Current Trends in Immunology
Vol. 14, 2013

Mini-Review

Therapeutic potential of endogenous heme oxygenase-1 activation in pathogenic infections

Subhash Dhawan[1,*], Alain Debrabant[1] and Kenneth M. Yamada[2]

[1]Division of Emerging and Transfusion Transmitted Diseases, Center for Biologics Evaluation and Research, Food and Drug Administration, [2]Laboratory of Cell and Development Biology, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA

ABSTRACT

The global prevalence of human immunodeficiency virus type-1 (HIV-1) and emerging infections with dengue, West Nile virus (WNV) and *Leishmania donovani* have increased greatly in recent decades, with rapidly growing numbers of people infected with these pathogens. Currently, no vaccine is available for the prevention of these infections, and no effective drugs are available to treat many of these blood-borne diseases. This article presents the novel concept of inducing an effective innate host defense response against these infections, which can be transmitted by blood transfusion or insect vectors. The proposed concept is supported by experimental evidence that the induction of an endogenous cytoprotective factor, heme oxygenase-1 (HO-1), by its physiological regulator hemin (the active component of an FDA-approved drug), promotes cellular protection against HIV-1, WNV, dengue, and *Leishmania donovani* infections. Thus, regardless of the type of pathogenic infection, HO-1 induction may provide a potentially novel, safe, and alternative natural therapeutic strategy for the treatment of a number of existing and emerging transfusion-transmitted diseases. In view of the continued challenges due to emerging drug-resistant mutants, this approach may particularly be useful for treating infections for which no vaccine is currently available, or no safe and effective drug is available to treat the diseases caused by such pathogens.

KEYWORDS: hemin, heme oxygenase-1, HIV-1, dengue virus, West Nile virus, *Leishmania donovani*, host factors

INTRODUCTION

Rapid increases in existing and emerging transfusion-transmitted/blood-borne diseases such as HIV-1, dengue, WNV, and *Leishmania donovani* pose serious challenges to human health [1-3]. Detection, identification, and successful treatment of infection by these pathogens have remained an ongoing challenge; importantly, safe and effective drugs for treating many of these infections are not available. Therefore, there is an urgent need for an alternative strategy that can induce innate protective mechanisms against most if not all pathogens.

There has been a recent burst of publications describing HO-1 as a pivotal protective gene, which, when induced, can restore homeostasis to many medical conditions by virtue of its anti-inflammatory and anti-apoptotic functions [4-9]. Despite this evidence for a protective role in many clinical conditions, the potential involvement of HO-1 in host defense against pathogenic infections has remained largely unexplored. This report provides the evidence for inducible HO-1 in cellular protection against infections by a variety of pathogens, including HIV-1, dengue, WNV, and *L. donovani*.

*Corresponding author: Dr. Subhash Dhawan, CBER, FDA, 1401 Rockville Pike (HFM-315), Rockville, MD 20852, USA.

The concept

Although initial attention was focused on the role of HO-1 in heme metabolism, recent studies have generated substantial interest concerning its regulation and function in cytoprotection [10]. We suggest that modulation of HO-1 may reduce pathogenesis by favorably influencing critical aspects of host defense against HIV-1 and other transfusion or insect-transmitted infectious agents. Delineating a role of HO-1 in protection against multiple blood-borne infectious agents for which either no vaccine or no safe and effective drug is currently available may provide an alternative approach for treating disease conditions caused by many deadly pathogens. This proof-of-concept report supports this hypothesis.

