Introduction
Urinary trypsin inhibitor, a serine protease inhibitor, has been widely used, particularly in Japan, as a drug for patients with acute inflammatory disorders such as pancreatitis, shock and disseminated intravascular coagulation. Previous in vitro studies have demonstrated that serine protease inhibitors may have anti-inflammatory properties beyond their inhibition of neutrophil elastase at sites of inflammation. However, the therapeutic effects of urinary trypsin inhibitor in vivo remain unclarified. In this review, we introduce the roles of urinary trypsin inhibitor in experimental systemic inflammatory responses induced by both the intraperitoneal and intratracheal administration of lipopolysaccharide using urinary trypsin inhibitor-deficient (−/−) and corresponding wild-type mice.

Conclusion
Urinary trypsin inhibitor may provide an attractive ‘rescue’ therapeutic option for systemic inflammatory response syndromes such as disseminated intravascular coagulation, acute lung injury and acute liver injury.

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Urinary trypsin inhibitor may provide an attractive ‘rescue’ therapeutic option for systemic inflammatory response syndromes such as disseminated intravascular coagulation, acute lung injury and acute liver injury.

Urinary trypsin inhibitor as a therapeutic option for inflammatory disorders
KI Inoue*, H Takano

Various serine proteases such as trypsin, chymotrypsin, neutrophil elastase and plasmin are reportedly inhibited by UTI. Based on the multivalent nature of protease inhibition, UTI appears to prevent organ injury by inhibiting the activity of these proteases. Although the therapeutic effects of UTI on circulatory shock have been recognized, especially in Japan, current understanding regarding the target mechanisms/pathways remains limited. The aim of this review was to discuss UTI as a therapeutic option for inflammatory disorders.

Discussion
The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964), and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

General characteristics of UTI
UTI is a multivalent Kunitz-type serine protease inhibitor found in human urine and blood. UTI, also referred to as ulinastatin, HI-30, ASPI or bikunin, is an acidic glycoprotein with a molecular weight of 30 kDa by SDS-polyacrylamide gel electrophoresis. It is composed of 143-amino acid residues and its sequence includes two Kunitz-type domains. UTI is produced by hepatocytes as a precursor in which UTI is linked to α₂-microglobulin. In hepatocytes, different types of UTI-containing proteins are formed by the assembly of UTI with one or two of the three evolutionarily related heavy chains (HC) 1, 2 and 3, through a chondroitin sulphate chain; these proteins comprise inter-α-inhibitor (IαI) family members, including IαI, pre-α-inhibitor (pαI), inter-α-like inhibitor (IαLI) and free UTI. IαI, pαI and IαLI are composed of HC1 + HC2 + UTI, HC3 + UTI and HC2 + UTI, respectively. During inflammation, UTI is cleaved from IαI family proteins through proteolytic cleavage by neutrophil elastase in the peripheral circulation or at the inflammatory site. Therefore, plasma UTI has been considered to be one of the acute-phase reactions, and indeed, the plasma UTI level and its gene expression alter in severe inflammatory conditions. Further, UTI is rapidly released into urine when infection occurs and is an excellent inflammatory marker, constituting most of the urinary trypsin activity. Various serine proteases such as trypsin; thrombin; chymotrypsin; kallikrein; plasmin; elastase; cathepsin and factors IXa, Xa, Xia and Xlla are inhibited by UTI. Furthermore, UTI can reportedly suppress urokinase-type plasminogen activator (uPA) expression through the inhibition of protein kinase C.

Clinical utility of UTI
Although its precise physiological role in normal subjects has not been fully clarified, clinically, UTI is widely used, especially in Japan, to treat acute pancreatitis including post-endoscopic retrograde cholangiopancreatography pancreatitis and septic shock, in which several proteases are considered to play a pathophysiological role. Also, in gynaecological
area, for instance, UTI therapy is reportedly effective in patients at risk for premature labour, with a moderately developed bulging membrane during the second trimester\(^{29}\), and can reduce meconium-induced chemical peritonitis and thereby facilitate intrauterine remission of foetal ascites\(^{24}\). Further, recently, it is used for the prevention of imminent abortion via vaginal administration.

