Non-heme Induction of Heme Oxygenase-1 Does Not Alter Cellular Iron Metabolism*

Received for publication, January 9, 2007 Published, JBC Papers in Press, January 22, 2007, DOI 10.1074/jbc.M700240200

Alex D. Sheftel†‡§, Sangwon F. Kim¶, and Prem Ponka‖§ 1

From the †Lady Davis Institute for Medical Research, Montréal, Québec H3T 1E2, Canada, ‡Department of Physiology, McGill University, Montréal, Québec H3G 1Y6, Canada, and §Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

The catabolism of heme is carried out by members of the heme oxygenase (HO) family. The products of heme catabolism by HO-1 are ferrous iron, biliverdin (subsequently converted to bilirubin), and carbon monoxide. In addition to its function in the recycling of hemoglobin iron, this microsomal enzyme has been shown to protect cells in various stress models. Implicit in the reports of HO-1 cytoprotection to date are its effects on the cellular handling of heme/iron. However, the limited amount of uncommitted heme in non-erythroid cells brings to question the source of substrate for this enzyme in non-hemolytic circumstances. In the present study, HO-1 was induced by either sodium arsenite (reactive oxygen species producer) or hemin or overexpressed in the murine macrophage-like cell line, RAW 264.7. Both of the inducers elicited an increase in active HO-1; however, only hemin exposure caused an increase in the synthesis rate of the iron storage protein, ferritin. This effect of hemin was the direct result of the liberation of iron from heme by HO. Cells stably overexpressing HO-1, although protected from oxidative stress, did not display elevated basal ferritin synthesis. However, these cells did exhibit an increase in ferritin synthesis, compared with untransfected controls, in response to hemin treatment, suggesting that heme levels, and not HO-1, limit cellular heme catabolism. Our results suggest that the protection of cells from oxidative insult afforded by HO-1 is not due to the catabolism of significant amounts of cellular heme as thought previously.

In the average adult, at equilibrium ~2 million red blood cells are being turned over every second. In this way, the process requires about 25 mg of iron (Fe) to be recycled from the hemoglobin of effete erythrocytes. Heme oxygenase 1 (HO-1)2 is the enzyme responsible for catabolizing the heme from senescent red blood cells to release Fe, as well as equimolar amounts of carbon monoxide (CO) and biliverdin, for its eventual reuse in erythropoiesis. Another noninducible isoform of heme oxygenase with heme catabolic properties similar to HO-1, HO-2 is present in substantial levels primarily in the central nervous system and testes (1, 2). Although the heme-degrading function of HO has been known since the 1960s (3, 4), only recently has it been shown that heme oxygenases may be involved in cytoprotection against cellular stresses (for review see Camara and Soares (5), Abraham and Kappas (6), Otterbein et al. (7), and Ryter et al. (8)). This newer discovery of the potential of these enzymes as a tissue defense mechanism has spawned a flurry of studies by numerous groups who have shown by several different approaches that induction or overexpression of heme oxygenase 1 can protect tissues from various insults, including oxidative stress and immune system attack.

The precise mechanism by which HO-1 mediates its protective function remains controversial. Breakdown of heme being the only known reaction catalyzed by the enzyme, extensive research has alleged that one or more of the reaction products mediate(s) this feature of HO-1. Biliverdin, besides having some intrinsic antioxidant properties, is rapidly converted to bilirubin by biliverdin reductase and is believed to redox cycle in the presence of biliverdin reductase and antioxidants to reduce reactive oxygen species (9). In addition, bilirubin possesses antioxidant activity (10). Carbon monoxide, which is chemically very similar to nitrogen monoxide (NO) (11), can bind to the heme groups in various proteins to modulate their function. By this property, it has been shown that CO may initiate intracellular signaling cascades related to cell survival, mimicking the effects of HO-1 induction (2, 5, 12, 13). Finally, when heme is provided to cells, the generation of Fe via the HO-1-catalyzed reaction induces ferritin (Ft; ubiquitous iron storage protein) synthesis (14), which Ferris et al. (15) demonstrated to decrease reactive oxygen species within cells by sequestering catalytic iron.

