Intensive Care Med (2008) 34:640–648
DOI 10.1007/s00134-008-1010-2

Michael Bauer
Klaus Huse
Utz Settmacher
Ralf A. Claus

The heme oxygenase – carbon monoxide system: regulation and role in stress response and organ failure

Received: 27 March 2006
Accepted: 5 November 2007
Published online: 20 February 2008

This article is discussed in the editorial available at: http://dx.doi.org/10.1007/s00134-008-1013-z.

M. Bauer (✉) · R. A. Claus
Friedrich Schiller University Hospital,
Department of Anesthesiology
and Intensive Care Medicine,
Erlanger Allee 101, 07740 Jena, Germany
e-mail: michael.bauer@med.uni-jena.de

K. Huse
Leibniz Institute for Age Research – Fritz Lipmann Institute,
Jena, Germany

U. Settmacher
Friedrich Schiller University Hospital,
Department of General, Visceral,
and Vascular Surgery,
Jena, Germany

Abstract Heme oxygenase (HO) breaks down heme, the iron-containing, oxygen-carrying constituent of red blood cells, yielding biliverdin, iron (II) ions, and carbon monoxide (CO). Among the isoenzymes cloned to date, only HO-1 can be induced by a panoply of stimuli linked by their ability to provoke oxidative stress. HO-1 induction protects against cell death in experimental models associated with ischemia/reperfusion or inflammation, making the gene a promising target for critical care medicine. Induction of HO-1 may confer protection by controlling intracellular levels of toxic heme, or by anti-inflammatory, anti-apoptotic, and blood flow-maintaining effects of its by-products biliverdin and CO. Although protective effects of upregulation of HO-1 have been reported for a variety of cells and tissues, evidence suggests that the protective action may be restricted to a rather narrow threshold of overexpression. In addition, there is substantial variation in gene expression depending on transcriptional control mechanisms such as a microsatellite length polymorphism. Genetic variability and the required use of cytotoxic inducers are hurdles for purposeful targeting of HO-1 gene expression in critical care, while administration of by-products of the pathway seems feasible at present.

Heme and heme oxygenase isozymes

Heme is a ubiquitous molecule with an active iron center carrying a high affinity for oxygen. The high affinity to oxygen allows for reversible binding and transport of oxygen in hemoglobin and myoglobin. Furthermore, by virtue of its cardinal function as an electron donator in oxidation/reduction cycles, the heme prosthetic moiety is of outstanding significance for electron transfer: heme groups serve as the catalytic site and act tightly bound to a variety of proteins involved in aerobic metabolism, including respiratory chain cytochromes and numerous cytochrome P450 isoenzymes [1]. "Free" cellular heme may derive from these ubiquitous heme proteins and may act as a pro-oxidant [2, 3]. Thus, free heme is potentially toxic and intracellular levels are vanishingly small and tightly controlled in most cells and tissues. While regulation of heme biosynthesis is accomplished through the modulation of δ-aminolevulinic acid synthase (ALA synthase; EC 2.3.1.37) activity [4], the enzymatic degradation of heme is controlled by microsomal heme oxygenase (HO; EC 1.14.99.3) isoenzymes which catalyze the initial and rate limiting step in heme catabolism [5]. Oxidative cleavage of the α-mesocarbon bridge of b-type heme molecules by HO yields equimolar quantities of biliverdin-IXα and carbon monoxide (CO), while divalent iron is released. Activities of ALA synthase and HO are both regulated by the cellular heme content [6].

Biliverdin-IXα is subject to further degradation to bilirubin which occurs through the action of the cytosolic

enzyme biliverdin reductase (bilirubin:NAD(P)+oxidoreductase; EC 1.3.1.24) [7]. The cellular fate of CO formed during heme degradation is only incompletely understood. CO may bind to oxyhemoglobin as well as to other heme-containing proteins, thereby affecting their heme prosthetic moieties and activity, as has been previously reported for nitric oxide (NO) [8, 9]. Thus, CO may activate soluble guanylate cyclase, leading to vasodilation. However, the much less pronounced effect of CO in stimulating GC compared to NO, and the presence of a dilatory effect despite administration of blockers of the cGMP pathway, suggest cellular mechanisms others than activation of sGC [10, 11]. In particular, there is evidence for a CO-induced dilation by BK_{Ca} channel activation, as blockade of BK_{Ca} inhibits CO-mediated vasodilation [12] and HO inhibitors derogate BK_{Ca} channel activity in smooth muscle cells [13]. It is important to note that modification of an externally located histidine residue with diethyl pyrocarbonate abrogates channel activation, suggesting an essential role of this amino acid [14]. Furthermore, the α-subunit of the channel is critically involved in its activation process. This subunit contains a heme-binding pocket, and binding of heme to the channel inhibits its activity [15]. By binding to protein-bound ferrous heme as a receptor, CO activates the channel by altering the interaction of heme with the intrinsic active protein, resulting in an increase in Ca^{2+} sensitivity [16, 17]. Under physiological conditions, in smooth muscle cells, channel activity is regulated by a local and transient increase of Ca^{2+} concentration in the micromolar range, resulting in membrane hyperpolarization via K^+ currents and subsequent reduced voltage-dependent Ca^{2+} channel activity [18]. In this context, endogenously generated or exogenously added CO elevate the amplitude and frequency of the channel dependent on varying Ca^{2+} concentrations, which may be an essential mechanism for CO-mediated vasodilatory actions [19]. Thus, the mode of action as well as the fate of endogenous CO is still a matter of debate. Ultimately, CO is exhaled by the lungs, and gas-chromatographic quantitation of exhaled CO can serve to assess HO activity in vivo [20].

