described.[11]

Western blotting analysis

Total protein samples were obtained from ischemic liver tissues using a total protein extraction kit (Applygen, Beijing, China) following the manufacturer's instructions. Protein concentrations were determined using bicinchoninic acid protein assay (Applygen). Equal amounts of protein were separated by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels and were transferred to polyvinylidene fluoride membranes (Millipore, Boston, MA, USA). The membranes were blocked with 3% bovine serum albumin in Tris-buffered saline for 2 h and then incubated with primary antibodies overnight at 4°C, respectively. After washing three times with Tris Buffered saline Tween, membranes were incubated with secondary antibodies at room temperature. Protein bands were detected using the enhanced chemiluminescence plus detection system (ImageQuant LAS4000mini, GE, Fairfield, CT, USA). The density of each band was quantified using Quantity One software (Toyobo, Japan). The data are expressed as ratios and were normalized to the amount of glyceraldehyde 3-phosphate.

Statistical analysis

All data were expressed as the mean ± standard deviation. The data were statistically analyzed using Student's *t* test or using a one-way analysis of variance for independent samples, using the GraphPad Prism 6.0 (GraphPad, San Diego, CA, USA). *P* values less than 0.05 were considered statistically significant.

Results

Sphingolipids including dhCer change with the progression of ACLF in rats

As shown in Figure 1A, LPS and D-gal treatment significantly increased the serum level of ALT and AST at 4 h and continued to increase at 8 h. PT gradually extended, as shown in Figure 1B, which suggested liver

