BBA - Molecular Basis of Disease 1865 (2019) 1192–1200

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

The damage-associated molecular pattern HMGB1 is released early after clinical hepatic ischemia/reperfusion

Rowan F. van Golen[a], Megan J. Reiniers[a], Gerben Marsman[b], Lindy K. Alles[a], Derrick M. van Rooyen[c], Björn Petri[d,e,f], Vincent A. Van der Mark[a,g], Adriaan A. van Beek[h], Ben Meijer[h], Martinus A. Maas[a], Sacha Zeerleder[b,i,j], Joanne Verheij[k], Geoffrey C. Farrell[c], Brenda M. Luken[b], Narci C. Teoh[c], Thomas M. van Gulik[a], Michael P. Murphy[l,m], Michal Heger[a,n,*]

[a] Department of Experimental Surgery, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
[b] Department of Immunopathology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
[c] Liver Research Group, Australian National University at The Canberra Hospital, Canberra, Australia
[d] Department of Microbiology, Immunology, and Infectious Diseases, Cumming School of Medicine, University of Calgary, Calgary AB T2N 1N4, Alberta, Canada
[e] Department of Physiology and Pharmacology, Cumming School of Medicine, University of Calgary, Calgary AB T2N 1N4, Alberta, Canada
[f] Snyder Institute for Chronic Diseases, University of Calgary, Calgary, Canada
[g] Tytgat Institute for Gastrointestinal and Liver Research, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
[h] Department of Cell Biology and Immunology, Wageningen University, Wageningen, the Netherlands
[i] Department of Hematology and Central Hematology Laboratory, Inselspital, Bern University Hospital, University of Bern, Switzerland
[j] Department for BioMedical Research, University of Bern, Switzerland
[k] Department of Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
[l] Medical Research Council Mitochondrial Biology Unit, Cambridge, United Kingdom
[m] Department of Medicine, University of Cambridge, Cambridge, United Kingdom
[n] Department of Pharmaceutics, College of Medicine, Jiaxing University, Jiaxing, Zhejiang, PR China

ARTICLE INFO

Keywords:
Damage-associated molecular patterns
Antioxidants
Intravital microscopy
Liver resection
Sterile inflammation
Mitochondrial DNA

ABSTRACT

Objective and background: Activation of sterile inflammation after hepatic ischemia/reperfusion (I/R) culminates in liver injury. The route to liver damage starts with mitochondrial oxidative stress and cell death during early reperfusion. The link between mitochondrial oxidative stress, damage-associate molecular pattern (DAMP) release, and sterile immune signaling is incompletely understood and lacks clinical validation. The aim of the study was to validate this relation in a clinical liver I/R cohort and to limit DAMP release using a mitochondria-targeted antioxidant in I/R-subjected mice.

Methods: Plasma levels of the DAMPs high-mobility group box 1 (HMGB1), mitochondrial DNA, and nucleosomes were measured in 39 patients enrolled in an observational study who underwent a major liver resection with (N = 29) or without (N = 13) intraoperative liver ischemia. Circulating cytokine and neutrophil activation markers were also determined. In mice, the mitochondria-targeted antioxidant MitoQ was intravenously infused in an attempt to limit DAMP release, reduce sterile inflammation, and suppress I/R injury.

Results: In patients, HMGB1 was elevated following liver resection with I/R compared to liver resection without I/R. HMGB1 levels correlated positively with ischemia duration and peak post-operative transaminase (ALT) levels. There were no differences in mitochondrial DNA, nucleosome, or cytokine levels between the two groups. In mice, MitoQ neutralized hepatic oxidative stress and decreased HMGB1 release by ± 50%. MitoQ suppressed

Abbreviations: ALT, alanine aminotransferase; ASA, American Society of Anesthesiologists; AST, aspartate aminotransferase; BMI, body mass index; CTRL, control (group); CRC, colorectal cancer metastasis; DAMP, damage-associated molecular pattern; dTPP, decyl-triphenylphosphonium; HCC, hepatocellular carcinoma; HMGB1, high-mobility group box 1; I/R, ischemia/reperfusion; ICAM, intercellular adhesion molecule; IHC, intrahepatic cholangiocarcinoma; IL, interleukin; INR, international normalized ratio; IQR, interquartile range; mtDNA, mitochondrial DNA; PHCC, perihilar cholangiocarcinoma; PVE, portal vein embolization; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; TLR, toll-like receptor; NAC, *N*-acetylcysteine; VIO, vascular inflow occlusion; VCAM, vascular cell adhesion protein

* Corresponding author at: Membrane Biochemistry & Biophysics, Department of Chemistry, Bijvoet Center for Biomolecular Research & Institute of Biomembranes, Utrecht University.
E-mail address: m.heger@uu.nl (M. Heger).

https://doi.org/10.1016/j.bbadis.2019.01.014
Received 10 June 2018; Received in revised form 21 December 2018; Accepted 11 January 2019
Available online 15 January 2019

transaminase release, hepatocellular necrosis, and cytokine production. Reconstituting disulfide HMGB1 during reperfusion reversed these protective effects.
Conclusion: HMGB1 seems the most pertinent DAMP in clinical hepatic I/R injury. Neutralizing mitochondrial oxidative stress may limit DAMP release after hepatic I/R and reduce liver damage.

1. Introduction

During major liver resection, the induction of liver ischemia by surgical clamping of the afferent hepatic vasculature is used to counter the risks of excessive blood loss. This surgical technique is known as vascular inflow occlusion (VIO) or the Pringle maneuver [1]. When employed within predefined time limits and in selected patients, this maneuver is considered safe. However, the transient lack of organ perfusion and oxygenation also inadvertently causes ischemia/reperfusion (I/R) injury [2–4]. The severity of hepatic I/R injury impacts the recovery of patients after major liver surgery.

Activation of sterile inflammation is a key feature of hepatic I/R injury. During ischemia, the lack of oxygen halts oxidative phosphorylation and leads to the build-up of citric acid cycle metabolites such as succinate [5,6]. Once the oxygen supply is restored, the consumption of accumulated succinate during the first minutes of reperfusion fuels a burst of reactive oxygen species (ROS) production by the mitochondrial electron transport chain [5,6]. The consequent wave of ROS-induced cell death triggers the release of damage-associated molecular patterns (DAMPs) by hepatocytes. DAMPs are innocuous intracellular constituents that become potent triggers of the innate immune system once released into the circulation [2,7]. Effector cells of the innate immune system such as neutrophils in turn confer the bulk of hepatic tissue injury. Based on this sequence of events, DAMPs occupy a crucial role in the onset of I/R injury as signal transducers and amplifiers of the sterile immune response.

Several DAMPs, including histones [8], DNA [9], and high-mobility group box 1 (HMGB1) [10] have been causally linked to hepatic I/R injury in animal studies. DAMP release has also been measured in clinical studies on sterile liver injury [11,12]. However, the link between mitochondrial oxidative stress and DAMP release has not been clinically elaborated in the context of hepatic I/R injury to date, and was therefore investigated in this study. To that end, DAMP release was studied in patients undergoing liver resection with or without being subjected to I/R during surgery.

It is shown that patients who underwent major liver surgery rapidly exhibit DAMP release after resection. Of the tested DAMPs, only HMGB1 levels increased specifically in I/R-subjected patients and not in the control group operated without I/R. HMGB1 release correlated positively with ischemia time and postoperative hepatocellular injury markers. The results were back-translated to a validated mouse model [13] to allow experimental confirmation and further elaboration. Decreasing mitochondrial oxidative damage during early reperfusion with the mitochondria-targeted antioxidant MitoQ in mice prevented HMGB1 release and attenuated the I/R immune response. Decreasing mitochondrial oxidative damage therefore may potentially improve outcomes in patients undergoing major liver surgery with prolonged ischemia times.

2. Materials and methods

References to the supplemental information are indicated with the prefix ‘S’.

2.1. Study participants and study design

The effect of liver surgery performed with or without intraoperative liver ischemia on DAMP release was investigated in a single-center observational trial registered at https://ClinicalTrials.gov under identifier NCT01700660. Eligible for participation were all patients scheduled for a major liver resection (removal of ≥3 Couinaud segments) that were ≥ 18 years old and had an American Society of Anesthesiology physical status score of ≤3. Patients were excluded from the study when considered unresectable during surgical exploration, when the employed ischemia time was < 20 min, when they underwent an emergency operation, or when the patient was pregnant or breast-feeding. Because the decision whether or not to use VIO and thereby subject patients to I/R was made at the discretion of the performing surgeon based on the actual or anticipated amount of blood loss, participants were non-randomly assigned to either the I/R group (N = 26) or the control group (N = 13). In the I/R group, intermittent VIO was typically performed in cycles comprising 20 min of ischemia followed by 10 min of reperfusion. In a minor fraction of patients, the operative cause necessitated the use of continuous VIO (see Supplemental Table S4). All patients were operated by the same primary surgeon (TvG).

