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Early resuscitation with exendin-4 alleviates acute lung injury after hemorrhagic shock in rats

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ABSTRACT

Background: Oxidative stress induced by hemorrhagic shock (HS) is known to initiate a systemic inflammatory response, which leads to subsequent acute lung injury. This study is aimed to assess the efficacy of exendin-4 (Ex-4) in attenuating lung injury in a rat model of HS and resuscitation (HS/R).

Methods: HS was induced in sodium pentobarbital–anesthetized adult male Wistar rats by withdrawing blood to maintain a mean arterial pressure of 30-35 mm Hg for 50 min. Then, the animals received Ex-4 (5 μg/kg) or vehicle (saline) intravenously and were resuscitated with a volume of normal saline 1.5 times that of the shed blood volume. Mean arterial pressure was measured throughout the experiment, and acid-base status, oxidative stress, inflammation, and lung injury were evaluated at 2 h after resuscitation.

Results: Ex-4 infusion reduced the methemoglobin content, the malondialdehyde content, the myeloperoxidase activity, and the expression of tumor necrosis factor-α and interleukin-6 in the lungs. The histologic injury was also markedly decreased in the Ex-4 group compared with the vehicle group.

Conclusions: Ex-4 ameliorates the oxidative stress, inflammatory response, and subsequent acute lung injury occurring after HS/R. Although future studies are required to elucidate the underlying mechanism, our results indicate that Ex-4 infusion may be a promising strategy for improving lung injury in the treatment of HS.

Introduction

Hemorrhagic shock (HS) remains a dominating cause of mortality after trauma.[1,2] The morbidity and mortality associated with HS are composite results of systemic inflammatory response syndrome, which subsequently triggers multiple organ failure.[3] As a major component of multiple organ failure,[4] acute lung injury is a common complication after severe HS and largely accounts for the late mortality.[5]

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Reperfusion after HS (HS/R) induces the formation of reactive oxygen species (ROS). Overwhelming ROS is closely correlated to tissue injury and plays an initial role in oxidative stress and cell death.[6] In addition, the increased neutrophil accumulation associated with lung injury can enhance local inflammation by infiltrating into pulmonary tissue and elevating the local of levels cytokines, such as interleukin-6 (IL-6).[7] Thus, effective strategies for mitigating this process during reperfusion are required.

Exendin-4 (Ex-4) is a glucagon-like peptide-1 receptor (GLP-1R) agonist belonging to the Exenatide family that can induce pancreatic B-cell proliferation, powerfully inhibit glucagon secretion and control blood glucose levels.[8] Previous studies have focused on the effects of Ex-4 on glucose tolerance, insulin secretion, and cell apoptosis inhibition.[9,10] In addition, Ex-4 was recently demonstrated to prevent ROS production through oxidative defense gene upregulation and to ameliorate renal injury after ischemia reperfusion.[11-13] We hypothesized that Ex-4 could mitigate lung injury and be used to improve pulmonary tissue conditions after HS/R.

We demonstrated that the intravenous administration of Ex-4 could attenuate oxidative stress, tissue inflammation, and lung injury. As such, the administration of Ex-4 is a promising therapeutic strategy for HS.

Materials and methods

Animals

This study was approved by the Institutional Animal Care and Use Committee of the Academy of Military Medical Sciences (IACUC No: AMMS-13-2015-003) and complied with the Guide for the Care and Use of Laboratory Animals. Twenty-four male Wistar rats weighing 280-310 g (Vital River Laboratories, Beijing, China) were kept on a 12-h light-dark cycle at 25°C and had free access to water and food during acclimation to a specific pathogen-free animal facility, according to standard laboratory procedures.

Surgical procedures

An HS model was performed as previously described.[3,14] In brief, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and placed on a warming pad (TMS-202; Softron, Beijing, China) that was maintained at 37° ± 0.1°C throughout the experiment. Then, the two femoral arteries and the left femoral vein were isolated and cannulated with sterile polyethylene catheters (PE-50). The left femoral arterial catheter was used to withdraw blood for the hemorrhage and blood gas measurements. The left femoral venous catheter was used for the drug administration and fluid infusion. The right femoral catheter was used for the continuous monitoring of mean arterial pressure (MAP). Heparin was administered intravenously (400 U/kg) to inhibit the coagulation of blood in the experimental equipment. Supplementary doses of pentobarbital were injected intraperitoneally when necessary.

HS model

The intubated rats were allowed to stabilize for 10 min before the baseline MAP was measured after the surgical operation. Two-thirds of the rats were used in the pressure-controlled HS model, which was initiated by bleeding at a rate of 0.4 mL/min to achieve a MAP of 30-35 mm Hg within 10 min using syringe pumps (LZS-AJ10; Softron). One-third of the rats were assigned to the sham group and did not undergo the hemorrhage procedures. The MAP was maintained for 50 min by further blood withdrawal. At the end of the HS, arterial blood samples were collected for analysis.

Treatment administration and resuscitation

After HS, the rats were randomly distributed into two groups and were administered either Ex-4 solution (5 μg/kg; GeneScript, Nanjing, China) or an equal volume of normal saline (NS; Shijiazhuang SiYao Ltd, Hebei, China) intravenously. After this administration, the animals were resuscitated with NS at 1.5 times the volume of shed blood. All infusions were driven by a pump at a constant rate of 0.3 mL/min. At the end of the experiment, the lung tissues were harvested and stored in liquid nitrogen for further processing.

Blood gas analysis

Arterial blood samples were collected at the baseline, after blood withdrawal and 2 h after resuscitation. Blood gas analyses of the partial pressure of oxygen (pO_2), the partial pressure of carbon dioxide (pCO_2), the blood lactate level, the base excess (BE) and the ionic concentration were performed by an analyzer (ABL90FLEX; Radiometer Copenhagen, Denmark).

Measurements of malondialdehyde level and myeloperoxidase activity

Fresh lung tissues were partly divided and placed in centrifuge tubes with 0.9% NS and protease inhibitors on ice (Roche, Mannheim, Germany). The homogenates were then centrifuged at 1000 × g for 6 min at 4°C. The supernatants were used for determining the MDA content and MPO activity via a colorimetric assay according to the manufacturer's recommendations, as previously reported (Jiancheng Biological Institute, Nanjing, China).[14]

Measurement of inflammatory cytokine levels

The IL-6, tumor necrosis factor-α (TNF-α), and IL-1β levels in the lung tissue were determined using an enzyme-linked immunosorbent assay kit (PeproTech, Rocky Hill, NJ) according to the manufacturer's instructions. The values are expressed as picogram/milligram protein.

Lung histopathology

Lung tissues were fixed with buffered 4% paraformaldehyde for 24 h. After being dehydrated and embedded in paraffin, the