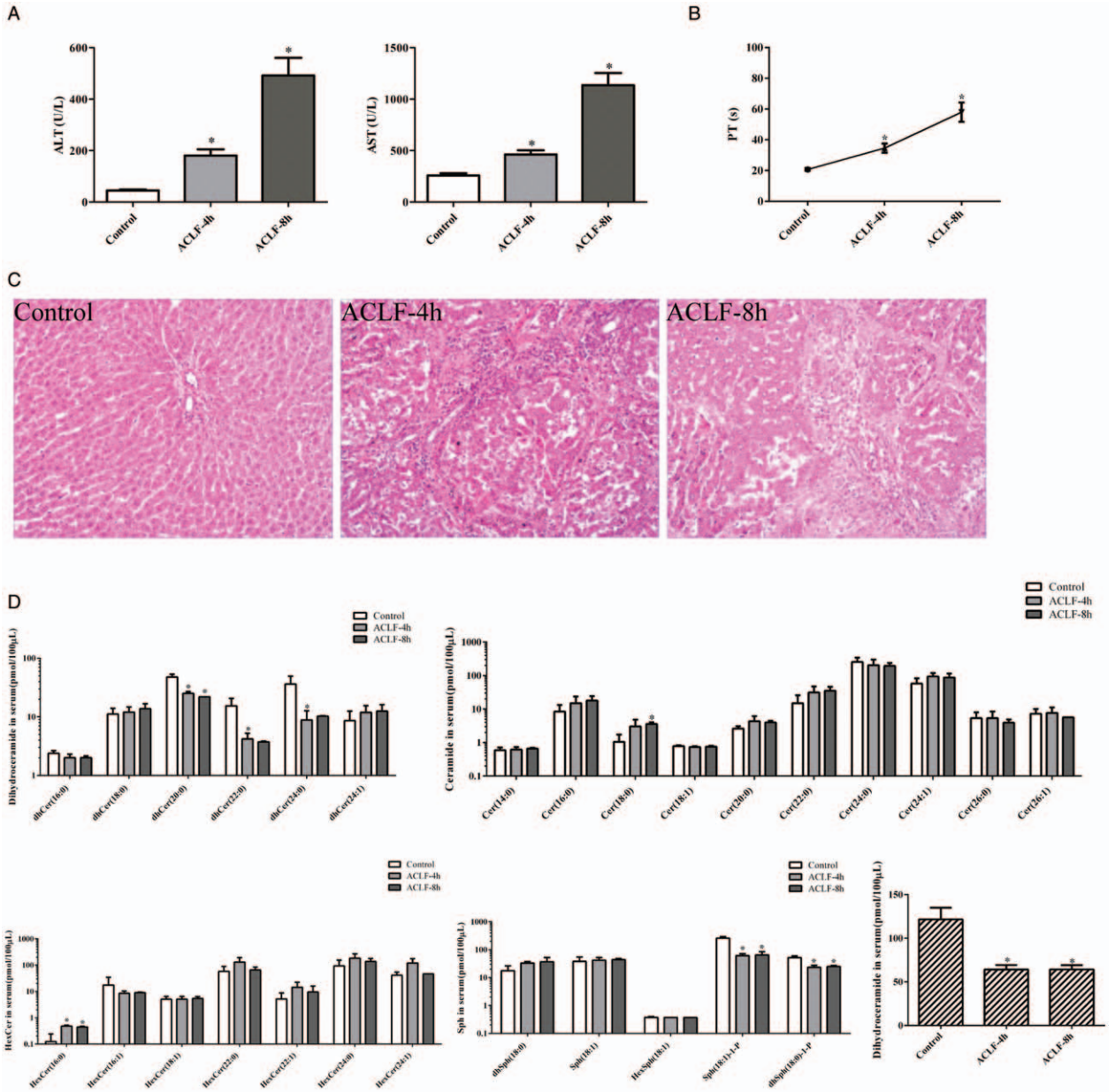

Figure 1: Sphingolipids including dihydroceramide change with the progression of ACLF in rats. (A) Change in serum levels of AST and ALT in rats with ACLF. (B) Change in prothrombin time in ACLF rats at 4 and 8 h post-D-galactosamine (D-gal/LPS administration. (C) Histopathological examination of ACLF rats (HE staining, original magnification: ×200). Representative photomicrographs are shown in the figure. (D) Changes in the serum sphingolipid composition of ACLF rats. $^{*}P < 0.05$ compared with control group. ACLF: Acute-on-chronic liver failure; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; D-gal: D-galactosamine; LPS: Lipopolysaccharide.

damage. To further verify the extent of liver injury, H&E staining was performed on the liver tissue sections. As shown in Figure 1C, tissue sections of control group showed no apparent abnormalities. In the tissue sections of ACLF group, inflammatory cell infiltration and numerous necrotic liver cells were observed. This observation was concurrent with the results of serum biochemical parameters and PT test. Thus, ACLF rat model was successfully established, consistent with our previous report.[11] The serum sphingolipid profiles of ACLF and control group were measured by HPLC-MS/MS. We observed a difference in the sphingolipid profiles between the two groups, particularly the dhCer levels. A significant decrease in the levels of dhCer (d18:0/24:0) in ACLF rat was observed. A similar result was observed in clinical samples.[10] Four hours or 8 h after LPS/D-gal administration, the levels of dhCer (d18:0/20:0) and dhCer (d18:0/22:0) also decreased markedly compared to their levels in the control. The serum levels of dhCer (d18:0/18:0) and dhCer (d18:0/24:1) in ACLF group increased slightly, which was not statistically significant ($P > 0.05$) when compared to their serum levels in control. The total dhCers in serum reduced significantly at 4 and 8 h post-LPS/D-gal administration [Figure 1D]. Serum levels of all ceramides (Cer), except Cer (d18:1/18:0), had no significant change in ACLF group when compared to their levels in the control group. At 8 h post-LPS/D-gal administration, Cer (d18:1/18:0) levels increased significantly compared to Cer (d18:1/18:0) levels

