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Heme Oxygenase-1 in Liver Transplant Ischemia-Reperfusion Injury: From Bench-to-Bedside

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Abstract

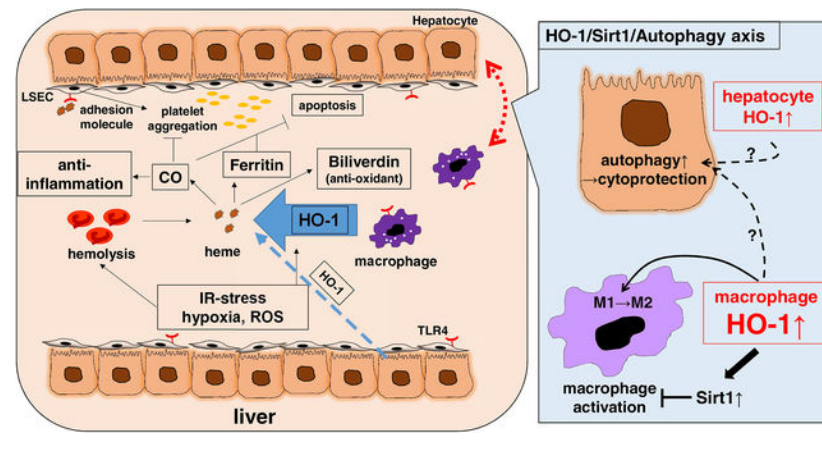
Hepatic ischemia-reperfusion injury (IRI), a major risk factor for early allograft dysfunction (EAD) and acute or chronic graft rejection, contributes to donor organ shortage for life-saving orthotopic liver transplantation (OLT). The graft injury caused by local ischemia (warm and/or cold) leads to parenchymal cell death and release of danger-associated molecular patterns (DAMPs), followed by reperfusion-triggered production of reactive oxygen species (ROS), activation of inflammatory cells, hepatocellular damage and ultimate organ failure. Heme oxygenase 1 (HO-1), a heat shock protein-32 induced under IR-stress, is an essential component of the cytoprotective mechanism in stressed livers. HO-1 regulates anti-inflammatory responses and may be crucial in the pathogenesis of chronic diseases, such as arteriosclerosis, hypertension, diabetes and steatosis. An emerging area of study is macrophage-derived HO-1 and its pivotal intrahepatic homeostatic function played in IRI-OLT. Indeed, ectopic hepatic HO-1 overexpression activates intracellular SIRT1/autophagy axis to serve as a key cellular self-defense mechanism in both mouse and human OLT recipients. Recent translational studies in rodents and human liver transplant patients provide novel insights into HO-1 mediated cytoprotection against sterile hepatic inflammation. In this review, we summarize the current bench-to-bedside knowledge on HO-1 molecular signaling and discuss their future therapeutic potential to mitigate IRI in OLT.

Graphical Abstract

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Introduction

Liver ischemia-reperfusion injury (IRI), a multifactorial process triggered by transient tissue deprivation of oxygen, followed by reoxygenation, may occur in clinical states such as shock, trauma, hepatic resection, procurement and orthotopic liver transplantation (OLT). Although OLT has become the standard life-saving strategy for patients with end-stage liver failure and those with malignancies, there are currently no therapeutics against or patient-specific diagnostics of peri-transplant hepatic IRI [1].

Although transplantation rates have improved progressively with a concomitant reduction of mortality, the limited supply of donor livers remains a major challenge [2]. According to Organ Procurement and Transplantation Network [US Department of Health and Human Services; <http://optn.transplant.hrsa.gov/data/>], in 2017, out of 13,239 patients on the liver transplant waiting list, only 8,082 received the transplant; 1,248 died while awaiting for the life-saving organ and another 1,329 individuals were removed from the list because they became too sick. Donor organ shortage has forced the use of extended criteria organs ranging from steatosis, fibrosis, or non-heart beating donors (NHBD), which may also suffer from prolonged periods of warm ischemia. These marginal grafts being highly susceptible to IR-stress, often suffer from primary non-function (PNF), early allograft dysfunction (EAD), as well as undergo more frequent acute and chronic rejection episodes. The incidence of EAD, ranging from 10.8% to 36.3% depending on the facility [3], often closely associates with the overall graft survival rate [4, 5]. As mechanisms that account for liver IRI are not well appreciated, novel biomarkers of hepatocellular graft function as well as strategies to improve clinical OLT outcomes and expand donor pool are warranted.