The enzyme systems regulating heme synthesis and degradation are not evenly distributed among organs and tissues, and HO activity is particularly high in spleen, testes, brain, and liver [21]. At present, cDNA of three isoenzymes, i.e. *HO-1*, *-2*, and *-3*[1], have been cloned and described to date [22–25]. While *HO-1* and *-2* have been extensively characterized for several species, cDNA of *HO-3* has been cloned in rat only. It was suggested that HO-3 exhibits a substantially lower catalytic activity than the isoenzyme 1 and 2 and plays a role in binding or transporting heme within the cell rather than degrading heme [23]. However, a recent study has questioned the existence of *HO-3*: The lack of *HO-3* transcription from two presumed genomic loci indicates that the *HO-3* gene might reflect a processed pseudo-gene of the *HO-2* locus [26]. Although HO-1 and HO-2 catalyze the same reaction and have similar cofactor requirements (NADPH, O_2), they substantially differ with respect to regulation and expression pattern. The isoenzymes are encoded by distinct genes located on chromosomes 22q12 (*HO-1*) and 16q13.3 (*HO-2*) in the human genome [27, 28]. HO-1 and -2 proteins differ in molecular weight and are immunologically distinct [29]. HO-2 – also referred to as the "constitutive" isoenzyme – does not seem to be inducible by either inflammatory or oxidative stress [30], although the promoter of the *HO-2* gene contains a glucocorticoid response element [31]. In any case, substantial increases in HO activity as observed in models pertinent to critical care are mediated by an increase in gene transcription rates for *HO-1* [30, 32]. HO-1 has been identified as the major 32-kDa heat shock (stress) protein hsp32 in the rat [33] while thermal stress did not increase HO-1 in cultured human liver cells [34]. This and other substantial differences regarding regulation of the human and rodent gene and their implications for critical care will be discussed in detail later.

HO-1 gene expression under stress conditions: heme-dependent and independent pathways

Formation of reactive oxygen intermediates is a hallmark of ischemia/reperfusion injury, and inflammatory conditions substantially contribute to organ failure in the critically ill. *HO-1* is highly inducible under these conditions and reflects one of the most prominent lines of defense of the cell against oxidative stress (Fig. 1). Moreover, deliberate induction of the gene prior to the stress event seems to be a promising stratagem to increase tolerance under conditions with anticipated formation of reactive oxygen species, e.g. in organ transplantation [35]. Similarly induction of the gene upon an insult confers protection in a variety of injury models pertinent to critical care medicine, as described in detail below. Thus, in-depth understanding of regulation of the *HO-1* gene is of outstanding importance if this pathway is to be utilized deliberately in critical care medicine. Unfortunately, there are substantial differences with respect to regulation of the gene in rodents and man, so that caution should be exercised in extrapolating promissory results from rodent models to the critical care setting [36]. Nevertheless, genetic variability in the human *HO-1* gene exists and geno- or haplotypes with higher transcription rates seem to favorably affect outcome (see below; for review [35, 37]). The *HO-1* gene – and thus presumably CO – is induced in critically ill patients in

[1]The HGNC approved gene symbols for the two isoenzymes are *HMOX1* (alias *HO-1* and *bK286B10*) and *HMOX2* (alias *HO-2*) respectively, thus we use *HO-1* and *HO-2* for convenience throughout the manuscript.

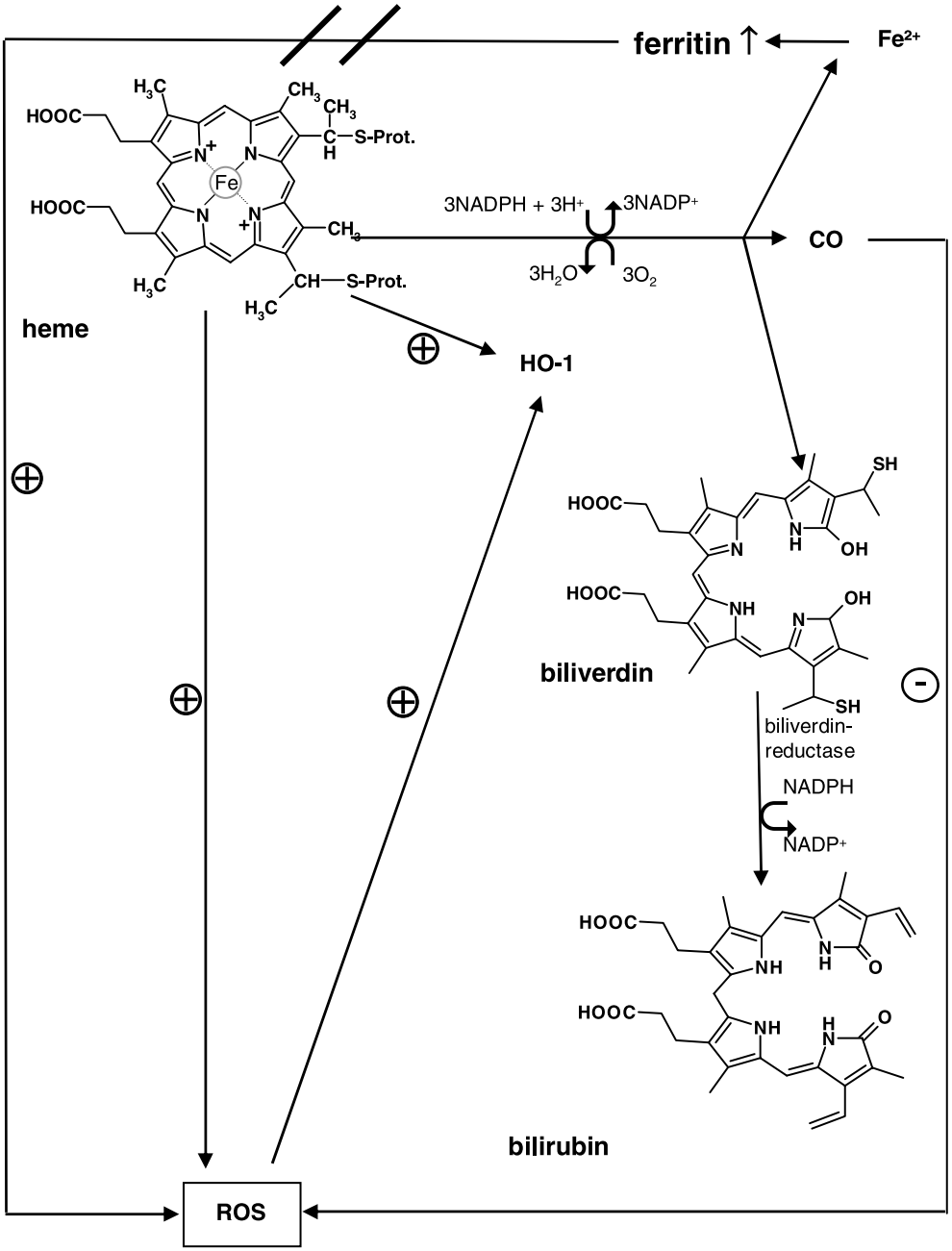

Fig. 1 Mutual interaction of the heme oxygenase pathway and the cellular response to oxidative stress (*ROS*, reactive oxygen species)

a broad range of conditions, as mentioned above, although the molecular mode of action will differ between inhaled and endogenously formed CO [36].