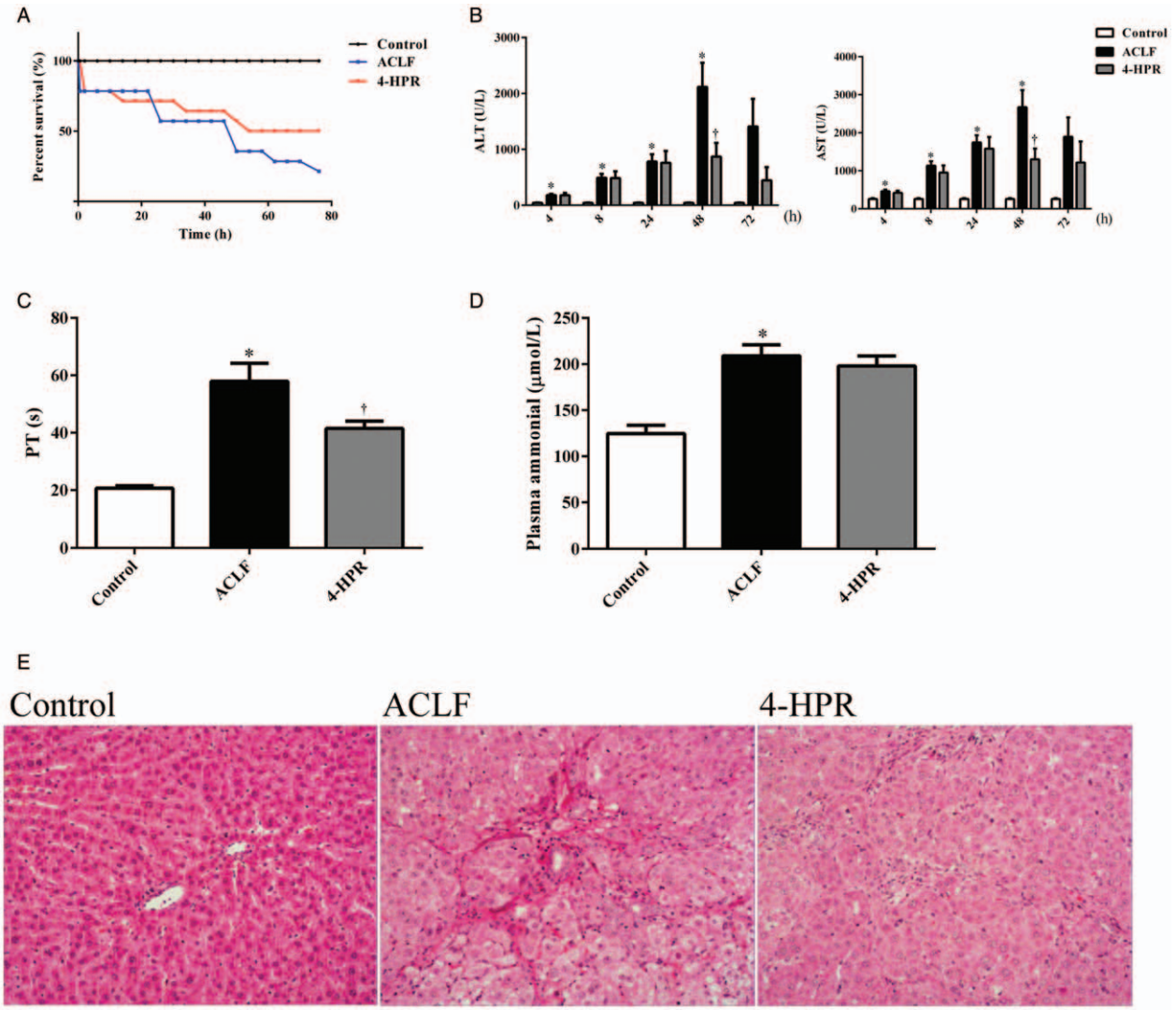

Figure 2: Alleviation of liver damage by 4-HPR in ACLF rats. (A) The effect of 4-HPR on the mortality rate of rats with ACLF. (B) The effect of 4-HPR on serum concentrations of AST and ALT in rats with ACLF. (C) The effect of 4-HPR on prothrombin time in rats with ACLF. (D) The effect of 4-HPR on serum plasma ammonia levels of rats with ACLF. (E) Histopathological examination of hepatic tissue sections to evaluate the effect of 4-HPR (HE staining, original magnification: ×200). Representative photomicrographs are shown in the figure. $^{*}P < 0.05$, compared with control group. $^{\dagger}P < 0.05$ compared with ACLF group. 4-HPR: *N*-(4-hydroxyphenyl); ACLF: Acute-on-chronic liver failure; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

in control group. In addition, the levels of HexCer (d18:1/16:0) increased significantly at 4 and 8 h post-LPS/D-gal administration. Additionally, at 4 and 8 h post-LPS/D-gal administration, the levels of Sph-1-P and dihydrosphingosine-1-phosphate decreased significantly compared to their levels in the control group.

