Effects of nicotinamide, an inhibitor of PARS activity, on gut and liver $O_2$ exchange and energy metabolism during hyperdynamic porcine endotoxemia

Abstract Objective: To investigate the effects of nicotinamide (NIC), an inhibitor of poly(ADP-ribose) synthetase (PARS), on intestinal and liver perfusion, $O_2$ kinetics, and energy metabolism over 24 h of hyperdynamic porcine endotoxemia. Design: Prospective, randomized, controlled experimental study with repeated measures. Setting: Animal laboratory in a university hospital. Subjects: Sixteen pigs, divided into two groups: nine endotoxemic animals without therapy (CON); seven animals treated with NIC. Interventions: Pigs were anesthetized, mechanically ventilated, and instrumented. Intravenous E. Coli LPS was continuously infused over 24 h concomitant with fluid resuscitation. After 12 h of endotoxemia continuous i.v. infusion of NIC (10 mg/kg per hour) was administered until the end of the experiment. Measurements and results: All animals developed hyperdynamic circulation with sustained increase in cardiac output and progressive fall in mean arterial pressure. NIC maintained blood pressure without affecting CO. Hepato-splanchnic macrocirculation was not modified by the treatment. Nevertheless, although NIC attenuated the progressive rise of ileal mucosal-arterial $PCO_2$ gap, it failed to improve portal venous L/P ratio, a marker of the overall energy state of the portal venous drained viscera. Similarly, neither the increased hepatic venous L/P ratio nor the simultaneous drop in hepatic lactate uptake were influenced by NIC. Conclusions: Although NIC maintained hemodynamic stabilization during long-term endotoxemia, it was unable to improve LPS-induced deterioration of the hepato-splanchnic energy metabolism. More potent and selective PARS inhibitors are needed to elucidate the role of a PARS-dependent pathway in a clinically relevant models of sepsis.

Key words Endotoxin · Septic · PARS · Liver · Gut · Metabolism · Lactate · Tonometry
Introduction

Sepsis and septic shock result in significant organ dysfunction and loss of critical cellular functions. There is now good evidence that excessive production of nitric oxide (NO), resulting from the induction of inducible NO synthase (iNOS), significantly contributes to several pathophysiological features that accompany acute inflammatory events such as endotoxia or sepsis [1]. In addition to its detrimental hemodynamic effects, NO can directly compromise cellular bioenergetics (even in the presence of adequate oxygen availability) via multiple actions, including inhibition of mitochondrial respiration [2]. Furthermore, a potential indirect noxious pathway of NO and its toxic reaction products has recently been proposed [3]: simultaneous production of NO and superoxide anion leads to the formation of peroxynitrite (ONOO\textsuperscript{-}), which may cause single strand breaks in DNA resulting in the activation of the highly energy-consuming DNA-repairing enzyme poly(ADP-ribose) synthetase (PARS). As a consequence, an excessive activation of PARS causes depletion of cellular high-energy phosphates with impairment of cellular metabolism [3]. The experimental data suggests that this “PARS suicide mechanism” is involved in many of fundamental disturbances of endotoxic shock, including endothelial dysfunction [4], loss of vascular energetic and contractile failure [5, 6], pulmonary endothelial and intestinal mesenteric hyperpermeability [7], and impairment of mitochondrial respiration [8, 9]. Moreover, strategies aimed at inhibiting PARS activity have exerted beneficial effects in various experimental models, including myocardial [10], renal [11], and splanchnic [12] ischemia-reperfusion injury or hemorhagic shock [13]. In a rat model of endotoxic shock treatment with the PARS inhibitor, nicotinamide prevented the delayed vascular failure and restored the endotoxic (LPS)-induced loss of vascular contractility [5]. Finally, PARS blockade with nicotinamide or 3-amino-benzamide was shown to prevent the development of multiple organ failure after zymosan-induced peritonitis in rats [14].

