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Intravenous glycine after cecal ligation and puncture has no effect on impaired hepatic microperfusion, leukocyte adhesion, and mortality in septic rats

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Abstract

Recent studies indicated that prefeeding of a glycine supplemented diet reduces the hepatic inflammatory response and liver damage in sepsis. We investigated the effect of a glycine-enriched infusion on hepatic microcirculatory disturbances and mortality in a rat model of sepsis after the onset of the disease. Male Wistar rats (240 ± 13 g) underwent cecal ligation and puncture (CLP) or laparotomy (LAP). A glycine (CLP + Gly, $n = 24$), valine (CLP + Val, $n = 24$), or sodium chlorid (CLP + Sc, $n = 24$) infusion was started 2 h after CLP. The LAP group received sodium chloride intravenously (LAP + Sc, $n = 18$). Five hours, 10 h, and 20 h after CLP or LAP intravital microscopy (IVM) was performed to investigate leukocyte–endothelial interaction (LEI) and mean erythrocyte velocity in liver sinusoids (sMEV) and postsinosoidal venules (vMEV). The portal blood flow (PBF), hepatic enzyme liberation, and glycine values in blood were measured. Immunohistochemical staining for ICAM-1 in liver tissue was performed and survival was observed. Glycine values were significantly elevated in the CLP + Gly vs. the CLP + Val and the CLP + Sc group at every timepoint of investigation. Glycine infusion had no beneficial effects on sMEV, vMEV, LEI, hepatic enzyme liberation, and survival. Heart rate and mean arterial pressure remained stable but PBF decreased significantly in all groups 20 h after CLP. Although glycine reduces the hepatic inflammatory response and liver damage in pretreatment of septic rats, there was no effect of intravenous glycine after the onset of sepsis in our experiments. Our animal model does not support the use of glycine in patients.

Keywords: Adhesion molecules; Sepsis; Liver; Glycine

Introduction

The hepatic microperfusion is impaired during the course of sepsis (Ring and Stremmel, 2000; Secchi et al., 2000; Smets et al., 1999; Wang et al., 1992). Due to its integral role in metabolism and host defense mechanisms, the liver is believed to be a major organ responsible for initiating the MODS (Koo et al., 1999). Lipopolysaccharides (LPS) activate Kupffer cells to produce cytokines like TNF-alpha, IL-1, and IL-6 which are leading to hepatocellular damage and the expression of endothelial adhesion molecules (Ayala et al., 1992; Bauer et al., 2000; Koo et al., 1999; Wang et al., 1993a, 1995). The rapid translocation of P-selectin from Weibel–Palade bodies to the endothelial surface initiates the transient and reversible adhesion of leukocytes (rolling) to the endothelium via L-selectin (Bauer et al., 2000; Hafezi-Moghadam and Ley, 1999; Hafezi-Moghadam et al., 2001; Ley, 2001; Norman et al., 1995; Parent and Eichacker,

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1999). During this process, activated endothelial cells produce chemoattractants such as IL-8 and platelet-activating factor (PAF) which may be secreted or remain surface bound. In leukocytes IL-8, PAF as well as C5a initiate a cascade of intracellular events which are leading to activation of β-integrins (LFA-1 and Mac-1) (Ley, 1993; Parent and Eichacker, 1999). β-integrins enable leukocytes to adhere to endothelial adhesion molecules like intercellular adhesion molecule-1 (ICAM-1, CD54) which initiates extravasation (Issekutz et al., 1999; Willam et al., 1999). The release of superoxid, arachidonic acid metabolites, and proteases of transendothelial migrated leukocytes injures hepatocytes (Issekutz et al., 1999; Sheth et al., 2000; Wheeler et al., 2000a).

Endothelial adhesion molecules are recognized as key determinants of the inflammatory response and are therefore potential targets for anti-inflammatory therapy (Mulligan et al., 1993a,b). ICAM-1 is constitutively expressed on unstimulated endothelial cells and is further increased by proinflammatory cytokines like TNF-alpha, IL-1, and interferon-γ (Ley, 1993; Willam et al., 1999).

