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Dual effect of hemin on renal ischemia-reperfusion injury

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ABSTRACT

Acute kidney injury (AKI) is a major public health concern, which is contributing to serious hospital complications, chronic kidney disease (CKD) and even death. Renal ischemia-reperfusion injury (IRI) remains a leading cause of AKI. The stress-responsive enzyme, heme oxygenase-1 (HO-1) mediates protection against renal IRI and may be preventively induced using hemin prior to renal insult. This HO-1 induction pathway called hemin preconditioning is largely known to be effective. Therefore, HO-1 might be an interesting therapeutic target in case of predictable AKI (e.g. partial nephrectomy or renal transplantation). However, the use of hemin to mitigate established AKI remains poorly characterized.

Mice underwent bilateral renal IRI for 26 min or sham surgery. After surgical procedure, animals were injected either with hemin (5 mg/kg) or vehicle. Twenty-four hours later, mice were sacrificed. Despite strong HO-1 induction, hemin-treated mice exhibited significant renal damage and oxidative stress as compared to vehicle-treated mice. Interestingly, higher dose of hemin is associated with more severe IRI-induced AKI in a dose-dependent relation. To determine whether hemin preconditioning remains efficient to dampen postoperative hemin-amplified IRI-induced AKI, we pretreated mice either with hemin (5 mg/kg) or vehicle 24 h prior to surgical procedure. Then, all mice (hemin- and vehicle-pretreated) received postoperative injection of hemin (5 mg/kg) to amplify IRI-induced AKI. In comparison to vehicle, prior administration of hemin to renal IRI mitigated hemin-amplified IRI-induced AKI as attested by fewer renal damage, inflammation and oxidative stress. In conclusion, hemin may have a dual effect on renal IRI, protective or deleterious, depending on the timing of its administration.

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1. Introduction

Acute kidney injury (AKI) is defined by the abrupt loss of renal function that is frequently associated with poor outcomes such as prolonged length of hospital stays, advanced chronic kidney disease (CKD), and even death [1]. Renal ischemia-reperfusion injury (IRI) is a cornerstone in many common causes of AKI including the use of iodinated contrast agents, partial nephrectomy, and renal transplantation. IRI combines major cell stress, significant burst of free radicals, and strong inflammatory responses leading to extensive cell injury, necrosis, and late interstitial fibrosis [2,3]. Therefore, the development of therapeutic or preventive strategies for IRI-induced AKI is an important public health concern [2,3].

In this context, the ubiquitous heme oxygenase (HO) cytoprotective pathway appears as a promising target for clinical intervention. HO isoform 1 (HO-1, encoded by *Hmox1*) is a stress-induced enzyme that metabolizes free heme into carbon monoxide, biliverdin, and iron. Through its byproducts, HO-1 exhibits cytoprotective, antiapoptotic, and immunomodulatory properties that provide resistance against IRI [4]. If HO-1 deficiency increases the susceptibility to both AKI and death upon renal IRI, preventive HO-1 induction with synthetic heme (i.e. hemin) does confer significant resistance against renal IRI [5–8]. Importantly, this pharmacological induction of HO-1 with hemin (i.e. hemin preconditioning) is effective in humans and FDA-approved for the treatment of acute intermittent porphyria, a disease characterized by heme deficiency [9].

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Although tubular epithelial cells are thought to be the main protective source of HO-1 in the kidney [10], our group and others have recently shown the importance of HO-1$^+$ myeloid cells in the mitigation of IRI-induced AKI [5,11–13]. Indeed, we have demonstrated that myeloid-restricted deletion of HO-1 leads to the loss of hemin-mediated protection [11].

Preemptive induction of HO-1 by hemin before renal IRI is known to be an efficient protective strategy against AKI in animal models [7,8,11]. However, the use of hemin as potential therapeutic strategy to mitigate established AKI remains poorly investigated. Therefore, we compare, herein, IRI in mice receiving hemin after IRI-induced AKI to control vehicle-treated mice in order to decipher whether hemin-induced HO-1 might be beneficial for established AKI.

2. Materials and methods

2.1. Mice

C57BL/6 wild-type mice were purchased from Harlan (Zeist, The Netherlands). Eight-to twelve-week-old male animals were used for all experiments, and animals were bred in our specific pathogen-free animal facility. All experiments were conducted in compliance with the Principles of Laboratory Animal Care formulated by the National Institute of Health (Guide for the Care and Use of Laboratory Animals, Eighth Edition, National Research Council, 2010) and were approved by the local committee for animal welfare (Commission d'éthique du Biopole ULB Charleroi).