The mechanism of hepatic tissue IRI encompasses innate immune cell activation and augmented oxidative stress responses. The latter can also be observed during metabolic disorders, such as obesity [6–8] or diabetes [9–11] due to a myriad of environmental stressors, which trigger hepatic sterile inflammation or metabolism disturbances. A key early derivative source of tissue injury is the ischemia phase of IRI [12] when ceasing the blood flow and accompanying lack of oxygen supply triggers cellular glycogen and/or ATP depletion. The resulting initial parenchymal cell death leads to release of danger-associated molecular patterns (DAMPs). During the reperfusion phase, cells recover from the ischemic

insult but now generate ROS that lead to further impairment of microcirculation and coagulation disorders with a simultaneous massive influx of inflammatory cells, all contributing to hepatocellular injury and ultimate organ failure. However, as discussed in this review, redox signaling may provide indispensable protection not only in terms of homeostatic maintenance but also for the prevention of several diseases.

The activation of immune cells, such as macrophages, liver-resident Kupffer cells (KCs), neutrophils and T cells leads to the release of pro-inflammatory ROS, cytokines/chemokines, complement activation, and vascular cell adhesion molecular programs, accelerating the hepatocellular damage. Macrophages are central in initiating these inflammatory cascades by activating pattern recognition receptors (PRRs), which further deteriorates the liver function. In particular, macrophage-derived heme oxygenase-1 (HO-1) has been shown to be crucial in the pathophysiology of organ IRI [13–16].

The heme oxygenase (HO) system is the rate-limiting step in the conversion of heme into ferrous iron (Fe^{2+}), biliverdin (BV) and carbon monoxide (CO). HO-1, a heat shock protein 32 (Hsp32), is the inducible HO isoform, the expression of which in mammalian tissues is relatively low under normal physiological conditions, except of the myeloid spleen cells [17]. Our group was the first to report on the protective role of HO-1 induction in the steatotic liver transplant rat model [18], and proposed that HO-1 may serve as a therapeutic target against oxidative stress in OLT recipients [19]. Numerous subsequent studies have confirmed the therapeutic potential of pharmacological or genetic HO-1 modulations against IR-stress in brain, heart, liver, kidney, and intestine [20–25]. Although cytoprotective, antioxidant and anti-inflammatory functions of HO-1 have long been associated with heme catabolism, recent studies have linked HO-1 with the autophagy intracellular pathway [15, 25, 26], a new and potent cytoprotective mechanism against peri-transplant organ damage.

In this review, we first focus on our current understanding of the cellular and molecular mechanisms of HO-1 function, emphasizing the emerging role of HO-1/SIRT1/autophagy in experimental IRI-OLT. Then, we discuss therapeutic prospects for the modulation of HO-1 expression in the clinical settings.

Liver IRI: An innate-dominated local inflammation response

The discovery of Toll-like Receptor 4 (TLR4) and identification of the high mobility group box 1 (HMGB1) and lipopolysaccharide (LPS) as TLR4 endogenous ligands have changed our appreciation of the innate immune system. Functionally, TLR4 is involved in multiple signaling pathways, influencing nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) or interferon regulatory factors (IRFs), which then enhance distinct cytokine and chemokine programs. As one of the key mediators in IR-triggered sterile inflammation, disruption of TLR4 prompted high resistance against IR-stress in mouse models of warm as well as extended cold ischemia, followed by OLT [27–29]. Of note, heme, as we discuss later, has been identified as one of the TLR4 ligands to exert pro-inflammatory effects.

HMGB1 is important for the function of transcription factor p53, one of the tumor suppressor genes that regulates cell cycle, apoptosis and DNA repair [30]. Damaged

hepatocytes and liver sinusoidal endothelial cells (LSECs) can also release HBGB1 during IRI, which once released outside the cell exerts DAMPs activity via macrophage TLR4 signaling axis. As a result, the expression of cell adhesion molecules, such as ICAM-1 or VCAM-1, increases in IR-stressed liver, coinciding with infiltration by neutrophils, monocyte-derived macrophages and T cells [31, 32]. Such a negative chain of well-orchestrated immune events may prolong IR-triggered acute liver inflammation cascade.