Glycine is a non-essential amino acid which has been shown to exert beneficial effects on organ damage in septic rats and animal models of liver ischemia and reperfusion (Grotz et al., 2001; Schemmer et al., 1999; Thurman et al., 1998; Wheeler et al., 2000b; Yang et al., 2001; Zhong et al., 1996). The release of TNF-alpha by monocytes and Kupffer cells and the production of superoxid by neutrophiles is reduced by glycine (Schemmer et al., 1999; Spittler et al., 1999; Wheeler and Thurman, 1999; Wheeler et al., 2000a). Glycine activates a glycine-gated chlorid channel in Kupffer cells, monocytes, and neutrophiles leading to hyperpolarization of the cell membrane through increased chlorid influx (Ikejima et al., 1997; Schemmer et al., 1999; Wheeler et al., 2000a). These mechanisms make voltage-dependent Ca^{2+} channels more difficult to open which is a prerequisite necessary for LPS-induced syntheses of TNF-alpha, proteases, and superoxid (Ikejima et al., 1997; Schemmer et al., 1999; Spittler et al., 1999; Wheeler et al., 2000a).

In most previous studies concerning effects of glycine in sepsis, glycine was applied by a prefeeding diet to rats before the onset of sepsis (Grotz et al., 2001; Yang et al., 2001). For the first time, we used an intravenous glycine infusion in a rat model of sepsis after the onset of the disease. The study was directed to survival and hepatic damage as reflected by liver microcirculation during the course of sepsis.

Materials and methods

Animals and protocol

All experimental procedures and protocols used in this investigation were approved by the Governmental Animal Protection Committee (Karlsruhe, Germany).

Male Wistar rats (240 ± 13 g) were anesthetized by intraperitoneal injection of 20 mg/kg body weight sodium pentobarbital (Nembutal; Sanofi, Düsseldorf, Germany) and 30 mg/kg body weight intramuscular injection of Ketamin. The right jugular vein was cannulated for the infusion of reagents. Sepsis was induced by cecal ligation and puncture (CLP) (Remick et al., 2000; Wichterman et al., 1980). Laparotomy of 2 cm in the lower abdomen was performed and the cecum was exteriorized. After non-obstructive ligation of the cecum, two stitches with an 18-G needle were performed. A control group underwent only laparotomy (LAP). Two hours after CLP or LAP rats received either a intravenous infusion of glycine 53 mg/kg BW/h (CLP + Gly, $n = 24$) or valine 53 mg/kg BW/h (CLP + Val, $n = 24$). One CLP group (CLP + Sc, $n = 24$) and the LAP group (LAP + Sc, $n = 18$) were treated with a similar amount of sodium chlorid intravenously.

The infusion of glycine, valine, or sodium chloride was maintained until the intravital microscopy investigation of the liver (IVM). Eight animals of each CLP group (CLP + Gly, CLP + Val, CLP + Sc) and six animals of the LAP + Sc group underwent IVM once 5 h, 10 h, or 20 h after CLP. The right carotid artery was cannulated for the measurement of heart rate and mean artery pressure. To maintain anesthesia during the observation period the left femoral vein was cannulated for continuous sodium pentobarbital (8 mg/h/kg body weight) and Ketamin (4 mg/h/kg body weight) infusion. Rectal temperature was measured and maintained at 37°C using a heating pad.

After IVM blood count in venous blood was performed, hepatocellular enzyme release (AST, ALT, LDH), heart rate, and mean arterial pressure were measured. The blood flow of the portal vein (PBF) was determined using the flow probe of a small animal ultrasonic flowmeter (Transonic Systems) (Secchi et al., 2000). Snap biopsies from the liver were taken and harvested in liquid nitrogen for immunohistochemical staining.

For the investigation of survival, 30 rats which were not included in the IVM investigations underwent CLP and 10 rats underwent LAP. The right jugular vein was cannulated as described above. Animals were randomized into three groups. An intravenous infusion of either glycine, valine, or sodium chloride was started 2 h after CLP and was maintained until the end of the investigation. The experiment was interrupted after an observation period of 48 h.

Intravital microscopy (IVM)

After placing the animal beneath the microscope, a 30-min stabilization period followed. The upper surface of the left liver lobe was exteriorized on a specially designed mechanical stage. To maintain body temperature, the liver lobe was continuously superfused by thermostat-controlled (37.0°C) Ringer-solution. Hepatic microcirculation was observed using a specially designed microscope for epi-