Biologicals 38 (2010) 552–556

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Ulinastatin-mediated protection against zymosan-induced multiple organ dysfunction in rats

Qiuping Yang [a], Xingang Liu [a], Min Liu [b], Ling Zhang [a], Yinghui Guan [c,*]

[a] Intensive Care Unit, First Hospital of Jilin University, 71 Xinmin Street, Changchun, Jilin130021, China
[b] Department of Emergency Medicine, 2nd Branch of First Hospital of Jilin University, 3302 Jilin Street, Changchun, Jilin 130011, China
[c] Respiratory Department, 2nd Branch of First Hospital of Jilin University, 3302 Jilin Street, Changchun, Jilin 130021, China

ARTICLE INFO

Article history:
Received 8 March 2010
Received in revised form
14 May 2010
Accepted 15 May 2010

Keywords:
Ulinastatin
Zymosan
Polysaccharide
Multiple organ dysfunction syndrome
MODS
Toll-like receptor-4
TLR4

ABSTRACT

The purpose of this study was to evaluate the potential organ-protective activity of ulinastatin (a urinary trypsin inhibitor) and to investigate the underlying mechanism(s) in a rat model of multiple organ dysfunction syndrome (MODS). When adult Wistar rats were challenged intraperitoneally with yeast polysaccharide (zymosan), they developed biochemical and histological abnormalities similar to those seen in human MODS as compared with the controls. Among these abnormalities were: 1) significant increases in serum concentrations of tumor necrosis factor-alpha (TNF-α) and soluble intercellular adhesion molecule-1 (sICAM-1); 2) aberrant values in the liver and kidney function tests; and 3) presence of evident pathology in the major organs (i.e. liver, kidney and lung). In addition, zymosan challenge resulted in an increase in toll-like receptor-4 (TLR4) mRNA abundance in all three organs tested. Ulinastatin treatment significantly decreased the zymosan-induced elevation in serum concentrations of TNF-α and sICAM-1 and tissue abundance of TLR mRNA in the liver, kidney and lung, effectively attenuated the development of the polysaccharide-induced biochemical and histological abnormalities and successfully reduced the MODS-associated death. In conclusion, ulinastatin is able to protect multiple organs from yeast polysaccharide-induced damage and function failure, at least partially, through a TLR4-dependent mechanism, suggesting a therapeutic potential against MODS.

1. Introduction

Multiple organ dysfunction syndrome (MODS) is the most common cause of death in critically ill patients admitted to intensive care units (ICU) and represents a significant challenge to emergency physicians worldwide. More recently refined as “the development of potentially reversible physiologic derangement involving two or more organ systems not involved in the disorder that resulted in ICU admission, and arising in the wake of a potentially life-threatening physiologic insult” [1], MODS is the final stage of a lesion progressed from systemic inflammatory response syndrome (SIRS) through to sepsis (a loosely classified condition in the past) and severe sepsis. Currently there is no consensus on the outcome target in the clinical care of patients with MODS [2] and some authors even argue that MODS is a ‘purposeful’ process evolved to ‘cease’ critically ill individuals who have limited chances to survive [3]. Nevertheless, it is believed that the MODS-associated mortality can be reduced through therapeutic interventions [2,4], particularly when multiple approaches are applied in combination [3].

Ulinastatin, also known as urinary trypsin inhibitor, was first identified in and purified from human urine by a Japanese group of researchers in 1982 [5]. It is known that ulinastatin is a glycoprotein liberated into blood and urine from its liver-derived precursor inter-α-trypsin inhibitor upon degradation by neutrophil elastase during inflammation [6]. As a serine protease, ulinastatin is able to inhibit various inflammatory proteases including trypsin, chymotrypsin, neutrophil elastase and plasmin [7]. Since many proteases are present in elevated amounts in infection and inflammation, the protease-inhibiting property of ulinastatin renders it an effective anti-inflammatory molecule [8]. Approved by the government authorities in Japan and China [9,10], synthetic ulinastatin has been widely used clinically to treat pancreatitis and rheumatoid arthritis in these two countries. Both animal studies and clinical data have shown that ulinastatin is capable of protecting multiple organs against inflammation- and/or injury-induced dysfunction [11–14]. However, the molecular mechanism(s) by which ulinastatin exerts its organ-protective activity is not fully understood.

* Corresponding author. Tel./fax: +86 13504315935.
E-mail address: gyhlhx@sina.com (Y. Guan).

1045-1056/$36.00
doi:10.1016/j.biologicals.2010.05.001

Toll-like receptor-4 (TLR4), a member of the TLR/IL-1R (interleukin-1 receptor) superfamily characterized by the presence of Toll/IL-1R (TIR) domains [15], was originally identified as the central receptor responsible for endotoxin lipopolysaccharide responsiveness [16]. Increasing evidence suggests that TLR4 is involved in the development of MODS [17]: the expression level of tissue TLR4 correlates with survival/mortality in animals with experimentally induced sepsis [18]; patients with TLR4 mutations have different levels of susceptibility to sepsis and MODS [19,20].