Alleviation of liver damage by 4-HPR in ACLF rats

The sphingolipid profile of ACLF rats further indicated that the levels of dhCer (d18:0/24:0) decreased significantly during the onset of ACLF, which was also observed previously in clinical samples.[10] Hence, we speculated that dhCer (d18:0/24:0) may play an important role in the pathogenesis of ACLF. To identify the role of dhCer (d18:0/24:0), we used 4-HPR, a dhCer DES inhibitor, to increase the levels of dhCer (d18:0/24:0). The results showed that the mortality rate of the ACLF group was 78%, and the median survival time was 49 h. The mortality rate of the 4-HPR group was 50%, and the median survival time was 53 h [Figure 2A], which indicated that 4-HPR could reduce the mortality rate and prolong the survival time of ACLF rats. The result of serum biochemical indices showed that 4-HPR could significantly reduce the levels of ALT and AST in ACLF rats, which indicated that 4-HPR could ameliorate liver injury of ACLF rats [Figure 2B]. Furthermore, 4-HPR could reduce PT of ACLF rats. The PT of 4-HPR group (41.49 s) was significantly shorter than the PT of ACLF group (57.96 s) [Figure 2C]. The effect of 4-HPR on the levels of plasma ammonia was also measured. The levels of plasma ammonia in ACLF group was significantly elevated compared to the plasma ammonia levels in control group. Treatment with 4-HPR attenuated the elevated plasma ammonia levels in ACLF rats [Figure 2D]. To further evaluate the effect of 4-HPR on ACLF rats, H&E staining was performed on the liver tissue sections. Consistent with other results, enhanced necrosis, ballooning degeneration of hepatocytes, and inflammatory cell infiltration were remarkably attenuated by 4-HPR treatment [Figure 2E].

These results suggest that 4-HPR treatment could alleviate the liver injury of ACLF rats.

Alleviation of liver damage by 4-HPR in ACLF rats by increasing serum dhCer (24:0) levels and inhibiting hepatocellular apoptosis

We detected the levels of dhCer by HPLC-MS/MS to validate whether 4-HPR could increase serum dhCer levels. As expected, 4-HPR significantly increased the levels of dhCer (d18:0/24:0) when compared to the dhCer (d18:0/24:0) levels in ACLF rats [Figure 3A]. Simultaneously, 4-HPR could significantly increase the levels of dhCer (d18:0/16:0), dhCer (d18:0/20:0), dhCer (d18:0/22:0), and dhCer (d18:0/24:1) when compared to their levels in the ACLF group [Figure 3B]. According to a previous report,[13] dhCer can inhibit the formation of Cer channels in mitochondria to inhibit apoptosis. Hence, we speculated that 4-HPR could alleviate liver injury by increasing the levels of dhCer and hence inhibit apoptosis. Western blot analysis revealed that 4-HPR treatment significantly suppressed the expression of cytochrome C, an apoptosis-related protein, compared to the cytochrome C expression in ACLF group [Figure 3C].

Discussion

In this study, we explored the role of sphingolipids especially dhCer (d18:0/24:0) in the progression of ACLF using a rat model based on the previous clinical results. As expected, HPLC-MS/MS results showed a difference in the sphingolipid profiles between control group and ACLF group. Consistent with the clinical result, the level of dhCer (d18:0/24:1) in ACLF group decreased significantly compared with the control group. Then we used 4-HPR, a DES inhibitor, to increase the levels of dhCer (d18:0/24:0) to further investigate the role of dhCer (d18:0/24:0) in ACLF. As expected, 4-HPR increased the level of dhCer (d18:0/24:0) compared with ACLF group. Meanwhile, 4-HPR significantly decreased the levels of ALT and AST, reduced the PT and plasma ammonia level. 4-HPR also alleviated liver injury and inhibited apoptosis in ACLF.

Cer are sphingolipids that form the backbone of other complex molecules such as sphingomyelins and glycosylceramides.[14] There are three metabolic pathways that synthesize Cer: the *de novo* pathway, the salvage pathway, and the sphingomyelinase pathway. However, the main contributor to their biosynthesis is the *de novo* pathway.[15] The *de novo* pathway takes place in endoplasmic reticulum, where serine palmitoyl-transferase catalyzes the conversion of L-serine and palmitoyl-CoA to 3-keto sphinganine which is further converted to sphinganine by 3-ketosphinganine reductase. Ceramide synthases attach acyl-CoA of different chain lengths to sphinganine to form different chain lengths of dhCers. Finally, DES reduces dhCer to form Cer. The sphingomyelinase pathway takes place in the plasma membrane via sphingomyelin hydrolysis. The salvage pathway takes place in lysosomes using hexosylceramides as its substrate.[16] Cer is a bioactive sphingolipid involved in mitochondria-mediated apoptosis. Cer can form channels to regulate mitochondrial outer membrane permeabilization.[17] In hepatocellular cancer, C6-Ceramide can increase tumor cell apoptosis, reducing tumor cell proliferation.[18] In our study, we observed that the levels of Cer (d18:1/18:0) increased post-LPS/D-gal administration. This may be related to extensive apoptosis in hepatocytes during the onset of ACLF.

