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Hepatic topographical changes of endoplasmic reticulum stress and unfolded protein response signaling after hemorrhagic shock and reperfusion

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ABSTRACT

Background: Endoplasmic reticulum (ER) stress plays a crucial role in cell death decisions in context of various diseases. Although it is known that ER stress occurs in livers subjected to hemorrhagic shock and reperfusion (HS/R), there is no understanding about the influence of the liver architecture on ER stress and the activation of the unfolded protein response (UPR).
Materials and methods: Mice were subjected to a pressure-controlled HS (30 ± 5 mmHg) for 90 min. Mice were sacrificed 2, 4, 6, 8, 10, 14, 18, and 24 h after shock induction. Plasma levels of inflammatory cytokines (IL-6, CXCL1, CXCL9, CXCL10, CCL2, CCL3) and transaminases were measured. Hematoxylin and eosin stains of paraffin-embedded liver tissue sections were evaluated for liver damage. Immunohistochemistry was used to analyze the hepatic topography of ER stress marker binding immunoglobulin protein and the activation of the three major pathways of the UPR.
Results: Compared with sham-operated mice, HS/R led to profound liver damage and an elevation of inflammatory cytokines. We found time-dependent topographical changes of ER stress in the livers. Furthermore, the three major pathways of the UPR represented by protein kinase RNA-like ER kinase, activating transcription factor 6, and inositol-requiring enzyme 1 were activated in differing ways dependent on the zonation within the liver acinus.
Conclusions: These findings show that the liver architecture must be taken into account when investigating the role of ER stress and the UPR in ischemia-reperfusion injury after HS/R.
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Introduction

Hemorrhagic shock (HS) is one of the most dire consequences of severe trauma.[1] Ischemia can cause organ damage, while reperfusion may induce stress by reactive oxygen species and cause generalized inflammation, together forming the so-called ischemia-reperfusion injury (IRI).[2] Although signaling pathways of this IRI have been studied before, the exact mechanisms are not fully understood. This hinders a target-orientated treatment of HS and IRI and demands further investigations.

The endoplasmic reticulum (ER) is a cellular subunit that plays an important role in the synthesis and folding of

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proteins. Previous investigations have shown that the ER is highly dependent on a homeostatic environment—requiring sufficient supply of blood with adequate oxygenation and redox state. HS and IRI can disturb the homeostatic balance, consequently causing protein chains to stay unfolded and to accumulate. This condition is called ER stress.[3] To restore normal ER function, mechanisms are activated, which are collectively referred to as the unfolded protein response (UPR). One central protein of the UPR is binding immunoglobulin protein (BiP). BiP is a chaperone protein, which is located in the lumen of the ER. In homeostasis, BiP supports the covalent folding of newly synthetized proteins.[4] In addition, it binds the luminal domain of the ER-transmembrane proteins inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and protein kinase RNA-like ER kinase (PERK) and keeps them in an inactive state.[5,6] Under ER stress, BiP dissociates from these transmembrane proteins and its expression is upregulated.[7] BiP dissociation allows the activation of IRE1, ATF6, and PERK, which initiates the known three major signaling pathways of the UPR. Downstream targets of these pathways promote general reduction of protein synthesis and gene expression of proteins that are involved in the folding machinery of the ER and degradation of misfolded proteins.[8] However, if the UPR is not able to restore homeostasis and adequate ER function, initiation of cell death might occur. Here, prolonged ER stress is described to be involved in cell death in brain ischemia,[9] cardiac failure,[10] and ischemia-reperfusion induced acute kidney injury.[11]

Although studies have proven the occurrence of ER stress after HS, almost nothing is known about the mechanism over a time course of topographical activation and changes of the UPR after HS.[12-14] For example, Jian *et al.* used a mouse model with a traumatic HS. They induced and maintained a mean arterial blood pressure (MAP) of 35 ± 5 mmHg for 90 min.[13] Twenty-four hours after resuscitation, they detected significantly elevated levels of ER stress marker BiP in liver tissue lysates of animals, which underwent shock procedure using Western blot analysis. Furthermore, the hepatic expression of the three ER-transmembrane proteins IRE1, PERK, and ATF6 was significantly increased as well.