The different inducers of *HO-1* act either via a metalloporphyrin-dependent (heme, the heme precursor ALA, phenobarbital, or other metalloporphyrins) or a metalloporphyrin/heme-independent (e.g. transition metals, heat shock, oxygen radicals) mechanism. Despite the differences, the effects of the diverse factors on hepatic *HO-1* gene expression appear to be mainly controlled at the transcriptional level [30, 32], and induction via classical stress pathways involves mitogen-activated protein kinases. The broad spectrum of inducing agents essentially reflects the presence of a variety of transcriptional enhancer elements including binding sites for activator protein-1 (AP-1) and nuclear factor κB (NFκB) as well as hypoxia response, cadmium response, heat shock response, metal response, and IL-6 response elements within the *HO-1* promoter (Fig. 2) [6]. Nevertheless, there is recent evidence to suggest that de-repression of the BACH-1 repressor (a bZip protein that forms heterodimers with small Maf proteins) is functionally significant to enhance *HO-1* transcription

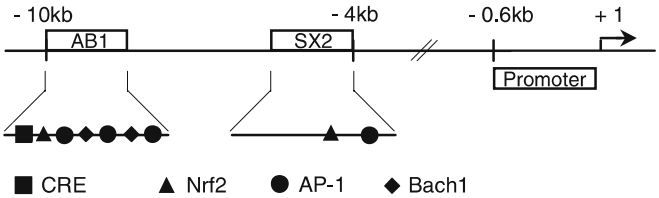

Fig. 2 Model of the human HO-1 gene and potential regulatory elements (*AB1*, distal enhancer element AB1; *SX2*, distal enhancer element SX2; *CRE*, cAMP responsive element; *Nrf2*, nuclear factor erythroid 2-related transcription factor 2; *AP-1*, activator protein-1)

in human cell lines: small interfering (si)RNAs targeted to *BACH-1* mRNA by itself induce upregulation of *HO-1* and further enhance dose-dependent upregulation by the prototypic inducer heme [38]. It is noteworthy that a wide variety of drugs used in the ICU setting, such as steroids [39], statins [40], and β1-agonists [41], can interfere with regulation of HO-1 and this might contribute to the salutary actions of these compounds [42].

Biological functions of by-products of HO-1: "The good, the bad, and the ugly"

Due to the potential toxic effects of free heme, a meticulous balance between its synthesis and catabolism is crucial to ensure cellular homeostasis. Thus, heme oxygenase has classically been viewed exclusively as a heme-degrading enzyme system, and heme itself has long been recognized as a potent inducer of *HO-1* [43]. On the other hand, two products of this pathway, i.e. biliverdin and CO, received little attention until the finding that they exert biological functions under physiological conditions [44, 45]. These functions are even more pertinent after induction of *HO-1*, e.g. after resuscitation from shock [46]. The observation that almost all of the stimuli for *HO-1* induction including the substrate heme are linked by their ability to provoke oxidative stress, in concert with the observation that bile pigments can function as endogenous antioxidants, has supported a role for HO-1 and its products biliverdin/bilirubin in the adaptive response to oxidative stress [47]. Among the stimuli pertinent to critical care medicine, (low-flow) ischemia and reperfusion secondary to shock and resuscitation is a particularly frequent clinical problem. Previous work from our laboratory regarding the role of HO1/hsp32 in the hepatic stress response in experimental models of hemorrhagic shock and subsequent resuscitation indicated that induction of *HO-1* reflects an adaptive response to reactive oxygen species (ROS) formation [48, 49], and confers delayed protection against tissue injury [50, 51].

Although the protective actions of CO and the bile pigments in vitro and in vivo during heme degradation are meanwhile well documented (see review by Scott et al. in this issue; [44, 52–54]), it is obvious that the long-known potential toxic effects of iron, CO, and the bile pigments [55, 56] are likely to limit the beneficial actions to a rather narrow threshold of overexpression. Iron is released in equimolar amounts when heme is degraded to yield biliverdin and carbon monoxide. Since iron catalyzes the formation of reactive oxygen intermediates, most notably the hydroxyl radical, it is obvious that this by-product may offset the anti-oxidative properties of the bile pigments if it is formed in significant amounts. Thus, *HO-1* expression as part of the cellular stress response may exhibit pro- and antioxidant properties [4]. Ferrous iron is rather effective as a strong oxidant; however, it stimulates the synthesis of ferritin through its regulatory protein binding and activation of iron response elements [57]. Ferritin is an intracellular iron-storage protein ensuring safe sequestration of free iron ions, thereby serving as an additional antioxidant [58]. However, the mechanisms that are involved in co-expression of HO and ferritin genes may involve additional pathways. For instance, in *HO-2* knock-out mice ROS may initiate transcriptional activation of the *HO-1* gene, but these animals fail to induce ferritin transcripts simultaneously [59]. Furthermore, there is evidence to suggest that iron ions can synergize with ROS to regulate the expression of oxidative stress response genes, including *HO-1* itself [60]. In any case, HO-1 seems to be of outstanding importance for iron reutilization in rodents [61] and humans [62] even under physiologic conditions, and HO-1 deficiency has been shown to result in iron deposition with inflammatory sequelae secondary to impaired reutilization. While HO-1-targeted mice exhibit a phenotype characterized by iron metabolic disorders with long survival, the first human autopsy case of HO-1 deficiency in a 6-year-old boy revealed predominant intravascular hemolysis, coagulation, and amyloidosis with substantially reduced life expectancy [63]. Whether increased release/deposition of free iron ions due to acute overexpression and increased HO activity may result from induction of the *HO-1* gene in vivo (as has been suggested in cultured cells [55]) has not been studied specifically.

Carbon monoxide has received much attention as a messenger molecule and as a potential therapeutic target [13]. HO as a potential endogenous source of CO co-localizes with soluble guanylate cyclase – as a potential target of CO actions – primarily in neuronal tissues, and inhibition of HO by false substrates or gene knock-out may adversely affect functions of the central and peripheral nervous system [65–68].

