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(Pro)renin receptor blocker improves survival of rats with sepsis

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ABSTRACT

Background: The renin-angiotensin system (RAS) affects inflammatory responses during sepsis. Nonproteolytic activation of prorenin by the (pro)renin receptor has recently been shown to stimulate the tissue RAS. In the present study, the effect of (pro)renin receptor blocker (PRRB) pretreatment on sepsis in a rat cecal ligation and puncture (CLP) model was investigated.

Materials and methods: Male Sprague-Dawley rats underwent CLP and were randomly divided into two groups: PRRB-treated group and control peptide–treated group. Survival was analyzed for 7 d after CLP. The serum concentrations of cytokines and high-mobility group box chromosomal protein 1 (HMGB1) were measured at three time points (0, 3, and 6 h after CLP). Hematoxylin-eosin staining and immunohistochemical staining for nonproteolytically activated prorenin and HMGB1 were performed on the cecum to assess pathologic changes found 6 h after CLP.

Results: Treatment with PRRB improved the survival rate of the post-CLP septic rats ($P = 0.023$). PRRB also significantly reduced serum tumor necrosis factor-α, interleukin-1β, and HMGB1 levels 6 h after CLP. In CLP rats that were treated with control peptide, the expression of activated prorenin was elevated in peritoneal foam cells. Moreover, expression of HMGB1 was increased in peritoneal inflammatory cells. In contrast, both were markedly suppressed in CLP rats that were treated with PRRB.

Conclusions: PRRB significantly improved the survival rate of rats with clinically relevant sepsis, possibly by attenuating a sepsis-induced systemic inflammatory response. We propose that overactivation of the RAS by activation of prorenin in foam cells may be a significant contributor to sepsis.

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1. Introduction

Sepsis remains a significant cause of morbidity and mortality throughout the world. Sepsis evokes acute respiratory failure, multiple organ failure, and disseminated intravascular coagulation [1]. While the pathologic sequelae of sepsis are characterized by a systemic inflammatory response, experimental therapeutics that target specific early inflammatory mediators have not proven efficacious in the clinic [1].

High-mobility group box 1 (HMGB1) was found to be a key mediator of sepsis [2]. HMGB1 is secreted from activated monocytes/macrophages as a cytokine mediator of inflammation [2]. We previously reported that HMGB1 was released systemically in murine models of sepsis, and have discussed its promise as a novel target for modulating stress responses [3–6].

The renin–angiotensin system (RAS) has an important role in maintaining blood pressure homeostasis, as well as fluid and salt balance [7]. During the last decade, the RAS has shown new facets, as additional components have been discovered. These include the involvement of the RAS in development, inflammation, and remodeling [8]. Recently, much attention has been focused on the involvement of the RAS in the pathogenesis of sepsis [9–12].

The discovery of a (pro)renin receptor ([P]RR) and the introduction of renin inhibitors in the clinic has brought prorenin, the inactive proenzyme form of renin, back into the spotlight [8]. Prorenin has a prosegment of 43 amino acid residues that are attached to the N terminus of mature (active) renin. The prosegment folds into the active site cleft of mature renin to prevent catalytically productive interaction with angiotensinogen. When a prorenin-binding protein interacts with the "handle region" of the prorenin prosegment, the prorenin molecule undergoes a conformational change to an enzymatically active state [13]. This phenomenon is called nonproteolytic activation, and recent studies have found that such binding proteins include the (P)RR [14,15]. We recently found that nonproteolytic activation of prorenin plays a pivotal role in the activation of the RAS and the development of nephropathy in diabetic rats. Furthermore, we showed that the (P)RR blocker (PRRB; formerly handle region decoy peptide) prevented the development of nephropathy. In fact, it suppressed an increase in renal angiotensins more than other RAS inhibitors, such as angiotensin converting enzyme (ACE) inhibitor [15].

Although studies of PRRB have targeted only chronic organ damage associated with chronic inflammatory diseases, including hypertension and diabetes, PRRB might also be effective in acute systemic inflammatory diseases such as sepsis or systemic inflammatory response syndrome. In the present study, we tested the effects of PRRB in the rat cecal ligation and puncture (CLP) model. We hypothesized that PRRB could reduce cytokine and HMGB1 levels in serum, thus improving survival. Macrophages are central players in the inflammatory response, providing an immediate defense against foreign agents. CLP-induced macrophages initiate the production of potent proinflammatory mediators, including tumor necrosis factor (TNF)-α, interleukin (IL)-6, and HMGB1 [3–6]. Therefore, in this study, the activated prorenin that was expressed in peritoneal inflammatory cells of CLP rats was evaluated by immunohistochemical staining to elucidate the mechanism underlying the effect of PRRB.

2. Materials and methods

2.1. Animals

Male, 8-wk-old, specific pathogen–free Sprague-Dawley rats, weighing 250–300 g, were purchased from CLEA Japan, Inc (Tokyo, Japan). The animals were allowed to acclimatize for 7 d before use. Rats were housed in the Laboratory Animals Center, Keio University School of Medicine, under standard temperature and light and dark cycles. Rats had access to chow and water *ad libitum* throughout the study. All procedures were performed under the approval of the Laboratory Animal Care and Use Committee at Keio University School of Medicine.

2.2. Cecal ligation and puncture

To establish live intra-abdominal infection and sepsis, we subjected the rats to the CLP procedure as previously described [3–5]. The animals were first anesthetized by intramuscular injection of ketamine (40 mg/kg of body weight), and a 20 mm midline incision was made to expose the cecum. The cecum was mobilized and ligated below the ileocecal valve while preserving the blood flow of the cecum, then a 5.0 mm blade incision was made at the tip of the cecum. The cecum was replaced in its normal intra-abdominal position and the wound closed with a running suture. All animals received saline solution (0.9% subcutaneously, 10 mL/kg of body weight) resuscitation immediately after the surgery. The procedure to induce sepsis was carried out in the morning.

2.3. Experimental design

Animals were randomly divided into two groups in a double-blinded fashion: those treated with PRRB and those treated with a control peptide. We previously designed the decapeptide PRRB (NH2-R10RILLKKMPSV19-COOH) (Gene Design Inc, Osaka, Japan), which includes the handle region sequence [15]. As a negative control for PRRB, we also prepared a control peptide, NH2-M30TRISAE36-COOH (Gene Design Inc) with an amino acid sequence outside the handle region, as described previously [15]. PRRB or control peptide was subcutaneously administered at 1 mg/kg/d by the osmotic minipump (model 2002 for 14 d use; Alzet, Cupertino, CA) from 7 d before until 7 d after the CLP procedure. We measured the body weight gain in rats before the CLP procedure to confirm that there was no difference between the PRRB-treated group and control peptide group, as previous studies had shown [15]. We used different rats in each study to avoid being biased by multiple factors. In all cases, technicians blinded to research group made assessments. The experimental unit was the cage of animals.