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Protective Effects of Urinary Trypsin Inhibitor on Systemic Inflammatory Response Induced by Lipopolysaccharide

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Summary **Urinary trypsin inhibitor (UTI), a serine protease inhibitor, has been widely used in Japan as a drug for patients with acute inflammatory disorders such as disseminated intravascular coagulation (DIC), shock, and pancreatitis. Recent *in vitro* studies have demonstrated that serine protease inhibitors may have anti-inflammatory properties beyond their inhibition of neutrophil elastase at the site of inflammation. However, the therapeutic effects of UTI *in vivo* remain unclear. In this review, we introduce the roles of UTI in the experimental systemic inflammatory response induced by both intraperitoneal and intratracheal administration of lipopolysaccharide using UTI deficient and wild-type mice. Our experiments suggest that UTI can protect against systemic inflammatory response and subsequent organ injury induced by bacterial endotoxin, at least partly, through the inhibition of proinflammatory cytokine and chemokine expression. UTI may therefore present an attractive "rescue" therapeutic option for systemic inflammatory response syndromes such as DIC, acute lung injury, and multiple organ dysfunction.**

Key Words**: urinary trypsin inhibitor, lipopolysaccharide, fibrinogen, cytokine**

Introduction

Bacterial infection can evoke shock, acute respiratory failure, multiple organ failure, and disseminated intravascular coagulation (DIC), resulting in a high mortality rate. Lipopolysaccharide (LPS), a major component of the outer membrane of gram-negative bacteria, initiates the cascade of pathophysiological reactions called endotoxin shock [*1*]. A number of mediators including lipid mediators, cytokines, free radicals, complement fragments, coagulatory factors, and proteases contribute to the pathogenesis of endotoxin shock [*2–6*]. Among them, the products generated from neutrophils are recognized to play important roles. Activated neutrophils release various types of mediators including proteases and oxygen radicals [*7*]. Protease-antiprotease imbalance is involved in a variety of inflammatory diseases [*8*, *9*]. Because neutrophil elastase exerts the most injurious effects on many substrates (e.g. elastin, type I–IV collagen, fibronectin, laminin, and proteoglycans), it can be a key mediator of tissue injury [*10*]. Indeed, neutrophil elastase and cathepsin G-deficient mice have shown resistance to lethal effects of LPS [*11*]. Further, deficiency of secretory leukoprotease inhibitor also reportedly caused higher mortality from endotoxin shock with higher production of IL-6 and high mobility group-1 [*12*]. In addition, Fitzal and co-workers have shown that blockade of pancreatic proteases in the intestinal lumen ameliorates systemic inflammation induced by intravenous administration of LPS [*13*]. Therefore, protease inhibitors may provide a therapeutic option for inflammatory diseases/conditions.

UTI is a multivalent Kunitz-type serine protease inhibitor

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that is found in human urine and blood. UTI is degenerated from pre-α-/inter-α-trypsin inhibitors during inflammation [*14*]. UTI reportedly inhibits neutrophil elastase activity *in vitro* [*15*, *16*] and trypsin activity in patients with pancreatitis [*17*]. Although the therapeutic effects of UTI on circulatory shock have been recognized, especially in Japan, the current understanding of the target mechanisms/pathways remains unsatisfactory. Tani and colleagues reported that UTI protects against septic shock induced by gram-negative bacteria *in vivo*, but only estimated clinical signs such as cardiac index, blood pressure, lactic acid, blood glucose, and blood base values [*18*]. Another group showed that UTI protects against hemorrhagic shock by preserving myocardial mitochondrial function [*19*]. However, these studies have a critical limit in that the animals were treated with human-derived UTI as a foreign protein; the direct effect of UTI on inflammatory diseases including systemic inflammatory response (SIRS) syndrome has never been examined in knock-out mice.

Role of UTI in Systemic Inflammation Induced by Intraperitoneal LPS Challenge

At first, the role of UTI in systemic inflammation using mice deficient in UTI gene [*20*] was investigated. Both UTI (–/–) and wild-type (C57/BL6: WT) mice were injected intraperitoneally (i.p.) with vehicle or LPS at a dose of 1 mg/kg body weight. Phosphate-buffered saline (PBS) was used as the vehicle for LPS. Evaluation of coagulatory and fibrinolytic parameters and white blood cell (WBC) counts at 72 h after i. p. challenge showed that fibrinogen levels were significantly greater in LPS-challenged WT mice ($p<0.05$) and LPS-challenged UTI (–/–) mice ($p<0.01$) than in vehicle-challenged mice with the same genotypes. In the presence of LPS, they were also significantly higher in UTI (–/–) mice than in WT mice ($p<0.05$). WBC counts significantly decreased after LPS challenge in UTI (–/–) mice ($p<0.01$ versus other groups). LPS appeared to shorten PT when compared to vehicle treatment in UTI (–/–) mice, although this difference did not reach significance. In the presence of LPS, prothrombin time was significantly shorter in UTI (–/–) mice than in WT mice ($p<0.05$).