Probably the greatest concern regarding the mechanism by which HO-1 protects tissues is the enigmatic source of substrate for the enzyme. Over 95% of the body’s heme is found in hemoglobin and myoglobin (16). Considering the relative contribution of the heme compartments to body mass, there are about 97,900 mg heme/kg tissue in the hemoglobin compartment (mostly composed of erythrocytes), 120 mg heme/kg tissue in the myoglobin compartment, and only 27 for that of all other compartments combined (subtracting bone mass from the remaining body weight) (Refs. 16 and 17 and Table 1). Thus, it becomes apparent that, outside of hemolytic conditions, there is a limited amount of heme available for HO-1 to cata-
olize into the implicated cytoprotective reaction products. In fact, it has been estimated that cellular “free heme” is likely to be at concentrations lower than 30 nm (18).

In the current study, we have compared the effects of HO-1 induction by hemin and a non-heme inducer (sodium arsenite) on iron metabolism in RAW 264.7 (macrophage-like) cells to investigate whether elevated HO-1 levels, which are sufficient to protect cells from oxidative stress, affect cellular iron metabolism.

**EXPERIMENTAL PROCEDURES**

**Materials and Cell Lines**—Unless specified otherwise, all reagents were purchased from Sigma. RAW 264.7 cells were purchased from the American Type Culture Collection (Manassas, VA) and grown at 37 °C in 75-cm² culture flasks (Invitrogen). The cells were incubated for 60 min and then washed, and the conditioned medium from before the sodium arsenite treatment was returned. We regarded the time at which we replaced the media as t = 1. 2) The sodium arsenate was added to the cells in full medium to a final concentration of 3 $\mu$M. This alternative protocol allowed us both to reach comparable levels of HO-1 protein as with 5 $\mu$M hemin treatment and to leave the arsenite present for the incubation period, thus more closely mimicking the conditions of the heme-treated samples. HO-1 mRNA and protein levels were determined by Northern blotting (full-length HO-1 cDNA probe) and Western blotting, respectively.

**Measurement of Ferritin Synthesis Rate**—Cells were labeled for 1 h with (100 $\mu$Ci/ml $^{35}$S)methionine in methionine-free Dulbecco’s modified Eagle’s medium and washed three times with cold phosphate-buffered saline after which they were lysed with radiolabeled precipitation assay buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) for 30 min at 4 °C. Anti-ferritin antibody was added to the lysates, which were incubated overnight at 4 °C, and then 60 $\mu$l of a 50% (v/v) suspension of protein A-Sepharose was added for 3 h at 4 °C to precipitate the immune complexes. The beads were washed three times with cold radiolabeled precipitation assay buffer and then boiled with SDS loading dye. Immunoprecipitated protein was resolved by 12.5% SDS-PAGE. The gel was dried and analyzed by autoradiography.

**Iron-regulatory Protein Binding Activity Assay**—Iron-regulatory protein (IRP) binding was determined using a band shift assay as described previously (23). Briefly, 5–10 million cells were washed with ice-cold phosphate-buffered saline and lysed at 4 °C in 80 $\mu$l of lysis buffer (10 mM HEPES, pH 7.5, 3 mM MgCl$_2$, 40 mM NaCl, 5% glycerol, 1 mM dithiothreitol, and 0.2% Nonidet P-40). After lysis, the samples were centrifuged for 5 min at 10,000 $\times$ g to remove the nuclei. Samples of cytoplasmic extract were diluted with 2 volumes of lysis buffer without Nonidet P-40 to a protein concentration of 1 $\mu$g/µl, and 10-$\mu$g aliquots were analyzed for IRP binding by incubating them with an excess amount of $^{32}$P-labeled pSR-T-ter RNA transcript, which contains one iron-responsive element (IRE) (24). This RNA was transcribed in vitro from linearized plasmid template using T7 RNA polymerase in the presence of $[^{32}$P]UTP. To form RNA-protein complexes, cytoplasmic extracts were incubated for 10 min at room temperature with excess amount of labeled RNA. Heparin (5 mg/ml) was added for another 10 min to prevent nonspecific binding. RNA-protein complexes were analyzed in 6% nondenaturing polyacrylamide gels. In parallel, duplicate samples were treated with 2% β-mercaptoethanol