HO-1 activation and inhibition of HIV-1 infection

Generally, HO-1 is not expressed constitutively in cells at a significant level; however, it can be induced rapidly by oxidative stress or by other inducers. Several substances such as heme, xenobiotics, and synthetic metalloporphyrins increase HO-1 expression in many cellular systems. The ability of some natural and synthetic flavone ring-containing molecules to induce HO-1 expression and their effects on HIV-1 infection of primary monocyte-derived macrophages (MDM) was tested. As shown in Figure 1A, all molecules efficiently induced HO-1 expression (panel a), and all suppressed HIV-1 replication in MDM (panel b). These findings suggest that the effective pharmacological modulation and magnitude of induction of this enzyme, which is widely distributed in tissues, combined with the unique biological activities of its catalytic byproducts, makes HO-1 an attractive therapeutic candidate if it can be demonstrated to play a key role(s) in mediating protection against pathogenic infections.

Choosing an optimal regimen of antiretroviral drugs is critical for treating individuals with HIV-1 infection to achieve long-term clinical success. Conventional antiretroviral therapy (ART) consisting of a cocktail of reverse transciptase, protease, and integrase inhibitors significantly reduces viral load in HIV-infected patients and minimizes progression to AIDS. However, if HIV-1 becomes resistant to one or more of these inhibitors, treatment becomes ineffective. In addition, because HIV-1 uses host cell factors for replication, designing safe and effective ART continues to pose difficult challenges. Moreover, many people worldwide still do not receive ART for various economic or logistical reasons. Therefore, a different approach is needed to address these complex practical issues.

Innate immune response is pivotal for host defense against pathogenic infections. It is, however, often ineffective in providing protection against a number of infections. Induction of a strong host response then becomes necessary to minimize the severity of infection or to reduce disease progression, especially for infections for which no effective drug therapy exists. This hypothesis was experimentally tested, and the results indicate that induction of the endogenous cytoprotective enzyme HO-1 by its natural substrate effectively inhibited HIV infection both *in vitro* and *in vivo* (Figure 1B and reference [11]), supporting a key role for HO-1 in host defense. These initial observations provided important clues on an alternative safe and effective therapeutic option for treating HIV infection.

The concept proposed in this article is novel in proposing the future use of a safe and effective drug, that is already FDA-approved for an unrelated disease, to induce HO-1 and treat a series of types of infections, even in the presence of an impaired immune response. In viral infections of T-cells and macrophages, for example, both virus and host gene transcription contribute to productive viral replication. Continuous emergence of drug resistance and frequent mutational changes in the viral genome further complicate virus-host defense interactions, resulting in difficult challenges to effective drug treatment; in fact, a number of non-bacterial infectious diseases have no good drug therapies. In these circumstances, induction of nascent cellular defense responses may provide a novel alternative or concurrent therapeutic strategy.

The use of hemin seemed appropriate to induce HO-1 expression because it is the active ingredient of the first FDA-approved formulation of hemin that was used for clinical use in the United States [12]. Activation of endogenous HO-1, likely through