Although CO and NO display some similarities, there are substantial differences between these two gaseous monoxides with respect to their mode of action. NO synthesis by the constitutive NOS isoforms is tightly regulated by physiological stimuli (coupled to Ca^{2+} release), and its half-life is highly limited due to an unpaired electron leading to rapid reactions with metal ions, ROS, or sulfhydryl groups in the cell. Thus, stimulation of the

constitutively expressed NOS isoenzymes (NOS1, NOS3) leads to a short-lived burst of NO production, which in turn results in a rapid and transient rise in local cGMP levels reflecting an approximately 100- to 400-fold activation of soluble guanylate cyclase (sGC). The substantial increase in sGC activity is due to binding of NO to the prosthetic heme moiety of sGC, leading to breaking of the proximal His–Fe bond and formation of a 5-coordinated nitrosyl heme complex [68, 69].

In contrast, CO is not a radical and its production by HO-1 is not tightly regulated in an "on-off" manner. Furthermore, binding of CO to the prosthetic heme group of sGC – at least under in vitro conditions – leads to formation of a 6-coordinated heme complex interacting with His–Fe bonds with only a fivefold increase in activity of the $\alpha_1\beta_1$ heterodimeric isoform of sGC [70]. However, mechanisms such as 'sensitization' of sGC to CO in biological systems [71] as well as control of NO production by HO [72] may result in a substantial increase in the impact of the HO pathway in the control of cGMP levels. Heme prosthetic moieties are abundantly present in enzymes catalyzing single electron-transferring reactions. Thus, CO might interfere with the activity of a variety of enzymes others than sGC. Evidence in this respect has been provided for a panel of enzymes including xanthine oxidase [73], NADPH oxidase [74, 75], and prostaglandin-H-synthase [76], underlining the significance of the HO system for modulation of the oxidative stress response.

Similar to the NOS system, which comprises constitutive and inducible isoforms, the HO system is characterized by constitutive and stress-inducible isoenzymes. The stress-induced production of NO by the inducible NOS isoform (NOS2) is independent of Ca^{2+}/calmodulin, which control NO production by the constitutive NOS isoforms. Thus, substantially higher amounts of NO are produced in a tonic fashion. Work from our laboratory suggests that similarities exist between the stress-inducible NOS/NO and the HO-1/CO pathway, at least for the portal circulation of the liver under conditions of septic and hemorrhagic shock [46, 51]. Blockade of HO activity with false substrates of the HO pathway produced a moderate, selective, and transient increase in portal vascular resistance but no decrease in portal blood flow in the normal rat liver. In contrast, a substantial, selective, and lasting increase in portal resistance was observed upon administration of Sn-protoprophyrin-IX, a false substrate leading to a long-lasting blockade of HO activity, after transcriptional activation of the *HO-1* gene by hemorrhage and resuscitation [46]. This augmented pressor response of false substrates of HO in the liver is paralleled by a decrease in portal blood flow and reflects unmasking of a parallel induction of vasoconstrictors, such as endothelin-1 [77]. Due to similarities between the gaseous monoxides CO and NO, sGC has been traditionally considered as the target of cellular actions of CO, although alternative modes of action of CO have been suggested. In addition, CO might regulate expression of *NOS2* in lung and liver, which is another important aspect regarding critical care in general and specifically sepsis [78]. These cGMP-independent effects may include activation of vascular 238pS K_{Ca} [79] and 105pS K_{Ca} channels, rendering smooth muscle cells less responsive to the actions of vasoconstrictors [80]. The protective effect of *HO-1* overexpression, e.g. in the lung, has been ascribed to CO-induced elevation of cGMP and/or activation of p38 MAPK [81]. Recently, it was shown that the expression and activity of the matrix metalloproteinases MMP-1 and MMP-2, which are regulated in a MAPK-dependent manner, are inhibited in the human lung epithelial cell line A549, implicating a crucial role of CO in the pathophysiology of emphysema and other diseases involving a protease/antiprotease imbalance [82]. In addition, CO at physiological concentrations inhibits the production of proinflammatory cytokines such as TNF-α, MIF, and IL-1 from macrophages and the secretion of interleukin-2 from activated T-cells via selective activation of the MAPK pathway [83, 84].

Induction of *HO-1* – a double-edged sword

Studies using *HO-1* knock-out mice, as well as the report of the first human case of HO-1 deficiency [63], suggest an important role for the inducible HO isoenzyme already under physiological conditions. Mice lacking *HO-1* were unable to modulate body iron stores properly and were more susceptible to tissue injury [61, 85]. In addition, stress conditioning including *HO-1* gene expression as well as *HO-1* gene transfer can render cells, tissues, and organisms less susceptible to subsequent stress [86, 87]. Although the bulk of literature available to date would suggest that *HO-1* gene expression confers protection in a variety of injury models pertinent to critical care medicine [88–91], there is evidence to suggest that the protective properties of this pathway are restricted to a rather narrow threshold of overexpression [55]. Thus, evidence available to date would suggest that HO-1 is neither exclusively cytoprotective nor exclusively cytotoxic. While most of the animal data were obtained after stress conditioning using cytotoxic inducers, e.g. heavy metal ions, this is not an intriguing option in the critical care setting. Nevertheless, these studies were conducted almost exclusively in rodent species and, thus, a fairly homogeneous induction of the gene in these animals can be assumed.

Differences between rodent and human *HO-1* genes – a plea for caution

Data from a flurry of studies mostly conducted in rodents provided evidence that antioxidant properties (presumably via biliverdin formation) and improved blood flow

(presumably via CO formation) contribute to the salutary effects of *HO-1* gene expression under conditions of shock and inflammation [46, 51, 92]. Thus, CO may confer additional protective effects via anti-inflammatory mechanisms [87, 93] particularly in models pertinent to surgical conditions, such as transplantation or infection.

