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Original Contribution

Sustained expression of heme oxygenase-1 alters iron homeostasis in nonerythroid cells

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ABSTRACT

Heme oxygenases initiate the catabolism of heme, releasing carbon monoxide, iron, and biliverdin. Sustained induction of heme oxygenase-1 (HO-1) in nonerythroid cells plays a key role in many pathological processes, yet the effect of long-term HO-1 expression on cellular iron metabolism in the absence of exogenous heme is poorly understood. Here we report that in a model nonerythroid cell, both transient and stable HO-1 expression increased heme oxygenase activity, but total cellular heme content was decreased only with transient enzyme expression. Sustained HO-1 activity increased the expression of both the mitochondrial iron importer mitoferrin-2 and the rate-limiting enzyme in heme synthesis, aminolevulinate synthase-1, and it augmented the mitochondrial content of heme. Also, the expression of transferrin receptor-1 and the activities of iron-regulatory proteins 1 and 2 decreased, whereas total labile iron and the regulatory activity of the heme-binding transcription factor Bach1 were unaltered. In addition, stable, but not transient, HO-1 expression decreased the activities of aconitase, as well as increasing proteasomal degradation of ferritin. Together, our results reveal a novel and coordinated adaptive response of nonerythroid cells to sustained HO-1 induction that has an impact on cellular iron homeostasis.

Introduction

Heme oxygenase-1 (HO-1)[2] plays a key role in many physiological processes, including the reutilization of iron during erythropoiesis and the response to stresses such as hypoxia. In addition, an increasing body of literature suggests that induction of HO-1 provides benefits in a number of pathological situations, including hematological disorders and cardiovascular disease [1–3]. These benefits are thought to be mediated by the anti-inflammatory and antioxidant actions of the products of HO-1 [4,5], as well as by its tissue-specific regulation of cell growth and survival [6]. It is therefore not surprising that the efficacy of a large range of clinical and experimental therapeutic compounds has been linked, at least in part, to their ability to induce HO-1 [1].

Whereas the beneficial effects of HO-1 induction are well established, much less is known about the underlying mechanism(s). Most research points to one or more of the products of the HO-1-catalyzed degradation of heme, particularly carbon monoxide and biliverdin, as effector molecules [2]. Biliverdin and its reduction product bilirubin, in particular, have been noted for their antioxidant [7], anti-inflammatory [8], and vasoprotective [9] activities. However, the role of the iron released during HO-1-induced heme degradation is controversial. Iron is essential for the function of many important proteins. On the other hand, iron can also be toxic if it participates in unwanted and uncontrolled redox reactions, and removal of iron has been demonstrated, in some circumstances, to mimic the protective effect of HO-1 (see, e.g., Ref. [8]). Although it is clear that HO-1 is essential for whole-body iron homeostasis [10], increased HO-1 activity has been reported to either decrease [8] or increase [11] the size of the intracellular pool of labile, and potentially redox-active, iron. Also, it is commonly assumed that induction of HO-1 is associated with increased expression of the iron-sequestering protein ferritin [12],

Abbreviations: ALAS1, δ-levulinate synthase; BvR, biliverdin reductase; CPR, cytochrome P450 reductase; HO-1, heme oxygenase-1; HO-2, heme oxygenase-2; ICP-MS, inductively coupled plasma mass spectrometry; IRE, iron-responsive element; IRP, iron-regulatory protein; ISC, iron–sulfur cluster; $O_2^{-\bullet}$, superoxide anion radical; TfR1, transferrin receptor-1

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which is thought to make iron derived from HO-1 activity unavailable for harmful redox reactions. However, ferritin is not always induced together with HO-1 [13], nor does it completely protect against iron-mediated damage in all circumstances [11].

One potential explanation for the apparently conflicting effects of iron released from HO-1 activity lies in the recognition that cellular responses might vary depending on the duration of increased HO-1 activity. For example, cells might respond differently to sustained vs transient HO-1 expression with regard to processes that contribute to cellular iron homeostasis [14]. A sustained nonheme induction of HO-1 in nonerythroid cells is probably relevant to the outcome of many pathological conditions, including atherosclerosis [15–17] and other chronic diseases [18]. However, the effect of long-term HO-1 expression on cellular homeostasis in such cells is poorly understood. In this work, we therefore investigated the impact of transient versus permanent expression of HO-1 on cellular iron metabolism and homeostasis in HEK293 cells, used as a model for nonerythroid cells.

Experimental procedures

Unless otherwise stated, all reagents were purchased from Sigma–Aldrich. Methods of cell culture and transfection, determination of superoxide anion radical ($O_2^{-\bullet}$), expression vector and gene cloning, immunoblotting, quantitative real-time PCR, and subcellular fractionation are detailed in the supplementary data. Primers used in real-time PCR are summarized in Table 1. The various antibodies used are listed in Table 2.