2.2. Renal IRI model

Renal IRI was performed as previously described [11]. Mice were anesthetized with an intraperitoneal injection (340 μl/25 g) of a solution containing Dormicum® (1 mg/ml; Roche), Fentanyl® (78 μg/ml; Janssen-Cilag), and Haldol® (5 mg/ml; Janssen-Cilag). Body temperature was maintained at 37 °C throughout the procedure. Kidneys were exposed through midline incision, and both renal pedicles were clamped for 26 min using nontraumatic microsurgical clamps (S&T Microsurgical Instruments). Evidence of ischemia was confirmed by visualizing dark color of clamped kidneys. After clamp removal, both kidneys were checked in order to see adequate reperfusion. The abdomen was closed in 2 layers, and all mice received a subcutaneous injection of Temgesic® (50 μg/kg; Schering-Plough) for analgesic purposes. The surgical procedure was performed on a heating pad to maintain the core temperature and mice were allowed to recover at 28 °C in a ventilated stove with food and water available. Sham-operated mice underwent the same procedure except for clamping of the pedicles. After surgical procedure, mice received an intraperitoneal injection of hemin (5 mg/kg) or saline. Mice were sacrificed 24 h after reperfusion and samples were collected. When specified, Mice also received an intraperitoneal injection of hemin or sterile saline 24 h prior to surgery.

2.3. Protoporphyrin preparation

Hemin (Ferriprotoporphyrin IX chloride, Sigma-Aldrich) was dissolved in 0.1 M NaOH, neutralized (to pH 7.2) with 1 M HCl and adjusted to concentration of 7.7 mM with distilled water. Aliquots were protected from light and stored at −80 °C until used. Hemin was then diluted in sterile saline (NaCl 0.9%) to appropriate concentration and filtered. Sterile saline was used as vehicle.

2.4. Renal function assessment and histopathology

Renal function was evaluated by measuring plasma creatinine as previously described [11]. Kidneys were fixed in 4% formaldehyde, embedded in paraffin, sectioned at 5-μm thickness, and stained with Periodic acid–Schiff–diastase. Renal damage was assessed in a blinded manner by the Tubular Injury Score [14].

2.5. Immunohistochemistry

Immunohistochemistry was performed as previously described [11]. Neutrophils were detected by using an anti–Ly-6G antibody (1:50; BD Biosciences) and Ly-6G$^+$ cells were counted in 10 non-overlapping fields (×400 magnification). Nitrotyrosine staining was performed using an anti-nitrotyrosine antibody (1:400; Abcam) and nitrotyrosine intensity in the renal cortex was quantified using NIH ImageJ software.

2.6. Enzyme-linked immunosorbent assay

HO-1 and KIM-1 enzyme-linked immunosorbent assay kits were purchased from Enzo Life Sciences and R&D Systems, respectively. Plasma HO-1 and KIM-1 were measured according to manufacturer's instructions.

2.7. RNA extraction and real-time quantitative reverse transcription PCR (qRT-PCR)

Total RNA was extracted from kidney tissues as previously described [11]. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a RNA loading control. The primers were custom ordered from Eurogentec as follows: GAPDH, forward, 5′-ATTGTCAGCAATGCATCCTG- 3′, reverse, 5′-CCTTCCA-CAATGCCAAAGTT-3′ and probe, 5′-FAM- CCCTGGCCAAGGTCATC-CATGA-TAMRA-3'; HO-1, forward, 5′- GCCGAGAATGCTGAGTTCAT-3′, reverse, 5′-AGGAAGCCATCACCAGCTTA-3′ and probe, 5′-FAM-AGAACTTTCAGAAGGGTCAGGTGTCCA-TAMRA-3'. Kidney tissue mRNA levels are expressed as $2^{-\Delta\Delta CT}$ in which CT represents “cycle of threshold”, $\Delta\Delta CT = \Delta CT_{\text{mouse of interest}} - \Delta CT_{\text{sham-operated WT C57BL/6 mouse}}$, and $\Delta CT = CT_{\text{gene of interest}} - CT_{\text{GAPDH}}$.

2.8. Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). A two-tailed nonparametric Mann-Whitney *U* test was used; *P*-values < 0.05 were considered to represent statistical significance. All graphs and statistical analyses were performed using GraphPad Prism 6.00 for Mac OS X (GraphPad Software, La Jolla California USA, www.graphpad.com).

3. Results

3.1. Postoperative injection of hemin induces HO-1 upon renal IRI

To evaluate whether postoperative injection of hemin would be able to mitigate established AKI through HO-1 induction, mice were injected either with hemin (5 mg/kg) or vehicle after surgical procedure. Twenty-four hours after hemin administration, hemin-treated mice exhibited significant increase in HO-1 expression in both kidney and plasma as compared to vehicle-treated mice (Fig. 1A and B).

3.2. Postoperative injection of hemin fails to mitigate established AKI

Despite strong HO-1 induction, postoperative hemin injection (5 mg/kg) did not preserve renal function upon IRI and even worsened it as attested by significant higher level of plasma creatinine (Fig. 2A). Moreover, hemin-treated mice displayed

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