In this light, alternative regulatory features of the human as compared to the mouse/rat gene give rise presumably to substantial variability in *HO-1* expression in humans, with potential implications for critical care medicine. One striking difference in the genomic organization of the genes in the species is the presence of a $(GT)_n$ repeat microsatellite in the human *HO-1* gene. Several lines of evidence point to a regulatory function of this motif. (1) Systematic analysis shows that rat and mouse genes completely lack the microsatellite (the same is true for the dog genome, while in the chimpanzee draft genomic sequence only a low-number repeat is present). (2) The non-inducible *HO-2* gene is not associated with such a $(GT)_n$ microsatellite [94]. (3) The length of this polymorphic repeat has been associated with a different outcome in a variety of diseased states. Interestingly, this has also been demonstrated for *NOS1*, where a similar genomic element at a similar position around the first exon is found and the repeat length influences the decline in lung function in patients with cystic fibrosis [95].

Whether this repeat influences gene transcription, for instance by spacing apart interacting binding sites for transcription factors, or may influence translation of the protein by forming a $(GU)_n$ stem–loop structure in the 5′-UTR of the mRNA has to be elucidated. There is at least evidence by two spliced ESTs (expressed sequence tags, BE407102, DA903962) from the EST database that this repeat region can be part of the *HO-1* transcript. If transcribed, such a structure may hinder the ribosome scanning and therefore efficient translation. A similar translational control mechanism has been described for glutamate receptor 2 [96].

In line with this concept, genotypes associated with higher transcriptional activity, such as a short GT repeat in the well-characterized microsatellite length polymorphism between –198 and –256, are associated with a better outcome in a variety of diseases pertinent to intensive care [97–102]. These include conditions associated with ischemia and reperfusion as well as inflammatory conditions, such as kidney transplantation and myocardial infarction but also lung emphysema. On the other hand, these differences would imply that strategies to induce the gene in humans deliberately, e.g. prior to ischemia/reperfusion associated with transplantation, will vary greatly with respect to the genetic background of the patient. Thus, assessment of the genotype prior to such interventions seems mandatory. Strategies to apply the by-products of the pathway, such as biliverdin or CO, directly to the patient seem more promising. It is noteworthy that – unlike data obtained in experimental models – the *HO-1* gene is induced in brain-dead organ donors as a result of the underlying trauma/injury [35, 37]. Thus deliberate induction of the gene prior to organ harvest is less likely to lead to substantial increases in activity. In any case, if the promising data from preclinical studies in rodents prove to be applicable to man, this pathway bears outstanding potential for medicine in general and in particular for the care of the critically ill.

References

1. Maines MD (1992) Heme oxygenase. Clinical applications and functions. CRC Press, Boca Raton, FL
2. Balla G, Vercellotti GM, Muller-Eberhard U, Eaton J, Jacob HS (1991) Exposure of endothelial cells to free heme potentiates damage mediated by granulocytes and toxic oxygen species. Lab Invest 64:648–655
3. Gutteridge JM, Smith A (1988) Antioxidant protection by haemopexin of haem-stimulated lipid peroxidation. Biochem J 256:861–865
4. Ryter SW, Tyrrell RM (2000) The heme synthesis and degradation pathways: role in oxidant sensitivity. Heme oxygenase has both pro- and antioxidant properties. Free Radic Biol Med 28:289–309
5. 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
6. Elbirt KK, Bonkovsky HL (1999) Heme oxygenase: recent advances in understanding its regulation and role. Proc Assoc Am Physicians 111:438–447
7. Kutty RK, Maines MD (1981) Purification and characterization of biliverdin reductase from rat liver. J Biol Chem 256:3956–3962
8. Maines MD (1997) The heme oxygenase system: a regulator of second messenger gases. Annu Rev Pharmacol Toxicol 37:517–554
9. Suematsu M, Wakabayashi Y, Ishimura Y (1996) Gaseous monoxides: a new class of microvascular regulator in the liver. Cardiovasc Res 32:679–686
10. Naik JS, Walker BR (2003) Heme oxygenase-mediated vasodilation involves vascular smooth muscle cell hyperpolarization. Am J Physiol Heart Circ Physiol 285:H2, 20–28
11. Hosein S, Marks GS, Brien JF, McLaughlin BE, Nakatsu K (2002) An extracellular source of heme can induce a significant heme oxygenase mediated relaxation in the rat aorta. Can J Physiol Pharmacol 80:761–765
12. Zhang F, Kaide J, Wei Y, Jiang H, Yu C, Balazy M, Abraham NG, Wang W, Nasjletti A (2001) Carbon monoxide produced by isolated arterioles attenuates pressure-induced vasoconstriction. Am J Physiol Heart Circ Physiol 281:H350–358
13. Wu L, Wang R (2005) Carbon monoxide: endogenous production, physiological functions, and pharmacological applications. Pharmacol Rev 57:585–630
14. Wang R, Wu L (1997) The chemical modification of KCa channels by carbon monoxide in vascular smooth muscle cells. J Biol Chem 272:8222–8226