To date, little is known regarding the effect of ulinastatin on TLR4 expression in MODS. The present study was therefore conducted to validate the organ-protective effect of ulinastatin as well as to investigate ulinastatin-mediated changes in TLR4 expression in a rat model of MODS.

2. Materials and methods

2.1. Animals, MODS model establishment and ulinastatin treatment

Wistar rats weighing 250 g or so were obtained from Jilin University Research Animal Laboratories. Prior to the experiments, the animals were allowed to adapt to their new environment for two days to a week. During this pre-experiment period as well as the experiment period that followed, standard rat chow and water were available *ad libitum*. All animal procedures were performed in accordance with the guidelines set forth by the Research Animal Committee at Jilin University.

The animals were divided into 3 groups, each with 4 males and 4 females. To induce the development of MODS, the animals in the first 2 groups were administered intraperitoneally with the yeast-derived polysaccharide zymosan (Sigma, St. Louis, MO, USA) suspended in liquid paraffin at 1 g/kg body weight, a dose optimal for induction of MODS in rats [21]. The animals in the third group were administered intraperitoneally with liquid paraffin suspension as vehicle controls.

To investigate the therapeutic effect of ulinastatin, the animals that had been subject to MODS induction with zymosan in the first 2 groups were given ulinastatin respectively at 0 and 1×10^5 units in 1× PBS through tail vein injection. The animals in the third group were given 1× PBS via tail vein injection and served as non-MODS and non-ulinastatin controls.

2.2. Blood tests

Venous blood was collected from animals in all three groups at 6 h, 12 h and 24 h after ulinastatin or vehicle (1× PBS) treatment. Serum was prepared and stored in aliquots at −85 °C before analyses. Serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine kinase (CK), CK-MB, lactate dehydrogenase (LDH), total bilirubin (TBIL), direct bilirubin (DBIL) and indirect bilirubin (IBL) were determined using a Hitachi Automatic Biochemical Analyzer as instructed.

2.3. Enzyme-linked immunosorbent assay (ELISA)

To determine the effect of zymosan and ulinastatin on endothelial injury and inflammatory cytokine production, serum levels of soluble intercellular adhesion molecule-1 (sICAM-1) and tumor necrosis factor-alpha (TNF-α) were determined by ELISA with commercially available kits (Gemmy Bio-Engineering Co., Ltd., Shengzhen, China) according to the manufacturer's instructions.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA was extracted from the liver, kidney and lung immediately after sacrifice of the animals. First strand cDNAs were synthesized by RT using a random primer and the extracted total cellular RNA as templates. The RT product (2 μl) was then amplified using a pair of primers specific for rat TLR4 and a pair of primers for the house-keeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Upon electrophoresis of the PCR products on an agarose gel, the cDNA bands for TLR4 and GAPDH were visualized by ethidium bromide under as ultraviolet light. The densitometric values of the TLR4 and GAPDH bands were obtained using a densitometer. After normalization against GAPDH, the differences in the TLR4 band density, presumably representing the differences in TLR4 mRNA abundance, between different samples were analyzed.

2.5. Histological examination

Immediately after the last blood sample collection at 24 h, the surviving animals were sacrificed. Tissue blocks were collected randomly from the liver, lungs and kidneys. Following fixation in buffered 10% formalin, the blocks were embedded in paraffin and sectioned at 5 μm. The sections were deparaffinized, rehydrated, stained with hematoxylin–eosin and examined under a light microscope for histological changes with digital images captured.

2.6. Statistical analyses

The data were expressed on mean ± SD and analyzed by ANOVA and multiple comparison *t* tests using the statistical software SPSS. The differences were considered significant when $P < 0.05$.

3. Results

3.1. Animal survival

Eight rats were initially included in each of the three groups. By the end of the experiment, all 8 rats survived; 5 and 2 rats died respectively in the zymosan-challenged group and the zymosan-challenged and ulinastatin-treated group. The death rate was significantly higher ($P < 0.05$) in the zymosan-challenged group than the other two groups and the difference between the control group and the zymosan-challenged and ulinastatin-treated group was not significant ($P > 0.05$).

3.2. Histopathology

To validate the zymosan-induced organ damage as well as to evaluate the protective property of ulinastatin against the zymosan-induced organ damage, we examined the histological changes in the liver, kidneys and lungs 24 h after the zymosan challenge with or without ulinastatin treatment by hematoxylin–eosin staining. When compared with the liquid paraffin control (Fig. 1A–C), 1 g/kg zymosan suspended in liquid paraffin induced organ damages similar to those in human sepsis and MODS: 1) in the liver as manifested by congestion, sinusoidal dilatation, and necrotic lesions with signs of hepatocyte atrophy and steatosis (Fig. 1D); 2) in the kidneys as manifested by necrosis of a large number of proximal tubular epithelial cells with edema in the renal cortex, presence of RBCs within the renal tubules, infiltration of the renal interstitium by inflammatory cells (Fig. 1E); and 3) in the lungs as manifested by infiltration of inflammatory cells around the interstitium and blood vessels as well as the presence of red blood cells (RBCs) largely in the bronchi and in a lesser extent in the alveoli (Fig. 1F). The treatment with 1×10^5 units of ulinastatin effectively and significantly reduced