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Acute ethanol intoxication suppresses pentraxin 3 expression in a mouse sepsis model involving cecal ligation and puncture

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ABSTRACT

Acute ethanol intoxication impairs immunological reactions and increases the risk of sepsis; however, the underlying mechanism remains unclear. Pentraxin (PTX) 3 is a humoral pattern recognition receptor whose levels rapidly increase in response to inflammation. PTX3 production is triggered by tumor necrosis factor (TNF)-α and is mediated by c-Jun N-terminal kinase (JNK). As PTX3 exerts protective effects against sepsis as well as acute lung injury, we investigated whether acute ethanol exposure exacerbates sepsis by altering PTX3 expression. Sepsis was induced in C57/BL6 mice by cecal ligation and puncture (CLP) after ethanol/saline administration. Survival rates were significantly lower in ethanol-treated than in saline-treated mice. Increased vascular permeability and attenuation of PTX3 expression were observed in the lungs of ethanol-treated mice 4 h after CLP. Concomitant with a delayed increase of plasma TNF-α in ethanol-treated mice, plasma PTX3 was also suppressed in the early phase of sepsis. Although TNF-α level in ethanol-treated mice exceeded that in saline-treated mice 16 h after CLP, PTX3 levels were still suppressed in the former group. JNK phosphorylation in lung tissue was suppressed in both groups 4 and 16 h after CLP. Furthermore, JNK phosphorylation in ethanol-treated human umbilical vein endothelial cells was suppressed even in the presence of exogenous TNF-α, resulting in inhibition of PTX3 mRNA and protein expression. Our results suggest that ethanol suppresses *de novo* PTX3 synthesis via two mechanisms – i.e., suppression of TNF-α production and inhibition of JNK phosphorylation. PTX3 suppression may therefore contribute to exacerbation of sepsis in acute ethanol intoxication.

Introduction

Sepsis is a leading cause of death among patients in intensive care units. Acute alcohol consumption impairs the innate and adaptive immune responses and undermines host defense against infection (Nelson & Kolls, 2002), resulting in an increased risk of mortality in animal models of sepsis (Pruett, Zheng, Fan, Matthews, & Schwab, 2004; Pruett et al., 2010; Woodman, Fabian, Beard, & Proctor, 1996). However, the mechanisms underlying these effects are not entirely clear.

When pathogenic microorganisms invade, pathogen-associated molecular patterns (PAMPs) stimulate the innate immune response and activate intracellular signal transduction pathways that lead to production of proinflammatory cytokines related to innate immunity (Harris & Raucci, 2006). Innate immunity is activated in the early phase of sepsis, and cytokine and chemokine levels increase rapidly. Uncontrolled production of proinflammatory cytokines leads to endothelial disruption as well as vascular leakage.

Pentraxin (PTX)3 has received considerable attention as a humoral mediator of innate immunity (Goodman et al., 1996). PTX3 belongs to a superfamily of proteins classified as long PTXs, in contrast to the short PTXs such as C-reactive protein (CRP). PTX3 is produced in cells throughout the body – including in endothelial cells – mainly in response to tumor necrosis factor (TNF)-α stimulation, whereas CRP is produced only in the liver in response to stimulation by interleukin (IL)-6 (Bottazzi et al., 1997). PTX3 plays an important role in the early phase of inflammation as a humoral pattern recognition receptor (PRR) (Garlanda, Bottazzi, Bastone, & Mantovani, 2005). PTX3 recognizes PAMPs expressed by microorganisms and is well known to exert favorable effects on systemic sepsis as well as local infection and inflammation, for instance upon acute lung injury (Daigo, Mantovani, & Bottazzi, 2014; Han et al., 2012).

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The effect of acute alcohol consumption on PTX3 production — which is primarily mediated by c-Jun N-terminal kinase (JNK) signaling (Dong et al., 2011; Han et al., 2005) — is unknown. We hypothesized that acute ethanol exposure alters PTX3 expression under septic conditions. Suppression of PTX3 production is presumed to exacerbate sepsis owing to disruption of lung endothelial integrity. Here, we propose an association between acute alcohol intoxication and sepsis risk in the context of PTX3 production.

Material and methods

Mouse model of acute ethanol intoxication and sepsis

Animal experiments were performed in accordance with the ARRIVE guidelines and were approved by the Nara Medical University Institutional Animal Care and Use Committee. Cecal ligation and puncture (CLP) was performed as previously described (Rittirsch, Huber-Lang, Flierl, & Ward, 2009), with slight modifications. Briefly, a 32% ethanol solution (v/v in saline; 6 g/kg) was administered by gavage to male C57/BL6 mice (10–12 weeks of age). The dose of ethanol was determined according to previous reports (Pruett et al., 2004, 2010). Control mice received saline. In a preliminary experiment, we confirmed that mice did not die from binge ethanol administration over a 48-h observation period. Mice were anesthetized with isoflurane 30 min after ethanol or saline administration, and CLP was performed. For some experiments, a sham operation was performed as a negative control.

