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Sabiporide improves cardiovascular function and attenuates organ injury from severe sepsis

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ABSTRACT

Background: The aim of the present study was to evaluate the efficacy of orally administered sabiporide, a selective Na^+/H^+ exchanger inhibitor on whole body protection from severe sepsis in rats.

Methods: Series 1: Sepsis was induced by cecal ligation and puncture (CLP). Animals received treatment of vehicle or sabiporide (10 mg/kg, p.o.). The experiment was terminated 20 h after CLP. Series 2: At 20 h after CLP, the necrotic cecum was excised and the abdominal cavity was washed. The animals were then returned to their cages. The experiment was terminated 7 d after CLP.

Results: Series 1: Compared with vehicle treatment, administration of sabiporide prevented hemodynamic derangement and improved cardiac function as evidenced by improved arterial pressure, left ventricle systolic pressure, ±dp/dt max, ejection fraction and fractional shorting, attenuated left ventricle end-diastolic pressure elevation, and wall motion abnormality. Furthermore, administration of sabiporide attenuated intestinal mucosal hyperpermeability and reduced accumulation of abdominal ascites. In addition, treatment with sabiporide also reduced plasma levels of tumor necrosis factor-α, interleukin 6, interleukin 10, cardiac troponin, aspartate aminotransferase, alanine aminotransferase, urea, and lactate, and attenuated neutrophil infiltration in the liver and gut. Series 2: Administration of sabiporide improved the 7-day survival rate after CLP in rats (42% in vehicle group *versus* 75% in sabiporide group).

Conclusions: Administration of sabiporide improved cardiovascular performance, lessened the inflammatory response, tissue hypoperfusion and multiorgan injury, and most importantly reduced mortality.

1. Introduction

Sepsis presents a major health care problem and remains one of the leading causes of death within the intensive care unit worldwide. The pathophysiology of sepsis involves a highly complex and integrated response, including metabolic acidosis, calcium overload, the activation of various cell types, inflammatory mediators, and the hemostatic system [1–3]. The development of metabolic acidosis in patients with sepsis and septic shock is often accompanied by impairment in organ function and an increase in morbidity and mortality [4,5]. Recent studies from our laboratory and that of others indicate that the detrimental effects of tissue hypoperfusion and metabolic acidosis have largely been attributed to changes in critical protein functions arising from alterations in extracellular and intracellular pH; and that the activity of

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Na^+/H^+ exchanger (NHE1), a ubiquitous plasma membrane transporter that regulates cytoplasmic pH, is an important determinant of cellular injury [5–7]. NHE1 activation is induced by intracellular acidosis through a proton-dependent regulation pathway resulting in intracellular sodium and calcium overload and tissue injury [8,9]. NHE1 activation is also induced by oxidative stress and endogenous mediators released from hypoxic cells including various autocrine and paracrine factors, such as endothelin 1, angiotensin II, and α_1-adrenergic agonists as well as toxic agents, such as hydrogen peroxide and lysophosphatidylcholine [8,10,11]. Furthermore, there is substantial evidence indicating that NHE1 regulates inflammatory processes, and inhibition of NHE1 attenuates neutrophil activation, chemokine production, and leukocyte-endothelial cell interactions, thus, providing protection from inflammation-related tissue damage [12–14].

Hypoxia, lactic acidosis, and systemic inflammation are hallmarks of severe sepsis. Thus, a variety of intracellular and extracellular factors produced during severe sepsis may contribute to tissue dysfunction through NHE1-dependent processes. Therefore, NHE1 inhibition may provide a novel approach to attenuate tissue injury caused by severe sepsis. Consistent with this concept, Sikes *et al.* [15] examined cardiac function, intracellular Na^+ and Ca^{2+} concentrations, myocardial pH, and high-energy phosphates in perfused hearts harvested from rats with or without sepsis. They showed that induction of sepsis causes a significant increase in serum lactate levels, more than twofold increase in intracellular myocardial Na^+ and Ca^{2+} concentrations, and a drop in left ventricular pressure of ~25%. In addition, they also showed that administration of amiloride to septic animals almost completely blunts the rise in myocardial Na^+ and Ca^{2+} concentrations, substantially attenuates the decrease in left ventricular pressure, and decreases the severity of lactic acidosis [15]. The efficacy of NHE1 inhibition has not been evaluated in a clinically relevant animal model of sepsis *in vivo*. Sabiporide is a potent orally active NHE1 inhibitor [7]. The aim of the present study was to evaluate the protective effects of NHE1 inhibition with sabiporide in a clinically relevant sepsis model of cecal ligation and puncture (CLP) in rats.