Because of its special architecture and zonation within the acinus, we chose to examine the topography of mouse livers in greater detail.[15] Here, we focused on topographic changes of ER stress marker BiP and the major pathways, represented by PERK, ATF6, and IRE1, using a detailed time trial to describe the topographical changes in ER stress after induced HS injury.

Materials and methods

Animal care

Eleven- to 14-week-old male C57BL/6 (wild type) mice (Charles River Laboratories GmbH, Sulzfeld, DE), weighing 20-30 g, were used for the experiments after at least 1 wk of acclimatization in the animal facility. Mice had free access to water and food at any time. We used only male mice to minimize hormonal fluctuations. All experimental protocols were approved by the Regional Council, Karlsruhe, Germany.

Experimental model

Mice were anesthetized with isoflurane 1.5 Vol% (Abbott Laboratories Ltd, Maidenhead, UK) via a mask and placed in a supine position on a heated operation table. Body temperature was measured using a rectal probe and kept at 37.0 ± 0.5°C. Local anesthetics, Bupivacaine hydrochloride (AstraZeneca GmbH, Wedel, DE), were applied before dissection of the groins. Both femoral arteries were cannulated with polyethylene-tubing. A blood pressure analyzer (BPA400; Micro-med Inc, Louisville) was connected to the right catheter. The left catheter was connected to a heparinized syringe. Induction of HS was performed by aspirating blood from the left groin up to MAP of 30 ± 5 mmHg and maintained for 90 min. Resuscitation with Ringer's solution (three times the shed blood volume) terminated the shock. After stabilization of the blood pressure, catheters were removed, vessels were ligated, and wounds were sutured. Isoflurane inhalation was stopped and mice were placed in a cage. Mice were sacrificed at different time points (2, 4, 6, 8, 10, 14, 18, and 24 h after shock induction) by cardiac puncture. Blood was drawn, centrifuged (2000 × g for 10 and 5 min), and aspartate aminotransferase (ASAT) as well as alanine aminotransferase (ALAT) plasma levels were measured (Fuji Dri-Chem NX500i; FUJIFILM Europe GmbH, Düsseldorf, DE). The livers were flushed with Ringer's solution, harvested, and embedded in paraffin for hematoxylin and eosin (H&E) staining and immunostaining.

Animals were randomly assigned to either shock or control groups. Animals of the shock group (hemorrhagic shock and reperfusion [HS/R]) were treated as described previously; sham controls (SCs) were anesthetized and cannulated but did not undergo shock procedure. Another control group was generated by sacrificing mice without any treatment but anesthesia for euthanasia and tissue harvesting (baseline controls [BCs]). Each group with respective time points consists of five to seven animals (HS/R: six to seven; SC: five to six; BC: six).

Assessment of cytokines

Blood was collected by cardiac puncture as described previously. After centrifugation, plasma was frozen with liquid nitrogen and stored at −80°C. Cytokine concentrations were measured after thawing and centrifugation of plasma samples by using Luminex multiplexing bead array platform (MCYTOMAG-70K; Merck Chemicals GmbH, Darmstadt, DE). The procedure was performed following the manufacture's protocol.

Histopathology

The liver samples were fixed in 4% paraformaldehyde neutral buffered in phosphate buffered saline (PBS) for 24 h at 4°C before being dehydrated through a series of alcohol with increasing concentrations. Then, the tissues were embedded in paraffin. The paraffin-embedded tissues were cut into 5 μm-sections and deparaffinized with xylene. After rehydration through a series of alcohol with decreasing concentrations and distilled water, the sections were stained with H&E. The slides were assessed for cell damage, and the percentage of damaged cells was quantified by evaluation of 20 representative visual fields (100 ×) of each time point using ImageJ (Version: 1.50i; Wayne Rasband, National Institutes of Health).

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