**Anti-inflammatory property of UTI**

Beyond its inhibition of inflammatory proteases mentioned above, UTI exhibits anti-inflammatory activity and suppresses the infiltration of neutrophils and release of elastase and chemical mediators from them\(^{13,22,23}\). As well, UTI reportedly inhibits the production of tumour necrosis factor (TNF)-\(\alpha\)\(^{24,25}\) and interleukin (IL)-1\(^{26}\) in lipopolysaccharide (LPS)-stimulated human monocytes and LPS- or neutrophil elastase-stimulated IL-8 gene expression in HL60 cells\(^{26}\) or bronchial epithelial cells\(^{27}\) in vitro. Matsuzaki et al. demonstrated that UTI inhibits LPS-induced TNF-\(\alpha\) and subsequent IL-1\(\beta\) and IL-6 induction by macrophages, at least partly, through the suppression of mitogen-activated protein kinase (MAPK) signalling pathways such as ERK1/2, JNK and p38 in vitro\(^{28}\). Nakatani and colleagues demonstrated that UTI inhibits neutrophil-mediated endothelial cell injury in vitro, suggesting that UTI can act directly on neutrophils and suppress the production and secretion of activated elastase from them\(^{29}\). Further, UTI downregulates stimulated arachidonic acid metabolism such as thromboxane B2 production in vitro\(^{30}\), which plays a role in the pathogenesis of sepsis syndrome\(^{30}\).

On the other hand, a large number of in vivo reports have provided evidence that UTI protects against pathological traits related to septic shock induced by gram-negative bacteria: UTI reduces LPS-induced hypotension\(^{31,33}\) through modulating TNF-\(\alpha\) production via the inhibition of early growth response factor (Egr)-1 in monocytes and pulmonary induction of inducible nitric oxide synthase\(^{31}\) and reduces mortality caused by sepsis\(^{34}\). Also, UTI can alleviate coagulatory disturbance accompanied by sepsis such as an increase in the serum level of fibrinogen and fibrinogen degradation products\(^{35}\). Another group showed that UTI protects against haemorrhagic shock by preserving the myocardial mitochondrial function\(^{36}\). Likewise, UTI has a protective effect against ischemia-reperfusion injury in the liver\(^{37}\), kidney\(^{38}\), heart\(^{39}\) and lung\(^{40}\) in vivo via the actions of its radical scavenging elements\(^{41}\). In addition, UTI reduces CX-C chemokine production during liver ischemia/reperfusion in vivo\(^{42}\). Intravenous pre-treatment with UTI was also suggested to have a neuroprotective action in a transient cerebral ischemia model\(^{43}\). In humans, pre-pump administration (5,000 U/kg) of UTI reportedly improves cardiopulmonary bypass-induced haemodynamic instability and pulmonary dysfunction through the attenuation of IL-6 and IL-8 release in humans\(^{44}\). Furthermore, UTI can inhibit coagulatory activation accompanied by severe inflammation such as tissue factor expression on monocytes in vitro and in vivo\(^{39}\) as well as coagulation and fibrinolysis during surgery in humans\(^{45}\). On the other hand, Kurosawa et al. clinically showed that (intravenous) pre-treatment with 300,000 IU of UTI prevents an increase in pulmonary artery pressure and shunting in patients after aortic unclamping during abdominal aortic aneurysmectomy\(^{46}\). Taken together, it is likely that UTI itself other than Iel/Pd also regulates hemodynamic stability during surgical stress. However, the detailed mechanisms and concise methodology for UTI treatment remain to be determined in the future. Also, further evaluation are needed using each gene-depleted mice for Iel and/or Pd.

In other studies, UTI reportedly had preventive effects on drug/metabolized nephrotoxicity by gentamycin, mercuric chloride and cisplatin\(^{47-51}\). The proposed mechanisms for this renal protection include reductions of lysosomal fragility and proximal tubule damage\(^{50,51}\). Koizumi and colleagues have shown that UTI prevents experimental crescentic glomerulonephritis in rats, at least in part, by inhibiting the intraglomerular infiltration of inflammatory cells\(^{52}\). On the other hand, Huang et al., employing both clinical and in vivo tests, showed that UTI reverses myocardial damage accompanied by severe burn via modulation of the inflammatory status with lipid peroxidation with cardiac apoptosis\(^{53}\). Interestingly, Tsujimura and colleagues reported a case of infectious interstitial pneumonia associated with systemic anaphylaxis in rabbits\(^{54}\). Further, UTI reportedly ameliorates refractory skin diseases such as Stevens–Johnson syndrome and toxic epidermal necrolysis\(^{55}\). Thus, these in vivo findings may pave the way for alternative therapeutic strategies for disorders other than sepsis and post-surgical haemodynamic abnormality, such as nephrotoxicity, nephritis, burn-associated tissue damage, interstitial pneumonia, anaphylactic shock and/or refractory skin diseases in the future.

