Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Effects of glycine, pyruvate, resveratrol, and nitrite on tissue injury and cytokine response in endotoxemic rats

Tim Hamburger, PhD,[a] *Martina Broecker-Preuss, PhD,*[b] *Matthias Hartmann, MD,*[c] *F. Ulrich Schade, PhD,*[d] *Herbert de Groot, MD, PhD,*[a] *and Frank Petrat, PhD*[a,*]

[a] *Institut für Physiologische Chemie, Universitätsklinikum, Universität Duisburg-Essen, Essen, Germany*
[b] *Klinik für Endokrinologie und Stoffwechselerkrankungen, Zentrallabor Bereich Laborforschung, Universitätsklinikum, Universität Duisburg-Essen, Essen, Germany*
[c] *Klinik für Anästhesiologie und Intensivmedizin, Universitätsklinikum, Universität Duisburg-Essen, Essen, Germany*
[d] *Klinik für Unfallchirurgie, Universitätsklinikum, Universität Duisburg-Essen, Essen, Germany*

ARTICLE INFO

Article history:
Received 4 October 2012
Received in revised form
15 December 2012
Accepted 11 January 2013
Available online 8 February 2013

Keywords:
Lipopolysaccharide
Cytokines
Ischemia
Granulocytes
Kupffer cells
Nitric oxide

ABSTRACT

Background: Glycine, pyruvate, resveratrol, and nitrite are well-known protective compounds among others in ischemic tissue injury. Here, we compared their effects in acute lipopolysaccharide (LPS)-induced shock in rats to assess whether inhibition of the proinflammatory cytokine response is a prerequisite for their protective actions.

Materials and methods: Rats (six or eight per group) were anesthetized, received LPS as an intravenous bolus (2.5 mg/kg), and were observed for 5 h. Glycine, sodium pyruvate, resveratrol, and sodium nitrite were continuously infused starting 30 min before LPS administration. Parameters included histopathologic changes, organ-specific cytokine levels, plasma nitrite and nitrate concentrations, and time courses of biomonitoring parameters, marker enzyme activities, and plasma cytokine concentrations.

Results: Glycine, pyruvate, resveratrol, and nitrite enhanced arterial blood pressure after LPS-induced shock. Also, parameters reflecting tissue ischemia were significantly improved and plasma markers of organ injury ameliorated by all substances. Of the plasma cytokine concentrations increased by LPS, some were differently decreased or even further increased by the substances. None of them reduced the elevated plasma nitrite and nitrate concentration. Glycine diminished the increases in tissue cytokine levels organ specifically, pyruvate decreased some cytokine concentrations in all organs, and nitrite significantly affected only a few cytokine concentrations in some organs, whereas the levels of many cytokines were raised by resveratrol. All substances except resveratrol decreased granulocyte infiltrates in the liver.

Conclusions: The present results demonstrate that glycine, pyruvate, resveratrol, and nitrite protect against LPS-induced shock and tissue injury (cell death) in rats and suggest that inhibition of the proinflammatory cytokine response is not mandatory for their protective actions.

* *Corresponding author*. Institut für Physiologische Chemie, Universitätsklinikum, Universität Duisburg-Essen, Hufelandstrasse 55, D-45122 Essen, Germany. Tel.: +49 201 723 4105; fax: +49 201 723 5943.
E-mail address: frank.petrat@uni-duisburg-essen.de (F. Petrat).
0022-4804/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2013.01.024

1. Introduction

Sepsis and septic shock are the most common cause of death in intensive care units around the world [1]. Septic shock is the final result of complex interactions between the host immune response and infecting pathogens and defined as sepsis accompanied by hypotension that persists despite adequate resuscitation with fluids [2].

Lipopolysaccharide (LPS), a glycolipid of the outer membrane of Gram-negative bacteria, is a well-known pathogenic compound in septic shock that has been used in numerous experimental studies to induce acute inflammatory shock reactions [3,4]. LPS induces a massive release of cytokines and other proinflammatory and anti-inflammatory mediators [5–7] and also leads to microvascular oxygen shortage because of an activation of the intravascular coagulation cascade [8–10] and arterial hypotension [11]. Consequently, tissue ischemia is a decisive pathogenic factor in LPS-induced shock.