TLR4 and interferon gamma (IFN- γ) signaling polarize liver-sequestered monocyte-derived macrophages into the M1 phenotype. This polarization stimulates the release of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF α), interleukin-6 (IL-6), IL-12, or ROS, which then induce Th1-type immune response. Although M1 macrophages exert strong antibacterial, antiviral and antitumor effects, they promote a pro-inflammatory phenotype in liver IRI [33, 34]. In contrast, macrophages stimulated with IL-4 and IL-13, Th2-type mediators, become M2 macrophages (arginase/mannose receptor positive), promoting tissue repair, angiogenesis and immunosuppressive effects by releasing IL-10. Although KCs are thought to initiate inflammatory responses via TLR4 signaling, they may also produce inhibitory cytokines, such as IL-10 or IL-4. More recently, resveratrol, a SIRT1 activator, and adiponectin have been reported to promote IL-10 secretion from M2-type KCs in alcoholic liver disorders (ALD) and non-alcoholic-steatohepatitis (NASH). Interestingly, IL-10 produced by M2-type KCs promoted selective M1 macrophage death by a mechanism involving arginase activation [35]. Clearly more studies are needed to better understand how polarized macrophages regulate sterile inflammation in liver IRI.

Hepatic IRI in OLT: A case for innate - adaptive immune interplay

IR-triggered tissue damage in OLT results from two, often overlapping types of cold and warm hepatocellular injury [36]. Cold IRI, characterized by damage to liver sinusoidal endothelial cells (LSECs) following microcirculation disturbance, can occur during the preservation stage after organ procurement [37]. By contrast, warm IRI initiates hepatocellular damage and occurs during the implantation of the cold-preserved liver into the recipient during revascularization. In cases of donation after cardiac death (DCD), donor livers suffer from additional extended periods of warm ischemic injury. In the ischemic stage, lack of oxygen supply leads to the initial hepatocyte cell death, and DAMPs release, such as HMGB1. After reperfusion, activated liver resident KCs produce cytokines and chemokines promoting infiltration of circulating monocyte-derived macrophages, neutrophils and T cells. Tissue-invading inflammatory immune cells further generate ROS and cytokine/chemokine programs, which lead to the increase of adhesion molecule expression and elaboration of other DAMPs.

The adaptive immune system has long been thought to be independent in the pathogenesis of liver IRI. By using adoptive cell transfer system in CD4 T cell-deficient mice, Zwacka et al. first documented the contribution of host T cell immune repertoire for activation and regulation of the hepatic tissue pro-inflammatory response to IR-stress [38]. As CD4 T cells can promote innate immune activation by two mechanisms, i.e., inflammatory cytokines (e.g. IFN γ) or co-stimulatory molecule signaling (e.g. CD28), we have reported that CD4 T

cells function in liver IRI via CD154 without de novo antigen-specific activation, while innate immunity-induced CD40 up-regulation may trigger the engagement of CD154-CD40 to facilitate tissue inflammation/injury [38, 39]. Subsequently, we have documented that T cell immunoglobulin mucin family (Tim) and programmed cell death 1 (PD-1), which are currently attracting major attention as immune checkpoint molecules, regulates IR-induced sterile inflammation, as well hepatocellular damage in a murine OLT model [40–44]. Thus, despite the absence of exogenous antigen in hepatic IRI under sterile conditions or in syngeneic liver transplantation, CD4⁺ T cells may readily promote the pro-inflammatory phenotype of innate immune activation via crucial co-stimulatory signaling. Figure 1 depicts a simplified scheme of intricate innate - adaptive immune interplay in the mechanism of hepatic IRI.

The “classical” protective functions of HO-1

Heme and its degradation

The biosynthesis of heme, a complex of iron and protoporphyrin IX, occurs in erythroid cells and in the liver [45]. Under physiological conditions, heme provides indispensable cellular functions, but once released into the blood stream, it functions as a cytotoxic protein. In small amounts, free heme promptly binds to haptoglobin and is engulfed by phagocytic cells [46]. The large amounts of heme when released during hemolysis, associate with erythrocyte abnormalities, such as sickle cell disease, sepsis, and ischemia-reperfusion injury [47, 48]. Indeed, excessive heme causes tissue damage due to its ability to produce oxygen free radicals (OFRs), which in turn activate inflammatory cell cascades. Of note, heme may also trigger TLR4 signaling in endothelial cells resulting in the impairment of sinusoidal perfusion through adhesion molecule expression [47]; and may serve as a TLR4 ligand in macrophage proinflammatory function, leading to the secretion of TNF- α in a TLR4, MyD88 and CD14 dependent mechanism [49]. Thus, excessive heme accelerates the inflammatory cascade in TLR4-dependent manner.

HO-1 catabolizes heme complex into its metabolic byproducts (biliverdin, free iron and CO) under the control of the microsomal NADPH-cytochrome P-450 reductase. In the absence of HO-1, heme can cause cell/tissue injury, while heme catabolism exerts cytoprotective functions against oxidative stress in IR-stressed liver [19, 50]. The expression of HO-1 is induced by the several stimuli, such as ROS, heme, loss of cellular glutathione, hydrogen peroxide, LPS and hypoxia [51]. The acute hypoxia also triggers the transcriptional response by hypoxia inducible factor 1 alpha (HIF1 α), which in turn induces HO-1 to attenuate IR-mediated cellular damage [52, 53].

