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Nitric oxide–supplemented resuscitation improves early gastrointestinal blood flow in rats subjected to hemorrhagic shock without late consequences

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Abstract

BACKGROUND: We have shown that hemorrhage/resuscitation altered gastrointestinal blood flow (GI-BF) and that gastric perfusion did not recover after resuscitation. This study aimed to determine the effect of nitric oxide (NO) supplemented resuscitation on the mean arterial blood pressure (MAP), GI-BF, and outcome after hemorrhagic shock.

METHODS: Rats were subjected to hemorrhage and resuscitation with/without the NO-donor S-nitroso human serum albumin (S-NO-HSA). GI-BF was determined using colored microspheres.

RESULTS: NO supplementation significantly decreased MAP at the end of resuscitation. At the same time point, the GI-BF has significantly increased in the stomach, duodenum, and colon. Two hours after treatment discontinuation, there was no difference in either MAP or GI-BF between NO-supplemented and control groups. The survival times indicated that S-NO-HSA treatment was noninferior compared with control.

CONCLUSIONS: NO-supplemented resuscitation improves the GI-BF during the early stage of resuscitation without a negative impact on short-/long-term survival despite a transient MAP decrease.

An increasing body of experimental and clinical data has emerged during the last few decades implicating the gut as a central organ in the development of multiple organ failure (MOF).[1,2] A breakdown of the mucosal barrier results in increased gut permeability and translocation of bacteria/bacterial products. The entry of bacteria/bacterial products (endotoxin) into the circulation and the release of inflammatory mediators including tumor necrosis factor (TNF), interleukin (IL), nitric oxide (NO), and radical species alters the immune function and may result in cell damage, tissue injury, and ultimately death.[3–6]

The systemic hemodynamic response to hypovolemic shock is characterized by a decrease in blood pressure and cardiac output and is associated with an increase in peripheral resistance, which are all typical features of circulatory shock.[7] The gastrointestinal (GI) tract is highly susceptible to diminished perfusion because the mucosal countercurrent microcirculation renders the villi particularly susceptible to ischemic insults.[8] The perfusion deficits after trauma and shock have been shown to be a central feature in the initiation of an inflammatory process, including Systemic In-

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flammatory Response Syndrome (SIRS) and the development of MOF.[9–11] Recently, we have shown that hemorrhage and resuscitation cause different degrees of alteration in gastric and intestinal blood flow and that gastric perfusion does not recover after resuscitation, predisposing this organ to further organ damage.[12]

Many different mechanisms including neural, humoral, and local cellular vasoactive substances control the GI circulation. However, their individual roles as well as possible synergistic effects are not yet fully understood. NO has been shown to be a critically important mediator in the autoregulation of vascular tone and blood pressure.[13] In addition to its vasodilatator properties, NO is also important in the regulation of platelet aggregation and neutrophil adhesion to the endothelium.[14,15]

The reports on the role of NO in hemorrhagic shock are controversial. It has been shown that early after the onset of hemorrhagic shock the release of endothelium-derived NO is reduced.[16] This phenomenon is most likely caused by the decreased activity of the constitutive NO synthase[17] and/or reduced conversion of L-arginine (substrate of nitric oxide synthase) to NO and citrulline.[18] L-arginine treatment during resuscitation has been shown to decrease inflammatory response and increase organ blood flow after trauma and hemorrhage.[19] Similarly, NO donor, such as S-nitroso-N-acetylpenicillamine, administered during the early stage of reperfusion has been described to attenuate hepatocellular injury after hemorrhagic shock.[20] In contrast, an excessive production of NO during hemorrhagic shock has been considered to contribute to vascular hyporeactivity,[21,22] cellular injury, and gut barrier failure.[23]

S-nitroso-human serum albumin (S-NO-human serum albumin [HSA]) has been reported to gradually increase NO release in vivo and to attenuate both early and late hepatic microcirculatory disturbances as well as the increase in leukocyte adherence.[24] Whether S-NO-HSA improves GI organ blood flow and survival after hemorrhage/resuscitation by directly increasing NO during resuscitation is not yet known. Thus, the aim of this study was to determine the effect of NO-supplemented resuscitation on the mean arterial blood pressure (MAP), GI blood flow, and outcome after hemorrhagic shock in rats.

Materials and Methods

Animals

The study was approved by the local Committee on Animal Experiments of Vienna, Austria, and all experiments were performed under the conditions described in the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health (Publication National Institutes of Health 86–23, revised 1985). Adult male Sprague-Dawley rats (Himberg, Austria) weighing between 450 g and 480 g were used. The animals were allowed to recover for at least 7 days after arrival to the facility before being used in this study. All animals had free access to standard laboratory chow and water. Before the experiment, animals were fasted overnight but allowed free access to water.

The colored microspheres (MSs), 15 μm suspended in saline + .05% Tween 80 containing 60 million spheres/20 mL suspension were purchased from Triton Technology, Inc, San Diego, CA. Reagents used for dye recovery from tissue and blood samples were obtained from Sigma Aldrich, Vienna, Austria. A membrane filter (22-mm diameter polyethylene PE, 10-μm pore size, Triton Technology, Inc) and filtration unit were purchased from Millipore (Bedford, MA).

Hemorrhagic shock procedure

Rats were randomized into 2 groups of 7 animals each before the induction of anesthesia with an intramuscular injection of a mixture of ketamine/xylazine 112/10 mg/kg body weight (BW). The animals were maintained under anesthesia by spontaneously breathing inhalation of isoflurane (.4%) on a temperature controlled surgical board (37° ± 1°C). Rectal temperature was continuously monitored in all animals.

A standardized and reproducible model of hemorrhagic shock in rats was used as described previously.[7,12] Briefly, after the induction of anesthesia, the right carotid artery was cannulated with a polyethylene catheter (PE-50) under aseptic conditions. The catheter was placed into the left ventricle under pressure control and connected to a blood pressure monitor Cardiosys (Experimetria, Budapest, Hungary) and an infusion pump (Harvard, Edenbridge, UK) for MSs infusion. A second catheter (PE-50) was placed into the right femoral artery and connected to the Cardiosys blood pressure monitor and to a second pump (Harvard) for reference blood withdrawal. This catheter was also used for shed blood withdrawal and resuscitation.

The rats were bled via the femoral artery for over 5 minutes until an MAP of 30 mm Hg to 35 mm Hg was reached and maintained for 180 minutes followed by resuscitation over a period of 60 minutes. Monitoring continued until 2 hours after the end of resuscitation. Subsequently, the animals were sacrificed to obtain organ samples for further processing.

Treatment

S-NO-HSA (10 μmol/kg/h BW) was infused during resuscitation with shed blood plus Ringer solution at twice the shed blood volume (Fig. 1). The dose of 10 μmol/kg/h S-NO-HAS selected in this study was chosen based on pilot experiments comparing the effect of 0.1 μmol/kg/h, 1.0 μmol/kg/h, 10.0 μmol/kg/h, 20.0 μmol/kg/h, and 50.0 μmol/kg/h BW on MAP in control rats. The control group received HSA at a dose of 10 μmol/kg/h BW.

The present experiment was performed with slight systemic heparinization during resuscitation. Before resuscita-