Table 1
Primers used for quantitative real-time PCR.

Gene	Sequence
δ-Aminolevulinate synthase 1	5′-GCGTTTCGTTTGGACTTCTC
	5′-CATCATCTTGGGGCAGTTTT
Biliverdin reductase	5′-CACTTTGGAAGAGCGAAAGG
	5′-CCAAGGACCCAGACTTGAAA
Ferritin heavy chain	5′-CTTTGACCGCGATGATGTGGCTTT
	5′-TTTGTCAGTGGCCAGTTTGTGCAG
Ferritin light chain	5′-CAGTGTTTGGACGGAACAGAT
	5′-CGGTGGAATAATTCTGACGAA
Heme oxygenase-1	5′-GTGTAAGGACCCATCGGAGA
	5′-ATGACACCAAGGACCAGAGC
Mitoferrin-2	5′-TTCCTTTCCAAGCCATTCAC
	5′-ACTCCTGGGTGTTGAGCAGT
NAD(P)H:quinone oxidoreductase	5′-TGCAGCGGCTTTGAAGAAGAAAGG
	5′-TCGGCAGGATACTGAAAGTTCGCA
Porphobilinogen deaminase	5′-GAGTGATTCGCGTGGGTACC
	5′-GGCTCCGATGGTGAAGCC
Thioredoxin reductase-1	5′-GCCCTGCAAGACTCTCGAAATTA
	5′-GCCCATAAGCATTCTCATAGACGA
Transferrin receptor-1	5′-AAAATCCGGTGTAGGCACAG
	5′-TTAAATGCAGGGACGAAAGG

Cellular labile iron

Cells were cultured in 96-well plates coated with poly-D-lysine (Becton–Dickinson) and loaded with 20 μM Phen Green SK diacetate (Invitrogen) for 30 min in Hanks' balanced salt solution. After being washed, the cells were treated with vehicle (dimethyl sulfoxide (DMSO); 1:1000) or 2 mM 1,10-phenanthroline, and the fluorescence increase ($Ex_{485\ nm}$, $Em_{535\ nm}$) was recorded over 30 min [19].

Enzyme assays

Heme oxygenase activity was determined as described [20]. Whole-cell and cytosolic aconitase activity was determined as described [21]. Succinate dehydrogenase activity was determined as described previously [22].

Determination of heme

Cells ($\sim 10^6$) or purified mitochondria were centrifuged and lysed in 2 M oxalic acid (250 μl). An aliquot of the resulting lysate (25 μl) was diluted with 2 M oxalic acid to 600 μl and then heated for 30 min at 105 °C before water was added to a final volume of 3 ml. After temperature equilibration, fluorescence ($Ex_{405\ nm}$, $Em_{602\ nm}$) was determined and the level of heme estimated from a standard curve generated from hemin. Corresponding fluorescence values of unheated lysates in oxalic acid were used as blanks and subtracted from the experimental values.

Determination of iron

Transfected cells were washed three times in 0.9% NaCl treated with Chelex-100 (Bio-Rad) and then lysed in 6 M metal-free nitric acid. Cellular iron was determined by inductively coupled plasma mass spectrometry (ICP-MS).

Table 2
Types and sources of antibodies used.

Protein	Antibody type	Supplier
Porin	Mouse monoclonal	Calbiochem
SOD2	Sheep polyclonal	Calbiochem
Actin	Goat polyclonal	Santa Cruz Biotechnologies
Bach1	Goat polyclonal	Santa Cruz Biotechnologies
Iron-regulatory protein-1	Goat polyclonal	Santa Cruz Biotechnologies
Iron-regulatory protein-2	Mouse monoclonal	Santa Cruz Biotechnologies
ISCU1/2	Rabbit polyclonal	Santa Cruz Biotechnologies
SOD1	Rabbit polyclonal	Santa Cruz Biotechnologies
Frataxin	Mouse monoclonal	Millipore
Lactate dehydrogenase	Goat polyclonal	Millipore
Heme oxygenase-1	Mouse monoclonal	Becton–Dickinson
Heme oxygenase-2	Rabbit polyclonal	Assay Designs
Biliverdin reductase	Rabbit polyclonal	Assay Designs
Cytochrome P-450 reductase	Rabbit polyclonal	Assay Designs
Tubulin	Mouse monoclonal	Sigma
δ-Aminolevulinate synthase 1	Rabbit polyclonal	Abcam
TATA-binding protein	Mouse monoclonal	Abcam
Ferritin	Sheep polyclonal	The Binding Site