Intensive Care Med (2008) 34:595–597
DOI 10.1007/s00134-008-1013-z

EDITORIAL

Gregory J. Quinlan
Anna L. Lagan
Timothy W. Evans

Haem oxygenase: A model for therapeutic intervention

Received: 3 January 2008
Accepted: 4 January 2008
Published online: 20 February 2008

This editorial refers to the articles available at:
http://dx.doi.org/10.1007/s00134-008-1010-2 and
http://dx.doi.org/10.1007/s00134-008-1011-1.

G. J. Quinlan · A. L. Lagan · T. W. Evans (✉)
Royal Brompton Hospital and Imperial College London,
Department of Critical Care Medicine,
Sydney Street, SW3 6NP London, UK
e-mail: t.evans@rbht.nhs.uk

The haem oxygenase (HO) enzyme system was first identified in 1968 by Tenhunen and colleagues [1]. Inducible (HO-1) and constitutive (HO-2) isoforms were characterised in 1974 [2] and 1986 [3] respectively. Subsequently, a third isoform, HO-3, has been demonstrated in rats, although the significance of this finding remains a matter for debate [4]. HO-1 and -2 form part of an enzymatic pathway for haem catabolism and are principally involved in the conversion of haem to biliverdin. Equimolar amounts of carbon monoxide (CO) and ferrous iron are also produced as by-products of this reaction (Fig. 1).

Considerable research efforts have been devoted to understanding the functional significance of the in vivo expression and activity of these enzymes. Although they are not distributed to all tissues, most cell types contain HO-2 and can induce HO-1, which suggests they fulfil a fundamental and important biological role (or roles). Moreover, as HO-1 is highly inducible by a diverse array of stimuli, the majority of which can be attributed to oxidative stress, the current consensus is that expression of this enzyme represents an endogenous antioxidant, and therefore protective, response. Thus, the removal of pro-oxidant haem [5] by haem oxygenase can be seen as an antioxidant function, as can the enzymatic conversion of biliverdin (produced by HO) to bilirubin by biliverdin reductase. Secondly, bilirubin has antioxidant properties and is chiefly functional in the lipid phase [6]. Thirdly, whilst beneficial effects have also been attributed to CO (see reviews in this issue by Bauer et al. and Foresti et al.) [7, 8], many not clearly linked to an antioxidant function, evidence suggests that it can prevent formation of reactive oxygen species (ROS) [9]. By contrast, the release of ferrous iron (Fe^{2+}) from haem can be viewed as a potentially pro-oxidant event, as iron in this form is an avid catalyst for the formation of damaging and aggressive ROS [10], although numerous protective mechanisms within cells under normal circumstances limit such deleterious reactions.

The review by Bauer et al. in this issue of the journal provides a balanced overview of the potential relevance of HO and CO to the pathophysiology of critical illness [7]. This review is timely, both in view of promulgated clinical trials and the often contradictory results of basic scientific studies. Thus, although many protective responses have been attributed to CO administration in the experimental setting, to date the nature of this protective response is unclear, thereby rendering predictions of clinical utility difficult. Secondly, understanding that HO-1 in humans is distinct from that identified in animal models is of particular importance. Thus, HO-1 is a heat shock protein in rats (HSP-32) but not in man. Thirdly, the dogma that pro-oxidant iron produced through haem breakdown is always safely sequestered within the iron storage protein ferritin is rightly questioned. Fourthly, and possibly most importantly, cis-acting regulation of HO-1 gene expression is evident in humans. A functional role has been postulated for a (GT)n microsatellite resident in the HO-1 5'UTR. Evidence suggests that HO-1 enzyme activity is dependant on the size of (GT)n repeat length [11]. Inheritance of shorter repeats is associated with increased

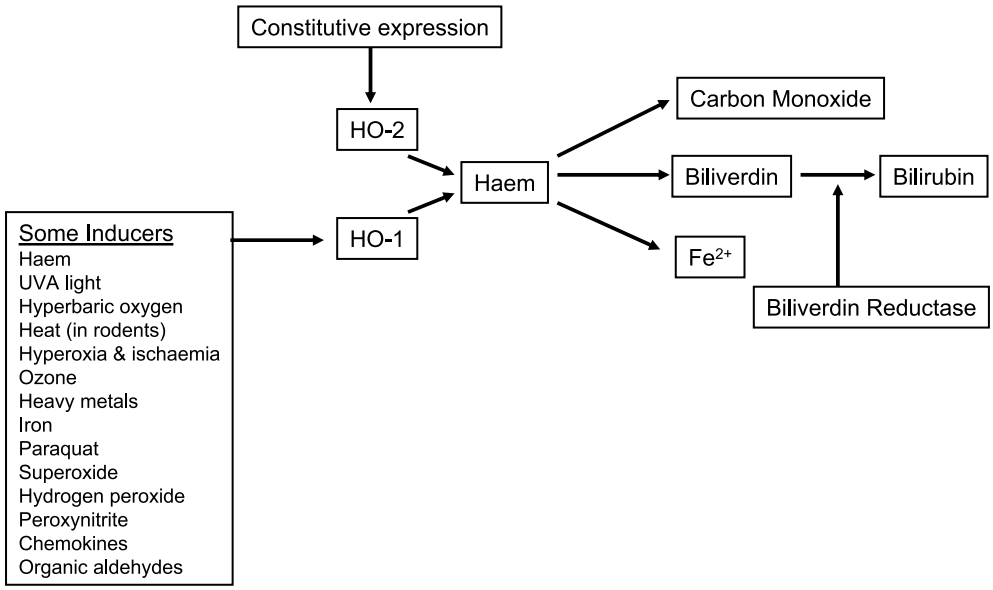

Fig. 1 The enzymatic pathway for haem catabolism

inducible expression of HO-1 mRNA [11, 12]. However, whether the microsatellite length polymorphism exerts its effect at the transcriptional or translational level, or indeed whether the functionality is due to tight linkage with the T-413A single nucleotide polymorphism, is unclear [13]. The HO-1 microsatellite is implicated in the pathophysiology of pulmonary disease [14]. Indeed, a role for polymorphisms in HO-2 has recently been postulated in susceptibility to acute respiratory distress syndrome (ARDS) [15]. Consequently, Bauer and colleagues are right to suggest that genotypic variation will need to be accounted for when considering therapeutic manipulation of HO-1 or its products [7].