Blood samples were drawn from a central venous catheter after the induction of general anesthesia (i.e., at baseline) and 1 h and 6 h after the start of reperfusion (I/R group) or completion of parenchymal transection (CTRL group). Plasma liver injury (ALT) and liver function (international normalized ratio (INR), total bilirubin) were determined as part of routine patient care. Post-operative complications were categorized according to the Clavien-Dindo grading system [14]. All experimental results were normalized to plasma protein content (Pierce BCA Protein Assay Kit, Life Technologies, Carlsbad, CA) to correct for hemodilution, as described [15]. All study protocols ware approved by the Institutional Review Board and written informed consent was obtained from all participants before undergoing any study-related procedures. The study design is summarized in Supplemental Fig. S1.

2.2. Circulating HMGB1

Plasma HMGB1 levels were determined in 10 µL of EDTA-anticoagulated plasma samples by ELISA (IBL International, Hamburg, Germany) according to the manufacturer's instructions. All samples were measured in duplicate in regular sensitivity mode on a Synergy HT microplate reader (BioTek Instruments, Winooski, VT).

2.3. Circulating nucleosomes and elastase-α1-antitrypsin complexes

Nucleosome levels were assessed by ELISA as previously described [16]. Briefly, monoclonal antibody CLB-ANA/60 (Sanquin, Amsterdam, the Netherlands) that recognizes histone 3 was used as capture antibody. Biotinylated F(ab)2 fragments of monoclonal antibody CLB-ANA/58 (Sanquin), which recognizes an epitope exposed on complexes of dsDNA histone 2A and histone 2B in combination with poly-horseradish peroxidase–labeled streptavidin (Sanquin) were used for detection. As a standard, we used culture supernatant of Jurkat cells (1×10^6 cells/mL), cultured for an additional week without refreshing the medium, to obtain 100% apoptotic cells. One unit is the amount of nucleosomes released by ≈100 Jurkat cells. The lower detection limit of the assay was 2.5 U/mL [17]. The reference range for circulating nucleosomes in healthy individuals is 0–10.3 U/mL.

Elastase-α1-antitrypsin complexes (E-AT) were measured by ELISA as described [18]. This assay was adapted from a previously described radioimmunoassay [19]. Briefly, plates were coated with a polyclonal rabbit anti-human neutrophil elastase antibody (1.5 µg/mL; Sanquin). Standard and samples were diluted in high-performance ELISA buffer

(HPE; Sanquin) containing 40 μg/mL bovine IgG. Bound complexes were detected with a biotinylated monoclonal anti–α1-antitrypsin antibody (1 μg/mL; Sanquin) in combination with poly-horseradish peroxidase–labeled streptavidin. Results are expressed in ng/mL by reference to a standard curve of normal human citrated plasma in which EA-T were generated by incubation with porcine elastase (final concentration 2 μg/mL; Sigma, Zwijndrecht, the Netherlands) for 15 min at room temperature. The detection limit of the assay was 2 ng/mL. The reference range for EA-T in healthy individuals is 8.5 to 55.7 ng/mL.

2.4. Circulating mitochondrial DNA

Plasma mitochondrial DNA levels were determined according to Nakahira et al. [20], with minor modifications. Total DNA was isolated from 190 μL of heparin-anticoagulated plasma using the QIAamp DNA Blood Mini Kit (cat. #51106, Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA was eluted in 100 μL of elution buffer. Levels of mitochondrial DNA (mtDNA) were analyzed in duplicate by real-time quantitative PCR on a Lightcycler 480 (Roche, Basel, Switzerland) using a reaction volume of 10 μL consisting of 2 μL of DNA, 2 μL of nuclease-free water (Qiagen), 1 μL of primer mix (0.5 μM final primer concentration), and 5 μL of SensiFAST SYBR No-ROX mix (Bioline, London, UK). The following primers were used: human mitochondrially-encoded NADH dehydrogenase 1 (MT-ND1): forward 5′-ATACCCATGGCCAACCTCCT-3′, reverse 5′-GGGCCTTTGCGTAGTTG TAT-3′ [12]. Melting curve analysis and ethidium bromide-stained agarose gel electrophoresis were used to validate primer specificity. A plasmid encoding a human cDNA clone of MT-ND1 was purchased from ORIGENE (SC101172) and was used as a logarithmic mtDNA standard in 10-fold serial dilutions (1.93×10^6 copies – 1.93×10^0 copies). Data were processed according to Ruijter et al. [21], fitted to the mtDNA standard, and normalized to plasma protein content.

2.5. Human plasma cytokine measurements

Interleukin (IL)-12p70, TNF, IL-10, IL-6, IL-1β, and IL-8/CXCL8 concentrations were determined in serum samples using the Cytometric Bead Array (CBA) Human Inflammatory Cytokines Kit (BD Biosciences, Franklin Lakes, NJ) according to the manufacturer's instructions. The samples were analyzed in an operator-blinded fashion and flow data were collected using a BD FACSCanto II (BD Biosciences). The results were analyzed with FCAP Array version 3.0 software (BD Biosciences). CCL2 plasma concentration was measured by ELISA (Duosets, R&D Systems, Minneapolis, MN). All reagents and solutions were prepared fresh each week according to the manufacturer's protocols and were sterile filtered using 0.2 μM bottle-top vacuum filters (Corning, Corning, NY). Bovine serum albumin was of the highest available purity (cat. #A7030, Sigma-Aldrich, St. Louis, MO) and ELISAs were performed using clear 96-well flat bottom polystyrene microplates (#9018, Corning). All cytokine levels were normalized to plasma total protein content.

2.6. Mouse hepatic ischemia/reperfusion experiments

The animal experiments were approved by the institute's animal welfare committee and surgical procedures were as described [13]. MitoQ was dissolved to a concentration of 6 mg/mL in sterile NaCl (Braun). After induction of anesthesia, 250 ng/kg – 3 mg/kg MitoQ or an equimolar amount of the inactive targeting moiety decyl-triphenylphosphonium (dTPP) in sterile NaCl was infused via the penile vein in a volume of 100 μL per 30 g body weight. After allowing MitoQ or dTPP to circulate for 10 min, partial (70%) liver ischemia was induced for 30 min [13]. As a control group, animals underwent sham surgery that entailed a laparotomy with mobilization of hilar structures, but without actual occlusion of the afferent vasculature. For every set of experiments, animals were randomly assigned to an experimental arm. For HMGB1 reconstitution experiments, 2.5 μg disulfide HMGB1 (IBL) in 100 μL sterile NaCl was administered intraperitoneally immediately after reperfusion was initiated. Disulfide HMGB1 was selected for the reconstitution experiments because (1) it is the predominant isoform released in mice subjected to hemorrhagic shock and resuscitation [22] - a model that pathophysiologically resembles liver I/R - and (2) the disulfide isoform activates the hepatic I/R-pertinent TLR-4 signaling axis [10,23,24]. Animals were sacrificed at 6 h or 24 h of reperfusion. Blood and liver samples were processed as described [13]. The number of animals per group per experiment is included in the figure legends. To account for variations in experimental conditions and animal batches, a new control group of vehicle-treated mice subjected to I/R was included in every new set of experiments.

2.7. Mouse liver histology

Mouse liver specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin as described [13]. The extent of confluent parenchymal necrosis was scored by a hepatopathologist (JV) according to the following grading system: 0 = no necrosis, 1 ≤ 25% necrosis, 2 = 25–50% necrosis, 3 = 51–75% necrosis, and 4 ≥ 76% necrosis.

2.8. Western blot

Frozen liver (−80 °C) samples were homogenized with an Omni Tissue Master 125 homogenizer (Omni International, Kennesaw, GA) in cell lysis buffer (50 mM HEPES, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 10% glycerol, and 0.1% Triton X-100 solution containing protease and phosphatase inhibitors). Cell debris was pelleted (12,000 ×*g*, 5 min), supernatant protein content determined (DC Protein Assay, Bio-Rad), and SDS-polyacrylamide gel electrophoresis (Bio-Rad Mini-PROTEAN) was performed with samples standardized to 30 μg protein/well. Proteins were separated using 7.5% gels, followed by electrophoretic elution onto PVDF membranes (Trans-Blot Turbo, Bio-Rad), as per manufacturer's instructions. Blots were run under reducing conditions. PVDF membranes were blocked (5% skim milk, 1 h), incubated with primary antibodies (Supplemental Table S1) for 16 h (4 °C) and stained with appropriate HRP-conjugated secondary antibodies (Santa Cruz). Immunoreactivity was detected using SuperSignal West Femto Substrate (Thermo Scientific) and digital chemiluminescence image capture (LAS-4000, FujiFilm, Tokyo, Japan). Densitometric analysis was performed with MultiGauge software (V3.0, FujiFilm, Tokyo, Japan) and all values were normalized to the expression of the housekeeping protein β-actin. All experiments were performed in triplicate.