Biliverdin

BV, an intermediate produced in the process of heme degradation, is converted into bilirubin (BR) by biliverdin reductase. Both BV and BR have strong antioxidant properties through their ability to scavenge ROS [54]. Several studies have shown that BV may exert potent anti-inflammatory effects against IR-induced cerebral [55], kidney [56], and heart [57] injury. We also reported the protective function of BV in an *ex vivo* perfusion hepatic IRI model [50, 58], and other attempts have been successfully made to expand the application of

BV treatment to large animal hepatic IR models [59]. Notably, BV suppressed T cell proliferation by inhibition of IL-2 synthesis through NFAT/NF- κ B pathway [60]. Hence, BV and upstream HO-1 have a prominent protective function against not only innate immune-driven IRI but also host acute rejection adaptive immune-responses. Some even suggest that BV and BR system may modulate late chronic allograft rejection [57].

Free Iron and Ferritin

Free iron is toxic to cells due to generated harmful ROS through the Fenton reaction, which prompts rapid expression of the iron-binding proteins [61]. Since Ferritin is responsible for generating pools of iron and can be induced by stress conditions like hypoxia, it is considered an acute reactive protein that is involved in the homeostasis of intracellular concentrations of iron [62, 63]. Indeed, Adenovirus (Ad)-mediated overexpression of the ferritin heavy chain (H-ferritin) protected rat livers from IRI through an anti-apoptotic mechanism [64]. Interestingly, a recent retrospective clinical study documented that high serum ferritin (SF) levels in the donor was associated with increased risk of IRI in OLT recipients, suggesting that SF could be useful as a predictive biomarker of IRI [65]. This may also explain why iron overload might be harmful and result in poor long-term prognosis. Likewise, HO-1 overexpression does not necessarily impose cytoprotection in some disease states, such as neurodegeneration or carcinogenesis [6, 66].

Carbon Monoxide

Carbon monoxide (CO), one of the metabolites of heme degradation by HO-1, is extremely harmful at high doses because of its ability to bind hemoglobin and prevent oxygen supply to the organs. Unlike carbon dioxide, CO is almost insoluble in water. However, one of the beneficial effect of CO has been thought to maintain microcirculation due to its ability to modulate thrombomodulin (TM) or protein C [67–69], as well as endothelium-dependent vasodilatation and inhibition of platelet aggregation [70–72]. We and others have reported the novel anti-apoptotic effects of CO signaling, dependent on the activation of mitogen-activated protein kinase (MAPK) pathway [73, 74]. Recently, Sun et al. have shown that CO treatment mitigated hepatic IRI via inhibition of HMGB1 translocation under the control of SIRT1, referred to as a “longevity gene” [75]. Moreover, CO may also induce immunosuppression by inhibiting T cell proliferation and blocking IL-2 production [76] or by inducing an anti-apoptotic phenotype in regulatory T cells [77]. Importantly, the application of CO has been successfully expanded to large animal models, as evidenced by reduced pulmonary [78] and hepatic IRI [79], as well as prolonged survival of swine lung transplants [80]. Growing body of evidence indicates CO treatment can be one of promising candidates for the clinical use. The development of chemical CO releasing compounds, as well as clinical use of low dose CO therapy, as recently reported in the acute respiratory distress syndrome patients [81], should pave the way to determine the therapeutic usefulness of this gas in human inflammatory liver diseases. Figure 2 illustrates the heme degradation cascade.

“Non-classical” HO-1 macrophage pathway

HO-1 cytoprotection in BMDM

The role of HO-1 system as a putative target against innate immune-driven IRI has been extensively studied [19, 82]. Our group was the first to document the cytoprotective functions of exogenous HO-1 induction by pharmacological or gene induction by cobalt-protoporphyrin (CoPP) and adenoviral gene transfer, respectively, in a fatty Zucker rat liver transplantation model [18]. Indeed, HO-1 overexpression was required to maintain tissue integrity, preserve organ function, and improve liver transplant survival, at least in part by sparing graft-infiltrating HO-1 expressing macrophages [83]. We have also shown that HO-1 selectively mitigated infiltration by proinflammatory macrophages, and depressed caspase-3 expression while simultaneously increasing the anti-apoptotic hepatic phenotype [84]. In addition, systemic upregulation of HO-1 by CoPP or gene transfer ameliorated warm hepatic IRI by depressing Type 1 interferon while promoting anti-apoptotic pathway [85–87]. Based on these findings, we proposed that macrophages-derived HO-1 signaling may control the severity of hepatic IRI [13].

