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Glycine selectively reduces intestinal injury during endotoxemia

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ABSTRACT

Background: Glycine is well known to protect the intestine against ischemia–reperfusion injury and during mechanical manipulation. Here, we studied whether glycine protects the small intestine during endotoxemia, even without being the site of the infection.

Materials and methods: Lipopolysaccharide (LPS) was infused at a rate of 1 mg/kg × h over a period of 7 h (subacute endotoxemia) in male Wistar rats. Glycine (single dose: 50 mg/kg × 15 min) was applied intravenously at 180 and 270 min after the beginning of the LPS infusion. Systemic parameters were periodically determined. The small intestine was analyzed for macroscopic (hemorrhages) and histopathologic changes (hematoxylin and eosin staining), and markers of inflammation (myeloperoxidase activity).

Results: Glycine neither decreased mortality nor beneficially affected vital parameters (e.g., mean arterial blood pressure and breathing rate), electrolytes, blood gases including pH and base excess, and plasma parameters of tissue injury such as lactate concentration, hemolysis, and aminotransferases activities during experimental endotoxemia. It, however, specifically diminished the LPS-induced small intestinal injury, as indicated by less intestinal accumulation of blood, less intestinal hemorrhages, and reduced intestinal hemoglobin content.

Conclusions: The present results demonstrate that glycine selectively protects the small intestine during subacute endotoxemia, even after manifestation of a severe systemic impairment. Because glycine is non-toxic at low doses, an administration of a moderate glycine dose (50–100 mg/kg) may be suitable to protect from intestinal damage during sepsis. Its true clinical potential, however, needs to be verified in further experimental studies and clinical trials.

1. Introduction

Sepsis is a complex systemic inflammatory host response to an infection defined by a combination of altered vital parameters and organ (dys) functions. A pre-shock state (sepsis or severe sepsis) and a shock state (septic shock = severe sepsis with simultaneous hypotension) can be distinguished [1,2]. The prognosis for patients with severe sepsis or septic shock, regardless of the advances in intensive care, remains poor [1,3,4].

It has long been hypothesized that the gut plays a crucial role in the progression of multiple organ failure. Critical illness, whether caused by trauma, burn, hemorrhage, and ischemia–reperfusion or sepsis, can result in pathologic

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changes of the intestine (e.g., due to intestinal hypoperfusion), which in turn causes multiple organ failure and death [5–11]. A subacute model of endotoxemia induced by a continuous intravenous infusion of lipopolysaccharide (LPS) [12,13] is particularly useful to simulate the intestinal damage during (normotensive) sepsis, without being the source of the infection, as is the case with the cecal ligation and puncture (CLP) model or following intraperitoneal injection of LPS or living bacteria.

The simplest amino acid glycine has been shown in many experimental studies to protect the intestine against ischemia–reperfusion injury [14–20] and during mechanical manipulation [21,22]. Further studies demonstrated that glycine reduces mortality and injury to lung, liver, or kidney during hemorrhagic shock [23,24], and in LPS and CLP models [25–29] when administered timely, that is, at the beginning of the resuscitation of hemorrhagic shock and before or directly after the onset of endotoxemia or sepsis. However, only little is known about the therapeutic potential of glycine regarding the small intestinal injury during sepsis. This study was performed to investigate the effect of glycine on LPS-induced intestinal injury during experimental endotoxemia in male Wistar rats, when administered far after the induction of endotoxemia. Glycine was given as intravenous infusion to ensure an immediate therapeutic glycine effect under these conditions.