2.9. Mouse plasma cytokine measurements

Mouse plasma samples were assayed for mouse GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL12p70, IL-13, IL-18, and TNF-α using the ProcartaPlex Mouse Th1/Th2 extended 11-plex kit and mouse BAFF, IL-10, IL-22, RANTES/CCL5, TSLP, and VEGF-A using ProcartaPlex simplex kits on the Luminex platform (Affymetrix, Santa Clara, CA) according to the manufacturer's instructions. The relevance of these inflammatory messengers for hepatic I/R injury is summarized in Supplemental Table S3. In brief, samples were thawed on ice. The antibody-coated beads were mixed and washed, and incubated overnight at 4 °C with 1:1 diluted standards or samples. After washing, the beads were incubated with detection antibody mix for 30 min at room temperature. The beads were subsequently washed and incubated for 30 min at room temperature with streptavidin-PE. After washing, the beads were measured with a Luminex instrument (Bio-Plex 200, Bio-Rad) that was calibrated using Bio-Rad calibration beads. Standard curves were calculated using 5-parameter logistic regression in Bioplex 5.0 software. Heatmaps were generated using GENE-E software (http://www.broadinstitute.org/cancer/software/GENE-E/) and show the

plasma cytokine levels of MitoQ-treated mice following 30 min of ischemia and 6 h or 24 h of reperfusion. Data per time point are expressed as fold-increase compared to the mean of the vehicle control group, whereby blue indicates a decrease and red indicates an increase in the MitoQ group versus control animals subjected to I/R only.

2.10. Intravital imaging and spectroscopic quantification of reactive oxygen species production during hepatic I/R injury in mice

The oxidation-sensitive fluorogenic probe 5(6)-carboxy-dichlorodihydrofluorescein was prepared from 5(6)-carboxy-dichlorodihydrofluorescein diacetate and encapsulated in hepatocyte-targeted liposomes according to Reiniers et al. [25]. Liposomes (0.1 μmol lipid/g body weight in 200 μL 10 mM HEPES, 0.88% NaCl, 0.292 osmol/kg, pH = 7.4) were injected via the penile vein and circulated for 35 min to allow intrahepatic accumulation. Hepatic I/R was subsequently performed as described [25]. To perform the surgical procedure with the mice positioned under the intravital imaging setup, ischemia was induced with a silicone sling placed around the hepatic pedicle instead of a microvascular clamp. During the first 10 min of reperfusion, hepatic probe conversion was quantified at 2-min intervals in real-time using a customized intravital fluorescence microscope (M165FC, Leica Microsystems, Wetzlar, Germany) equipped with a spectrometer (QE65000, Ocean Optics, Dunedin, FL). Spectroscopic data were integrated over the entire spectral width (λ = 250–1050 nm) and normalized to baseline.

2.11. Statistical analyses

Statistical analyses were performed using Graphpad Prism 6 (La Jolla, CA) and SPSS 21.0 (Chicago, IL), abiding by a significance level (α) of 0.05 unless otherwise indicated. Normal distribution of data sets with ≥8 values was assessed using a D'Agostino and Pearson omnibus test. Normally distributed data were tested for intragroup and intergroup differences using a student's *t*-test, a one-way ANOVA with Dunnet's post-hoc test, or a repeated measure ANOVA with Geisser-Greenhouse correction and Tukey's post-hoc correction. All continuous numerical variables that failed the normality test were log transformed and re-analyzed. Log-transformed data that followed a Gaussian distribution were analyzed parametrically as described. If the transformed data failed the normality test, non-parametric tests (Mann Whitney U, Kruskall-Wallis with Dunn's post-hoc test, or Friedman with Dunn's correction) were performed on the non-transformed data. Data sets with < 8 values group were tested parametrically. Categorical data were analyzed using a Fisher's exact test (binary data) or a Chi-squared test (> 2 variables) and correlations were tested using Spearman's rho.

3. Results

3.1. DAMP release after major liver resection in patients

To investigate DAMP release after clinical liver I/R, a total of 74 patients were enrolled in an observational study. Of the 74 study participants, 35 were excluded due to unresectable disease during surgical exploration (N = 30), withdrawal of consent (N = 1), an unanticipated change in primary surgeon (N = 1), or because a minor instead of a major liver resection was performed (N = 3). The relationship between hepatic I/R injury and DAMP release was therefore studied in 39 patients who underwent a major liver resection with (N = 26, 'I/R') or without (N = 13, 'CTRL') the intraoperative use of VIO. The study design is summarized in Supplemental Fig. S1. The baseline patient characteristics are shown in Table 1 and the clinical outcomes are presented in Table 2.

The baseline patient characteristics were comparable between the two study arms. VIO use was associated with longer operating time, resection time, and hospital stay compared to the control group (Table 2). A trend towards more extensive liver resection was seen in the I/R group (Table 2). The severity of postoperative liver injury did not differ between the groups when judged by liver damage (i.e., the postoperative ALT peak) or liver function parameters such as INR and bilirubin (Table 2). There were also no differences in transfusion requirements, postoperative complications, or mortality between the control and I/R group (Table 2). As the aim of the study was to explore DAMP release and activation of sterile inflammation in I/R-subjected patients, it should be underscored that the study was neither designed nor powered to detect differences in patient outcomes between the control and I/R group.

Systemic DAMP levels were next determined in the complete cohort at baseline and at 1 h and 6 h after surgery. HMGB1 and nucleosomes were assessed based on preclinical hepatic I/R work [8,24]. Circulating mitochondrial DNA (mtDNA) was assayed because hepatic I/R injury is thought to originate in ROS-generating mitochondria [5] and because mtDNA release has been documented in both animal and clinical studies on sterile liver injury [11,12]. Fig. 1A–B show that systemic HMGB1 and nucleosomes concentrations increased within 1 h after surgery in the combined cohort, which was accompanied by a rise in neutrophil activation (Fig. 1D). Systemic mtDNA levels remained unchanged during the study period (Fig. 1C). These data demonstrate that the DAMPs HMGB1 and nucleosomes are released into the circulation after major liver resection.

To determine whether DAMP release was caused by ischemia-mediated hepatocyte injury or by surgical trauma per se, which has been noted previously [26], the patient data were stratified into an I/R and a control group. Fig. 2A–D show that, of the tested DAMPs, only HMGB1 levels increased significantly at 1 h of reperfusion in the I/R group but not in the control group. The release of the cytokines IL-1β and IL-6 was also more pronounced in the I/R group than in the control

Table 1
Baseline characteristics.

	CTRL (N = 13)	I/R (N = 26)	p-Value
Age (years, median ± IQR)	62 (45–73)	66 (53–70)	0.670
Gender, male (N, %)	8 (62)	18 (69)	0.725
BMI (median ± IQR)	25.2 (22.9–26.3)	23.9 (20.9–26.8)	0.418
ASA score (N, %)			
I	3 (23)	6 (23)	0.153
II	10 (77)	14 (54)	
III	0 (0)	6 (23)	
Diagnosis (N, %)			
CRC metastasis	1 (9)	4 (15)	0.132
PHCC	3 (23)	13 (50)	
IHCC	2 (15)	2 (8)	
HCC	2 (15)	3 (12)	
Benign	3 (23)	3 (12)	
Other	2 (15)	1 (3)	
Preoperative chemotherapy, yes (N, %)	2 (15)	4 (15)	1.000
Biliary drainage, yes (N, %)	3 (23)	9 (35)	0.714
PVE, yes (N, %)	1 (8)	1 (4)	1.000
ALT baseline, U/L (median ± IQR)	30 (25–52)	53 (24–71)	0.294
AST baseline, U/L (median ± IQR)	32 (28–52)	45 (28–73)	0.178
Total bilirubin baseline, μM/L (median ± IQR)	9 (6–14)	7 (5–14)	0.471
INR baseline (median ± IQR)	1.0 (1.0–1.1)	1.0 (1.0–1.1)	0.676

ALT = alanine aminotransferase; ASA = American Society of Anesthesiologists; AST = aspartate aminostransferase; BMI = body mass index; CRC = colorectal cancer metastases; HCC = hepatocellular carcinoma; IHCC = intrahepatic cholangiocarcinoma; INR = international normalized ratio; IQR = interquartile range; PHCC = perihilar cholangiocarcinoma; PVE = portal vein embolization. Categorical data were analyzed using Fisher's exact test (binary data) and Chi-square test (> 2 variables). Differences between numerical variables were assessed using the student *t*-tests.

Table 2
Clinical outcomes.

