

Biliverdin and Heme Oxygenase Antiviral Activity Against Hepatitis C Virus

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Article commented:

Zhu Z, Wilson AT, Luxon BA, Brown KE, Mathahs MM, Bandyopadhyay S, McCaffrey AP, Schmidt WN. Biliverdin Inhibits Hepatitis C Virus Nonstructural 3/4A Protease Activity: Mechanism for the Antiviral Effects of Heme Oxygenase? *Hepatology* 2010; 52:1897-1905.

Original Abstract:

Induction of heme oxygenase-1 (HO-1) inhibits hepatitis C virus (HCV) replication. Of the products of the reaction catalyzed by HO-1, iron has been shown to inhibit HCV ribonucleic acid (RNA) polymerase, but little is known about the antiviral activity of biliverdin (BV). Herein, we report that BV inhibits viral replication and viral protein expression in a dose-dependent manner in replicons and cells harboring the infectious J6/JFH construct. Using the SensoLyte 620 HCV Protease Assay with a wide wavelength excitation/emission (591 nm/622 nm) fluorescence energy transfer peptide, we found that both recombinant and endogenous nonstructural 3/4A (NS3/4A) protease from replicon microsomes are potently inhibited by BV. Of the tetrapyrroles tested, BV was the strongest inhibitor of NS3/4A activity, with a median inhibi-

tory concentration (IC₅₀) of 9 μM, similar to that of the commercial inhibitor, AnaSpec (Fremont, CA) #25346 (IC₅₀ 5 μM). Lineweaver-Burk plots indicated mixed competitive and noncompetitive inhibition of the protease by BV. In contrast, the effects of bilirubin (BR) on HCV replication and NS3/4A were much less potent. Because BV is rapidly converted to BR by biliverdin reductase (BVR) intracellularly, the effect of BVR knockdown on BV antiviral activity was assessed. After greater than 80% silencing of BVR, inhibition of viral replication by BV was enhanced. BV also increased the antiviral activity of an interferon in replicons. Conclusion: BV is a potent inhibitor of HCV NS3/4A protease, which likely contributes to the antiviral activity of HO-1. These findings suggest that BV or its derivatives may be useful in future drug therapies targeting the NS3/4A protease.

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Comment:

Hepatitis C virus is classified into the genus Hepacivirus of the Flaviviridae family. It is a spherical virus of approximately 50 nm of diameter. It has a glycoproteic envelope that contains lipids and a positive chain genome of 9600 nucleotides of length that codifies a precursor of large-sized lipoproteins, segmented simultaneously with the processes of cotranslation and posttranslation to produce particular structural and nonstructural proteins. The coding genes order is 5'-C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-3'. The C gene, consisted of 190 amino acids, is the basic subunit of the nucleocapsid protein. A characteristic of the E gene is the presence of a hypervariable region that codifies two envelope

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glycoproteins (E1 and E2), involved in the union to cellular receptors and the entrance of the virus to the host cell. NS2 and NS3 genes codify two proteases that are involved in the processing of the nonstructural region, while NS4A gene acts as a cofactor for the activity of a NS3 serin-protease enzyme. NS5A derive two phosphorylated products (whose function is unknown), NS5B contains the RNA dependent-RNA polymerase domain, essential for the viral replication. This RNA virus is characterized for a high genomic heterogeneity degree as a consequence of RNA polymerase to correct replication mistakes.