Until now, few studies were focused on the effect of HexCer molecules. Evidence from a 20-year cohort study showed that plasma HexCer (d18:1/18:1) might be related to enhanced levels of viral replication in chronic hepatitis C (CHC) virus infection, especially in CHC patients with genotype 2.[19] Another study indicated that HexCer (d18:1/12:0) may be a potential marker of severe hepatic fibrosis in CHC.[20] In our study, we observed elevated levels of HexCer during the onset of ACLF. In this study, we mainly focused on dhCer, there is not much discussion

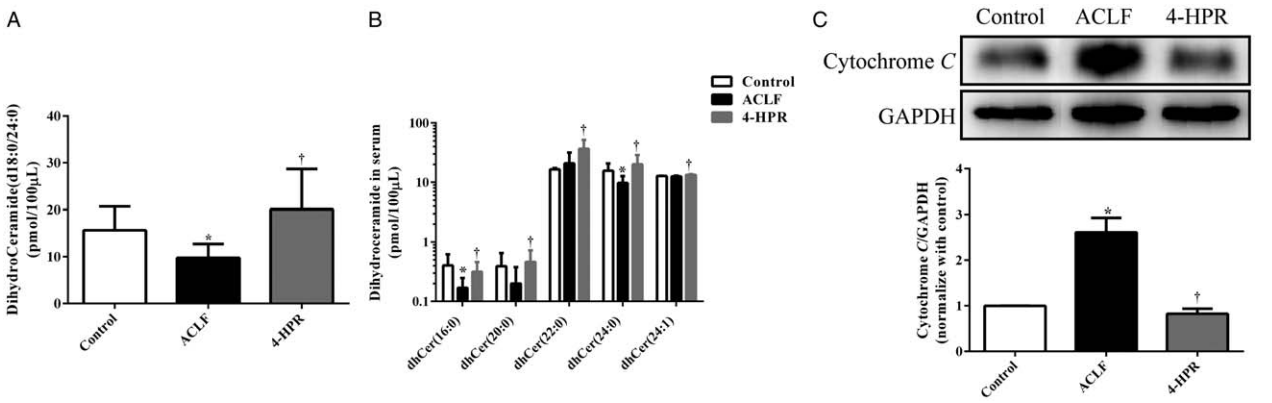

Figure 3: Alleviation of liver damage by 4-HPR in ACLF rats by increasing serum dhCer (24:0) levels and inhibiting hepatocellular apoptosis. (A) The effect of 4-HPR on dhCer (d18:0/24:0) levels in the serum of rats with ACLF. (B) The influence of 4-HPR on dihydroceramides levels in serum of rats with ACLF. (C) The influence of 4-HPR on the expression of cytochrome *C* analyzed by western blotting. The expression is normalized to the housekeeping protein GAPDH. $^{*}P < 0.05$ compared with control group. $^{\dagger}P < 0.05$ compared with ACLF group. 4-HPR: *N*-(4-hydroxyphenyl); ACLF: Acute-on-chronic liver failure; dhCer: Dihydroceramide; GAPDH: Glyceraldehyde 3-phosphate.

about the role of HexCer, but our results provide a reference for subsequent research.