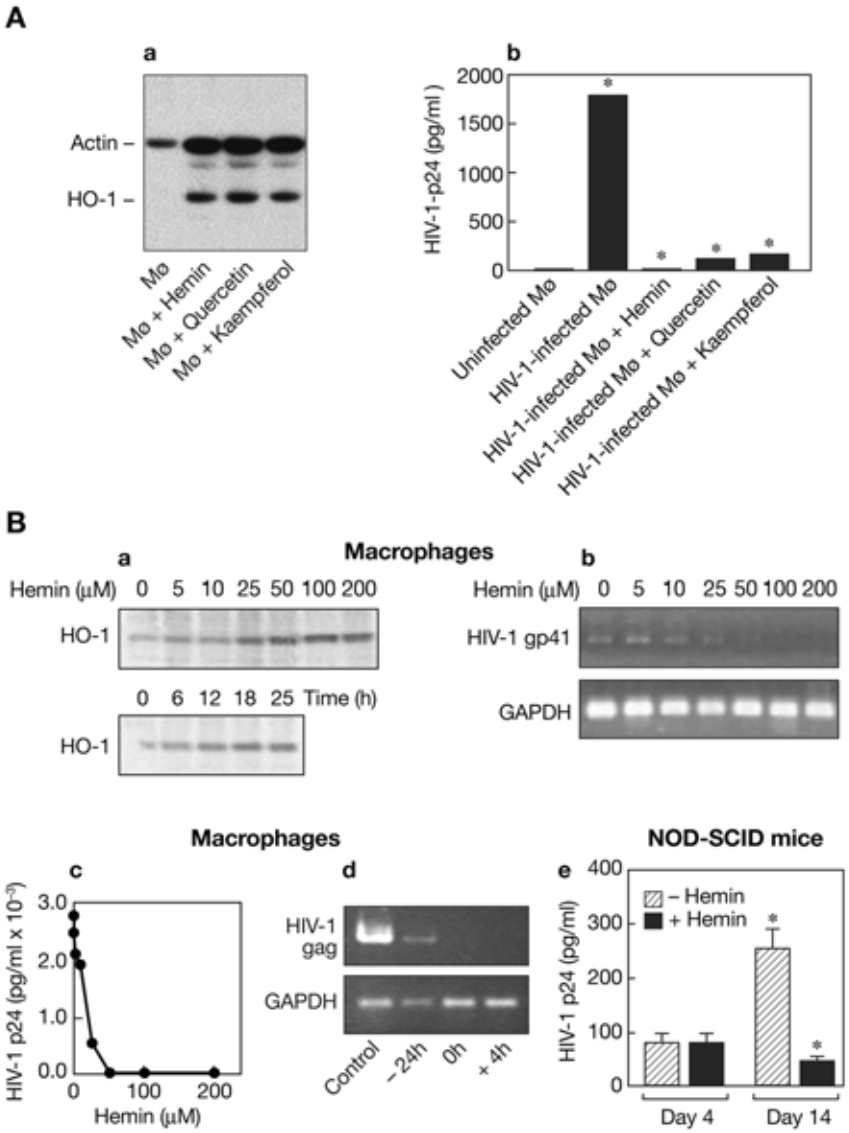

Figure 1. (A) Correlation between HO-1 induction by various HO-1-inducing substances and inhibition of HIV-1 replication in MDM. (a) Western blot analysis of total protein from cells treated with various reagents. (b) HIV-1-p24-antigen measurement in culture supernatant from HIV-infected cells cultured in the absence or presence of various HO-1-inducing agents by ELISA. **(B)** HO-1 induction inhibits HIV-1 infection. (a) Hemin-induced HO-1 expression in MDM by Western blot analysis. (b) RT-PCR analysis of genomic RNA from HIV-1-infected monocytes treated with various concentrations of hemin. (c) Inhibition of HIV-1 replication in MDM by hemin treatment as measured by HIV-1-p24 levels in culture supernatants. (d) RT-PCR analysis of genomic RNA from HIV-1-infected monocytes treated with hemin 24 h before, at the time of, and 4 h after infection. (e) HIV-1 p24 in serum of HIV-infected humanized NOD-SCID mice with or without hemin administration 3 days after infection. $^{*}P<0.05$. (Reproduced with permission from The Journal of Immunology, Vol. 176, pp. 4252-4257, 2006, by Devadas, K. and Dhawan, S. Copyright 2006. The American Association of Immunologists, Inc.).

other host genes, enhanced cellular defense against viral transcription and productive replication. Therefore, given that a hemin formulation has already met FDA safety requirements for use in humans, the protective role of HO-1 induction could provide a basis for potential clinical use of hemin in treating HIV-1 and many emerging blood-borne infections such as dengue, WNV, and

Leishmania donovani for which safe and effective therapeutic interventions are quite challenging. Thus, the induction of HO-1 gene expression may represent a critical event in innate cellular responses. A recently published first-in-human report describing safe and effective induction of HO-1 by hemin in healthy volunteers further rules out other possible regulatory hurdles [13]. Furthermore, hemin administration at a dose similar to the amount of a dietary iron supplement may be considered as an additional precautionary alternative.