Consistent with the idea that macrophage HO-1 might regulate innate immune inflammation in OLT, we have shown that adoptive transfer of genetically HO-1 overexpressing BMDMs prior to the transplant, suppressed pro-inflammatory responses and promoted anti-apoptotic effects in a rat syngeneic OLT model [88]. We have identified macrophages (both infiltrating and Kupffer cell resident) in IR-stressed liver grafts as the main HO-1 producers not only in the rat [83] but also in human liver transplant patients [15]. Considering their inherent phagocytic functions, it seems reasonable that macrophages acquire a strong HO-1 production ability for self-protection [89]. These findings confirm the validity of a novel investigative tool in which native macrophages can be transfected *ex vivo* with cytoprotective HO-1 gene, and then infused, if needed, to prospective recipients to mitigate IR-mediated sterile inflammation, such as during liver transplantation, resection or trauma. As decreasing donor organ quality represents one of the most challenging problems in transplantation, one may then investigate a new and clinically attractive HO-1-based gene targeted strategy to “rejuvenate” extended criteria donor livers and thus improve their function in OLT recipients.

HO-1/SIRT1/Autophagy axis in liver IRI

Activation of silent information regulator factor 2-related enzyme 1 (SIRT1), a NAD⁺-dependent type III histone/protein deacetylase, is essential for tissue protection against IR-stress [90]. SIRT1 expression, up-regulated during starvation, under oxidative stress or DNA damage, plays a key role in the autophagy induction [91, 92]. Autophagy, an intracellular “self-cleaning” mechanism that has been in the limelight in recent years, helps to better understand basic hepatic functions, such as glycogenolysis, gluconeogenesis and β -oxidation [93]. Although autophagy was suppressed in hepatocytes under some conditions like metabolic disorders or oxidative stress, pharmacological activation of autophagy alleviated steatosis and hepatic injury in fatty liver mouse models [94, 95].

In our recent studies, macrophage – SIRT1 signaling axis was critical for HO-1 driven anti-inflammatory programs and M2 polarization in IR-stressed livers [15, 16]. Moreover, we have documented that HO-1 enhanced autophagy in IR-stressed livers, both in a clinically relevant mouse model and in human OLT patients [26]. In addition, disruption of SIRT1 depressed the autophagy enhancement in mouse liver grafts subjected to Ad-HO-1 gene transfer, suggesting that HO-1 regulates autophagy in a SIRT1-dependent manner. As a novel anti-inflammatory function of HO-1, we have since elucidated an essential role of macrophage HO-1 in the regulation of IRI-OLT via a SIRT1-autophagy pathway. We have reported that post-reperfusion HO-1 levels in liver grafts positively correlated with microtubule-associated protein light chain 3B (LC3B), one of the established autophagy markers. The expression of LC3B was observed mainly in hepatocytes, while HO-1 was predominantly expressed by infiltrating macrophages in IR-stressed livers. Moreover, bone marrow-derived macrophages overexpressing HO-1 by *ex vivo* gene transfer significantly increased liver graft LC3B protein levels. As the manipulation of HO-1 expression was limited primarily to non-parenchymal cells, these findings indicate that myeloid cell-derived HO-1 regulates autophagy pathway in IR-stressed hepatocytes. However, how macrophage HO-1 regulates the hepatic autophagy program awaits future investigation. Figure 3 depicts putative function of HO-1/SIRT1/ autophagy axis in liver IRI.