2. Methods

2.1. Animals

All animal studies were approved by the Institutional Animal Care and Use Committee and complied with the Animal Welfare Act. Male Sprague–Dawley rats weighing 300–350 g were used in all experiments. The rats were housed under controlled light–dark conditions and fed with standard rat food and water *ad libitum*. All animals were observed daily for general health, and all invasive procedures were performed under aseptic conditions.

2.2. Induction of sepsis

Animals were anesthetized with ketamine (60 mg/kg, intramuscular [i.m.]) plus xylazine (10 mg/kg, i.m.). Sepsis was induced by CLP as previously described by Miksa *et al.* [16]. Briefly, a 2-cm midline abdominal incision was performed. The cecum was then exposed, ligated just distal to the ileocecal valve to avoid intestinal obstruction, punctured twice with an 18-gauge needle, and returned to the abdominal cavity. The incision was then closed in layers. Sham-operated animals underwent the same procedure with the exception that the cecum was neither ligated nor punctured. The animals were resuscitated with 3 mL/100 g body weight normal saline subcutaneously immediately after surgery. The animals were then returned to their cages.

2.3. Experimental protocol

After CLP, animals were randomly assigned to two study groups, and received treatment of vehicle (0.5% Natrosol + 0.01% TWEEN 80, $n = 8$) or sabiporide (selective NHE1 inhibitor, 10 mg/kg, oral administration, $n = 8$) at 1, 10, and 19 h after CLP. The dose was selected based on preclinical pharmacokinetic data. The experiment was terminated 20 h after CLP.

2.4. Echocardiography

Twenty hours after CLP, animals were anesthetized with ketamine (60 mg/kg, i.m.) plus xylazine (10 mg/kg, i.m.). Echocardiography was performed with a Hewlett–Packard echocardiographic system SONOS 2000 with a 7.5/5.5 MHz transducer (Hewlett-Packard Company, Palo Alto, CA). For each animal, a two-dimensional short-axis view was taken at the midpapillary muscle level to obtain left ventricular ejection fraction. Linear dimensions were measured from two-dimensionally guided M-mode tracing, and fractional shortening was obtained. An electrocardiography tracing was recorded simultaneously with the echocardiogram. Wall motion score index was the sum of wall motion scores divided by the number of visualized segments. In this scoring system, higher scores indicate more severe wall motion abnormalities: 1 = normal, 2 = hypokinesis, 3 = akinesis, 4 = dyskinesis, and 5 = aneurysm [17]. All measurements were repeated three times, and the results represent the average of these measurements.

2.5. Hemodynamic assessment and tissue harvest

After echocardiography analysis, the left external jugular vein was cannulated for right atrial pressure measurement. A catheter was inserted into the right common carotid artery for quantifying arterial blood pressure with a Powerlab data acquisition system (ADInstruments Inc, CO). Heart rate was derived from the blood pressure signal. After arterial blood pressure was measured, the catheter was introduced into the left ventricle through the right carotid artery to monitor left ventricular pressure (LVP) and its first derivative (±dp/dp max). At the end of the experiment, blood samples were collected and centrifuged. Plasma samples were stored at −80°C until assayed. Abdominal ascites were also collected and volumes were determined.

Liver and intestinal tissues were collected for immediate assay or snap-frozen until later analysis.