We and others have demonstrated that the amino acids glycine, the end product of aerobic glycolysis, pyruvate, the plant polyphenol resveratrol, and nitrite have anti-inflammatory, cytoprotective, and immunomodulatory properties providing protection in several models of ischemia–reperfusion [12–18] and/or endotoxemia [19–25]. However, although many promising qualities have been reported for these compounds, no attempt has been made so far to compare their protective potentials in acute LPS-induced shock and assess whether inhibition of the proinflammatory cytokine response is a prerequisite for their protective actions when administered at low intravenous doses.

To this end, in the present study, we compare the tissue protective and systemic effects, as well as influences on the cytokine network of low doses of glycine, pyruvate, resveratrol, and nitrite, that is, doses we recently found to be most protective against mesenteric ischemia–reperfusion injury, in a rat model of acute systemic inflammation and shock induced by intravenous LPS.

2. Materials and methods

2.1. Chemicals

Sodium pyruvate, resveratrol, and LPS (*E. coli*, serotype O111:B4; LOT: 099K4025) were obtained from Sigma-Aldrich (St. Louis, MO). Glycine was obtained from SERVA (Heidelberg, Germany), sodium nitrite from Merck (Darmstadt, Germany), isoflurane (Florene) from Abbott (Wiesbaden, Germany), ketamine 10% from CEVA (Düsseldorf, Germany), lidocaine (Xylocain 1%) from Astrazeneca (Wedel, Germany), and Ringer's solution from Fresenius Kabi (Bad Homburg, Germany). Portex catheters (0.58 mm inner diameter and 0.96 mm outer diameter) were purchased from Smiths Medical International (Hythe, UK). Paraffin (Paraplast Tissue Embedding Medium REF 501006) was obtained from McCormick Scientific (St. Louis, MO), normal saline from B. Braun (Melsungen, 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 Essen University Hospital. Animals were kept under standardized conditions of temperature (22°C ± 1°C), humidity (55% ± 5%), and 12/12-h light/dark cycles with free access to food (ssniff-Spezialdiäten, Soest, Germany) and water. All animals received human care according to the standards of Annex III of the directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010, on the protection of animals used for scientific purposes. The experimental protocol received ethical approval by the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, according to the German Animal Welfare Act.

2.3. Anesthesia, analgesia, and surgical procedure

Rats were anesthetized with isoflurane (1.3%–2.0% in 100% medical O_2 at 1.0 L/min) and received ketamine (50 mg/kg, subcutaneously) and lidocaine (5 mg/kg, subcutaneously) for analgesia as described previously [14]. The right femoral artery and vein were surgically prepared and cannulated with a Portex catheter. At the end of the experiment (300 min after LPS administration; see in the following section), animals were killed by resecting the heart (and other organs) under deep isoflurane anesthesia.

2.4. Experimental groups

The study was performed in two consecutive series with a total of 84 rats. In the first series with eight rats per group, the LPS-induced histopathologic changes at the end of the experiments, final (at the end of the experiments) organ-specific cytokine levels, and concentrations of free hemoglobin (Hb) and nitrite and nitrate (as a measure of nitric oxide [NO]) in the final blood plasma were determined. The second series, which underwent the same experimental protocol, was performed with six animals per group and intended to obtain time courses of biomonitoring and blood gas analysis parameters, marker enzyme activities, and cytokine concentrations in blood plasma.

LPS (2.5 mg) was dissolved in 1 mL of 0.9% NaCl solution and then filtered through a bacteria-tight filter (Minisart 0.2 μm; Sartorius, Göttingen, Germany). Rats received 2.5 mg LPS/kg (1 mL/kg) within 15 s into the femoral vein, and the catheter was flushed immediately with 0.5 mL of 0.9% NaCl solution thereafter. Sodium pyruvate (214.3 mg) and glycine (85.7 mg) were freshly dissolved in 30 mL and resveratrol (1.7 mg) in 200 mL of sterile 0.9% NaCl solution, followed by pH adjustment to 7.35 with NaOH or HCl, respectively. Because of its pH-dependent decay, sodium nitrite (5 mg) was dissolved in 50 mL of pH-adjusted 0.9% NaCl solution (pH, 7.5–8.0), and 2 mL of this stock solution was added to 33 mL of 0.9% NaCl solution (pH, 7.5–8.0). All solutions were filtered through bacteria-tight filters and infused with a syringe pump (Perfusor-Secura FT; B. Braun) into the *vena femoralis* at a rate of 7 mL/(kg × h) over a total period of 330 min starting 30 min before LPS administration. The doses administered per hour