| Hemoprotein | Total heme content | In situ tissue weight | Heme/tissue |
|-------------|--------------------|----------------------|-------------|
| Hemoglobin  | 23,500             | 2.4                  | 97,900      |
| Myoglobin   | 3,300              | 28                   | 120         |
| Other (–bone) | 820                | 30                   | 27          |

**Effects of Heme Oxygenase on Cellular Iron Metabolism**

Induction and Measurement of HO-1—RAW 264.7 cells were seeded in full medium for 24 h prior to HO-1 induction. Hemin solution was prepared by first dissolving hemin chloride powder in 0.15 N NaOH followed by a 10× dilution in 0.15 M Tris-HCl, pH 7.0. This solution was added to the cells in full medium to achieve a 5 $\mu$M hemin concentration. For non-heme induction of HO-1, sodium arsenite dissolved in distilled water was used in one of two ways. 1) Full medium was removed from the cells before the addition of 20 $\mu$M sodium arsenite in Dulbecco’s modified Eagle’s medium without supplemental serum. The cells were incubated for 60 min and then washed, and the conditioned medium from before the sodium arsenite treatment was returned. We regarded the time at which we replaced the media as t = 1. 2) The sodium arsenite was added to the cells in full medium to a final concentration of 3 $\mu$M. This alternative protocol allowed us both to reach comparable levels of HO-1 protein as with 5 $\mu$M hemin treatment and to leave the arsenite present for the incubation period, thus more closely mimicking the conditions of the heme-treated samples. HO-1 mRNA and protein levels were determined by Northern blotting (full-length HO-1 cDNA probe) and Western blotting, respectively.
Effects of Heme Oxygenase on Cellular Iron Metabolism

before the addition of the RNA probe to reveal the total levels of IRP1.

Degradation of Exogenous Hemin or Endogenous Heme—Exogenous heme-RAW 264.7 cells were incubated in the presence or absence of 3 μM sodium arsenite for 6 h followed by treatment with 50 μM 59Fe-hemin for 1 h.

Endogenous heme-RAW 264.7 cells were incubated in the presence of 1 μM 59Fe-transferin. After 3 days, the cells were washed and treated with or without 3 μM sodium arsenite for 6 h.

Heme and non-heme fractions were isolated by washing the cells and then performing a previously described acid lysis and trichloroacetic acid precipitation method (25). The radioiron in each fraction was evaluated by counting in a Cobra II gamma counter (Packard Instruments).

Data Analysis—All of the data presented are representative of at least three identical experiments. Error bars represent standard deviations.

Measurement of Reactive Oxygen Species—One-half million RAW 264.7 or RAW-HO1 cells were seeded per well in 6-well tissue culture plates and incubated under standard culture conditions overnight. The cells were incubated at 37 °C for 30 min in the presence of 50 μg/ml reactive oxygen species indicator, 2’,7’-dichlorodihydrofluorescein diacetate (DCF-DA; Invitrogen). Where indicated, lipopolysaccharide (100 ng/ml; Sigma) was added to the cells after the DCF-DA treatment, and/or SnPP (250 μM) was added to the cells 30 min before the DCF-DA treatment. Cellular fluorescence was determined by flow cytometry on a FACSDiVa (BD Biosciences).