15. Tang XD, Xu R, Reynolds MF, Garcia ML, Heinemann SH, Hoshi T (2003) Haem can bind to and inhibit mammalian calcium-dependent Slo1 BK channels. Nature 425:531–535
16. Jaggar JH, Li A, Parfenova H, Liu J, Umstot ES, Dopico AM, Leffler CW (2005) Heme is a carbon monoxide receptor for large-conductance Ca2+-activated K+ channels. Circ Res 97:805–812
17. Xi Q, Tcheranova D, Parfenova H, Horowitz B, Leffler CW, Jaggar JH (2004) Carbon monoxide activates KCa channels in newborn arteriole smooth muscle cells by increasing apparent Ca2+ sensitivity of alpha-subunits. Am J Physiol Heart Circ Physiol 286:H610–618
18. Jaggar JH, Porter VA, Lederer WJ, Nelson MT (2000) Calcium sparks in smooth muscle. Am J Physiol Cell Physiol 278:C235–256
19. Jaggar JH, Leffler CW, Cheranov SY, Tcheranova D, Shuyu E, Cheng X (2002) Carbon monoxide dilates cerebral arterioles by enhancing the coupling of Ca2+ sparks to Ca2+-activated K+ channels. Circ Res 91:610–617
20. Vreman HJ, Stevenson DK (1988) Heme oxygenase activity as measured by carbon monoxide production. Anal Biochem 168:31–38
21. Maines MD (1988) Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. FASEB J 2:2557–2568
22. Cruse I, Maines MD (1988) Evidence suggesting that the two forms of heme oxygenase are products of different genes. J Biol Chem 263:3348–3353
23. 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
24. Shibahara S, Muller R, Taguchi H, Yoshida T (1985) Cloning and expression of cDNA for rat heme oxygenase. Proc Natl Acad Sci USA 82:7865–7869
25. Shibahara S, Yoshizawa M, Suzuki H, Takeda K, Meguro K, Endo K (1993) Functional analysis of cDNAs for two types of human heme oxygenase and evidence for their separate regulation. J Biochem 113:214–218
26. Hayashi S, Omata Y, Sakamoto H, Higashimoto Y, Hara T, Sagara Y, Noguchi M (2004) Characterization of rat heme oxygenase-3 gene. Implication of processed pseudogenes derived from heme oxygenase-2 gene. Gene 336:241–250
27. Kutty RK, Kutty G, Rodriguez IR, Chader GJ, Wiggert B (1994) Chromosomal localization of the human heme oxygenase genes: heme oxygenase-1 (HMOX1) maps to chromosome 22q12 and heme oxygenase-2 (HMOX2) maps to chromosome 16p13.3. Genomics 20:513–516
28. Kuwano A, Ikeda H, Takeda K, Nakai H, Kondo I, Shibahara S (1994) Mapping of the human gene for inducible heme oxygenase to chromosome 22q12. Tohoku J Exp Med 172:389–392
29. Trakshel GM, Maines MD (1989) Multiplicity of heme oxygenase isozymes. HO-1 and HO-2 are different molecular species in rat and rabbit. J Biol Chem 264:1323–1328
30. Bauer I, Wanner GA, Rensing H, Alte C, Miescher EA, Wolf B, Pannen BH, Clemens MG, Bauer M (1998) Expression pattern of heme oxygenase isoenzymes 1 and 2 in normal and stress-exposed rat liver. Hepatology 27:829–838
31. Maines MD, Eke BC, Zhao X (1996) Corticosterone promotes increased heme oxygenase-2 protein and transcript expression in the newborn rat brain. Brain Res 722:83–94
32. Sonin NV, Garcia-Pagan JC, Nakanishi K, Zhang JX, Clemens MG (1999) Patterns of vasoregulatory gene expression in the liver response to ischemia/reperfusion and endotoxemia. Shock 11:175–179
33. Shibahara S, Muller RM, Taguchi H (1987) Transcriptional control of rat heme oxygenase by heat shock. J Biol Chem 262:12889–12892
34. Bauer I, Rensing H, Florax A, Ulrich C, Pistorius G, Redl H, Bauer M (2003) Expression pattern and regulation of heme oxygenase-1/heat shock protein 32 in human liver cells. Shock 20:116–122
35. Bauer M (2003) Heme oxygenase in liver transplantation: heme catabolism and metabolites in the search of function. Hepatology 38:286–288
36. Mayr FB, Spiel A, Leitner J, Marsik C, Germann P, Ullrich R, Wagner O, Jilma B (2005) Effects of carbon monoxide inhalation during experimental endotoxemia in humans. Am J Respir Crit Care Med 171:354–360
37. Bauer M, Bauer I (2002) Heme oxygenase-1: redox regulation and role in the hepatic response to oxidative stress. Antioxid Redox Signal 4:749–758
38. Shan Y, Lambrecht RW, Ghaziani T, Donohue SE, Bonkovsky HL (2004) Role of Bach-1 in regulation of heme oxygenase-1 in human liver cells: insights from studies with small interfering RNAS. J Biol Chem 279:51769–51774
39. Deramaudt TB, da Silva JL, Remy P, Kappas A, Abraham NG (1999) Negative regulation of human heme oxygenase in microvessel endothelial cells by dexamethasone. Proc Soc Exp Biol Med 222:185–193
40. Grosser N, Hemmerle A, Berndt G, Erdmann K, Hinkelmann U, Schurger S, Wijayanti N, Immenschuh S, Schroder H (2004) The antioxidant defense protein heme oxygenase 1 is a novel target for statins in endothelial cells. Free Radic Biol Med 37:2064–2071
41. Rensing H, Bauer I, Kubulus D, Wolf B, Winning J, Ziegeler S, Bauer M (2004) Heme oxygenase-1 gene expression in pericentral hepatocytes through beta1-adrenoceptor stimulation. Shock 21:376–387
42. Raddatz A, Kubulus D, Winning J, Bauer I, Pradarutti S, Wolf B, Kreuer S, Rensing H (2006) Dobutamine improves liver function after hemorrhagic shock through induction of heme oxygenase-1. Am J Respir Crit Care Med 174:198–207
43. Kubulus D, Rensing H, Paxian M, Thierbach JT, Meisel T, Redl H, Bauer M, Bauer I (2005) Influence of heme-based solutions on stress protein expression and organ failure after hemorrhagic shock. Crit Care Med 33:629–637
44. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN (1987) Bilirubin is an antioxidant of possible physiological importance. Science 235:1043–1046
45. Suematsu M, Goda N, Sano T, Kashiwagi S, Egawa T, Shinoda Y, Ishimura Y (1995) Carbon monoxide: an endogenous modulator of sinusoidal tone in the perfused rat liver. J Clin Invest 96:2431–2437
46. Bauer M, Pannen BH, Bauer I, Herzog C, Wanner GA, Hanselmann R, Zhang JX, Clemens MG, Larsen R (1996) Evidence for a functional link between stress response and vascular control in hepatic portal circulation. Am J Physiol 271:G929–935
47. Applegate LA, Luscher P, Tyrrell RM (1991) Induction of heme oxygenase: a general response to oxidant stress in cultured mammalian cells. Cancer Res 51:974–978