Histopathological changes in the lung, kidney, and liver of both genotypes at 72 h after LPS challenge revealed severe neutrophilic inflammation in UTI (–/–) lungs challenged with LPS. In contrast, little neutrophilic infiltration was found in LPS-treated WT mice. Vehicle treatment caused no histopathological changes in either genotype. LPS challenge induced neutrophilic infiltration around glomeruli and in the interstinum of the kidneys of both genotypes. However, the severity was more prominent in UTI (–/–) mice than in WT mice in the presence of LPS. Vehicle treatment caused no histopathological changes in either genotype. LPS caused wide spread centrilobular vacuolation of hepatocytes and neutrophilic infiltration in livers of both genotypes. In the presence of LPS, there were no significant differences between genotypes. Vehicle treatment caused no histopathological changes in either genotype.

The protein expression of interleukin (IL)-1β, macrophage inflammatory protein (MIP)-1α, MIP-2, macrophage chemoattractant protein (MCP)-1, and keratinocyte chemoattractant [*21*] in the lung, kidney, and liver 72 h after LPS administration was evaluated. In the lung, LPS challenge caused significant elevation of IL-1β, MIP-1α, MIP-2, MCP-1, and keratinocyte-derived chemoattractant (KC) levels in UTI (–/–) mice when compared to vehicle challenge ($p<0.01$). In WT mice, LPS treatment significantly enhanced the expression of IL-1β, MIP-1α, MIP-2, MCP-1, and KC when compared to vehicle challenge. In the presence of LPS, the lung expression of MCP-1 was significantly greater in UTI (–/–) mice than in WT mice ($p<0.05$). The results in the kidney and liver were similar to those in the lung. These results suggest that UTI protects against systemic inflammation induced by intraperitoneal administration of LPS, at least partly, through the inhibition of proinflamatory cytokine production/release [*22*].

Role of UTI in Acute Lung Inflammation Induced by Intratracheal Challenge of LPS

In another series of studies, the role of UTI in another type of SIRS, acute lung inflammation induced by pulmonary exposure to LPS, was examined. In brief, UTI (–/–) and WT mice were intratracheally treated with vehicle or LPS, and sacrificed 24 h later. In both genotypes, LPS treatment induced significant increases in the numbers of total cells and neutrophils in bronchoalveolar lavage (BAL) fluid as compared with vehicle treatment ($p<0.01$). LPS treatment caused greater and significant increases in the numbers of BAL total cells ($p<0.05$) and neutrophils ($p<0.01$) in UTI (–/–) mice than in WT mice. Also, LPS treatment increased the lung water content in both genotypes of mice ($p<0.05$) when compared to vehicle treatment. UTI (–/–) mice, however, showed a significantly greater increase in lung water content when compared to WT mice ($p<0.05$) following LPS treatment. Lung specimens stained with hematoxylin and eosin 24 h after intratracheal instillation showed that in the presence of LPS, WT mice showed moderate infiltration of neutrophils. In UTI (–/–) mice, LPS treatment led to a marked recruitment of neutrophils and interstitial edema. Vehicle administration alone caused no histological changes in either WT or UTI (–/–) mice. LPS treatment induced significant elevation of the protein levels of IL-1β, MIP-1α, MCP-1, and KC in lung homogenates when compared to vehicle treatment in both genotypes ($p<0.01$). In the presence of LPS, the local expression of