RESULTS

Heme or Sodium Arsenite Induces HO-1 Expression in RAW 264.7 Cells—To establish a reliable HO-1 induction protocol, we treated RAW 264.7 cells with heme or sodium arsenite and measured both HO-1 mRNA expression and protein levels by Northern and Western blotting, respectively. We observed a rapid increase in HO-1 mRNA levels within 1 h of treatment with 20 μM sodium arsenite, which decreased back to base-line levels within 12 h (Fig. 1A). Treatment with 5 μM hemin led to a more sustained elevation of HO-1 mRNA levels, because heme was allowed to remain in the medium throughout the time period, whereas exposure to sodium arsenite was only a 1-h pulse (Fig. 1A). Protein levels rapidly followed the increased message levels, with the most robust expression apparent around 12 h for both heme and sodium arsenite (Fig. 1B, i). In an alternative induction procedure, we determined that a continuous treatment with 3 μM sodium arsenite or a 6-h incubation with 5 μM hemin yielded similar HO-1 protein levels (Fig. 1B, ii).

HO-1 Induction by Heme, but Not Sodium Arsenite, Induces an Increase in Ferritin Synthesis—When intracellular, chelatable iron levels rise, ferritin synthesis is activated in order for cells to store the excess of the metal. Additionally, it has been demonstrated that HO-1 induction in the presence of heme will increase Ft levels via increased iron liberation from the metalloporphyrin (14). However, it remains unclear whether increases in HO-1 in the absence of exogenous heme will lead to iron liberation and subsequent increases in Ft synthesis. Therefore, we treated RAW 264.7 cells with either hemin (5 μM, continuously) or sodium arsenite (20 μM, 1-h pulse) and measured the rate of ferritin synthesis by metabolic labeling and immunoprecipitation. (In general, the heavy (21 kDa) and light (19 kDa) chain Ft subunits can be resolved on the polyacrylamide gel; however, in some gels the two bands overlapped.) Fig. 2 shows that heme treatment led to a robust increase in ferritin synthesis that reached a maximum around 6 h and decreased to slightly elevated levels by 12 h. In contrast, incubation of the cells in the presence of sodium arsenite had no...
When the cells were treated with 5 μM hemin after (and in the presence of) the inhibitor, a significant concentration-dependent decrease in Ft synthesis rate was apparent. Additionally, when the highly permeant iron chelator salicylaldoxime was present (100 μM; in the absence of SnPP), the level of Ft synthesis was barely detectable either in the presence or absence of hemin. Together, these data show that iron released via heme oxygenase causes elevated Ft synthesis when cells are exposed to hemin (Fig. 3).

Heme Oxygenase-dependent Release of Iron from Hemin Causes a Decrease in IRP Activity—Acute ferritin protein up-regulation is controlled primarily by a post-transcriptional mechanism involving the inactivation of IRPs, which block translation of the ferritin mRNA by binding to a stem-loop structure on the 5′-untranslated region of the message known as an IRE. IRP binding activity can be measured by an electrophoretic mobility shift assay. Thus, we determined the IRP binding activity in our samples to verify that iron released from hemin by HO was responsible for the increases in Ft synthesis that we observed. As illustrated in Fig. 4A, hemin treatment caused a considerable decrease in both IRP1 and IRP2 activities, whereas sodium arsenite treatment had no effect. This inhibition of IRP binding is abrogated in a concentration-dependent manner if cells are pretreated with SnPP, as shown in Fig. 4B. By Northern blotting, using a Ft heavy chain probe, we determined that there was no contribution of transcriptional regulation to the increase in Ft levels by hemin (Fig. 4C).

**HO-1 Induction by Sodium Arsenite Has No Effect on Endogenous Heme Levels**—Although we observed no evidence that HO-1 induction by sodium arsenite releases Fe from heme in the absence of exogenous substrate, there remains the possibility that Ft synthesis rate changes below our measurement sensitivity or that the small amount of released iron is rapidly sequestered in the already present Ft. For this reason, we per-
formed experiments to determine whether endogenous heme levels are decreased when HO-1 levels are elevated. To label all of the heme in RAW 264.7 cells, we grew the cells in the presence of 1 μM 59Fe2-transferrin for 3 days. Control cells contained about 6.5% of their cell-associated radioactivity in heme (Fig. 5, right panel). Treatment with sodium arsenite had no effect on this value. A control experiment was performed in which exogenous 59Fe-hemin was applied to RAW 264.7 cells, with or without sodium arsenite treatment, confirming that the chemical induction of HO will lead to the catabolism of heme if it is exogenously available for the enzyme (Fig. 5, left panel).