HO-1 in LT donor vs. recipient

Our interest in establishing HO-1-based application in clinical organ transplantation has led us to address whether HO-1 should be induced in the donor liver or OLT recipient. We analyzed the hepatic HO-1 expression level in fifty-one adult primary human OLT recipients. Liver biopsies were collected during cold storage (prior to implantation) and 2 hours after portal reperfusion (prior to skin closure). Consistent with our previous experimental data [15], post-operative but not pre-operative liver HO-1 expression negatively correlated with IRI severity in OLT patients. Although a statistically significant difference could not be obtained, when HO-1 expression before and after transplantation was compared between high and low groups in the median, pre-transplant HO-1 low group tended to have a better prognosis, whereas the post-transplant HO-1 low group showed worse clinical outcome. Of note, we found a strong correlation between pre-transplant and post-transplant HO-1 expression levels. In addition, when the post-transplant/pre-transplant HO-1 ratio, representing an index of peri-transplant HO-1 enhancement in the graft was analyzed, we discovered there was a positive correlation with post-transplant HO-1 expression. We hypothesized that pre-transplant HO-1 expression affected post-transplant HO-1 levels, and perioperative HO-1 increases but not basal levels of HO-1 were essential for post-transplant HO-1 as well as hepatoprotection in IR-stressed OLT. In parallel, taking into account that intrahepatic HO-1 expression is derived mostly from macrophages (donor-derived KCs or recipient-derived macrophages), we utilized myeloid-specific HO-1 deficient mice to explore as to whether and how recipient-derived HO-1 may affect liver graft HO-1 levels and hepatic function. Interestingly, recipient myeloid-specific HO-1 deficiency exacerbated the hepatocellular damage and accelerated pro-inflammatory cytokine programs as compared to WT counterparts with proficient myeloid-specific HO-1 expression. Although pre-operative HO-1 expression in the donor liver showed no correlation with the

recipient outcome in our study, Geuken et al. reported high expression of HO-1 in the donor liver negatively correlated with recipient outcomes [96]. In addition, Saat et al. demonstrated that both DCD and DBD rat livers were characterized by enhanced hepatic HO-1 gene expression at the time of procurement, while DBD livers exhibited higher pro-inflammatory gene expression, such as IL-1 β , TNF- α or IL-6. Interestingly, after 12 hours of cold storage, HO-1 expression in the DCD donor livers returned to that seen in the control group, whereas DBD donor livers still exhibited higher gene expression [97]. As for the effect of pre-transplant HO-1 on clinical OLT outcomes, further in-depth investigations are warranted.

Although there is no doubt about hepatoprotective effects of macrophage-derived HO-1, recent studies showed LSECs and hepatocytes have the ability to generate HO-1 against oxidative stress [98–100]. Indeed, HO-1 down-regulation by siRNA inhibited autophagy leading to hepatocyte apoptosis in vitro [101, 102], while HO-1 up-regulation by gene transfer protected hepatocytes from TNF- α induced cell death after ethanol stimulation [103]. Thus, despite relatively low expression levels in IR-stressed livers, HO-1 produced by hepatocytes or LSECs may certainly contribute to hepatoprotection in IR-stressed liver. To date, however, no HO-1 specific conditional knockout mice (e.g. hepatocyte-specific or LSECs-specific) has been tested in IRI-OLT settings.

Future prospects: From bench to bedside

The accruing clinical data supports the idea that selectively inducing HO-1 in host's macrophages may mitigate peri-transplant hepatic IR-stress and improve OLT outcomes. Other factors, such as HO-1 overexpression level and its duration should be carefully considered though, based on the pathophysiological condition as well as the recipient background. Indeed, HO-1 overexpression did not result in cytoprotection in some clinical settings [6]; while the toxicity of excessive HO-1 overexpression has been described in hamster fibroblasts [102]. In nerve cells, neurodegeneration can occur if HO-1 is overexpressed independent of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway [104]. One needs to keep in mind that HO-1 expression may be causatively associated with carcinogenesis/tumorigenesis or induce cytochrome P-450-centered hepatic dysfunction [105]. In our own experiments, successful chemical induction of HO-1 in the rat livers after a single dose of CoPP, could still be observed more than 100 days after OLT [18]. In the clinical setting, application of HO-1 still poses significant limitations because there is no evidence of its optimal expression levels or duration. Recently, promising autologous cell therapies with ex vivo gene engineered macrophages devoid of viral gene modulation and few side effects have been carried out to treat a variety of human tumors [106]. Experimental HO-1 macrophage-targeted therapies using Hb-Hp complex, which binds to a scavenger receptor CD163; or Heme-Hx complex, which is taken-up through a receptor mediated endocytosis [107] await confirmation in clinical trials.

Diverse HO-1 functions

A major obstacle to the clinical application of HO-1 is its diverse biological function. For instance, in liver transplant patients due to terminal malignant disease, anti-inflammatory HO-1 effects against IR-stress may prove detrimental and counterproductive as HO-1

overexpression can spoil the anti-tumor immunity, as shown by suppressing ICAM-1 signaling in colorectal carcinoma cells [108], inhibit apoptosis in renal cell carcinoma [109] or prompt cell proliferation in pancreatic cancers [110]. On the other hand, Zhou et al. has recently reported stable HO-1 expression suppressed the progression of hepatocellular carcinoma [111]. Moreover, Norberto et al. documented HO-1 expression negatively correlated with poor prognosis of breast cancer and its cytoplasm rather than nucleus localization being crucial for HO-1 mediated antitumor effects [112].