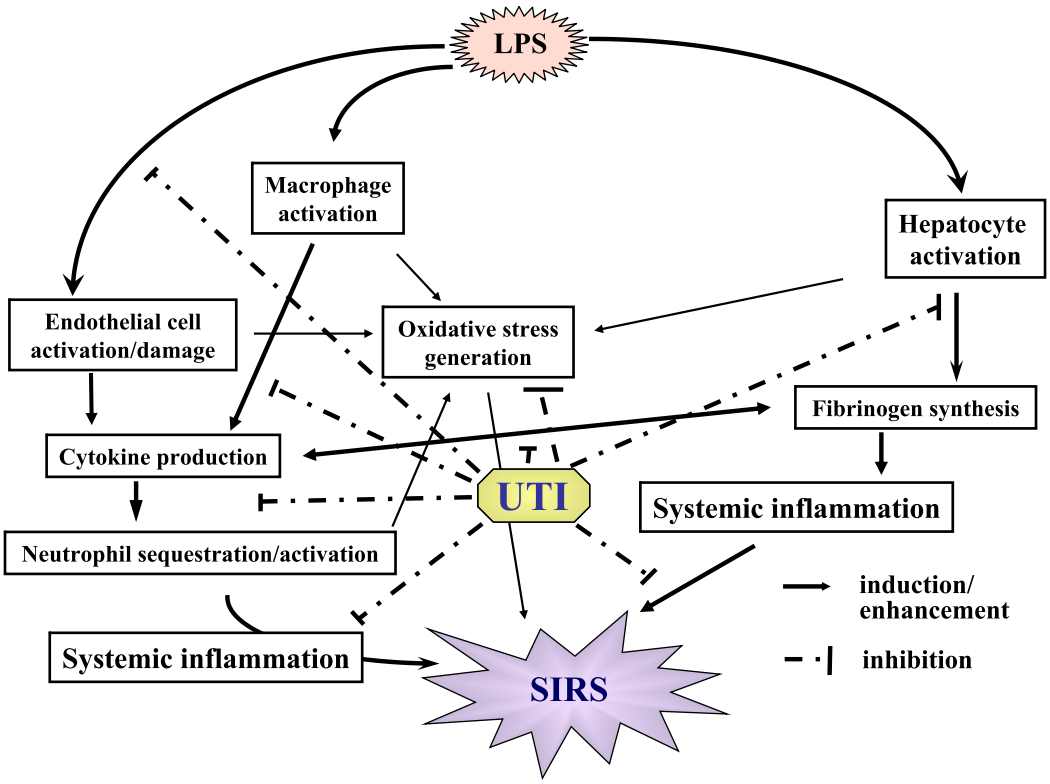

Fig. 1. Shematic representation of the protective role of UTI against SIRS induced by LPS in mice. Our data suggest that UTI is protective against 1) endothelial activation/damage, 2) proinflammatory cytokine and chemokine production and/or release, 3) fibrinogen synthesis, 4) neutrophil recruitment into organs, and/or 5) organ injury.

MCP-1 and KC was significantly higher in UTI (–/–) mice than in WT mice ($p<0.05$ for KC, $p<0.01$ for MCP-1). Furthermore, immunohistochemical examination showed that in the presence of LPS, immunoreactive 8-hydroxy-2'-deoxyguanosine was detected in the lungs of both strains of mice, but the staining was more prominent in UTI (–/–) mice than in WT mice. On the other hand, immunoreactive nitrotyrosine was strongly detected only in UTI (–/–) mice challenged with LPS. Quantitative gene expression analyses of lung homogenates obtained 4 h after intratracheal challenge showed that compared to vehicle treatment, LPS treatment resulted in significant elevation of gene expression for inducible nitric oxide synthase (iNOS) in both genotypes of mice ($p<0.05$). In the presence of LPS, the local expression of iNOS was higher in UTI (–/–) mice than in WT mice. These results suggest that UTI is also protective against acute lung inflammation induced by intratracheal administration of LPS, at least in part, via the local suppression of proinflammatory cytokines [*23*] and antioxidation [*24*].

In conclusion, UTI protects against SIRS pathophysiology and subsequent organ damage induced by LPS in mice, at least partly, via the modulation of the proinflammatory cytokine IL-1β, as well as chemokines such as MIP-2, MCP-1, and KC (Fig. 1). These *in vivo* results provide direct and novel molecular evidence for the "rescue" therapeutic potential of UTI against systemic inflammatory responses syndrome such as DIC, acute lung injury, and multiple organ dysfunction syndrome.

Abbreviations

UTI, urinary trypsin inhibitor; LPS, lipopolysaccharide; UTI (–/–), UTI-deficient; WT, wild type; MCP, macrophage chemoattractant protein; KC, keratinocyte chemoattractant; DIC, disseminated intravascular coagulation; PHS, prostaglandin H2; TX, thromboxane; IL, interleukin; TNF, tumor necrosis factor; i.p., intrperitoneally; PBS, phosphate-buffered saline; PT, prothrombin time; FDP, fibrinogen/fibrin degradation; WBC, white blood cell; ELISA, enzyme-linked immunosorbent assays; MIP, macrophage inflammatory protein.

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