**HO-1 Overexpression Does Not Increase Ferritin Synthesis**—Our experiments thus far demonstrate that, without exogenous hemin, HO-1 induction will not lead to a significant increase in heme degradation. To demonstrate that the enzyme is nonetheless capable of protecting the cells from oxidative insult, we stably transfected RAW 264.7 cells with a mouse HO-1 construct and examined whether the overexpression of HO-1 would decrease intracellular ROS. After confirming that the basal expression of HO-1 was elevated (Fig. 6A) in the transfectants, we verified that there was no consequence of this overexpression on the base-line Ft synthesis rate (Fig. 6B, 5th lane). Although steady-state Ft synthesis was unaffected by HO-1 overexpression, an increase in Ft synthesis rate was observed in the cells after exogenous hemin supplementation, providing further evidence that there was no significant amount of endogenous heme available for the enzyme.

**Heme Oxygenase Overexpression Protects Cells from ROS**—Treatment of RAW 264.7 cells with LPS induces an oxidative burst that increases intracellular ROS with a consequential decrease in cell viability (26). Measurable changes in intracellular ROS in these cells can be detected using DCF-DA following a 30-min exposure to LPS (27). Using flow cytometry, we observed a consistent increase in fluorescence in RAW 264.7 cells treated with DCF-DA and then LPS (Fig. 6C, left panel). In contrast, when RAW-HO1 cells were treated with LPS, there was no detectable increase in fluorescence (Fig. 6C, right panel), suggesting that the presence of elevated HO-1 levels protects the cells from intracellular ROS.

**DISCUSSION**

Overwhelming evidence supports the hypothesis that HO-1 performs a protective role in the face of various cellular insults (5–7). However, the mechanism through which it executes this function remains unknown. In certain pathophysiological conditions, damage to tissues, namely erythrocyte or muscle tissue, leads to release of copious amounts of hemoprotein, which can then supply substrate to heme oxygenase in the affected tissues.
Hemoglobin, when free in the plasma, will become oxidized to methemoglobin, which can readily abdicate its heme moiety (28, 29). Although plasma hemoglobin and heme-binding proteins such as haptoglobin, hemopexin, and albumin are able to prevent the liberation and toxicity of heme, excessive release of hemoprotein will presumably saturate these buffering systems, allowing heme to enter endothelial cells. This is the case in rhabdomyolysis, a disease in which Nath et al. (30) have demonstrated that HO-1 induction provides protection to tissues presumably through the subsequent increases in Fe and bilirubin. Ischemia-reperfusion injury will also bring about significant intravascular hemolysis (31, 32), again supplying substrate for HO. In these cases, the detoxifying effects of heme oxygenase include the clearance of heme, which in itself is cytotoxic (33–35). In contrast, it has been shown that heme oxygenase overexpression or induction can confer cytoprotection in the absence of aberrant, extracorpuscular hemoproteins (5, 13, 36–38). Additionally, the ability of HO-1 to be up-regulated by a barrage of non-heme inducers strongly, although indirectly, implies a function for this enzyme in instances where extracellular heme is absent. Collectively, the results of our study suggest that the heme catabolic activity of HO-1 is irrelevant or subordinate to the ability of the enzyme to protect cells against cellular stressors.