HO-1 in emerging transfusion-transmitted and blood-borne infections

Although macrophages are principal effector cells of the immune system that function in an immunologic crisis to kill invading infectious agents, they can also provide a safe haven to many microorganisms that bypass the lysosomal degradation pathway. While these pathogens may use distinct routes of cellular entry, they productively infect and replicate in macrophages and impair immune function; they all can be potentially transmitted by blood transfusion, as well as by insect vectors.

Recognizing the challenges and opportunities described above, we hypothesized that drug treatment involving activation of HO-1 expression in primary human macrophages could play a host protective role against emerging transfusion-transmitted/blood-borne pathogens such as dengue, WNV, and *Leishmania donovani*. Validity of this concept of the protective role of inducible HO-1 against these pathogens was tested directly. Indeed, macrophages in which HO-1 was induced became largely refractory to productive infection by all three of these diverse microorganisms, indicating that this endogenous enzyme is a hallmark of common intracellular host defense activities irrespective of the type of invading pathogen. Thus, HO-1 appears to be an indicator or biomarker for effective drug responsiveness and modulating cellular function, suggesting new host-defense strategies for the development of therapeutic interventions against a variety of pathogenic infections.

HO-1 in dengue virus infection

The global prevalence of dengue infections has greatly increased in recent decades. With frequent travel to endemic areas and changing climate conditions, spread and transmission of deadly dengue viral infections continue to present an immense global health concern. Diverse viral strains and constant genomic mutations have challenged the development of effective vaccines against dengue virus. Vector methods to control transmission are largely ineffective. There is no vaccine against dengue virus, or drugs to treat dengue infections. This failure of conventional approaches for treating dengue infection warrants exploring new ideas as viable options.

Exposure of primary macrophages to dengue, WNV, or *Leishmania donovani* in the absence of hemin did not induce HO-1 expression (Figure 2A, panel a). Consistent with the observations described above, treatment of macrophages with hemin significantly induced HO-1 expression that was unaffected by the presence of dengue virus, WNV, or *Leishmania donovani* infections (Figure 2A, panel b).

The correlation between the HO-1 induction and dengue virus infection was examined by infecting untreated and hemin-treated macrophages with dengue-1. Virus replication was determined by plaque assay, and the intracellular virus was visualized by immunofluorescence four days after infection. Hemin treatment reduced virus production in dengue-infected MDM by ten-fold (not shown). Immunostaining of cells using an antibody specific for dengue-1 revealed substantially reduced expression of viral antigen in hemin-treated MDM (Figure 2B). These results mirrored those obtained with HIV infection, further substantiating a role of HO-1 in host defense.

HO-1 in West Nile virus infection

Infections with WNV are the leading cause of arboviral encephalitis in the United States. WNV can be transmitted via blood transfusion, besides its well-known route of transmission by mosquitoes. Although much insight into WNV pathogenesis has been gained since the first WNV case was identified in the U.S. in 1999, there is still no vaccine or therapeutic treatment available for WNV disease. Thus, prevention of new infections and treatment of WNV have become a global public health issue.

To test whether induction of endogenous HO-1 expression can suppress WNV infection, macrophages were treated for 24 hours with hemin and then challenged with WNV. Virus replication quantified by plaque assays 4 days after infection revealed approximately four-fold reduction of infectious WNV produced by hemin-treated cells compared to untreated infected MDM (Figure 2C). WNV does not induce visible cytopathic effects in primary MDM. Therefore, the monkey kidney cell line LLC was cultured in the presence of conditioned media from WNV-infected and hemin-treated WNV-infected MDM and examined for cytopathic effects four days after incubation. Morphology of Wright-stained LLC incubated with culture fluids from WNV-infected MDM demonstrated typical characteristic cytopathic effects characterized by multiple vacuoles in the cytoplasm (not shown). Treatment of MDM with hemin also profoundly reduced WNV-associated cytopathic effects in LLC cells. Analogous to our findings for dengue infection, these results indicate an important role for HO-1 in suppressing the pathogenesis of WNV infection.

