Research Article

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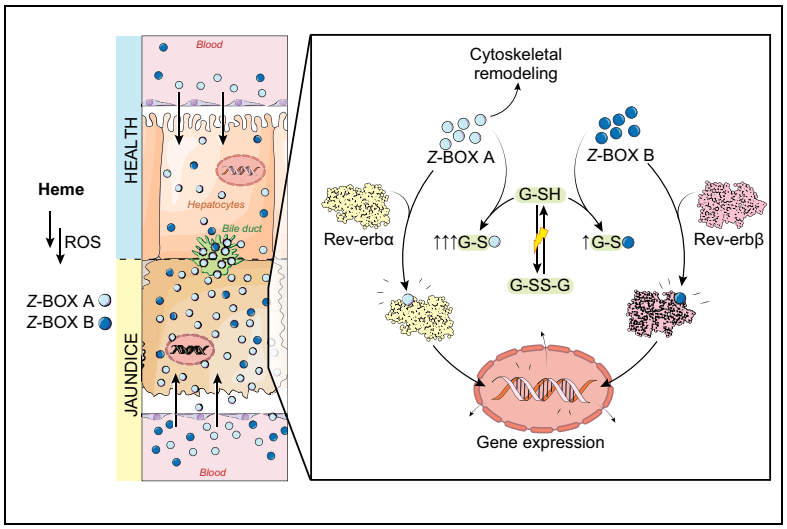
Impact of higher-order heme degradation products on hepatic function and hemodynamics

Graphical abstract



Highlights

- Plasma levels of bilirubin oxidation products rise in cholestatic liver disease.
- The regio-isomers *Z*-BOX A and B exhibit distinct pharmacokinetics and -dynamics.
- *Z*-BOX A and B differentially affect the hepatocellular glutathione redox state.
- *Z*-BOX A and B differentially modulate the activity of Rev-erbα and Rev-erbβ.

Authors

Raphael A. Seidel, Thierry Claudel, Franziska A. Schleser, ..., Georg Pohnert, Michael Trauner, Michael Bauer

Correspondence

michael.bauer@med.uni-jena.de (Michael Bauer)

Lay summary

Degradation of the blood pigment heme yields the bile pigment bilirubin and the oxidation products *Z*-BOX A and *Z*-BOX B. Serum concentrations of these bioactive molecules increase in jaundice and can impair liver function and integrity. Amounts of *Z*-BOX A and *Z*-BOX B that are observed during liver failure in humans have profound effects on hepatic function when added to cultured liver cells or infused into healthy rats.

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Impact of higher-order heme degradation products on hepatic function and hemodynamics

Raphael A. Seidel[1,2], Thierry Claudel[3], Franziska A. Schleser[1], Navin K. Ojha[4], Matthias Westerhausen[5], Sandor Nietzsche[6], Christoph Sponholz[1], Frans Cuperus[3,7], Sina M. Coldewey[1], Stefan H. Heinemann[4], Georg Pohnert[2], Michael Trauner[3,†], Michael Bauer[1,*,†]

[1]*Department of Anesthesiology and Intensive Care Medicine/Center for Sepsis Control and Care, Jena University Hospital, Germany;* [2]*Institute of Inorganic and Analytical Chemistry, Bioorganic Analytics, Friedrich Schiller University Jena, Germany;* [3]*HansPopper Laboratory of Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Austria;* [4]*Center for Molecular Biomedicine, Department of Biophysics, Friedrich Schiller University Jena & Jena University Hospital, Germany;* [5]*Institute of Inorganic and Analytical Chemistry, Inorganic Chemistry I, Friedrich Schiller University Jena, Germany;* [6]*Electron Microscopy Center, Jena University Hospital, Germany;* [7]*Pediatric Gastroenterology and Hepatology, Center for Liver, Digestive, and Metabolic Diseases, University Medical Center Groningen, The Netherlands*

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Background & Aims: Biliverdin and bilirubin were previously considered end products of heme catabolism; now, however, there is evidence for further degradation to diverse bioactive products. *Z*-BOX A and *Z*-BOX B arise upon oxidation with unknown implications for hepatocellular function and integrity. We studied the impact of *Z*-BOX A and B on hepatic functions and explored their alterations in health and cholestatic conditions.
Methods: Functional implications and mechanisms were investigated in rats, hepatocytic HepG2 and HepaRG cells, human immortalized hepatocytes, and isolated perfused livers. *Z*-BOX A and B were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in acute and acute-on-chronic liver failure and hereditary unconjugated hyperbilirubinemia.
Results: *Z*-BOX A and B are found in similar amounts in humans and rodents under physiological conditions. Serum concentrations increased ~20-fold during cholestatic liver failure in humans ($p < 0.001$) and in hereditary deficiency of bilirubin glucuronidation in rats ($p < 0.001$). Pharmacokinetic studies revealed shorter serum half-life of *Z*-BOX A compared to its regio-isomer *Z*-BOX B ($p = 0.035$). While both compounds were taken up by hepatocytes, *Z*-BOX A was enriched ~100-fold and excreted in bile. Despite their reported vasoconstrictive properties in the brain vasculature, BOXes did not affect portal hemodynamics. Both *Z*-BOX A and B showed dose-dependent cytotoxicity, affected the glutathione redox state, and differentially modulated activity of Rev-erbα and Rev-erbβ. Moreover, BOXes-triggered remodeling of the hepatocellular cytoskeleton.
Conclusions: Our data provide evidence that higher-order heme degradation products, namely *Z*-BOX A and B, impair hepatocellular integrity and might mediate intra- and extrahepatic cytotoxic effects previously attributed to hyperbilirubinemia.
Lay summary: Degradation of the blood pigment heme yields the bile pigment bilirubin and the oxidation products *Z*-BOX A and *Z*-BOX B. Serum concentrations of these bioactive molecules increase in jaundice and can impair liver function and integrity. Amounts of *Z*-BOX A and *Z*-BOX B that are observed during liver failure in humans have profound effects on hepatic function when added to cultured liver cells or infused into healthy rats.

Introduction

Heme (iron protoporphyrin IX) serves as a prosthetic group in a variety of proteins involved in oxygen transport, redox reactions, and signaling.[1] When released extracellularly, labile heme acts as an alarmin[2] and cytotoxic agonist[3] that is encountered by scavenger systems.[4] Intracellular heme concentrations are tightly controlled by its enzymatic degradation via heme oxygenases (HO),[5] yielding the first-order degradation products biliverdin, ferrous iron, and carbon monoxide (CO).[6,7] The induction of HO-1 reflects a hallmark of the cellular response to oxidative stress and confers tissue protection during infection and inflammation.[8,9] Bilirubin, generated from biliverdin by biliverdin reductases, contributes to tissue integrity via its anti-oxidative properties.[10,11] Nevertheless, above a certain threshold bilirubin is held responsible for intra- and extrahepatic dysfunction,

Keywords: Heme degradation; Bilirubin oxidation end products (BOXes); Bilirubin toxicity; Rev-erb; Glutathione; Cholestasis; Hemodynamics; Pharmacokinetics; Cytoskeleton; Reactive oxygen species.
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* Corresponding author. Address: Department of Anesthesiology and Intensive Care Medicine, Jena University Hospital, Am Klinikum 1, 07747 Jena, Germany; Tel.: +49 3641 9 323101; fax: +49 3641 9 323102.
E-mail address: michael.bauer@med.uni-jena.de (M. Bauer).
† These authors share senior authorship.