Two recent studies by different groups have demonstrated that overexpression of HO, mutated to be devoid of catalytic activity (with respect to heme catabolism), will still confer protection against oxidative stress. Hori et al. (39) transfected U937 macrophage-like cells with catalytically inactive HO-1, finding that these cells were still able to withstand peroxide challenge. Dore’s laboratory demonstrated a protective effect of HO-2 during heme challenge both in vitro (40) and in vivo (41). Interestingly, this group showed that catalytically inactive HO-2 will also protect cells against peroxide toxicity (40). Thus HO-2 possesses an antioxidant function independent of its ability to degrade heme. It is likely that the inducible form of heme oxygenase, HO-1, functions similarly in tissues that do not express significant levels of HO-2 (i.e. most tissues besides testes and the central nervous system); the potent cytoprotective effects of HO-1, therefore, may be completely independent of the ability of the enzyme to degrade heme. Alternatively, it is possible that rather than catabolizing copious amounts of heme to produce sufficient levels of potentially protective heme metabolites, HO-1 plays its protective role by reducing the levels of a specific pro-oxidant or pro-apoptotic hemoprotein. The release of Fe^{3+} by the heme oxygenase-catalyzed reaction cannot be ignored as a possible source of a potent pro-oxidant. Taille et al. (42) have demonstrated that HO-1 overexpression in RAW 264.7 cells reduces cellular levels of NAD(P)H oxidase, which generates most of the superoxide (O_2^-) in these cells. Total hepatic cytochrome levels have also been documented in rats with chemically induced (arsenic species) heme oxygenase (43, 44). In contrast, the Sinclair laboratory (45) has shown that, although the levels of specific cytochromes P450 decrease with sodium arsenite induction of HO-1, those effects are not the result of increased heme oxygenase activity. Recent studies have also implicated HO-1 in mediating the anti-inflammatory effects of interleukin-10, indicating that HO-1 may also exert its defensive properties via an as yet unexplored mechanism (46, 47). Interestingly, Suttner et al. (48) found significant migration of HO-1 into the nucleus of lung cells, which was associated with an increase in cell viability under hypoxia, suggesting that HO-1 may affect the cellular stress response at a genetic level. It is noteworthy that this study also found that there were no changes to reactive iron levels in HO-1 overexpressing cells (48).

In showing that the effect of hemin on ferritin synthesis was through the liberation of iron by HO-1, we have confirmed the original findings of Eisenstein et al. (14), who showed that chemical inhibition of heme oxygenase prevented the induction of ferritin synthesis by 50 μM hemin. However, here we have used a significantly lower concentration of hemin (5 μM). In addition, we also demonstrated that the IRE-IRP system is responsible for this effect of hemin. Interestingly, although we were able to recover the IRE binding activity of IRP1 following hemin treatment by inhibiting heme oxygenase activity, IRP2 was not recoverable. These results are consistent with recent evidence that hemin may inhibit IRP2 activity independently of iron (49, 50), although the physiological relevance of this effect is questionable (51), especially in light of the fact that the pool of “uncommitted” heme in non-erythroid tissues is so minute.

We observed that HO-1 overexpression protected cells from endogenously produced ROS (elicited by LPS treatment of the macrophages). Importantly, the level of HO-1 in these cells, although elevated in comparison to untreated/untransfected cells, was considerably lower than those achievable via hemin treatment. Additionally, we found that hemin treatment brought about a greater increase in ferritin synthesis in HO-1-overexpressing cells than in controls, whereas base-line Ft synthesis was unchanged by transfection. Together these results strongly suggest that the protection of cells by HO-1 does not require the catabolism of significant amounts of heme.

In conclusion, we have shown that under normal conditions, there is virtually no heme substrate available for HO-1 in macrophages or fibroblasts. The protection of tissues by this enzyme is therefore likely to be via a heretofore unknown mechanism.

Acknowledgments—We thank Dr. J. Bommer for technical assistance, Dr. S. Shibahara for the generous gift of HO-1-containing plasmid, and Dr. J. Alam for assistance with the HO-1 transfectants.

REFERENCES
1. Trakshel, G. M., Kutty, R. K., and Maines, M. D. (1986) J. Biol. Chem. 261, 11131–11137
2. Maines, M. D. (1997) Annu. Rev. Pharmacol. Toxicol. 37, 517–554
3. Tenhunen, R., Marver, H. S., and Schmid, R. (1968) Proc. Natl. Acad. Sci. U. S. A. 61, 748–755
4. Maines, M. D. (1988) FASEB J. 2, 2557–2568
5. Camara, N. O., and Soares, M. P. (2005) Free Radic. Biol. Med. 38, 426–435
6. Abraham, N. G., and Kappas, A. (2005) Free Radic. Biol. Med. 39, 1–25
7. Otterbein, L. E., Soares, M. P., Yamashita, K., and Bach, F. H. (2003) Trends Immunol. 24, 449–455
8. Ryter, S. W., Alam, J., and Choi, A. M. (2006) Physiol. Rev. 86, 583–650
9. Baranano, D. E., Rao, M., Ferris, C. D., and Snyder, S. H. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 16093–16098
Effects of Heme Oxygenase on Cellular Iron Metabolism