DhCers are the intermediate in the *de novo* pathway. For many years dhCers were considered inactive Cer. However, recent research demonstrated that they are important bioactive molecules.[8] Our previous clinical results showed that dhCer (d18:0/24:0) was significantly lower in non-surviving ACLF patients than in surviving ACLF patients, which indicated that dhCer (d18:0/24:0) may be a beneficial factor for ACLF.[10] Based on our previous study, we further examined the role of dhCer (d18:0/24:0) in an ACLF rat model, to propose a possible preventive measure for ACLF. As expected, the levels of dhCer (d18:0/24:0) were significantly decreased in ACLF rats compared to the levels of dhCer (d18:0/24:0) in control. In addition, the levels of dhCer (d18:0/20:0) and dhCer (d18:0/22:0) also decreased significantly. DhCers are the precursor of Cer in the *de novo* pathway. DES converts dhCer to ceramide. We speculated that pharmacological inhibition of DES may increase the levels of dhCer, which could alleviate liver injury in ACLF. A recent study demonstrated that 4-HPR (or fenretinide), a vitamin A analog, is an inhibitor of DES.[21] The drug fenretinide has entered clinical trials for the treatment of breast cancer.[22] In our results, 4-HPR decreased the mortality rate of ACLF rats and prolonged their survival time. Additionally, 4-HPR significantly reduced the levels of ALT and AST. PT also decreased significantly by 4-HPR treatment. Treatment with 4-HPR also reduced the levels of plasma ammonia. Histopathological examination results indicated that 4-HPR alleviated liver damage in ACLF rats. Importantly, HPLC-MS/MS analysis revealed that the levels of dhCer (d18:0/24:0) were significantly increased by 4-HPR treatment. Furthermore, dhCer (d18:0/24:0) was the only dhCer that decreased significantly in both experiments, suggesting that dhCer (d18:0/24:0) plays an important role in ACLF. A recent study suggested that dhCer could inhibit the formation of ceramide channels in mitochondria and thus, inhibit apoptosis.[13] Another study demonstrated that downregulating DES1 expression could inhibit caspase 9 and caspase 3 activity and ameliorate palmitic acid-mediated apoptosis and cell growth inhibition.[23] In our study, 4-HPR treatment downregulated the expression of an apoptosis-related protein, cytochrome C. Hence, we speculated that the protective effect of 4-HPR on ACLF may be related to its effect in elevating the levels of dhCer (d18:0/24:0) and consequently inhibiting apoptosis in ACLF.

In summary, our results indicated that dhCer (d18:0/24:0) has the potential to serve as a biomarker of ACLF. In addition, DES inhibitors, such as 4-HPR, could alleviate liver damage in ACLF rats by increasing serum dhCer (d18:0/24:0) levels and inhibiting apoptosis of hepatic cells. These findings provide novel insights into the mechanism of ACLF, in this regard, 4-HPR may be a potential drug target for the treatment of ACLF. However, it still some limitations in this study. We proved that dhCer (d18:0/24:0) could be a biomarker for ACLF prognosis, targeting dhCer could be a promising target for the treatment of ACLF, but the underlying mechanism require further investigation. What's more, further verification with more animal models is required.

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Conflicts of interest

None.

