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Cytokine and radical inhibition in septic intestinal barrier failure

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ABSTRACT

Background: Breakdown of the intestinal barrier is a driving force of sepsis and multiple organ failure. Radical scavengers or cytokine inhibitors may have a therapeutic impact on intestinal failure. Therapeutic effects on different sites of small intestine and colon have not been compared. Therefore, we investigated time-dependent intestinal permeability changes and their therapeutic inhibition in colon and small intestine with an *ex vivo* model.

Methods: Male Sprague–Dawley rats were either pretreated for 24 h with lipopolysaccharide (LPS) intraperitoneally alone or in combination with a radical scavenger (pyruvate or Tempol) or a cytokine inhibitor (parecoxib or vasoactive intestinal peptide). The gastrointestinal permeability was measured by time-dependent fluorescein isothiocyanate inulin diffusion using washed and everted tube-like gut segments. Blood and tissue samples were taken to investigate the development of inflammatory cytokine level (interleukin 6) in the context of cytokine inhibition and reactive oxygen species level via nicotinamide adenine dinucleotide phosphate oxidase activity in radical scavenger groups.

Results: After LPS treatment, mucosal permeability was enhanced up to 170% in small intestine and colon. In the small intestine the most significant reduction in permeability was found for pyruvate and parecoxib. Treatment with vasoactive intestinal peptide and parecoxib resulted in the most pronounced reduction of permeability in the colon.

Conclusions: Our data suggest that cytokine inhibitors and radical scavengers have pronounced effects in LPS-induced disrupted intestinal barrier of the colon and small intestine. Our novel model comparing different anatomic sites and different points in time after the onset of sepsis may contribute to gain new insight into mechanisms and treatment options of sepsis-related gut mucosal breakdown.

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1. Introduction

Multiple organ failure and its frequent initial pathophysiological mechanism and the disruption of the intestinal barrier function are of major concern in critically ill surgical patients.

In clinical studies and animal experiments, it could be shown that an increase of intestinal permeability (permeable bowel syndrome) as a consequence of damaged intestinal mucosa is an early risk factor for the development of multiple organ failure. On the one hand, the intestine is "victim" being damaged by hypoxia and microcirculatory dysfunction, and on the other hand, it is a driving force due to the systemic release of bacteria and toxic mediators by the disrupted barrier. Increase of intestinal permeability as a marker for intestinal damage was shown in humans with sepsis [1,2], as well as multiple trauma [3], and burns [4]. In a patients study with severe acute pancreatitis, it could be demonstrated that patients developing multiple organ failure had a significantly increased intestinal permeability [5]. Bacterial translocation due to a hyperpermeable intestinal barrier is considered to be the main cause of superinfection of pancreatic necrosis, resulting in a fatal source of unfavorable clinical outcome in this disease [6]. Changes in intestinal permeability can be studied *ex vivo* with well-established models based on the transport of metabolic inert substances such as fluorescein isothiocyanate (FITC)-inulin through the gut [7]. However, methods published to date do not allow to measure intestinal permeability at different points in time in a single experimental setting, and they do not consider the fact that different anatomic areas of the intestine might react differently to the applied stimulus. We investigated changes in reactive oxygen species (ROS) production and inflammatory cytokines separately to facilitate the interpretation of the complex impact of these mediators on the different gut segments [8,9]. We assume that the mechanical function of the gut barrier and the development of sepsis are closely related.

Multiple mechanisms of the pathophysiology of the destruction of the intestinal barrier function and its therapeutical preservation have been investigated so far. Currently, none of such therapeutics entered clinical use for this indication. Therefore, it is warranted to gain information on this clinical field by experimental studies. Generation of oxygen radicals may be one important cause of intestinal barrier damage, which might be influenced by novel pharmacologic therapies.

Pyruvate as a radical scavenger reduces ROS production and inhibits mucosal injury [10]. Venkataraman *et al.* [11] demonstrated in a rat animal model that ethyl pyruvate infusions after sepsis induction lead to a significant reduction of nitrite and/or nitrate release and prolonged survival of the rats. At the same time, serum levels of the proinflammatory interleukin (IL)-6 were found to be reduced with an increase of the anti-inflammatory IL-10.

Tempol also is a prominent radical scavenger. It is able to ameliorate ROS-induced tissue damage with similar effects as the other radical scavenger mentioned previously. Liaw *et al.* [12] demonstrated in rats with intraperitoneally induced endotoxemia a reduced mortality rate, degraded plasma cytokine levels (IL-6, nitric oxide, and IL-1), and reduced migration of defense cells in tissue.

On the other hand, increased expression of cyclooxigenase 2 (COX-2) has been shown to be related to a disturbed intestinal barrier function and bacterial translocation [13]. Osterberg *et al.* [14] demonstrated in an animal model that the administration of selective COX-2 inhibitors lead to less mucosal cell damage than in controls. Vasoactive intestinal peptide (VIP) is a neuropeptide and has anti-inflammatory properties [15]. VIP appears to be an important mediator in sepsis balancing anti-inflammatory mediators and proinflammatory mediators and shows clearly effects in studies as a potential therapeutic drug in serve sepsis [16,17]. In the present study, we therefore investigated the potential inhibition of intestinal permeability at two different anatomic sites using either free radical scavengers (pyruvate or Tempol) or cytokine inhibitors (VIP and COX-2 inhibitor) and their effects on the ROS-generating system or inflammatory cytokines in plasma, respectively.

2. Methods

2.1. Animals

For animal experiments, we used male Sprague–Dawley rats (obtained from Charles River; Germany) with a weight of 300–420 g. In a controlled environment, the rats were kept in standard cages in groups of two animals (ambient temperature was 22 ± 1°C, rhythm of 12-h light–darkness). Access to food and water was *ad libitum*. All procedures complied strictly with the National Institutes of Health guidelines for care and use of laboratory animals and were approved by the appropriate governmental agency (reference number: G-08-22, Regierungspräsidium Freiburg im Breisgau, Germany). All animals developed a sepsis-like systemic inflammatory response after lipopolysaccharide (LPS) injection (1 mg/kg intraperitoneally). Our study stopped monitoring the progress of the systemic inflammatory damage after 24 h by euthanization of the animals by a dorsal cut through the diaphragm into the heart during deep anesthesia. There were no lethal dropouts.

2.2. Treatment protocol

For establishment of the new *ex vivo* model, we could demonstrate in preliminary tests injecting LPS 3, 6, and 24 h before measurement a significant increase of intestinal permeability after intraperitoneal injection of a gradual sub-lethal single dose of LPS (1 mg/kg) only 24 h before measurement.

Therefore, experiments were performed on 24-h pretreated rats. They received an intraperitoneal injection of a single dose LPS (1 mg/kg, [18–20]) or LPS (1 mg/kg) plus either pyruvate (100 mg/kg, [21–23]), VIP (10 μg/kg, [24]), or parecoxib (Dynastat) (25 μg/kg, [14,25,26]) 24 h before surgery and measurement. The control group received an intraperitoneal injection of saline at an equal volume compared with the LPS administration. Tempol (also known as 4-Hydroxy-TEMPO) (30 mg/kg/d in drinking water, [27,28]) was applied 1 week before the administration of LPS (*ex vivo* time course: Fig. 1). Size of the groups: control, $n = 9$; LPS 24 h: $n = 9$; LPS + Tempol: $n = 5$; LPS + pyruvate: $n = 6$; LPS + parecoxib: $n = 8$; LPS + VIP: $n = 9$. To