10. Stocker, R., Yamamoto, Y., McDonagh, A. F., Glazer, A. N., and Ames, B. N. (1987) Science 235, 1043–1046
11. Marks, G. S., Brien, J. F., Nakatsu, K., and McLaughlin, B. E. (1991) Trends Pharmacol. Sci. 12, 185–188
12. Brouard, S., Otterbein, L. E., Anrather, J., Tobiasch, E., Bach, F. H., Choi, A. M., and Soares, M. P. (2000) J. Exp. Med. 192, 1015–1026
13. Sato, K., Balla, J., Otterbein, L., Smith, R. N., Brouard, S., Lin, Y., Cizmadia, E., Sevigny, J., Robson, S. C., Vercellotti, G., Choi, A. M., Bach, F. H., and Soares, M. P. (2001) J. Immunol. 166, 4185–4194
14. Eisenstein, R. S., Garcia-Mayol, D., Pettingell, W., and Munro, H. N. (1979) Iron Metabolism in Man, p. 2, Blackwell Scientific Publications, Oxford
15. Ferris, C. D., Jaffrey, S. R., Sawa, A., Takahashi, M., Brady, S. D., Barrow, R. K., Tysoe, S. A., Wolosker, H., Baranano, D. E., Dore, S., Poss, K. D., and Snyder, S. H. (1999) Nat. Cell Biol. 1, 152–157
16. Bothwell, T. H., Charlton, R. W., Cook, J. D., and Finch, C. A. (1979) Iron Metabolism in Man, p. 2, Blackwell Scientific Publications, Oxford
17. Snyder, W. S., Cook, M. J., Karhausen, L. R., Nasset, E. S., Howells, G. P., and Tipton, I. H. (1974) International Commission on Radiological Protection No. 23: Report of the Task Group on Reference Man, Pergamon Press, Toronto
18. Sassa, S. (2004) Antioxid. Redox. Signal. 6, 819–824
19. Ponka, P., Brouard, S., Otterbein, L., Smith, R. N., Brouard, S., Lin, Y., Cizmadia, E., Sevigny, J., Robson, S. C., Vercellotti, G., Choi, A. M., Bach, F. H., and Soares, M. P. (2001) J. Immunol. 166, 4185–4194
20. Zhang, A. S., Sheftel, A. D., and Ponka, P. (2005) Blood 105, 368–375
21. Martinez-Medellin, J., and Schulman, H. M. (1972) Biochim. Biophys. Acta 264, 272–274
22. Labbe, R. F., and Nishida, G. (1957) Biochim. Biophys. Acta 26, 437
23. Kim, S., and Ponka, P. (1999) J. Biol. Chem. 274, 33035–33042
24. Mulliner, E. W., Neupert, B., and Kuhn, L. C. (1989) Cell 58, 373–382
25. Borova, J., Ponka, P., and Neuwirt, J. (1979) Biochim. Biophys. Acta 320, 143–156
26. Srisko, K., and Cha, Y. N. (2004) Biochem. Pharmacol. 68, 1709–1720
27. Woo, C. H., Lim, J. H., and Kim, J. H. (2004) J. Immunol. 173, 6973–6980
28. Bann, H. F., and Jandl, J. H. (1968) J. Biol. Chem. 243, 465–475
29. Balla, J., Jacob, H. S., Balla, G., Nath, K., and Vercellotti, G. M. (1992) Trans. Assoc. Am. Physicians 105, 1–6
30. Nath, K. A., Balla, G., Vercellotti, G. M., Balla, J., Jacob, H. S., Levitt, M. D., and Rosenberg, M. E. (1992) J. Clin. Investig 90, 267–270
31. Leff, J. A., Kennedy, D. A., Terada, L. S., Emmett, M., McCutchan, H. J., Walden, D. L., and Repine, J. E. (1991) J. Lab. Clin. Med. 118, 352–358