Common underlying mechanisms in liver IRI and pandemic metabolic disorders

The number of OLTs in Hepatitis C virus (HCV) patients is decreasing, while alcoholic liver disorders (ALD) and nonalcoholic-steatohepatitis (NASH) have been dramatically increasing [113]. The major problem with NASH, characterized by macrovesicular steatosis and lobular hepatitis with necrosis or ballooning degeneration and fibrosis, is that there are no subjective symptoms until the end stage, and when diagnosed, no effective treatment except for liver transplantation exists. Hence, there is an unmet need for novel preventive and treatment strategies against fatty liver disease in human patients. In a limited patient cohort, the HO-1 expression was significantly increased in NASH patients and the increase reflected the severity of the disease [114]. In addition, Jais et al. demonstrated that mice with hepatocyte or macrophage conditional HO-1 deletion evoked resistance to diet-induced insulin resistance and inflammation, dramatically reducing secondary disease such as steatosis and liver toxicity [9]. Surprisingly, in matched biopsies from “healthy” versus insulin-resistant obese human subjects, HO-1 expression was among the strongest positive predictors of metabolic disease [9]. Consistent with the latter, livers suffering from cirrhosis showed higher HO-1 expression in both animals and humans [115–117]. To the contrary, others have shown that CoPP-induced HO-1 prompted resistance to high fat diet and prevented liver steatosis in mice [118]. Collectively, these conflicting data provide insights as to how environmental stresses in human donor livers with increased HO-1 levels may dictate the donor liver tissue quality.

As discussed, HO-1 therapeutic effects in liver transplant inflammation are mediated/controlled by TLR4, SIRT1 and/or autophagy signaling pathways. However, TLR4/HMGB1 signaling, as well as SIRT1 gene and autophagy functions are closely interrelated to the pathogenesis of metabolic disorders, including liver steatosis following steatohepatitis [93, 119, 120]. Indeed, Li et al. demonstrated that liver specific SIRT1 deficient mice fed with high fat diet (HFD) exhibited severe steatosis with increased expression of genes regulating fatty acid oxidation, as compared with wild-type littermates [121]; while Pedersen et al. reported that SIRT1 mRNA levels were lower in adipose tissue of obese patients as compared with normal-weight controls [122]. It remains to be determined whether hepatic IR-inflammation, and NASH do indeed share a common underlying etiology via a putative HO-1 dependent mechanism.

Unlike in animal models, ‘normal’ human donor livers do not necessarily reflect a ‘healthy’ hepatic status. In other words, the human liver is often stressed (alcohol, high-fat diet,

various environmental factors, etc.) with inconsistent basal HO-1 levels, making it difficult to define what is truly ‘normal’. As discussed [123], relative changes of HO-1 expression, rather than the hepatic HO-1 steady state, may be crucial to exert ultimate cytoprotection against IR-stress in OLT. Moreover, one needs to keep in mind most experiments include HO-1 induction in ‘normal’ livers of inbred animals. This may contribute to apparent dissociation between human and murine centered HO-1 studies. For personalized and precise organ matching to reduce risks of IRI in OLT, HO-1 induction should be tailored to fit an individual’s immune status.

Concluding remarks

Recent bench-to-bedside translational studies provide new insights into HO-1 signaling in the mechanism of macrophage activation and hepatocellular damage in IRI-OLT. The immune phenotypes and pathways reviewed here are likely to be applicable to other types of liver inflammatory diseases, such as NASH, an emerging global epidemic projected to become the leading indication for liver transplantation. Thus, better understanding of the crosstalk between HO-1 signaling in the context of host innate – adaptive immune interface should open infinite possibilities to regulate redox signaling beyond the IR-stress in OLT.

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Abbreviations:

Ad	Adenovirus
BMDM	bone marrow-derived macrophage
BR	bilirubin
BV	biliverdin
CO	carbon monoxide
DAMPs	danger-associated molecular patterns
DBD	donation after brain death
DCD	donation after cardiac death
EAD	early allograft dysfunction
Hb	hemoglobin
HMGB1	high-mobility group box 1
HO-1	heme oxygenase-1
Hp	haptoglobin
Hsp	heat shock protein

Hx	hemopexin
IRI	ischemia-reperfusion injury
KCs	Kupffer cells
LPS	lipopolysaccharide
LSECs	liver sinusoidal endothelial cells
MAPK	mitogen-activated protein kinase
NHBD	non-heart beating donor
NPC	non parenchymal cell
OLT	orthotopic liver transplantation
PNF	primary non-function
PRRs	pattern recognition receptors
ROS	reactive oxygen species
SF	serum ferritin
TNF	tumor necrosis factor