In the study entitled *Biliverdin Inhibits Hepatitis C Virus Nonstructural 3/4A Protease Activity: Mechanism for the Antiviral effects of Heme Oxygenase?* published in the December issue of the *Hepatology* by Zhu et al., it is reported that biliverdin has a strong inhibitory activity against recombinant and endogenous NS3/4A protease.¹ Several studies have shown that HCV infection is associated with an oxidative injury, and for this reason modulation of host cells antioxidant activity has been suggested as a potential therapeutic target for HCV infection.² Dionisio et al. found that the two main players in the oxidative stress caused by HCV are the hepatitis C core protein and non structural protein NS5A.³

Heme oxygenase (HMOX) is the enzyme which degrades heme and produces carbon monoxide, biliverdin and iron. Biliverdin is then converted into bilirubin by biliverdin reductase, HMOX is upregulated not only by heme, but also by oxidative stress stimuli, and its overexpression constitutes a protective cellular response against oxidative stress.⁴

HMOX has anti-inflammatory and antiapoptotic activities both *in vivo* and *in vitro*, and the induction of this enzyme has been found to have a protective role in ischemic acute renal failure,⁵ cerebral ischemia,⁶ organ transplantation⁷ and apoptotic damage.⁸ Interestingly, HMOX has been found to have important antiviral effects against hepatitis B virus (HBV). Protzer and colleagues reported that the induction of HMOX by cobalt-protoporphyrin-IX treatment significantly reduced the levels of HBV core protein.⁹ In regards to human immunodeficiency virus (HIV), McPhee et al. found that biliverdin and bilirubin inhibited recombinant HIV-1 protease *in vitro* and also HIV-2 and simian immunodeficiency virus proteases¹⁰. Other pyrrolic pigments, such as stercobilin, urobilin, biliverdin dimethyl ester and xanthobilirubic acid, also showed similar inhibitory activity at low concentrations.¹⁰ There is ac-

cumulating body of evidence that bile pigments have other multiple and in general anti-inflammatory properties. They exert anti-complement action, block leukocyte adhesion and activation, monocyte migration or inhibic adhesion molecule signalling.¹¹ In 2008, Zhu et al. evaluated the effect of HMOX on HCV replication and found that overexpression of HMOX decreased HCV DNA replication in full length and nonstructural replicons, without affecting DNA synthesis and cellular growth.² More recently they observed that biliverdin, and in to a lesser extent its metabolite, bilirubin, have antiviral activity in the human hepatoma cell line (Huh5-15) and Huh7.5 cells harboring full length (Huh7.5FL) Con1 replicons. Biliverdin showed antiviral activity in concentrations as low as 20 μ M (1.17 mg/dL); by contrast, bilirubin-IX- α and other mixed isomers showed antiviral activity at concentrations of 200 μ M (11.7 mg/dL) or higher. Qin found that deconjugated bile pigments inhibit serine-activated pancreatic proteases such as chymotrypsin and trypsin, which led the group to investigate the effects of biliverdin and other tetrapyrrolic derivates on the activity of HCV NS 3/4A.¹² When Zhu et al. measured the activity of the protease and compared the inhibitory activity of the biliverdin with that of a known competitive inhibitor of the NS3/4A (AnaSpec #25346) they observed that biliverdin activity was similar to that of the inhibitor. Although still unsettled, the mechanism of biliverdin inhibition on proteases might be related to the helical porphyrin-like conformation of this pyrrolic derivative affecting also lipophilicity of this pigment.

It remains to be elucidated what is the biological relevance of the findings that biliverdin and other tetrapyrrolic pigments inhibit the activity of NS3/4A as well as NS5B. Serum concentrations of heme are much lower^{13,14} than those used by *Zhu et al.*¹, and serum levels of biliverdin are practically undetectable.¹⁵ It also should be noted that in a clinical study on US population no correlation was found between *HMOX1* promoter sequence variations modulating enzymatic activity and HCV infection outcomes.¹⁶ These results are in line with our own results (LV, unpublished data) demonstrating no prognostic role in genetic variations of both *HMOX1* and bilirubin UDP-glucuronosyl transferase (*UGT1A1*) genes on HCV clinical status. Although the gap between *in vitro* and *in vivo* data still remains rather large, the demonstration of inhibitory effects of tetrapyrrolic pigments on HCV proteases may unlock a wide range of possibilities to create new potential therapies for HCV infection.

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