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Original Article

# Ascorbate lacks significant influence in rats with bile duct ligation-induced liver injury

Hsin-Ling Ho [a,d,e], Teh-Ia Huo [a,d], Ting Chang [b], Wen-Shin Lee [b,e], I-Fang Hsin [c,d,e], Fa-Yauh Lee [a,e], Hui-Chun Huang [a,b,e,*], Ming-Chih Hou [a,e], Shou-Dong Lee [e,f]

[a] *Division of Gastroenterology and Hepatology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, ROC*
[b] *Division of General Medicine, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, ROC*
[c] *Division of Endoscopy Center for Diagnosis and Treatment, Taipei Veterans General Hospital, Taipei, Taiwan, ROC*
[d] *Department and Institute of Pharmacology, National Yang-Ming University School of Medicine, Taipei, Taiwan, ROC*
[e] *Faculty of Medicine, National Yang-Ming University School of Medicine, Taipei, Taiwan, ROC*
[f] *Division of Gastroenterology, Department of Medicine, Cheng Hsin General Hospital, Taipei, Taiwan, ROC*

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**Abstract**

*Background*: Liver inflammation may induce fibrogenesis, cirrhosis and portal hypertension. Liver cirrhosis is characterized by increased intrahepatic resistance and enhanced vasoconstrictive response. The splanchnic vasodilatation, angiogenesis and portosystemic collaterals formation further bring about lethal complications. Ascorbate is a potent antioxidant with anti-inflammation, anti-fibrosis, and anti-angiogenesis effects. However, the relevant influences in chronic liver injury have not been sufficiently explored.
*Methods*: Chronic liver injury was induced in Spraque-Dawley rats with common bile duct ligation (BDL). Ascorbate (250 mg/kg/day, oral gavage) or vehicle was administered starting on the 1st day after operation. On the 8th (hepatitis) and 29th (cirrhosis) day, serum biochemistry parameters, hepatic concentrations of lipid peroxidation-related substances, protein expressions of α-SMA, TGF-β, iNOS, eNOS, p-eNOS-Ser1177, p-eNOS-Thr496, VEGF, VEGFR2, p-VEGFR2, and liver histology were evaluated. In three series of paralleled groups, rats treated with 28-day ascorbate or vehicle received hemodynamic measurements, hepatic and collateral vasoresponsiveness perfusion experiments, mesenteric CD31 immunofluorescence staining, and Western blot analyses of mesenteric VEGF, VEGFR2, pVEGFR2, PDGF, PDGFβ, COX1, COX2, eNOS, p-eNOS-Thr495, p-eNOS-Ser1177 protein expressions. In another series, the severity of portosystemic shunting was evaluated.
*Results*: Ascorbate did not influence hepatitis, oxidative stress, fibrosis, and hemodynamic parameters in BDL rats. The intrahepatic and collateral vasoresponsiveness were not affected, either from direct incubation or acute treatment with ascorbate. Furthermore, the mesenteric angiogenesis and severity of shunting were not influenced.
*Conclusion*: The oxidative stress, fibrosis, hemodynamic derangements, angiogenesis and vascular functional changes in BDL-induced chronic liver injury may be too overwhelming to be modulated by ascorbate.
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*Keywords:* Ascorbate; Hepatitis; Liver cirrhosis; Portal hypertension

## 1. Introduction

Chronic liver injury may elicit a cascade of fibrogenesis and ultimately, liver cirrhosis and portal hypertension. Liver cirrhosis is characterized by increased intrahepatic resistance owing to fibrotic bands and regeneration nodules (structural component) and enhanced intrahepatic vasoconstrictive

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* Corresponding author: Dr. Hui-Chun Huang, Division of General Medicine, Department of Medicine, Taipei Veterans General Hospital, 201, Section 2, Shi-Pai Road, Taipei 112, Taiwan, ROC.
*E-mail address:* hchuang2@vghtpe.gov.tw (H.-C. Huang).

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response (functional component). The insufficient intrahepatic bioavailability of vasodilators such as nitric oxide (NO) is also noted.[1] On the other hand, the peripheral and splanchnic vasodilatation lead to decreased systemic vascular resistance, compensatorily increased heart rate and cardiac output, increased splanchnic blood flow, portal inflow and portal pressure (PP). The difficulty of the portal blood flow in entering the liver induces formation of portosystemic collaterals,[2] in which angiogenesis also plays a role.[3] Recent studies have revealed that vascular endothelial growth factor (VEGF) is overexpressed in splanchnic organs from portal hypertensive rats. Furthermore, angiogenesis triggers the development and maintenance of splanchnic hyperemia and portosystemic collaterals, which can be ameliorated by VEGFR2 (VEGF receptor 2) blockade.[4,5] These derangements, actually, are responsible for dreadful complications such as gastroesophageal variceal hemorrhage.