Blood samples

Blood samples were collected by cardiac puncture at the indicated times after CLP under anesthesia with isoflurane. Whole blood was anticoagulated with 3.8% sodium citrate (9:1 v/v), and plasma samples were prepared by centrifugation at 3000×*g* for 15 min.

Blood alcohol concentration

Blood alcohol concentration (BAC) was measured by headspace gas chromatography (GC-8A, Shimadzu, Kyoto, Japan) as described elsewhere (Shi et al., 2015).

Lung histology

Immediately after anesthesia and exsanguination, lungs were dissected and inflated via a tracheal cannula with 10% formalin and immersed in formalin overnight. Tissue sections were stained with hematoxylin and eosin, and alveolar septal wall thickness was measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA) as previously described (Sureshbabu et al., 2015). For immunohistochemistry, fixed lung tissue sections were stained with anti-PTX3 antibody (Santa Cruz, Dallas, TX, USA).

Lung vascular permeability assay

Lung vascular permeability was evaluated as previously described (Tressel et al., 2011). Briefly, Evans Blue dye (200 μL at 20 mg/kg; Sigma–Aldrich, St. Louis, MO, USA) was injected into the jugular vein 4 h after CLP. After allowing the dye to circulate for 30 min, mice were sacrificed and their lungs flushed with phosphate-buffered saline (PBS) and weighed. The dye was extracted overnight at 60 °C with formamide and the absorbance at 620 nm was measured. The optical density at 620 nm was corrected by lung weight.

Detection of thrombin-antithrombin (TAT) complex

TAT levels in mouse plasma were determined with a mouse-specific enzyme-linked immunosorbent assay (ELISA) kit (Abcam, Cambridge, UK) according to the manufacturer's instructions.

Measurement of nitrite and nitrate levels

To determine nitric oxide (NO) levels in mouse plasma, plasma samples were centrifuged with an Amicon Ultra-0.5 Centrifugal Filter Device (Millipore, Billerica, MA, USA) for deproteinization. Concentrations of nitrite and nitrate (NOx), the stable NO metabolites, were determined by the Griess reaction (NO_2/NO_3 Assay Kit-C II; Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions.

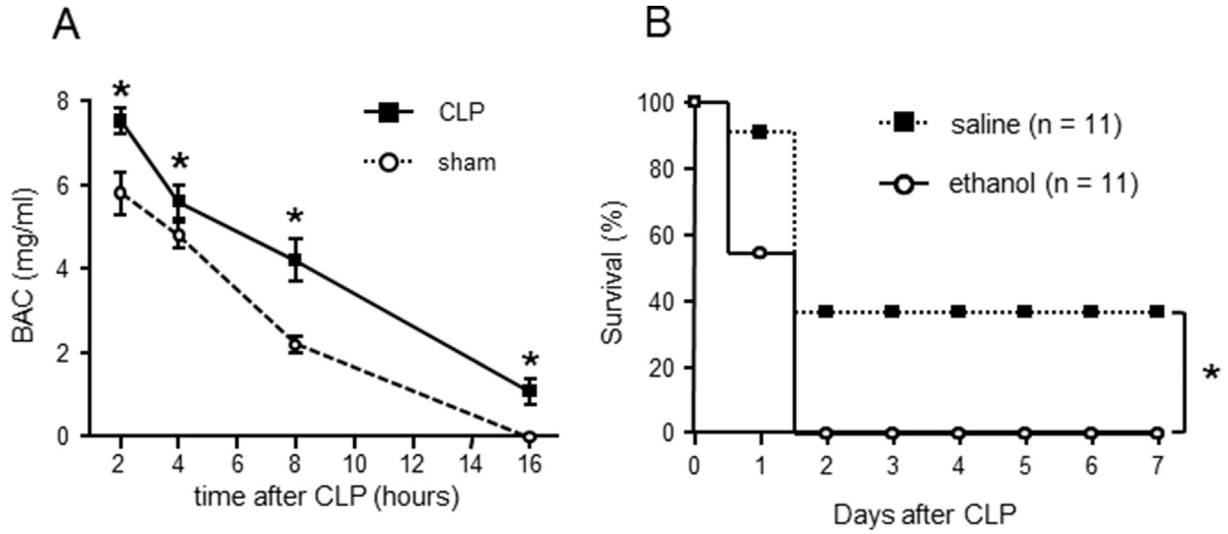

Fig. 1. **(A)** Time course analysis of BAC. Blood samples were collected at indicated time points, and plasma was obtained by centrifugation (n = 4–6). *$p < 0.05$, CLP (■) vs. sham (○) mice (Student's *t* test). **(B)** Effects of acute ethanol intoxication on survival rates of CLP-induced septic mice. Mice were administered ethanol (○, 6 g/kg) or saline (■) by gavage (n = 11). Survival rates differed between the two groups ($p = 0.011$, log-rank test).