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Valproic acid protects septic mice from renal injury by reducing the inflammatory response

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ABSTRACT

Background: Valproic acid (VPA), a histone deacetylase inhibitor, has extensive activities against inflammation, oxidation, and malignancy. This study was designed to investigate the protective effect of VPA on the systemic inflammatory response and renal injury in septic mice.

Materials and methods: The septic model of mice was established using a cecal ligation –puncture technique. A single dose of VPA (300 mg/kg) was administered at 30 min postoperatively.

Results: We found that VPA reduced the tubular swelling and lowered the serum levels of blood urea nitrogen, creatinine, and C-reactive protein. After treatment with VPA, the renal level of malondialdehyde and the activity of myeloperoxidase decreased markedly; the activity of superoxide dismutase and the glutathione content increased accordingly; and the serum levels of tumor necrosis factor α, interleukin 1β, and interleukin 6 decreased markedly. Furthermore, VPA suppressed the renal expression of cyclooxygenase 2 and inducible nitric oxide synthase and repressed the release of prostaglandin E2 and nitric oxide.

Conclusions: Our results demonstrate that VPA reduces the inflammatory response in a septic model and protects mice from renal injury, showing substantial potential in the treatment of sepsis.

1. Introduction

Sepsis is defined as a type of systemic inflammatory response syndrome caused by infections or highly suspected infectious lesions [1], and it is a stepwise amplified uncontrolled inflammatory response involving the activation of a vast number of inflammatory factors [2]. Updated epidemiologic data show that there are more than 18 million cases of serious sepsis reported annually, and this number is increasing by 1.5%–8.0% every year. As one of the most common causes of death in infectious diseases, up to 14,000 deaths associated with sepsis are reported daily [3]. The morbidity and mortality of sepsis is dependent of the effectiveness of anti-inflammatory therapy [4], and it is important to develop novel anti-inflammatory agents for sepsis.

Valproic acid (VPA), a short-chain fatty acid comprised eight carbon atoms, was extracted and synthesized initially by Burton in 1882 from valeric acid. VPA is a histone deacetylase inhibitor approved for the clinical treatment of seizures and other neurologic disorders [5–7]. In addition to its

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anti-inflammatory effects resulting from the inhibition of the release of inflammatory factors [8–11], VPA also possesses extensive activities against fibrosis [12,13], oxidation [14,15], and malignancy [16–18].

Sepsis is a condition that could induce acute renal injury as a result of uncontrolled systemic inflammatory response, greatly increasing the mortality [19]. Recent publications reported protective effects of VPA on the pulmonary, hepatic, and renal function of septic rats [20], the efficacy on adriamycin-induced nephropathy [21], and the inhibitory effect on renal fibrosis in mice [13], but the detailed underlying mechanisms should be further explored. This study was designed to investigate the protective effect of VPA on the sepsis-associated acute renal injury using a septic model in mice established by cecal ligation and puncture (CLP) to provide evidence for the development of new agents for this condition.

2. Materials and methods

2.1. Animals

Eight-week-old C57BL/6 mice (weight: 18–22 g) were commercially obtained from the Laboratory Animal Center of China Medical University. Before the study, all animals were acclimated in an animal room with prespecified conditions (temperature: 22°C; relative humidity: 40%–50%; 12-h light–dark cycles) for 1 wk, with diet and water supplied *ad libitum*. The care and management of the animals in this study were approved by the Laboratory Animal Ethics Committee of China Medical University.

2.2. Experimental groups

A total of 24 mice were randomized into four groups: sham group, VPA group, CLP group, and VPA + CLP group ($n = 6$ each). The animals in the VPA + CLP and CLP groups were subjected to CLP to establish a septic model and were given VPA (300 mg/kg, Sigma, St. Louis, MO) or an equal volume of normal saline at 30 min postoperatively. The animals in the VPA and sham groups underwent the same surgical procedure as the aforementioned animals with the exception of CLP, and they were given VPA (300 mg/kg, Sigma) or an equal volume of normal saline at 30 min postoperatively.

2.3. Septic model establishment in mice

The septic model in mice was established using CLP procedure [22,23]. After the animals were anesthetized with pentobarbital sodium (30 mg/kg) administered intraperitoneally, the cecum was exposed via a 1-cm longitudinal abdominal midline incision made on the prepared skin. The cecum was ligated with 4-0 suture just below the ileocecal valve and punctured twice with 18-ga needles before being returned to the abdominal cavity. The wound was closed routinely. Postoperatively, all animals were given 1 mL of normal saline subcutaneously and were placed on a warm pad until they recovered from the anesthesia. At 24 h after the surgery, the animals were sacrificed, and the appropriate peripheral blood was collected for the examination of serum chemistry parameters. The left kidney was harvested, fixed in 10% formalin and embedded in paraffin for histopathologic analysis. The right kidney was frozen at −80°C for future use.

2.4. Histopathology

The 5-μm sections made from the paraffin-embedded tissue block were prepared in Periodic acid–Schiff stains (Sigma) and counterstained with hematoxylin. After a routine process, including dehydration, hyalinization, and sealing, the section was subjected to microscopy and radiography to obtain the data.

2.5. Hematology and serum chemistry

The serum levels of C-reactive protein (CRP), blood urea nitrogen (BUN), and creatinine (Cr) were determined using commercially obtained kits (Jiancheng Bioengineering Institute, Nanjing, China), and the determinations were performed strictly following the instructions for the use of the supplied kits. Each sample was examined in triplicate.

2.6. Determination of oxidative stress parameters in renal tissue

Ten percent of the tissue preparation was made from renal tissue that was homogenized with normal saline, and the preparation was centrifuged at 3000 rpm at 4°C for 10 min. The resultant supernatant was equilibrated with phosphate-buffered saline and used to determine the levels of proteins in the tissue homogenates. The levels of malondialdehyde (MDA), myeloperoxidase (MPO), superoxide dismutase (SOD), and glutathione (GSH) were determined using commercially obtained kits (Jiancheng Bioengineering Institute). The level of nitric oxide (NO) was determined using the Griess assay (Beyotime, Haimen, China). The determinations were performed strictly following the instructions for the use of the supplied kits. Each sample was examined in triplicate.

2.7. Enzyme-linked immunosorbent assay

The serum levels of tumor necrosis factor α (TNF-α), interleukin (IL)-1β, IL-6, and prostaglandin E2 (PGE_2) were determined using commercially obtained enzyme-linked immunosorbent assay kits (USCN, Wuhan, China) strictly following the instructions for the use of the supplied kits.

2.8. Western blot analysis

After the concentration of the total protein of the renal tissue in each group that was extracted using radioimmunoprecipitation lysis buffer was measured using a bicinchoninic acid assay, an aliquot of the total protein in each group (40 μg) was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis, and the protein was electrically transferred to a polyvinylidene fluoride membrane (Millipore, Bedford, MA). After the membrane was blocked in 5% nonfat milk at room temperature, and the diluted primary antibodies were added (inducible nitric oxide synthase [iNOS],