HO-1 in *Leishmania donovani* infection

The *Leishmania* are protozoan parasites that infect and replicate only within macrophages of infected mammalian hosts. More than 12 million individuals worldwide are infected with *Leishmania* parasites,

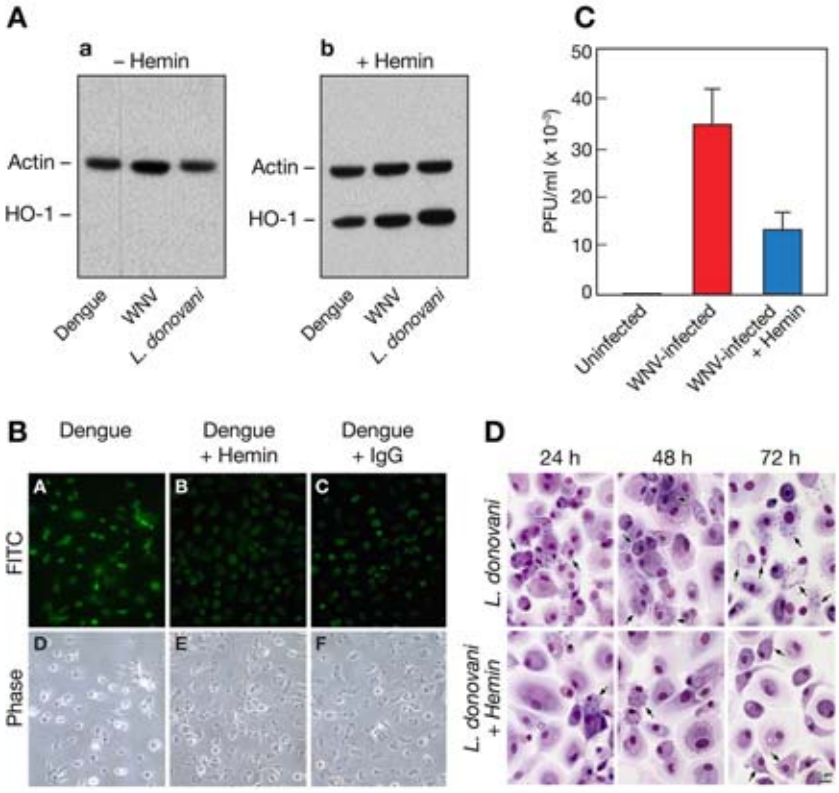

Figure 2. (A) Hemin-induced HO-1 expression in dengue, WNV, and *L. donovani*-infected macrophages. (a) Immunoblot of total proteins of primary macrophages infected in the absence of hemin; or (b) cultured for 24 h in the presence of 100 μM hemin and then infected with the indicated pathogens. Blots were simultaneously probed with anti-HO-1 and anti-β-actin antibodies. **(B)** Immunofluorescence assays of dengue-1-infected macrophages. Dengue-1-infected cells labeled with FITC-conjugated secondary antibodies. Lower panels represent phase contrast microscopy of corresponding fields. **(C)** Virus titer in WNV-infected macrophages cultured in the absence or presence of 100 μM hemin. **(D)** Primary human macrophages pre-treated for 24 h with 100 μM hemin, infected with *L. donovani*, and cultured for 24, 48, and 72 h after infection in the absence (top panels) or presence of hemin (bottom panels). *L. donovani*-infected macrophages are shown by arrows.

and 2 million new cases emerge each year. *Leishmania* promotes HIV-1 production in HIV-infected patients and, as a result, more rapid progression to AIDS; conversely, HIV-1 increases the risk of developing visceral leishmaniasis by 100 to 2,300 times in endemic areas [14]. These two pathogens exert synergistically detrimental effects in co-infections. Currently, no vaccine is available for the prevention of *Leishmania* infection, and drugs used to treat leishmaniasis are not only highly toxic, but patients develop resistance to such drugs after prolonged use.