	CTRL (N = 13)	I/R (N = 26)	p-Value
Resected segments[a] (%)			0.131
3	6 (46.2%)	5 (19.2%)	
≥4	7 (53.8%)	21 (80.8%)	
Resection time, min (median ± IQR)	59 (41.5–71.5)	82 (60–130)	**0.006**
Duration of ischemia[b] min (median ± IQR)	N/A	48 (31–68)	
Duration of surgery, min (median ± IQR)	306 (261–373.5)	460 (380–503)	**0.001**
Transfusion requirement, units (%)			0.455
0	9 (62.9)	12 (46.2)	
1–2	2 (15.4)	6 (23.1)	
≥3	2 (15.4)	7 (26.9)	
Hospital stay, days (median ± IQR)	9 (7.5–11)	11.5 (8–22)	**0.036**
Grade III-V complications[c] (%)	7 (53.8)	13 (50.0)	1.000
ICU admission (%)	3 (23.1)	7 (26.9)	1.000
In-hospital mortality (%)	1 (7.7)	2 (7.7)	1.000
Peak ALT, U/L (median ± IQR)	273 (143–642)	456 (289–784)	0.152
Peak INR (median ± IQR)	1.19 (1.12–1.31)	1.30 (1.17–1.41)	0.411
Peak total bilirubin, μmol/L (median ± IQR)	17 (14–34)	32 (19–47)	0.129

A histopathological assessment of the resection specimens is included in Supplemental Table S2. CTRL = control group; I/R = ischemia/reperfusion group; ALT = aspartate alanine aminotransferase; ICU = intensive care unit; INR = international normalized ratio; IQR = interquartile range; min = minutes. Categorical data were analyzed using Fisher's exact test (binary data) and Chi-square test (> 2 variables). Differences between numerical variables were assessed using the student's *t*-test.

[a] Designates the number of resected Couinaud liver segments.

[b] Patients were subjected to continuous or intermittent vascular inflow occlusion as specified in Supplemental Table S4. Details on the resected liver segments can be found in Supplemental Tables S4 and S5.

[c] Complications were scored according to the Clavien-Dindo classification [14].

group at 1 h of reperfusion (Fig. S2). The early intergroup differences in HMGB1 and cytokine release resolved 6 h after surgery (Fig. 2A, S2). Nucleosome release and neutrophil activation were comparable between groups at all time points (Fig. 2B–C). In line with the post-operative release of HMGB1 in only the I/R group, systemic HMGB1 levels after surgery correlated positively to the postoperative ALT peak (Fig. 2E) and the duration of hepatic ischemia (Fig. 2F). Such a relationship was typically absent for cytokine levels or neutrophil activation (Fig. S3). Collectively, these results suggest that HMGB1 is the DAMP that is most pertinent to clinical hepatic I/R injury.

3.2. Neutralizing mitochondrial oxidative stress limits HMGB1 release after mouse liver I/R

After establishing that HMGB1 is released in patients after hepatic I/R, the therapeutic efficacy of inhibiting HMGB1 release was investigated in a validated mouse hepatic I/R model [13].

Because mitochondrial oxidative injury is considered the most proximal trigger of I/R injury and therefore may cause the release of DAMPs, it was tested whether the mitochondria-targeted antioxidant MitoQ could limit HMGB1 release and, thereby, attenuate hepatic I/R injury in mice. The cytoprotective efficacy of MitoQ was established first. The intravenous administration of MitoQ reduced plasma ALT levels at 6 h and 24 h of reperfusion in mice over a 0.25–1 mg/kg dose range (Fig. 3A), whereas non-specific MitoQ toxicity was seen at higher dosages. Based on this pharmacodynamic profile, 1 mg/kg of MitoQ was used in all in vivo experiments.

In concordance with a previous report demonstrating the antioxidant efficacy of MitoQ in I/R-subjected mice [27], MitoQ reduced oxidation of the fluorogenic probe 5(6)-carboxy-dichlorofluorescein in hepatocytes during the first 10 min of reperfusion, as measured by intravital spectroscopy (Fig. 3B). The fluorogenic probe was delivered specifically to hepatocytes using a hepatotargeted delivery system [25]. MitoQ did not affect the number of leukocytes in the hepatic microcirculation during the first 90 min of reperfusion (Fig. S6, S7). The early reduction in oxidative stress (Fig. 3B) translated to a drop in hepatocellular necrosis and transaminase release at 24 h of reperfusion (Fig. 3C–D), indicating a reduction in hepatic I/R injury. Animals that received the inactive targeting moiety of MitoQ, dTPP, were not protected from I/R injury (Fig. 3C–D), reaffirming that the antioxidant properties of the ubiquinol moiety convey the hepatoprotective effects of MitoQ.

After demonstrating that MitoQ was able to reduce I/R injury, the effect of neutralizing mitochondrial oxidative stress on HMGB1 release after mouse liver I/R was explored. Fig. 3E shows that MitoQ reduced plasma HMGB1 levels after I/R by approximately 50% at 6 h of reperfusion. Quantification of HMGB1 in liver biopsies by Western blot showed similar HMGB1 levels in MitoQ-treated and untreated mice subjected to I/R (Fig. 3F). This finding may relate to differences in resolving capacity between the techniques used for plasma and whole-liver HMGB1 quantification. To examine whether HMGB1 release was associated with the documented hepatoprotective effect of MitoQ, DAMP reconstitution experiments were performed. Infusing the pro-inflammatory disulfide isoform of HMGB1 [28] at the start of reperfusion nullified the protective potential of MitoQ (Fig. 3G), which supports the hypothesis that I/R injury sequentially proceeds via mitochondrial oxidative injury, cell death, and DAMP release..

Systemic HMGB1 alerts the immune system via RAGE and/or TLR-4 [24,29], which drive cytokine production by activating various pro-inflammatory transcription factors. After establishing that neutralizing mitochondrial oxidative stress with MitoQ decreased HMGB1 release and reduced hepatic I/R injury, it was determined whether MitoQ treatment also attenuated inflammatory signaling following mouse liver I/R. Levels of chemotactic and cytotoxic messengers such as TNF-α and IL-1β were lower in the MitoQ group at 6 h of reperfusion, whereas a stronger induction of anti-inflammatory IL-10 was noted at the 24 h time point (Fig. 3J). This favorable effect of MitoQ on cytokine profiles may have resulted in the downregulation of the leukocyte receptor VCAM-1, even though the expression of the principal sinusoidal neutrophil receptor ICAM-1 was unaffected (Fig. 3H–I).

4. Discussion

This study shows that (i) the DAMP HMGB1 seems most pertinent in clinical liver I/R injury, (ii) that HMGB1 levels positively correlate with liver injury markers in I/R-subjected patients, and (iii) that treating mitochondrial oxidative injury with MitoQ prevents HMGB1 release and consequent sterile inflammation, ultimately attenuating I/R injury in mice.

Major liver resection remains associated with considerable mortality, exceeding 10% in patients with high-risk tumors [30]. The ramifications of liver ischemia therefore still influence surgical practice on a daily basis. Part of the challenge is that supportive care is the only current treatment for hepatic I/R injury, and in that respect, several observations can be made based on the current work. It is the first report that shows DAMP release directly after major liver resection, and additionally identifies HMGB1 as the DAMP most pertinent to clinical I/R injury. Liver I/R in patients was characterized by an early rise in HMGB1 levels 1 h after surgery, whereas HMGB1 returned to baseline 6 h after surgery. The early release of HMGB1 fits previous reports showing that HMGB1 from hepatocytes is already propagated by ischemia, and persists throughout the reperfusion phase in mice subjected to I/R [31]. It is also consistent with the finding that HMGB1 is found in the caval effluent immediately after liver transplantation [32].