48. Paxian M, Rensing H, Rickauer A, Schonhofen S, Schmeck J, Pannen BH, Bauer I, Bauer M (2001) Kupffer cells and neutrophils as paracrine regulators of the heme oxygenase-1 gene in hepatocytes after hemorrhagic shock. Shock 15:438–445
49. Rensing H, Bauer I, Peters I, Wein T, Silomon M, Jaeschke H, Bauer M (1999) Role of reactive oxygen species for hepatocellular injury and heme oxygenase-1 gene expression after hemorrhage and resuscitation. Shock 12:300–308
50. Pannen BH, Kohler N, Hole B, Bauer M, Clemens MG, Geiger KK (1998) Protective role of endogenous carbon monoxide in hepatic microcirculatory dysfunction after hemorrhagic shock in rats. J Clin Invest 102:1220–1228
51. Rensing H, Bauer I, Datene V, Patau C, Pannen BH, Bauer M (1999) Differential expression pattern of heme oxygenase-1/heat shock protein 32 and nitric oxide synthase-II and their impact on liver injury in a rat model of hemorrhage and resuscitation. Crit Care Med 27:2766–2775
52. Dennery PA, McDonagh AF, Spitz DR, Rodgers PA, Stevenson DK (1995) Hyperbilirubinemia results in reduced oxidative injury in neonatal Gunn rats exposed to hyperoxia. Free Radic Biol Med 19:395–404
53. Hayashi S, Takamiya R, Yamaguchi T, Matsumoto K, Tojo SJ, Tamatani T, Kitajima M, Makino N, Ishimura Y, Suematsu M (1999) Induction of heme oxygenase-1 suppresses venular leukocyte adhesion elicited by oxidative stress: role of bilirubin generated by the enzyme. Circ Res 85:663–671
54. Stocker R, McDonagh AF, Glazer AN, Ames BN (1990) Antioxidant activities of bile pigments: biliverdin and bilirubin. Methods Enzymol 186:301–309
55. Suttner DM, Dennery PA (1999) Reversal of HO-1 related cytoprotection with increased expression is due to reactive iron. FASEB J 13:1800–1809
56. Wennberg RP (1991) Cellular basis of bilirubin toxicity. N Y State J Med 91:493–496
57. Eisenstein RS (2000) Iron regulatory proteins and the molecular control of mammalian iron metabolism. Annu Rev Nutr 20:627–662
58. Balla J, Vercellotti GM, Jeney V, Yachie A, Varga Z, Eaton JW, Balla G (2005) Heme, heme oxygenase and ferritin in vascular endothelial cell injury. Mol Nutr Food Res 49:1030–1043
59. Dennery PA, Spitz DR, Yang G, Tatarov A, Lee CS, Shegog ML, Poss KD (1998) Oxygen toxicity and iron accumulation in the lungs of mice lacking heme oxygenase-2. J Clin Invest 101:1001–1011
60. Ryter SW, Si M, Lai CC, Su CY (2000) Regulation of endothelial heme oxygenase activity during hypoxia is dependent on chelatable iron. Am J Physiol Heart Circ Physiol 279:H2889–2897
61. Poss KD, Tonegawa S (1997) Heme oxygenase 1 is required for mammalian iron reutilization. Proc Natl Acad Sci USA 94:10919–10924
62. Yachie A, Niida Y, Wada T, Igarashi N, Kaneda H, Toma T, Ohta K, Kasahara Y, Koizumi S (1999) Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. J Clin Invest 103:129–135
63. Kawashima A, Oda Y, Yachie A, Koizumi S, Nakanishi I (2002) Heme oxygenase-1 deficiency: the first autopsy case. Hum Pathol 33:125–130
64. Ingi T, Cheng J, Ronnett GV (1996) Carbon monoxide: an endogenous modulator of the nitric oxide-cyclic GMP signaling system. Neuron 16:835–842
65. Hartsfield CL (2002) Cross talk between carbon monoxide and nitric oxide. Antioxid Redox Signal 4:301–307
66. Verma A, Hirsch DJ, Glatt CE, Ronnett GV, Snyder SH (1993) Carbon monoxide: a putative neural messenger. Science 259:381–384
67. Baranano DE, Ferris CD, Snyder SH (2001) Atypical neural messengers. Trends Neurosci 24:99–106
68. Kharitonov VG, Sharma VS, Pilz RB, Magde D, Koesling D (1995) Basis of guanylate cyclase activation by carbon monoxide. Proc Natl Acad Sci USA 92:2568–2571
69. Andrew CR, George SJ, Lawson DM, Eady RR (2002) Six- to five-coordinate heme-nitrosyl conversion in cytochrome c' and its relevance to guanylate cyclase. Biochemistry 41:2353–2360
70. Stone JR, Marletta MA (1994) Soluble guanylate cyclase from bovine lung: activation with nitric oxide and carbon monoxide and spectral characterization of the ferrous and ferric states. Biochemistry 33:5636–5640
71. Friebe A, Schultz G, Koessling D (1996) Sensitizing soluble guanylate cyclase to become a highly CO-sensitive enzyme. EMBO J 15:6835–6868
72. Xue L, Farrugia G, Miller SM, Ferris CD, Snyder SH, Szurszewski JH (2000) Carbon monoxide and nitric oxide as coneurotransmitters in the enteric nervous system: evidence from genomic deletion of biosynthetic enzymes. Proc Natl Acad Sci USA 97:1851–1855
73. Kweon MH, Park YI, Sung HC, Mukhtar H (2006) The novel antioxidant 3-O-caffeoyl-1-methylquinic acid induces Nrf2-dependent phase II detoxifying genes and alters intracellular glutathione redox. Free Radic Biol Med 40:1349–1361
74. Ryter SW, Choi AM (2002) Heme oxygenase-1: molecular mechanisms of gene expression in oxygen-related stress. Antioxid Redox Signal 4:625–632
75. Chang SH, Garcia J, Melendez JA, Kilberg MS, Agarwal A (2003) Haem oxygenase 1 gene induction by glucose deprivation is mediated by reactive oxygen species via the mitochondrial electron-transport chain. Biochem J 371:877–885
76. Suh GY, Jin Y, Yi AK, Wang XM, Choi AM (2006) CCAAT/enhancer-binding Protein Mediates Carbon Monoxide-induced Suppression of Cyclooxygenase-2. Am J Respir Cell Mol Biol 35:220–226
77. Rensing H, Bauer I, Zhang JX, Paxian M, Pannen BH, Yokoyama Y, Clemens MG, Bauer M (2002) Endothelin-1 and heme oxygenase-1 as modulators of sinusoidal tone in the stress-exposed rat liver. Hepatology 36:1453–1465
78. Sarady JK, Zuckerbraun BS, Bilban M, Wagner O, Usheva A, Liu F, Ifedigbo E, Zamora R, Choi AM, Otterbein LE (2004) Carbon monoxide protection against endotoxic shock involves reciprocal effects on iNOS in the lung and liver. FASEB J 18:854–856
79. Wang R, Wu L, Wang Z (1997) The direct effect of carbon monoxide on KCa channels in vascular smooth muscle cells. Pflugers Arch 434:285–291
80. Kaide JI, Zhang F, Wei Y, Jiang H, Yu C, Wang WH, Balazy M, Abraham NG, Nasjletti A (2001) Carbon monoxide of vascular origin attenuates the sensitivity of renal arterial vessels to vasoconstrictors. J Clin Invest 107:1163–1171
81. Tsuburai T, Suzuki M, Nagashima Y, Suzuki S, Inoue S, Hasiba T, Ueda A, Ikehara K, Matsuse T, Ishigatsubo Y (2002) Adenovirus-mediated transfer and overexpression of heme oxygenase 1 cDNA in lung prevents bleomycin-induced pulmonary fibrosis via a Fas-Fas ligand-independent pathway. Hum Gene Ther 13:1945–1960
82. Desmard M, Amara N, Lanone S, Motterlini R, Boczkowski J (2005) Carbon monoxide reduces the expression and activity of matrix metalloproteinases 1 and 2 in alveolar epithelial cells. Cell Mol Biol (Noisy-le-grand) 51:403–408

83. Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, Davis RJ, Flavell RA, Choi AM (2000) Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. Nat Med 6:422–428
84. Pae HO, Oh GS, Choi BM, Chae SC, Kim YM, Chung KR, Chung HAT (2004) Carbon monoxide produced by heme oxygenase-1 suppresses T cell proliferation via inhibition of IL-2 production. J Immunol 172:4744–4751
85. Poss KD, Tonegawa S (1997) Reduced stress defense in heme oxygenase 1-deficient cells. Proc Natl Acad Sci USA 94:10925–10930
86. Amersi F, Buelow R, Kato H, Ke B, Coito AJ, Shen XD, Zhao D, Zaky J, Melinek J, Lassman CR, Kolls JK, Alam J, Ritter T, Volk HD, Farmer DG, Ghobrial RM, Busuttil RW, Kupiec-Weglinski JW (1999) Upregulation of heme oxygenase-1 protects genetically fat Zucker rat livers from ischemia/reperfusion injury. J Clin Invest 104:1631–1639
87. Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, Davis RJ, Flavell RA, Choi AM (2000) Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. Nat Med 6:422–428
88. Yu HP, Choudhry MA, Shimizu T, Hsieh YC, Schwacha MG, Yang S, Chaudry IH (2006) Mechanism of the salutary effects of flutamide on intestinal myeloperoxidase activity following trauma-hemorrhage: up-regulation of estrogen receptor-{beta}-dependent HO-1. J Leukoc Biol 79:277–284
89. Sarady-Andrews JK, Liu F, Gallo D, Nakao A, Overhaus M, Ollinger R, Choi AM, Otterbein LE (2005) Biliverdin administration protects against endotoxin-induced acute lung injury in rats. Am J Physiol Lung Cell Mol Physiol 289:L1131–1137
90. Gorbunov NV, Asher LV, Ayyagari V, Atkins JL (2006) Inflammatory leukocytes and iron turnover in experimental hemorrhagic lung trauma. Exp Mol Pathol 80:11–25
91. Akagi R, Takahashi T, Sassa S (2005) Cytoprotective effects of heme oxygenase in acute renal failure. Contrib Nephrol 148:70–85
92. Wunder C, Brock RW, Frantz S, Gottsch W, Morawietz H, Roewer N, Eichelbronner O (2005) Carbon monoxide, but not endothelin-1, plays a major role for the hepatic microcirculation in a murine model of early systemic inflammation. Crit Care Med 33:2323–2331
93. Willis D, Moore AR, Frederick R, Willoughby DA (1996) Heme oxygenase: a novel target for the modulation of the inflammatory response. Nat Med 2:87–90
94. HMOX2 locus is found in the human genome assembly hg18 at chr16:4,466,447–4,500,348
95. Texereau J, Marullo S, Hubert D, Coste J, Dusser DJ, Dall'Ava-Santucci J, Dinh-Xuan AT (2004) Nitric oxide synthase 1 as a potential modifier gene of decline in lung function in patients with cystic fibrosis. Thorax 59:156–158
96. Myers SJ, Huang Y, Genetta T, Dingledine R (2004) Inhibition of glutamate receptor 2 translation by a polymorphic repeat sequence in the 5′-untranslated leaders. J Neurosci 24:3489–3499
97. Dick P, Schillinger M, Minar E, Mlekusch W, Amighi J, Sabeti S, Schlager O, Raith M, Endler G, Mannhalter C, Wagner O, Exner M (2005) Haem oxygenase-1 genotype and cardiovascular adverse events in patients with peripheral artery disease. Eur J Clin Invest 35:731–737
98. Li P, Elrayess MA, Gomma AH, Palmen J, Hawe E, Fox KM, Humphries SE (2005) The microsatellite polymorphism of heme oxygenase-1 is associated with baseline plasma IL-6 level but not with restenosis after coronary in-stenting. Chin Med J (Engl) 118:1525–1532
99. Ullrich R, Exner M, Schillinger M, Zuckermann A, Raith M, Dunkler D, Horvat R, Grimm M, Wagner O (2005) Microsatellite polymorphism in the heme oxygenase-1 gene promoter and cardiac allograft vasculopathy. J Heart Lung Transplant 24:1600–1605
100. Li P, Sanders J, Hawe E, Brull D, Montgomery H, Humphries S (2005) Inflammatory response to coronary artery bypass surgery: does the heme-oxygenase-1 gene microsatellite polymorphism play a role? Chin Med J (Engl) 118:1285–1290
101. Geuken E, Buis CI, Visser DS, Blokzijl H, Moshage H, Nemes B, Leuvenink HG, de Jong KP, Peeters PM, Slooff MJ, Porte RJ (2005) Expression of heme oxygenase-1 in human livers before transplantation correlates with graft injury and function after transplantation. Am J Transplant 5:1875–1885
102. Exner M, Bohmig GA, Schillinger M, Regele H, Watschinger B, Horl WH, Raith M, Mannhalter C, Wagner OF (2004) Donor heme oxygenase-1 genotype is associated with renal allograft function. Transplantation 77:538–542