On the basis of these promising data from rodent models, we investigated the effects of PARS inhibition in a porcine model of long-term, well-resuscitated, hyperdynamic and hypermetabolic endotoxia as described recently [15, 16, 17]. Considering the postulated role of the hepato-splanchnic region in the pathogenesis of septic shock and multiple organ failure [18], we focused on the intestinal and liver perfusion, O\textsubscript{2} kinetics, and energy metabolism. As a PARS inhibitor we used nicotinamide, which causes negative feedback inhibition of PARS. To mimic clinically relevant situation, the compound was administered as a continuous infusion in a “post-treatment” fashion after full-blown sepsis had been established.

Materials and methods

Animals and preparations

The experiment was performed in adherence to National Institutes of Health Guidelines on the Use of Laboratory Animals. The study protocol was approved by the University Animal Care Committee as well as the federal authorities for animal research (Regierungspräsidium Tübingen, Baden-Württemberg, Germany). The present report describes results that are part of a series of experiments investigating the effects of NIC as well as of selective iNOS inhibition in our model of porcine endotoxia. All these experiments were performed in random order within the same time period. Therefore, the results for the control group infused with endotoxin alone are the same as those presented in a complementary report (Matejovic et al., Shock in press). This allowed us to minimize the number of animals needed for an experiment as requested by the legal regulations for animal research. Sixteen domestic pigs of either sex (40–55 kg) were investigated. The anesthesia as well as the surgical preparation have been previously described in detail [15, 16, 17]. Briefly, the pigs were anesthetized with pentobarbital (200–300 mg/kg) and paralyzed with alcuronium (14 mg/h). Depth of anesthesia was controlled by EEG. The pigs were mechanically ventilated and the tidal volume and respiratory rate were adjusted to maintain arterial PCO\textsubscript{2} between 35–45 mmHg. A pulmonary artery thermistor dilution catheter was placed via the right jugular vein. In one femoral artery, a catheter was introduced for continuous blood pressure recordings and blood sampling, in the other one a thermistor-tipped fiberoptic catheter for thermal-dye double indicator dilution measurements. Ringer’s Lactate solution was infused i.v. as baseline fluid. A midline laparotomy was performed, and precalibrated ultrasound transit-time flow probes (Transonic Systems, Ithaca, N.Y., USA) were placed around the portal vein and the common hepatic artery, and flows were continuously recorded. Then, catheters were introduced into the portal and hepatic vein. A loop-ileostomy was performed for the insertion of a fiberoptic PCO\textsubscript{2} sensor (Paratrend 7, Biomedical Sensors, High Wycombe, Bucks., UK). The abdominal wall was closed. A postsurgical stabilization period of 8 h was allowed before baseline measurements were obtained.

Measurements and calculations

Cardiac output (CO) was determined by thermodilution (66 S Monitor, Hewlett Packard, Palo Alto, Calif., USA) as the mean of 4–5 injections of 10 ml ice-cold saline randomly spread over the respiratory cycle. The intrathoracic blood volume was measured by arterial thermal-green dye double-indicator dilution (COLD Z-021, Pulson, Munich, Germany) after injection of 10 ml of cold indocyanine green (2.5 mg ml\textsuperscript{-1}). The continuously recorded portal venous (Qpv) and hepatic arterial blood flow (QhA) rates were summed to obtain the total hepatic blood flow (Qh). Arterial, portal, and hepatic venous blood samples were analyzed for PO\textsubscript{2}, PCO\textsubscript{2}, and pH (NOVA Stat Profile Ultra, NOVA Biomedical, Waltham, Mass., USA) as well as total hemoglobin and hemoglobin O\textsubscript{2} saturation (IL 482 CO-Oximeter, Instrumentation Laboratories, Luxembourg, Mass., USA; calibrated for pig blood). Systemic O\textsubscript{2} delivery (DO\textsubscript{2}SYS) and uptake (VO\textsubscript{2}SYS) were calculated from the standard formulas. Intestinal O\textsubscript{2} (iO\textsubscript{2}-ER) extraction was calculated as the quotient of arterial-portal venous O\textsubscript{2} content difference divided by the arterial O\textsubscript{2} content. Liver DO\textsubscript{2} (hDO\textsubscript{2}) and O\textsubscript{2} uptake (hVO\textsubscript{2}) were calculated as the product of Qpv and QhA times the portal venous and hepatic arterial O\textsubscript{2} content, respectively, and the portal-hepatic venous and the arterial-hepatic venous