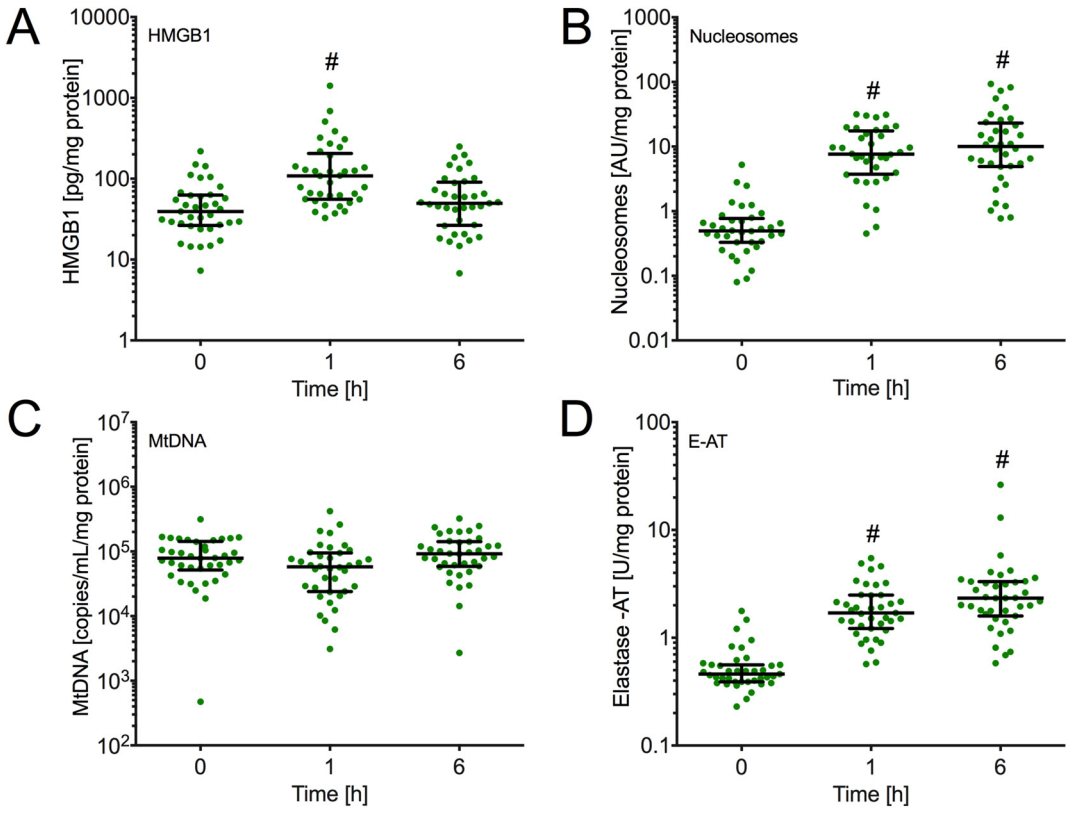

Fig. 1. DAMP release and neutrophil activation after clinical liver resection.
A–D show plasma levels (median ± IQR) of the DAMPs HMGB1, nucleosomes, and mtDNA, and the marker for neutrophil activity E-AT at 1 h and 6 h after liver surgery in patients. Shown are the pooled data of the control and I/R group. Note that the *y*-axes are scaled logarithmically. E-AT = elastase-α1-antitrypsin complex; HMGB1 = high mobility group box 1; mtDNA = mitochondrial DNA. # indicates $p < 0.05$ versus $t = 0$.

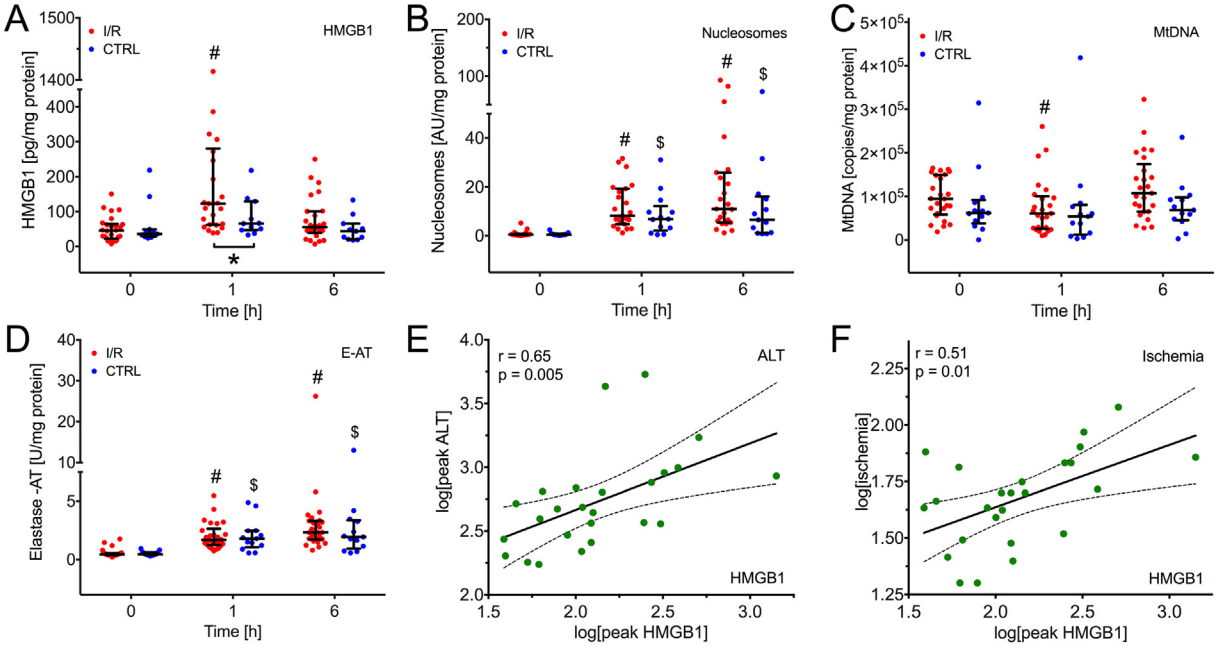

Fig. 2. HMGB1 release correlates to postoperative liver injury after major liver resection.
A–D show systemic DAMP levels (median ± IQR) for patients operated with VIO (I/R, red) or without VIO (control, blue). **E–F** display the correlation between circulating HMGB1 and liver ischemia time and the post-operative hepatocellular injury peak. The dashed lines indicate the 95% confidence interval of the regression line. Additional correlation data are presented in Supplemental Fig. S3. # indicates $p < 0.05$ versus $t = 0$ in the I/R group, $ indicates $p < 0.05$ versus $t = 0$ in the control group, and * indicates $p < 0.05$ between the groups indicated by the horizontal bracket. ALT = aspartate alanine aminotransferase; E-AT = elastase-α1-antitrypsin complex; HMGB1 = high mobility group box 1; I/R = ischemia/reperfusion; IQR = interquartile range; mtDNA = mitochondrial DNA; VIO = vascular inflow occlusion.