References

1. Hernaez R, Solà E, Moreau R, Ginès P. Acute-on-chronic liver failure: an update. Gut 2017;66:541–553. doi: 10.1136/gutjnl-2016-312670.
2. Duseja A, Singh SP. Toward a better definition of acute-on-chronic liver failure. J Clin Exp Hepatol 2017;7:262–265. doi: 10.1016/j.jceh.2017.05.002.
3. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, *et al*. Acute-on-chronic liver failure is a distinct syndrome that develops inv patients with acute decompensation of cirrhosis. Gastroenterology 2013;144:1426–1437.e1-9. doi: 10.1053/j.gastro.2013.02.042.
4. Xue R, Meng Q, Li J, Wu J, Yao Q, Yu H, *et al*. The assessment of multipotent cell transplantation in acute-on-chronic liver failure: a systematic review and meta-analysis. Transl Res 2018;200:65–80. doi: 10.1016/j.trsl.2018.05.006.
5. Wu J, Zheng SS. Liver transplantation in China: problems and their solutions. Hepatobiliary Pancreat Dis Int 2004;3:170–174.
6. Young MM, Kester M, Wang HG. Sphingolipids: regulators of crosstalk between apoptosis and autophagy. J Lipid Res 2013;54:5–19. doi: 10.1194/jlr.R031278.
7. Ahn EH, Schroeder JJ. Induction of apoptosis by sphingosine, sphinganine, and C(2)-ceramide in human colon cancer cells, but not by C(2)-dihydroceramide. Anticancer Res 2010;30:2881–2884.
8. Rodriguez-Cuenca S, Barbarroja N, Vidal-Puig A. Dihydroceramide desaturase 1, the gatekeeper of ceramide induced lipotoxicity. Biochim Biophys Acta 2015;1851:40–50. doi: 10.1016/j.bbalip. 2014.09.021.
9. Lee AY, Lee JW, Kim JE, Mock HJ, Park S, Kim S, *et al*. Dihydroceramide is a key metabolite that regulates autophagy and promotes fibrosis in hepatic steatosis model. Biochem Biophys Res Commun 2017;494:460–469. doi: 10.1016/j.bbrc.2017.10.110.
10. Qu F, Zheng SJ, Liu S, Wu CS, Duan ZP, Zhang JL. Serum sphingolipids reflect the severity of chronic HBV infection and predict the mortality of HBV-acute-on-chronic liver failure. PLoS One 2014;9:e104988. doi: 10.1371/journal.pone.0104988.
11. Li F, Miao L, Sun H, Zhang Y, Bao X, Zhang D. Establishment of a new acute-on-chronic liver failure model. Acta Pharm Sin B 2017;7:326–333. doi: 10.1016/j.apsb.2016.09.003.
12. Qu F, Wu CS, Hou JF, Jin Y, Zhang JL. Sphingolipids as new biomarkers for assessment of delayed-type hypersensitivity and response to triptolide. PLoS One 2012;7:e52454. doi: 10.1371/journal.pone.0052454.
13. Stiban J, Fistere D, Colombini M. Dihydroceramide hinders ceramide channel formation: Implications on apoptosis. Apoptosis 2006;11:773–780. doi: 10.1007/s10495-006-5882-8.
14. Grösch S, Schiffmann S, Geisslinger G. Chain length-specific properties of ceramides. Prog Lipid Res 2012;51:50–62. doi: 10.1016/j.plipres.2011.11.001.
15. Barbarroja N, Rodriguez-Cuenca S, Nygren H, Camargo A, Pirraco A, Relat J, *et al*. Increased dihydroceramide/ceramide ratio mediated by defective expression of degs1 impairs adipocyte differentiation and function. Diabetes 2015;64:1180–1192. doi: 10.2337/db14-0359.
16. Chavez JA, Summers SA. A ceramide-centric view of insulin resistance. Cell Metab 2012;15:585–594. doi: 10.1016/j.cmet.2012.04.002.

17. Chang KT, Anishkin A, Patwardhan GA, Beverly LJ, Siskind LJ, Colombini M. Ceramide channels: destabilization by Bcl-xL and role in apoptosis. Biochim Biophys Acta 2015;1848:2374–2384. doi: 10.1016/j.bbamem.2015.07.013.
18. Li G, Liu D, Kimchi ET, Kaifi JT, Qi X, Manjunath Y, *et al*. Nanoliposome C6-ceramide increases the anti-tumor immune response and slows growth of liver tumors in mice. Gastroenterology 2018;154:1024–1036.e9. doi: 10.1053/j.gastro.2017.10.050.
19. Zhang JY, Qu F, Li JF, Liu M, Ren F, Zhang JY, *et al*. Up-regulation of plasma hexosylceramide (d18: 1/18: 1) contributes to genotype 2 virus replication in chronic hepatitis C: a 20-year cohort study. Medicine 2016;95:e3773. doi: 10.1097/MD.0000000000003773.
20. Li JF, Qu F, Zheng SJ, Ren F, Wu HL, Liu M, *et al*. Plasma sphingolipids: potential biomarkers for severe hepatic fibrosis in chronic hepatitis C. Mol Med Rep 2015;12:323–330. doi: 10.3892/mmr.2015.3361.
21. Rahmaniyan M, Curley RW Jr, Obeid LM, Hannun YA, Kraveka JM. Identification of dihydroceramide desaturase as a direct in vitro target for fenretinide. J Biol Chem 2011;286:24754–24764. doi: 10.1074/jbc.M111.250779.
22. Siddique MM, Li Y, Wang L, Ching J, Mal M, Ilkayeva O, *et al*. Ablation of dihydroceramide desaturase 1, a therapeutic target for the treatment of metabolic diseases, simultaneously stimulates anabolic and catabolic signaling. Mol Cell Biol 2013;33:2353–2369. doi: 10.1128/MCB.00226-13.
23. Zhu Q, Yang J, Zhu R, Jiang X, Li W, He S, *et al*. Dihydroceramide-desaturase-1-mediated caspase 9 activation through ceramide plays a pivotal role in palmitic acid-induced HepG2 cell apoptosis. Apoptosis 2016;21:1033–1044. doi: 10.1007/s10495-016-1267-9.

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