Indeed, apart from managing complications of liver cirrhosis and portal hypertension, the control of hepatic injury at the beginning is pivotal, and the substances easily obtained and inexpensive without significant toxicity are ideal candidates. It has been identified that oxidative stress with lipid peroxidation is a common pathological mechanism contributing to the initiation and progression of hepatic damage.[6] Upon inflammation, lipid peroxidation products recruit neutrophil and aggravates liver injury.[7] They also launch fibrogenesis by enhancing fibrogenic gene expression in activated stellate cells.[8,9] Therefore, antioxidant administration is a potentially feasible therapeutic strategy to delay the process of liver damage.[10]

Among the food-derived antioxidants, ascorbate (ascorbic acid, vitamin C) may be the most well-known and prevalent. Ascorbate provides *in vivo* antioxidant protection primarily as an aqueous phase peroxyl and oxygen radical scavenger. It is concentrated in tissues with a high potential for free radical generation, such as the eye, brain, liver, lung, and heart. Some evidence indicates that ascorbate protects against lipid peroxidation by regenerating the reduced form of α-tocopherol, the primary lipid-phase antioxidant.[11,12]

Regarding the levels of ascorbate in patients with chronic liver diseases, it has been reported that antioxidant vitamin levels, especially vitamin C, were significantly lower in patients with primary biliary cirrhosis.[13] In another study, a significant decrease in plasma ascorbate level was observed in patients with chronic active hepatitis and liver cirrhosis,[14] indicating that these patients were subject to oxidative stress.

Ascorbate has also aroused much attention due to its beneficial vascular effects. Ascorbate reversed NO-dependent endothelial dysfunction in coronary or peripheral arteries of patients with atherosclerosis.[15] Heitzer et al.[16] measured forearm microvascular responses to acetylcholine in long-term smokers, and observed a marked improvement in acetylcholine-induced vasodilation with intra-arterial high dose infusion of vitamin C. Regensteinera et al. also found that ascorbate improved flow-mediated brachial artery dilation (FMD) in patients with type 2 DM.[17] Similarly, Motoyama et al. observed a significant short-term improvement in brachial artery endothelial function after acute parenteral infusion of vitamin C in smokers. At the same time, a significant decrease was seen in the plasma levels of thiobarbituric acid–reactive substances, suggesting an acute reduction in oxidative stress.[18]

The vascular effect of ascorbate may be mediated via the modulation of NO activities. L-ascorbic acid potentiates agonist-induced endothelial NO synthesis.[19,20] Decreased endothelial NO activity is a common feature of type 2 diabetes and may contribute to the development of vascular complications, which can be improved by intra-arterial administration of vitamin C.[21] Ascorbate also potentiates the tetrahydrobiopterin-dependent endothelial NOS (eNOS) activation via enhancing the affinity of tetrahydrobiopterin for eNOS.[22] Regarding chronic treatment, Hornig et al.[23] observed a significant improvement in radial artery FMD in patients with chronic heart failure after four weeks of high dose oral vitamin C supplementation (1 g twice daily).[24] Recently, Gokce et al. reported a sustained beneficial effect on endothelial function after one-month treatment with ascorbate.[25]

It is worth noting that ascorbate inhibits angiogenesis as well.[26] The level of VEGF was significantly higher in mice challenged with melanoma B16FO cells than in ascorbate-supplemented mice.[27] A previous study indicated a significant inverse correlation between ascorbate and VEGF protein levels.[28] It has also been found that L:-ascorbate inhibited the secretion of vascular VEGF and the growth of xenograft pancreatic tumor in athymic mice.[29]

The distinct features of ascorbate may be beneficial for various stages of liver injury, but the relevant survey has not yet been performed. In addition, ascorbate is water-soluble without significant toxicity, which makes it feasible for patients with liver diseases. This study, therefore, aimed to investigate if ascorbate modulates the aforementioned aspects in rats with common bile duct ligation (BDL)-induced chronic liver injury.

## 2. Methods

### 2.1. Animal model: common bile duct ligation (BDL)

Bile duct ligation is a well-established animal model to induce liver injury via cholestasis. Typically, liver inflammation and cirrhosis develop beginning on the 5th and 28th day after BDL,[30,31] respectively, which makes this model appropriate for the survey of various stages of liver injury. In this study, male Sprague–Dawley rats weighing 240–270 g at the time of surgery were used. BDL was performed as previously described[32] under ketamine anesthesia (100 mg/kg, intramuscularly). To avoid the coagulation defects, weekly vitamin K injection (50 μg/kg intramuscularly) was applied.[30] All the procedures were conducted in accordance with the principles of laboratory animal care [Guide for the Care and Use of Laboratory Animals, DHEW publication No. (NIH) 85-23, rev. 985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD., USA.]. The Taipei Veterans General Hospital

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