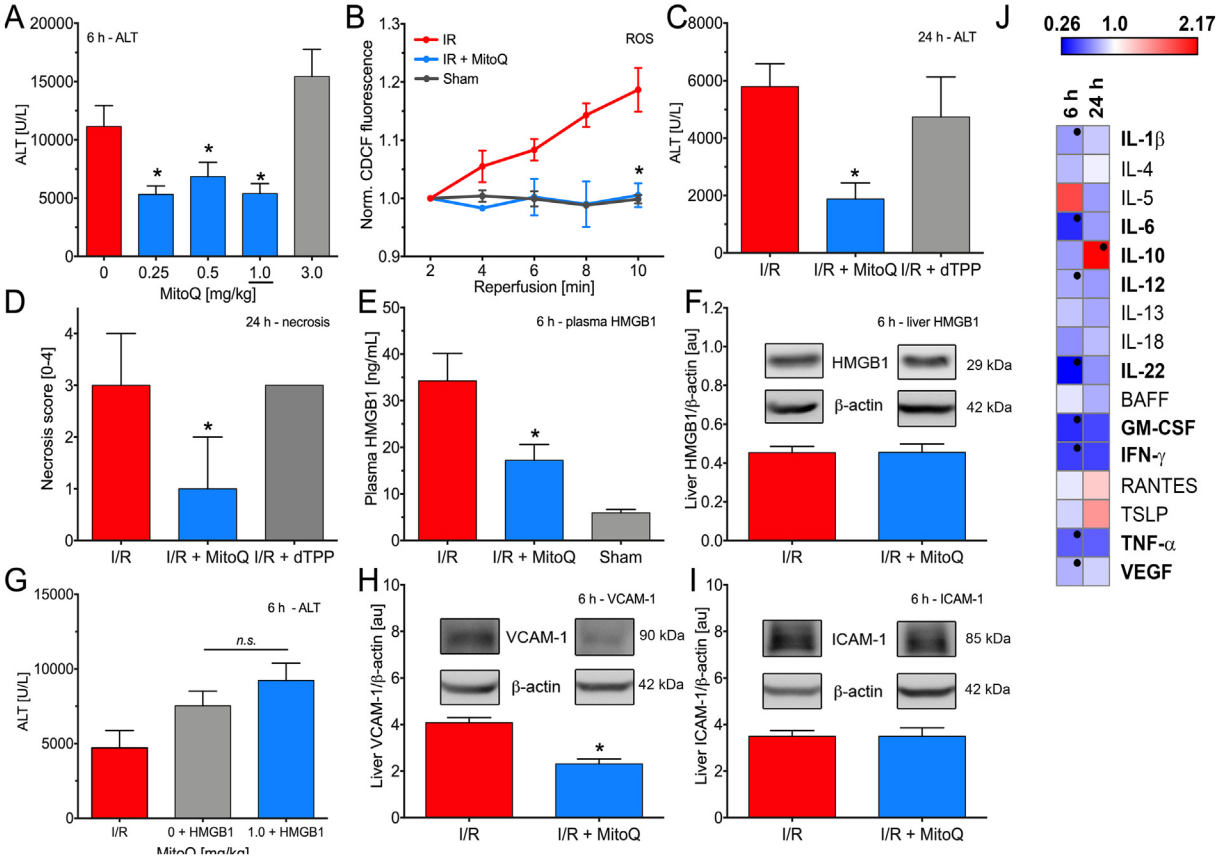

Fig. 3. MitoQ attenuates hepatic I/R injury in mice by suppressing HMGB1 release.
A shows the dose-response relationship between MitoQ and hepatocellular injury. The 1 mg/kg MitoQ dosage was used in all subsequent experiments (solid line). **B** demonstrates that MitoQ mitigated hepatic oxidative stress during early reperfusion as measured by real-time in vivo microscopy/spectroscopy. **C–D** show that MitoQ decreased ALT release and hepatocellular necrosis at 24 h reperfusion, whereas redox-inactive MitoQ (dTPP) was not protective (also see Fig. S4). **E–F** indicate that MitoQ attenuated HMGB1 release but did not affect intracellular HMGB1 levels. **G** demonstrates that reconstitution of disulfide HMGB1 reverses the protective effects of MitoQ. **H–I** show that MitoQ decreased expression of the leukocyte receptor VCAM-1 but did not affect ICAM-1 expression. The heat map **(J)** depicts a decrease (blue) or increase (red) in plasma cytokine concentration in MitoQ-treated animals subjected to I/R versus vehicle-treated animals subjected to I/R. The dots indicate a statistically significant difference between the MitoQ and control group. Full hepatocellular damage and cytokine results are included in Supplemental Fig. S5 and Supplemental Table S6 and a functional description of measured cytokines is included in Supplemental Table S3. Results are shown as mean ± SEM, except for **D** (median ± range). Western blot results are presented as densitometric analysis. One representative blot per group is included per graph. Group sizes are ≥6 animals/group, except for **B** (3–4 mice/group). Area under the curve analysis was used to assess differences in ROS production (**B**). * indicates $p < 0.05$ in the MitoQ versus the I/R group. au = arbitrary unit; ALT = aspartate alanine aminotransferase; CDCF = 5(6)-carboxy-dichlorofluorescein; dTPP = decyl-triphenylphosphonium; ROS = reactive oxygen species; HMGB1 = high mobility group box 1; ICAM-1 = intercellular adhesion molecule 1; VCAM-1 = vascular cell adhesion molecule 1.

The notion that HMGB1 levels correlated to transaminase release and the duration of ischemia indicates that HMGB1 may hold prognostic or even therapeutic value, as HMGB1 is an active mediator of immune activation that could serve as an interventional target. Small-molecule inhibitors of the HMGB1 receptor RAGE are being clinically evaluated for ancillary indications [33], whereas inhibition of TLR receptors has been proposed to treat inflammatory disorders [34]. Direct HMGB1 inhibition has also shown promise in treating drug-induced liver injury in mice [35]. This starkly contrasts the liver injury markers such as ALT or bilirubin, which are 'passive' markers for hepatocellular injury that do not modulate immune responses. The latter also applies to other hepatic I/R biomarkers such as keratin 18 [36]. DAMP-targeted interventions could for instance be used on an on-demand basis to control I/R injury in patients with anticipated (or unexpected) extensive ischemia times. A similar rationale has driven the introduction of in situ liver cooling techniques [37,38].

HMGB1 could theoretically also derive from other cells or organs after liver I/R, such as the intestines [39]. It is however most plausible that hepatocytes are the source of HMGB1, for several reasons. First, the postoperative rise in HMGB1 is not seen in hepatocyte-specific HMGB1 knockout mice subjected to liver I/R [40]. Similar results have been obtained with mice deficient in hepatocyte TLR-4, an innate immune receptor that mediates HMGB1 release after I/R [24,41]. Second, HMGB1 levels were more prominent in caval than in portal blood after liver transplantation [32], whereas no differences were noted between systemic and portal HMGB1 concentrations. The latter excludes the bowel as a source of HMGB1 after liver transplantation. Last, in vitro studies have shown that hepatocytes rendered hypoxic or exposed to the oxidant hydrogen peroxide release HMGB1 into the culture supernatant [24]. An unanswered question is which HMGB1 isoform is released after liver I/R, as the biological effects of HMGB1 depend on the oxidation status of the protein [28]. In addition, it should be elucidated how HMGB1 is inactivated and/or regulated at sites of inflammation. This is imperative given the transient nature of postoperative HMGB1 surges noted both after liver transplantation [32] and in the current study (Fig. 2).

The finding that mtDNA levels were unaffected by liver I/R is unexpected, given that mtDNA release was seen in mice and patients with acetaminophen (APAP) hepatotoxicity, which pathophysiologically resembles I/R in terms of oxidative injury to hepatocyte mitochondria

[12,42,43]. The discrepancy may relate to several differences between I/R injury and APAP overdose. First, the mechanistic pathways culminating in mitochondrial damage are different. In case of APAP, cytoplasmic glutathione stores are depleted, leading to the accumulation of the toxic NAPQI that associates with mitochondrial proteins and leads to mitochondrial permeability transition (MPT) and necrotic cell death [43]. Accordingly, APAP causes cytoplasmic redox stress that subsequently migrates to the mitochondria. In case of I/R, the depletion and subsequent repletion of the terminal substrate of the electron transport chain (ETC) - molecular oxygen - leads to an oxidative burst and ROS production that perturbs ETC proteins by redox modification and causes MPT and mainly necrotic cell death [44]. Mitochondrial damage by I/R therefore has a mitochondrial origin, which could translate to differential mtDNA kinetics versus APAP-triggered mtDNA kinetics. Corroboratively, mtDNA release seems to be a tightly regulated process rather than a mere consequence of necrosis inasmuch as rendering livers necrotic with furosemide instead of APAP did not trigger mtDNA release [12]. Second, hepatocellular injury in patients with APAP toxicity was considerably more severe than in our I/R cohort based on ALT levels [12]. The proposition that mtDNA is released mainly in severe liver injury is also in line with a later report showing that mtDNA release is more pronounced in patients with poor outcome after APAP overdose [11]. One could further argue that ischemia by itself is the factor that differentiates APAP toxicity from I/R injury. Indeed, mtDNA release has been predominantly reported in patients with non-ischemic causes of sterile injury, which in addition tot APAP hepatotoxicity includes inflammatory bowel disease [45] and trauma [46]. This line of reasoning, however, does not align with the fact that we also did not find mtDNA release in patients who underwent a major hepatectomy without intraoperative VIO use (i.e., non-ischemic sterile liver injury).

The current data also highlight that mitochondrial oxidative stress may be an even more proximal target for intervention [47], as this may limit DAMP release. The finding that MitoQ was able to suppress HMGB1 release fits the earlier notion that the glutathione precursor *n*-acetyl-cysteine (NAC) reduced HMGB1 release after in vitro hepatocyte anoxia/reoxygenation [24]. Antioxidants, including NAC, lack efficacy in various clinical scenarios, including hepatic I/R injury [48]. MitoQ differs from these compounds in that it is designed to target the site of oxidant production after I/R (i.e., mitochondria) and also detoxifies the most relevant oxidant (i.e., superoxide) [5]. MitoQ has been used previously in mice to successfully treat hepatic I/R injury [27]. In the latter report, MitoQ efficacy was assessed using surrogate markers for oxidative injury such as mitochondrial protein carbonylation and hepatic 3-nitrotyrosine content [27]. Using a direct intravital fluorescence-based method [25] it was confirmed that MitoQ reduces hepatocyte oxidative stress early after I/R. In addition, MitoQ has already been employed in phase II studies where it lowered transaminase levels in patients with hepatitis C [49], which is an encouraging follow-up to the preclinical notion that MitoQ is generally well-tolerated and not toxic [50]. The clinical investigation and implementation of mitochondria-targeted antioxidants such as MitoQ therefore seems a realistic objective.