32. Losonczy, G., and Harsing, L. (1982) Nephron 32, 180–184
33. Balla, J., Vercellotti, G. M., Jeney, V., Yachie, A., Varga, Z., Eaton, J. W., and Balla, G. (2005) Mol. Nutr. Food Res. 49, 1030–1043
34. Wyckoff, E. E., Lopreato, G. F., Tipton, K. A., and Payne, S. M. (2005) J. Bacteriol. 187, 5658–5664
35. Graca-Souza, A. V., Maya-Monteiro, C., Paiva-Silva, G. O., Braz, G. R., Paes, M. C., Sorgine, M. H., Oliveira, M. F., and Oliveira, P. L. (2006) Insect. Biochem. Mol. Biol. 36, 322–335
36. Tsui, T. Y., Wu, X., Lau, C. K., Ho, D. W., Xu, T., Siu, Y. T., and Fan, S. T. (2003) Circulation 107, 2623–2629
37. Bouche, D., Chauveau, C., Roussel, J. J., Mathieu, P., Braudeau, C., Tesson, L., Souillou, J. P., Iyer, S., Buelow, R., and Anequin, I. (2002) Transpl. Immunol. 9, 235–238
38. Vile, G. F., and Tyrrell, R. M. (1993) J. Biol. Chem. 268, 14678–14681
39. Hori, R., Kashiba, M., Toma, T., Yachie, A., Goda, N., Makino, N., Soejima, A., Nagasawa, T., Nakabayashi, K., and Suematsu, M. (2002) J. Biol. Chem. 277, 10712–10718
40. Kim, Y. S., and Dore, S. (2005) Free Radic. Biol. Med. 39, 558–564
41. Wang, J., Zhuang, H., and Dore, S. (2006) Neurobiol. Dis. 22, 473–476
42. Taille, C., El Benna, J., Lanone, S., Dang, M. C., Ogier-Denis, E., Aubier, M., and Boczkowski, J. (2004) J. Biol. Chem. 279, 28681–28688
43. Sardana, M. K., Drummond, G. S., Sassa, S., and Kappas, A. (1981) Pharmacology 23, 247–253
44. Albros, A., Cebrian, M. E., Connelly, J. C., Bach, P. H., and Bridges, I. W. (1992) Xenobiotica 22, 591–597
45. Jacobs, J., Roussel, R., Roberts, M., Marek, D., Wood, S., Walton, H., Dwyer, B., Sinclair, P., and Sinclair, J. (1998) Toxicol. Appl. Pharmacol. 150, 376–382
46. Lee, T. S., and Chau, L. Y. (2002) Nat. Med. 8, 240–246
47. Chen, S., Kapturczak, M. H., Wasserfall, C., Glushakova, O. Y., Campbell-Thompson, M., Deshane, J. S., Joseph, R., Cruz, P. E., Hauswirth, W. W., Madsen, K. M., Croker, B. P., Berns, K. I., Atkinson, M. A., Flotte, T. R., Fisher, C. C., and Agarwal, A. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 7251–7256
48. Suttner, D. M., Sridhar, K., Lee, C. S., Tomura, T., Hansen, T. N., and Demery, P. A. (1999) Am. J. Physiol. 276, L443–L451
49. Jeong, J., Rouault, T. A., and Levine, R. L. (2004) J. Biol. Chem. 279, 45450–45454
50. Yamanaka, K., Ishikawa, H., Megumi, Y., Tokunaga, F., Kanie, M., Rouault, T. A., Morishima, I., Minato, N., Ishimori, K., and Iwai, K. (2003) Nat. Cell Biol. 5, 336–340
51. Haile, D. J., Rouault, T. A., Harford, J. B., and Klausner, R. D. (1990) J. Biol. Chem. 265, 12786–12789