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Effect of ascorbic acid on hepatic vasoregulatory gene expression during polymicrobial sepsis

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Abstract

The aim of this study was to investigate the effects of ascorbic acid on hepatic vasoregulatory gene expression during polymicrobial sepsis. Rats were subjected to polymicrobial sepsis by cecal ligation and puncture (CLP). Rats received either vehicle (n = 10) or ascorbic acid (AA, 100 mg/kg, n = 10) intravenously immediately after the CLP procedure. Serum aminotransferase levels and hepatic lipid peroxides markedly increased 24 h after CLP and this increase was attenuated by AA treatment. The hepatic concentrations of reduced glutathione decreased in CLP animals. This decrease was inhibited by AA. CLP significantly increased the mRNA level of ET-1 ($p < 0.01$) and ET_B receptor ($p < 0.01$) in livers; an increase that was prevented by AA treatment. There were no significant changes in ET_A mRNA expression among any of the experimental groups. There were significant increases in the mRNA expression of nitric oxide synthases ($p < 0.01$) and heme oxygenase-1 ($p < 0.01$) in livers from CLP animals. This increase was prevented by AA treatment. The expression of tumor necrosis factor-α and cyclooxygenase-2 mRNAs significantly increased 4.9-fold ($p < 0.01$) and 4.4-fold ($p < 0.01$) in livers from CLP animals, respectively. This increase was attenuated by AA treatment. Our data suggest that AA reduces oxidative stress and lipid peroxidation, regulates the hepatic vasoregulatory gene expression in polymicrobial sepsis and thus it could reduce hepatic microvascular dysfunction during sepsis.

Keywords: Ascorbic acid; Polymicrobial sepsis; Oxidative stress; Hepatic vasoregulatory gene expression

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Introduction

Sepsis and the related systemic inflammatory syndrome and multiple organ dysfunction syndrome (MODS) continue to be the most common causes of morbidity and mortality in intensive care units, despite various therapeutic advances in the management of sepsis (Barriere and Lowry, 1995). In these disorders, it is well established that outcome correlates with the number of dysfunctional organ systems and that hepatic insufficiency is an especially important indicator of poor outcome (Knaus et al., 1985). However, the nature of hepatic dysfunction and the processes that mediate its development remain poorly understood.

Several lines of evidence suggest that the embarrassment of microvascular blood flow during sepsis may contribute to the subsequent development of MODS (Baveja et al., 2002). Liver microcirculation is normally maintained under the fine balance of vasoconstrictors and vasodilators, of which endothelin-1 (ET-1), nitric oxide (NO), and carbon monoxide have been reported to be the prominent vasomediators (Pannen et al., 1996, 1998). Although posttranscriptional modifications can occur, these vasomediators are primarily controlled at the level of transcription (Beck and Sterzel, 1996). There is evidence of changes in transcripts of the genes related to the vascular mediators in the rat liver in response to an acute dose of endotoxin (Sonin et al., 1999), however, it is unknown if a similar mechanism operates in a more complicated scenario of polymicrobial sepsis.

There is convincing evidence to suggest that severe oxidative stress occurs in patients with sepsis (Macdonald et al., 2003). Reactive oxygen species (ROS) have provoked considerable interest in recent years as major contributors to endotoxin-induced tissue injury (Rackow and Astiz, 1993). Oxidative damage to biomolecules can be inhibited by antioxidants (Berger et al., 1997). Ascorbic acid (AA) has been reported to preserve cardiac tissue and reduce the oxidative indices after ischemia/reperfusion (Barta et al., 1991). More recently, it was reported that AA prevents microvascular dysfunction in septic animals (Armour et al., 2001).

Therefore, the purpose of this study was to investigate the effect of AA on the imbalanced hepatic vasoregulatory gene expression observed in response to sepsis.

Materials and methods

Chemicals

L-Ascorbic acid, isopropanol, diethyl pyrocarbonate (DEPC), and ethidium bromide (EtBr) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). RNA PCR kit R019A (AMV) and Ex Taq® DNA polymerase were purchased from TaKaRa Schuzo Co. (Shiga, Japan). All other chemicals used were of reagent grades and were locally and commercially available.

Cecal ligation and puncture (CLP)

Polymicrobial sepsis in rats was induced by CLP according to the method of Chaudry et al. (1979). Male Sprague–Dawley rats (275–300 g) were fasted overnight but allowed to drink tap water ad libitum. All animals were treated humanely under Sungkyunkwan university Animal Care committee guidelines. After anesthetized by the intraperitoneal injection of pentobarbital sodium (30 mg/kg), and a 2-cm