Several limitations of the current study should be considered when interpreting the results. The observational nature of the current study means that it is neither designed nor powered to detect differences in clinical outcomes. The similarity in post-operative liver injury parameters between the I/R and control group (Table 2) therefore does not mean that liver ischemia is innocuous, but that VIO in experienced hands is a safe salvage procedure when used to counteract the harm of excessive blood loss. The relatively favorable post-operative transaminase and bilirubin values recorded in the I/R group also relates to the fact that only patients with sufficient (future) remnant liver size and function are eligible for major liver resection. This is supported by the fact that the noted extent of liver injury (i.e., ALT release) is comparable to previous clinical liver I/R cohorts [51,52]. The interpretation of the clinical data is further hindered by the inability to separate the contribution of ischemic liver injury from the effects of surgical trauma on DAMP release. It has for instance been reported that the DAMP ATP is released from the resection plane after partial hepatectomy [26]. In the latter study, patients were not exposed to ischemia during liver resection. Also, the duration of liver surgery and hepatic manipulation during surgery both influence the release of liver injury markers [53]. The longer operating time and trend towards more extensive resections in the I/R group (Table 1) may therefore also add to the higher HMGB1 levels in the I/R group rather than VIO use only, although this does not per se disqualify the conclusions when contextualized to the mouse data. Last, it should be noted that, in line with available evidence [54], the majority of patients were operated using intermittent VIO (Table S4). This technique allows for brief periods of parenchymal (re)perfusion in between cycles of liver ischemia. In contrast, most animal models of hepatic I/R, including ours, employ continuous liver ischemia. As the anoxic period primes mitochondria for the post-ischemic burst of ROS production [6], it is conceivable that intermittent VIO lessens the extent of mitochondrial oxidative injury after I/R. Accordingly, it also means that it remains to be shown whether I/R injury resulting from intermittent VIO is amenable to treatment with mitochondria-targeted antioxidants such as MitoQ.

In conclusion, it is shown that HMGB1 release is related to clinical liver I/R injury. The finding that the mitochondria-targeted antioxidant MitoQ limited HMGB1 release and reduced I/R injury in mice may pave the way for the clinical use of targeted antioxidants against early-onset radicals and oxidants to attenuate hepatic I/R injury.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgements

Readers wishing to receive raw study data should contact the corresponding author. The authors are grateful to Wouter van Riel, Noor Hagemeijer, Marieke Smit, and Annemiek Dekker for assisting with the collection of patient samples and to Albert van Wijk for analytical support. Lori Zbytnuik and Michelle Wilson from the Kubes lab (Calgary) are acknowledged for technical and infrastructural support. Katarzyna Stevens and Pina Colarusso from the Live Cell Imaging Facility (Snyder Institute, Calgary) are acknowledged for assistance with intravital imaging and image analysis. The authors thank Edward Chouchani, Tracy Prime, and Sebastian Rogatti from the MRC Mitochondrial Biology Unit in Cambridge for technical and infrastructural help with the MitoQ experiments.

Financial support

RVG was supported by a PhD Scholarship and The Young Talent Fund, both from the Academic Medical Center in Amsterdam. MH was supported by grants from the Dutch Anti-Cancer Foundation (Stichting Nationaal Fonds Tegen Kanker) in Amsterdam, the Phospholipid Research Center in Heidelberg, the Nijbakker-Morra Foundation in Leiden, and Stichting Technologische Wetenschap (STW). MH is currently supported by the Dutch Cancer Society (project # 10666). This work was also supported by the Live Cell Imaging Facility (Snyder Institute, Calgary) funded by an equipment and infrastructure grant from the Canadian Foundation Innovation (CFI), the Alberta Science and Research Authority, and the Mouse Phenomics Resources Laboratory funded by the Snyder Institute for Chronic Diseases at the University of Calgary.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://

doi.org/10.1016/j.bbadis.2019.01.014.