Haem oxygenase seems to be operational over a narrow therapeutic range of expression, in that both under- and pronounced over-expression appear to be associated with death in the ICU setting [16]. Together with an understanding of individual variability at the genetic level, this implies that attempts to induce HO-1 for therapeutic advantage may be difficult. Utilising products of enzymatic activity with established therapeutic effect in a controlled fashion may prove a more fruitful approach. The review by Foresti et al. published in this issue of *Intensive Care Medicine* discusses the merits of just such an approach [8]. A critical appraisal of the literature that demonstrates generally impressive results in preclinical studies employing CO is balanced by mostly observational studies in human subjects, the conclusions from which are much more ambiguous. Importantly, the authors suggest that although such an approach may be feasible, the need to achieve therapeutic impact at organs distant from the lungs may limit potential benefits, as the level of CO administration needed to achieve this may be locally toxic. A feasible alternative is suggested in the form of CO-releasing molecules (CO-RMs). When administered systemically, these are able to carry and release CO in a controlled fashion, thereby overcoming potential toxicity issues. Although CO-RMs are some way from clinical use, results in biological models using these systems have been generally encouraging and justify further pharmaceutical development.

Much remains to be learned about the functional effects of the HO enzymes. These important reviews provide grounds for cautious optimism that novel therapies designed around the beneficial responses attributable to them may emerge in due course.

References

1. Tenhunen R, Marver HS, Schmid R (1968) The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. Proc Natl Acad Sci USA 61:748–755
2. Maines MD, Kappas A (1974) Cobalt induction of hepatic heme oxygenase; with evidence that cytochrome P-450 is not essential for this enzyme activity. Proc Natl Acad Sci USA 71:4293–4297
3. Maines MD, Trakshel GM, Kutty RK (1986) Characterization of two constitutive forms of rat liver microsomal heme oxygenase. Only one molecular species of the enzyme is inducible. J Biol Chem 261:411–419

4. McCoubrey WK Jr, Huang TJ, Maines MD (1997) Isolation and characterization of a cDNA from the rat brain that encodes hemoprotein heme oxygenase-3. Eur J Biochem 247:725–732
5. Reeder BJ, Wilson MT (2005) Hemoglobin and myoglobin associated oxidative stress: from molecular mechanisms to disease states. Curr Med Chem 12:2741–2751
6. Ollinger R, Wang H, Yamashita K, Wegiel B, Thomas M, Margreiter R, Bach FH (2007) Therapeutic applications of bilirubin and biliverdin in transplantation. Antioxid Redox Signal 9:2175–2185
7. Bauer M, Huse K, Settmacher U, Claus RA (2008) The heme oxygenase–carbon monoxide system: regulation and role in stress response and organ failure. Intensive Care Med DOI 10.1007/s00134-008-1010-2
8. Foresti R, Bani-Hani MG, Motterlini R (2008) Use of carbon monoxide as a therapeutic agent: promises and challenges. Intensive Care Med DOI 10.1007/s00134-008-1011-1
9. Wang X, Wang Y, Kim HP, Nakahira K, Ryter SW, Choi AM (2007) Carbon monoxide protects against hyperoxia-induced endothelial cell apoptosis by inhibiting reactive oxygen species formation. J Biol Chem 282:1718–1726
10. Halliwell B, Gutteridge JM (2007) Free radicals in biology and medicine, 4th edn. Oxford University Press, Oxford, UK
11. Hirai H, Kubo H, Yamaya M, Nakayama K, Numasaki M, Kobayashi S, Suzuki S, Shibahara S, Sasaki H (2003) Microsatellite polymorphism in heme oxygenase-1 gene promoter is associated with susceptibility to oxidant-induced apoptosis in lymphoblastoid cell lines. Blood 102:1619–1621
12. Yamada N, Yamaya M, Okinaga S, Nakayama K, Sekizawa K, Shibahara S, Sasaki H (2000) Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. Am J Hum Genet 66:187–195
13. Ono K, Mannami T, Iwai N (2003) Association of a promoter variant of the haeme oxygenase-1 gene with hypertension in women. J Hypertens 21:1497–1503
14. Exner M, Minar E, Wagner O, Schillinger M (2004) The role of heme oxygenase-1 promoter polymorphisms in human disease. Free Radic Biol Med 37:1097–1104
15. Lagan AL, Quinlan GJ, Mumby S, Melley DD, Goldstraw P, Bellingan GJ, Hill MR, Briggs D, Pantelidis P, du Bois RM, Welsh KI, Evans TW (2008) Variation in iron homeostasis genes between patients with acute respiratory distress syndrome and healthy controls. Chest (in press)
16. Melley DD, Finney SJ, Elia A, Lagan AL, Quinlan GJ, Evans TW (2007) Arterial carboxyhemoglobin level and outcome in critically ill patients. Crit Care Med 35:1882–1887