2. Materials and methods

2.1. Chemicals and materials

Glycine and lipopolysaccharide (LPS; *Escherichia coli*, serotype O111:B4, LOT: 012M4098 V) were purchased from Sigma–Aldrich (St. Louis, MO), Ringer solution and 0.9% sodium chloride solution from Fresenius Kabi (Bad Homburg, Germany), and Portex catheters (0.58 mm i.d./0.96 mm once daily) from Smiths Medical International (Hythe, Kent, United Kingdom). Isoflurane (florene) was obtained from Abbott (Wiesbaden, Germany), ketamine 10% from Ceva (Düsseldorf, Germany), lidocaine (xylocain 1%) from AstraZeneca (Wedel, Germany), and medical oxygen from Air Liquide (Düsseldorf, Germany).

2.2. Animals

Male Wistar rats (450–500 g) were obtained from the central animal unit of the University Hospital Essen. Animals were kept under standardized conditions of temperature (22 ± 1°C), humidity (55 ± 5%), and 12-h light–dark cycles with free access to food (ssniff-Spezialdiäten, Soest, Germany) and water. All animals received humane care according the standards of the Federation of European Laboratory Animal Science Association. The experimental protocol has been approved based on the local animal protection act.

2.3. Anesthesia, analgesia, and surgical procedure

Rats were anesthetized with isoflurane (1.5%–2.0% in 100% medical O_2 at 1.0 L/min) and received ketamine (50 mg/kg, subcutaneously) and lidocaine (5 mg/kg, *s.c.*) for analgesia as described previously [14,25]. The right *A. femoralis* and *V. femoralis* were surgically prepared and cannulated with Portex catheters. At the end of the experiment (at T = 420 min), animals were sacrificed by cardiac incision under deep isoflurane anesthesia, unless they died within the experimental time.

2.4. Study groups

The study was performed with eight animals per group (except for the sham and glycine control group: $n = 4$; overall 24 animals). LPS was freshly dissolved in 0.9% sodium chloride solution and filtered through a bacteria-tight filter (Minisart 0.2 μm; Sartorius, Göttingen, Germany). Rats received an intravenous infusion of LPS into the *V. femoralis* at a rate of 7 mL/kg × h over a total period of 420 min (1 mg LPS/kg × h, subacute endotoxemia model) using a syringe pump (Perfusor-Secura FT; B. Braun, Melsungen, Germany) [12]. Glycine was freshly dissolved in sterile 0.9% sodium chloride solution, filtered through a bacteria-tight filter following adjustment of the pH to 7.35, and infused 180 and 270 min after starting the LPS infusion at a rate of 12 mL/kg × h (50 mg GLY/kg × 15 min). Outside the periods of glycine administration, an infusion of 0.9% sodium chloride was applied to keep the catheter functional. The following experimental groups were compared:

- Sham (sham control group): no LPS, no glycine;
- GLY (glycine control group): no LPS, 2 × 50 mg glycine/kg × 15 min;
- LPS (lipopolysaccharide group): 1 mg LPS/kg × h, no glycine;
- LPS + GLY (lipopolysaccharide and glycine group): 1 mg LPS/kg × h, 2 × 50 mg glycine/kg × 15 min.

2.5. Survival studies

The survival rate of each group was calculated at the end of the studies. Survival curves were analyzed by the Kaplan–Meier method. The median survival time was determined for a Kaplan–Meier estimator at 50% for the respective groups within the observation period.

2.6. Assessment of vital parameters

Systolic, diastolic, and mean arterial blood pressures (MAP) were recorded *via* the femoral artery catheter in 10-min intervals. An infusion bag containing Ringer's solution was used to keep the catheter functional. Heart rates were determined from systolic blood pressure spikes. The breathing rates were determined based on the breathing movements in 10-min intervals. The core body temperature of the rats was continuously monitored using a rectal sensor; cooling below 37°C was prevented by an underlying thermostated operating table and by covering the animals with aluminum foil.

2.7. Assessment of blood and plasma parameters

Using 2.0-mL self-filling arterial samplers (Pico50; Radiometer Medical, Brønshøj, Denmark) containing 80 IU electrolyte-balanced heparin, blood samples (0.5 mL) were taken from the femoral artery catheter immediately after its insertion