References

[1] J.H. Pringle, V. Notes on the arrest of hepatic hemorrhage due to trauma, Ann. Surg. 48 (1908) 541–549.
[2] R.F. van Golen, T.M. van Gulik, M. Heger, The sterile immune response during hepatic ischemia/reperfusion, Cytokine Growth Factor Rev. 23 (2012) 69–84.
[3] W.G. van Riel, R.F. van Golen, M.J. Reiniers, M. Heger, T.M. van Gulik, How much ischemia can the liver tolerate during resection? Hepatobiliary Surg. Nutr. 1 (2016) 58–71.
[4] R.F. van Golen, T.M. van Gulik, M. Heger, Mechanistic overview of reactive species-induced degradation of the endothelial glycocalyx during hepatic ischemia/reperfusion injury, Free Radic. Biol. Med. 52 (2012) 1382–1402.
[5] E.T. Chouchani, V.R. Pell, A.M. James, L.M. Work, K. Saeb-Parsy, C. Frezza, et al., A unifying mechanism for mitochondrial superoxide production during ischemia-reperfusion injury, Cell Metab. 23 (2016) 254–263.
[6] E.T. Chouchani, V.R. Pell, E. Gaude, D. Aksentijević, S.Y. Sundier, E.L. Robb, et al., Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS, Nature 515 (2014) 431–435.
[7] M.J. Reiniers, R.F. van Golen, T.M. van Gulik, M. Heger, Reactive oxygen and nitrogen species in steatotic hepatocytes: a molecular perspective on the pathophysiology of ischemia-reperfusion injury in the fatty liver, Antioxid. Redox Signal. 21 (2013) 1119–1142.
[8] H. Huang, J. Evankovich, W. Yan, G. Nace, L. Zhang, M. Ross, et al., Endogenous histones function as alarmins in sterile inflammatory liver injury through toll-like receptor 9, Hepatology 54 (2011) 999–1008.
[9] Z.M. Bamboat, L.M. Ocuin, V.P. Balachandran, H. Obaid, G. Plitas, R.P. Dematteo, Conventional dcs reduce liver ischemia/reperfusion injury in mice via IL-10 secretion, J. Clin. Invest. 120 (2010) 559–569.
[10] A. Tsung, R. Sahai, H. Tanaka, A. Nakao, M.P. Fink, M.T. Lotze, et al., The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion, J. Exp. Med. 201 (2005) 1135–1143.
[11] M.R. McGill, V.S. Staggs, M.R. Sharpe, W.M. Lee, H. Jaeschke, Acute Liver Failure Study Group, Serum mitochondrial biomarkers and damage-associated molecular patterns are higher in acetaminophen overdose patients with poor outcome, Hepatology 60 (2014) 1336–1345.
[12] M.R. McGill, M.R. Sharpe, C.D. Williams, M. Taha, S.C. Curry, H. Jaeschke, The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation, J. Clin. Invest. 122 (2012) 1574–1583.
[13] R.F. van Golen, M.J. Reiniers, M. Heger, J. Verheij, Solutions to the discrepancies in the extent of liver damage following ischemia/reperfusion in standard mouse models, J. Hepatol. 62 (2014) 975–977.
[14] D. Dindo, N. Demartines, P.A. Clavien, Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey, Ann. Surg. 240 (2004) 205–213.
[15] M. Rehm, D. Bruegger, F. Christ, P. Conzen, M. Thiel, M. Jacob, et al., Shedding of the endothelial glycocalyx in patients undergoing major vascular surgery with global and regional ischemia, Circulation 116 (2007) 1896–1906.
[16] S. Zeerleder, B. Zwart, H. te Velthuis, R. Manoe, I. Bulder, I. Rensink, L.A. Aarden, A plasma nucleosome releasing factor (NRF) with serine protease activity is instrumental in removal of nucleosomes from secondary necrotic cells, FEBS Lett. 581 (2007) 5382–5388.
[17] A.E. van Nieuwenhuijze, T. van Lopik, R.J. Smeenk, L.A. Aarden, Time between onset of apoptosis and release of nucleosomes from apoptotic cells: putative implications for systemic lupus erythematosus, Ann. Rheum. Dis. 62 (2003) 10–14.
[18] M.L. van Montfoort, F. Stephan, M.N. Lauw, B.A. Hutten, G.J. Van Mierlo, S. Solati, et al., Circulating nucleosomes and neutrophil activation as risk factors for deep vein thrombosis, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 147–151.
[19] J.H. Nuijens, J.J. Abbink, Y.T. Wachtfogel, R.W. Colman, A.J. Eerenberg, D. Dors, et al., Plasma elastase alpha 1-antitrypsin and lactoferrin in sepsis: evidence for neutrophils as mediators in fatal sepsis, J. Lab. Clin. Med. 119 (1992) 159–168.
[20] K. Nakahira, S.Y. Kyung, A.J. Rogers, L. Gazourian, S. Youn, A.F. Massaro, et al., Circulating mitochondrial DNA in patients in the ICU as a marker of mortality: derivation and validation, PLoS Med. 10 (2013) e1001577(discussion e1001577).
[21] J.M. Ruijter, C. Ramakers, W.M. Hoogaars, Y. Karlen, O. Bakker, M.J. van den Hoff, A.F. Moorman, Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data, Nucleic Acids Res. 37 (2009) e45.
[22] Q. Sun, P. Loughran, R. Shapiro, I.H. Shrivastava, D.J. Antoine, T. Li, et al., Redox-dependent regulation of hepatocyte absent in melanoma 2 inflammasome activation in sterile liver injury in mice, Hepatology 65 (2017) 253–268.
[23] H. Yang, H. Wang, Z. Ju, A.A. Ragab, P. Lundbäck, W. Long, et al., MD-2 is required for disulfide hmgb1-dependent TLR4 signaling, J. Exp. Med. 212 (2015) 5–14.
[24] A. Tsung, J.R. Klune, X. Zhang, G. Jeyabalan, Z. Cao, X. Peng, et al., HMGB1 release induced by liver ischemia involves toll-like receptor 4 dependent reactive oxygen species production and calcium-mediated signaling, J. Exp. Med. 204 (2007) 2913–2923.
[25] M.J. Reiniers, R.F. van Golen, S. Bonnet, M. Broekgaarden, T.M. van Gulik, M.R. Egmond, M. Heger, Preparation and practical applications of 2′,7′-dichlorodihydrofluorescein in redox assays, Anal. Chem. 89 (2017) 3853–3857.
[26] E. Gonzales, B. Julien, V. Serrière-Lanneau, A. Nicou, I. Doignon, L. Lagoudakis, et al., ATP release after partial hepatectomy regulates liver regeneration in the rat, J. Hepatol. 52 (2010) 54–62.
[27] P. Mukhopadhyay, B. Horváth, Z. Zsengellèr, S. Bátkai, Z. Cao, M. Kechrid, et al., Mitochondrial reactive oxygen species generation triggers inflammatory response and tissue injury associated with hepatic ischemia-reperfusion: therapeutic potential of mitochondrially targeted antioxidants, Free Radic. Biol. Med. 53 (2012) 1123–1138.
[28] E. Venereau, M. Casalgrandi, M. Schiraldi, D.J. Antoine, A. Cattaneo, F. De Marchis, et al., Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release, J. Exp. Med. 209 (2012) 1519–1528.
[29] P. Huebener, J.P. Pradere, C. Hernandez, G.Y. Gwak, J.M. Caviglia, X. Mu, et al., The HMGB1/RAGE axis triggers neutrophil-mediated injury amplification following necrosis, J. Clin. Invest. 125 (2015) 539–550.
[30] J.K. Wiggers, B.G. Koerkamp, K.P. Cieslak, A. Doussot, D. van Klaveren, P.J. Allen, et al., Postoperative mortality after liver resection for perihilar cholangiocarcinoma: development of a risk score and importance of biliary drainage of the future liver remnant, J. Am. Coll. Surg. 223 (2016) 321–331.e1.
[31] J. Evankovich, S.W. Cho, R. Zhang, J. Cardinal, R. Dhupar, L. Zhang, et al., High mobility group box 1 release from hepatocytes during ischemia and reperfusion injury is mediated by decreased histone deacetylase activity, J. Biol. Chem. 285 (2010) 39888–39897.
[32] M. Ilmakunnas, E.M. Tukiainen, A. Rouhiainen, H. Rauvala, J. Arola, A. Nordin, et al., High mobility group box 1 protein as a marker of hepatocellular injury in human liver transplantation, Liver Transpl. 14 (2008) 1517–1525.
[33] A.H. Burstein, I. Grimes, D.R. Galasko, P.S. Aisen, M. Sabbagh, A.M. Mjalli, Effect of TTP488 in patients with mild to moderate alzheimer's disease, BMC Neurol. 14 (2014) 12.
[34] E.J. Hennessy, A.E. Parker, L.A. O'Neill, Targeting toll-like receptors: emerging therapeutics? Nat. Rev. Drug Discov. 9 (2010) 293–307.
[35] P. Lundbäck, J.D. Lea, A. Sowinska, L. Ottosson, C.M. Fürst, J. Steen, et al., A novel high mobility group box 1 neutralizing chimeric antibody attenuates drug-induced liver injury and postinjury inflammation in mice, Hepatology 64 (2016) 1699–1710.
[36] M. Yang, D.J. Antoine, J.L. Weemhoff, R.E. Jenkins, A. Farhood, B.K. Park, H. Jaeschke, Biomarkers distinguish apoptotic and necrotic cell death during hepatic ischemia/reperfusion injury in mice, Liver Transpl. 20 (2014) 1372–1382.
[37] M.J. Reiniers, R.F. van Golen, M. Heger, B. Mearadji, R.J. Bennink, J. Verheij, T.M. van Gulik, In situ hypothermic perfusion with retrograde outflow during right hemihepatectomy: first experiences with a new technique, J. Am. Coll. Surg. 218 (2013) e7–16.
[38] M.J. Reiniers, P.B. Olthof, R.F. van Golen, M. Heger, A.A. van Beek, B. Meijer, et al., Hypothermic perfusion with retrograde outflow during right hepatectomy is safe and feasible, Surgery 162 (2017) 48–58.
[39] M. Kojima, M. Tanabe, M. Shinoda, S. Yamada, T. Miyasho, K. Suda, et al., Role of high mobility group box chromosomal protein 1 in ischemia-reperfusion injury in the rat small intestine, J. Surg. Res. 178 (2012) 466–471.
[40] H. Huang, G.W. Nace, K.A. McDonald, S. Tai, J.R. Klune, B.R. Rosborough, et al., Hepatocyte specific HMGB1 deletion worsens the injury in liver ischemia/reperfusion: a role for intracellular HMGB1 in cellular protection, Hepatology 59 (2013) 1984–1997.
[41] G.W. Nace, H. Huang, J.R. Klune, R.E. Eid, B.R. Rosborough, S. Korff, et al., Cellular specific role of toll-like receptor 4 in hepatic ischemia-reperfusion injury, Hepatology 58 (2013) 374–387.
[42] Y. He, D. Feng, M. Li, Y. Gao, T. Ramirez, H. Cao, et al., Hepatic mitochondrial DNA/toll-like receptor 9/microrna-223 forms a negative feedback loop to limit neutrophil overactivation and acetaminophen hepatotoxicity in mice, Hepatology 66 (2017) 220–234.
[43] A. Ramachandran, L. Duan, J.Y. Akakpo, H. Jaeschke, Mitochondrial dysfunction as a mechanism of drug-induced hepatotoxicity: current understanding and future perspectives, J. Clin. Transl. Res. 4 (2018).
[44] H. Jaeschke, J.J. Lemasters, Apoptosis versus oncotic necrosis in hepatic ischemia/reperfusion injury, Gastroenterology 125 (2003) 1246–1257.
[45] R.K. Boyapati, D.A. Dorward, A. Tamborska, R. Kalla, N.T. Ventham, M.K. Doherty, et al., Mitochondrial DNA is a pro-inflammatory damage-associated molecular pattern released during active IBD, Inflamm. Bowel Dis. 24 (2018) 2113–2122.
[46] Q. Zhang, M. Raoof, Y. Chen, Y. Sumi, T. Sursal, W. Junger, et al., Circulating mitochondrial damps cause inflammatory responses to injury, Nature 464 (2010) 104–107.
[47] M. Heger, M.J. Reiniers, R.F. van Golen, Mitochondrial metabolomics unravel the primordial trigger of ischemia/reperfusion injury, Gastroenterology 148 (2015) 1071–1073.
[48] R. Bahde, H.U. Spiegel, Hepatic ischaemia-reperfusion injury from bench to bedside, Br. J. Surg. 97 (2010) 1461–1475.
[49] E.J. Gane, F. Weilert, D.W. Orr, G.F. Keogh, M. Gibson, M.M. Lockhart, et al., The mitochondria-targeted anti-oxidant mitoquinone decreases liver damage in a phase II study of hepatitis C patients, Liver Int. 30 (2010) 1019–1026.
[50] S. Rodriguez-Cuenca, H.M. Cochemé, A. Logan, I. Abakumova, T.A. Prime, C. Rose, et al., Consequences of long-term oral administration of the mitochondria-targeted antioxidant mitoq to wild-type mice, Free Radic. Biol. Med. 48 (2010) 161–172.
[51] M.A. van den Broek, J.G. Bloemen, S.A. Dello, M.C. van de Poll, S.W. Olde Damink, C.H. Dejong, Randomized controlled trial analyzing the effect of 15 or 30 min intermittent pringle maneuver on hepatocellular damage during liver surgery, J. Hepatol. 55 (2011) 337–345.
[52] B. Beck-Schimmer, S. Breitenstein, J.M. Bonvini, M. Lesurtel, M. Ganter, A. Weber, et al., Protection of pharmacological postconditioning in liver surgery: results of a prospective randomized controlled trial, Ann. Surg. 256 (2012) 837–845.
[53] M.C. van de Poll, J.P. Derikx, W.A. Buurman, W.H. Peters, H.M. Roelofs, S.J. Wigmore, C.H. Dejong, Liver manipulation causes hepatocyte injury and precedes systemic inflammation in patients undergoing liver resection, World J. Surg. 31 (2007) 2033–2038.
[54] J. Belghiti, R. Noun, R. Malafosse, P. Jagot, A. Sauvanet, F. Pierangeli, et al., Continuous versus intermittent portal triad clamping for liver resection: a controlled study, Ann. Surg. 229 (1999) 369–375.