Alternatively, UTI may be applicable in combination with other drugs. For example, UTI with amphotericin B can ameliorate invasive pulmonary aspergillosis\(^{57}\). On the other hand, a combined therapeutic strategy with low-dose dopamine, gabexate mesilate and UTI improves the water balance and reduces the incidence of pulmonary complications in thoracic esophagectomy patients\(^{58}\).
Subsequent studies indicate that UTI can inhibit the invasiveness of tumour cells of various histologic origins. Moreover, UTI has been shown to downregulate the expression of the cancer metastasis-associated molecules uPA and uPA receptor possibly through MAPK-dependent signalling cascades in vitro and in vivo. In addition, UTI has anti-inflammatory effects against several forms of malignancy in vitro. These studies suggest that UTI is a candidate anti-cancer drug, although further studies are required in the future.

In order to confirm its anti-inflammatory potential in vivo, we conducted a series of studies on the role of UTI in the inflammation related to bacterial endotoxin (LPS) using UTI (−/−) mice. In one study, both UTI (−/−) and wild-type (C57/B6: WT) mice were injected intraperitoneally with vehicle or LPS at a dose of 1 mg/kg body weight. Evaluation of the coagulatory and fibrinolytic parameters and white blood cell (WBC) counts at 72 h after intraperitoneal (i.p.) challenge showed that fibrinogen levels were significantly greater in LPS- than in vehicle-challenged mice with the same genotypes. In the presence of LPS, however, they were also significantly higher in UTI (−/−) than in WT mice. WBC counts significantly decreased after LPS challenge in UTI (−/−) mice. LPS appeared to shorten prothrombin time (PT) when compared to vehicle treatment in UTI (−/−) mice, although this difference did not reach significance. In the presence of LPS, the PT was significantly shorter in UTI (−/−) than in WT mice. Further, histopathological changes in the lung, kidney and liver of both genotypes after LPS challenge revealed severe neutrophilic inflammation in UTI (−/−) lungs challenged with LPS, whereas little neutrophilic infiltration was found in LPS-treated WT mice. The overall trend was similar regarding findings in the kidney and liver.

The protein expression levels of proinflammatory molecules such as macrophage chemoattractant protein (MCP)-1 in the lungs; MCP-1 and keratinocyte-derived chemoattractant (KC) in the kidneys and IL-1β, macrophage inflammatory protein (MIP)-2, MCP-1 and KC in the livers were significantly greater in UTI (−/−) than in WT mice after LPS challenge. These results indicate that UTI protects against systemic inflammation induced by the i.p. administration of LPS, at least partly, through the inhibition of proinflammatory cytokine production/release, suggesting that UTI may be therapeutic against sepsis in humans.

A previous study showed that UTI improves acute lung injury in vivo; however, no evidence has been reported using a genetic approach. In another series of studies, therefore, we also showed that UTI also protects against acute lung inflammation induced by the intratracheal administration of LPS, at least in part, via the local suppression of proinflammatory cytokines and antioxidation, suggesting that UTI may be a useful therapeutic tool for acute lung injury.

One study has shown that plasma UTI levels increase in patients with acute hepatitis and markedly decrease in those with fulminant hepatitis, suggesting that the plasma UTI level is closely linked to the severity of liver damage. Further, the plasma UTI level is reportedly correlated with the degree of liver damage in patients with chronic liver diseases such as liver cirrhosis and hepatocellular carcinoma. Using UTI (−/−) mice, we showed that UTI protects against the local inflammatory response accompanied by severe liver injury, which supports its anti-inflammatory properties in vivo, implicating a therapeutic potential of UTI in fulminant hepatitis in humans. In this regard, Nobuoka and colleagues have recently implicated UTI in normal liver regeneration using UTI (−/−) mice via the regulation of systemic (serum) levels of cytokines such as IL-6 and IL-10 and chemokines such as MCP-1 and MIP-1α.

Conclusion

Our experiments employing genetic approach suggest that UTI can protect against the systemic inflammatory response and subsequent organ injury induced by LPS, at least partly, through the inhibition of proinflammatory cytokine and chemokine expression, which provide important in vivo evidence and understanding about a protective role of UTI in inflammatory conditions. UTI may therefore provide an attractive ‘rescue’ therapeutic option for systemic inflammatory response syndromes such as disseminated intravascular coagulation, acute lung injury and acute liver injury.

Acknowledgement

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Abbreviations list

HC, heavy chain; IL, interleukin; Iol, inter-α-inhibitor; IaI, inter-α-like inhibitor; KC, keratinocyte-derived chemoattractant; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCP, macrophage chemoattractant protein; MP, macrophage inflammatory protein; PT, prothrombin time; pαI, pre-α inhibitor; TNE, tumour necrosis factor; uPA, urokinase-type plasminogen activator; UTI, urinary trypsin inhibitor; WBC, white blood cell

References


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