After having discovered the effectiveness of HO-1 activation in reducing viral infections of MDM, the next obvious question was whether HO-1 induction could even protect macrophage infection by a parasite. As shown in Figure 2D, infection of MDM with *Leishmania donovani* revealed a large number of intracellular amastigote parasites observed at 24 h, 48 h, and 72 h post-infection. Hemin activation of macrophages markedly reduced the number of the parasites, suggesting that HO-1 induction affects the survival of parasites in the macrophages. Although more evidence is needed, the proposed unique regulation of host factors by HO-1 and/or HO-1-dependent pathways is a likely general mechanism for host cell protection.

CONCLUSION

The proof-of-concept study reported in this article demonstrates correlation between hemin-induced HO-1 expression and protection of primary macrophages against multiple pathogenic infections and strongly supports the concept of therapeutically activating this inducible innate response mechanism as a pivotal step in host defense. Consequently, inhibition of viral and parasitic infections by hemin may provide an economical, broad-spectrum therapeutic with potential to alleviate a public health crisis involving existing as well as neglected tropical infectious diseases.

ACKNOWLEDGEMENTS

We thank Dr. Jay Epstein and Dr. Basil Golding for valuable suggestions. We also thank Dr. Xue Wang, Dr. Ragupathy Viswanath, Dr. Indira Hewlett and Dr. Robin Biswas for critical review of the manuscript. The findings and conclusions in this paper have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy. Supported in part by intramural FDA and NIDCR Intramural Research Programs.

REFERENCES

1. www.cdc.gov/dengue/
2. Hayes, E. B. and Gubler, D. J. 2006, Annu. Rev. Med., 57, 181.
3. www.cdc.gov/parasites/leishmaniasis/
4. Schmidt, W. N., Mathahs, M. M. and Zhu, Z. 2012, Frontiers in Pharmacol., 3, 129.
5. Constantin, M., Choi, A. J. S., Cloonan, S. M. and Ryter, S. W. 2012, Int. J. Hypertension, Article ID 859235 (doi: 10.1155/2012/859235).
6. Pamplona, A., Ferreira, A., Balla, J., Jeney, V., Balla, G., Epiphanio, S., Chora, A., Rodrigues, C. D., Gregorie, I. P., Cunha-Rodrigues, M., Portugal, S., Soares, M. P. and Mota, M. M. 2003, Nat. Med., 13, 703.
7. Seixas, E., Gozzelino, R., Chora, A., Ferreira, A., Silva, G., Larsen, R., Rebelo, S., Penido, C., Smith, N. R., Coutinho, A. and Soares, M. P. 2009, Proc. Natl. Acad. Sci. (USA), 106, 15837.
8. Deshane, J., Wright, M. and Agarwal, A. 2005, Acta Biochimica Polonica, 52, 273.
9. Zhu, Z., Wilson, A. T., Mathahs, M. M., Wen, F., Brown, K. E., Luxon, B. A. and Schmidt, W. N. 2008, Hepatology, 48, 1430.
10. Correa-Costa, M., Amano, M. T. and Camara, O. S. 2012, World J. Nephrol., 6, 4.
11. Devadas, K. and Dhawan, S. 2006, J. Immunol., 176, 4252.
12. Siegert, S. W. and Holt, R. J. 2008, Adv. Ther., 25, 842.
13. Bharucha, A. E., Kulkarni, A., Choi, K. M., Camilleri, M., Lempke, M., Brunn, G. J., Gibbons, S. J., Zinsmeister, A. R. and Farruguia, G. 2010, Clin. Pharmacol. Ther., 87, 187.
14. Ezra, N., Ochoa, M. T. and Craft, N. 2010, J. Glob. Infect. Dis., 2, 248.