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HIGHLIGHTS

- Hepatic ischemia-reperfusion injury (IRI) is a major risk factor in orthotopic liver transplantation (OLT), which affects early and late clinical outcomes.
- Heme oxygenase 1 (HO-1), induced under IR-stress, exerts potent cytoprotective and homeostatic functions in IRI-OLT.
- By activating intracellular SIRT1/autophagy molecular signaling axis, HO-1 overexpression is essential for self-defense mechanism in experimental and clinical OLT settings.
- Bench-to-bedside translational approach on the crosstalk between HO-1 signaling in the context of host innate – adaptive immune interface may offer novel insights to regulate redox signaling beyond the IRI in OLT.

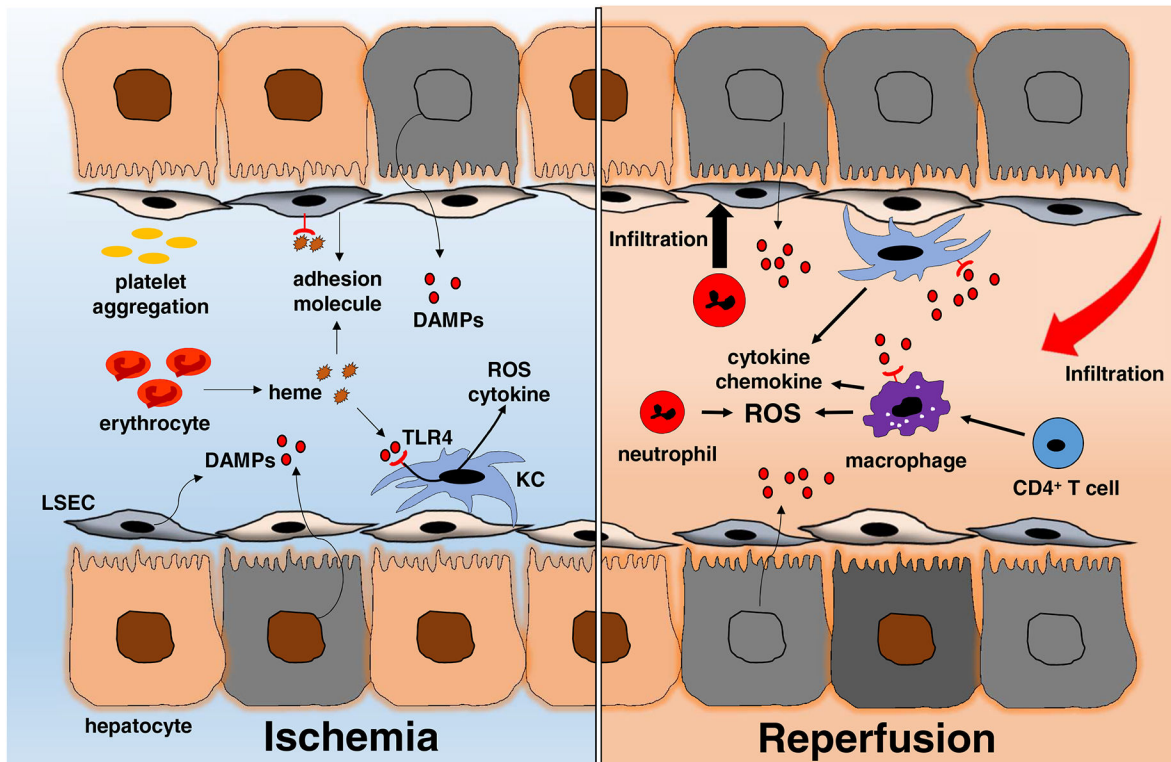


Figure 1:

The distinct stages of liver IRI. The ischemia insult, an initial process of hepatic disturbances, results from the lack of oxygen supply. Subsequently, DAMPs released by dead cells up-regulate the expression of adhesion molecules by liver sinusoidal endothelial cells (LSEC) and production of ROS by activated Kupffer cells. Excessive heme released from damaged erythrocytes may trigger inflammatory responses through TLR4-heme signaling axis. Reperfusion injury, triggered by oxygenation, accelerates hepatic IR-immune response via Kupffer cell activation in concert with monocyte-derived macrophage, T cell and neutrophil infiltration. The IRI proinflammatory immune cascade sustains itself by recruiting peripheral immune cells from the circulation.

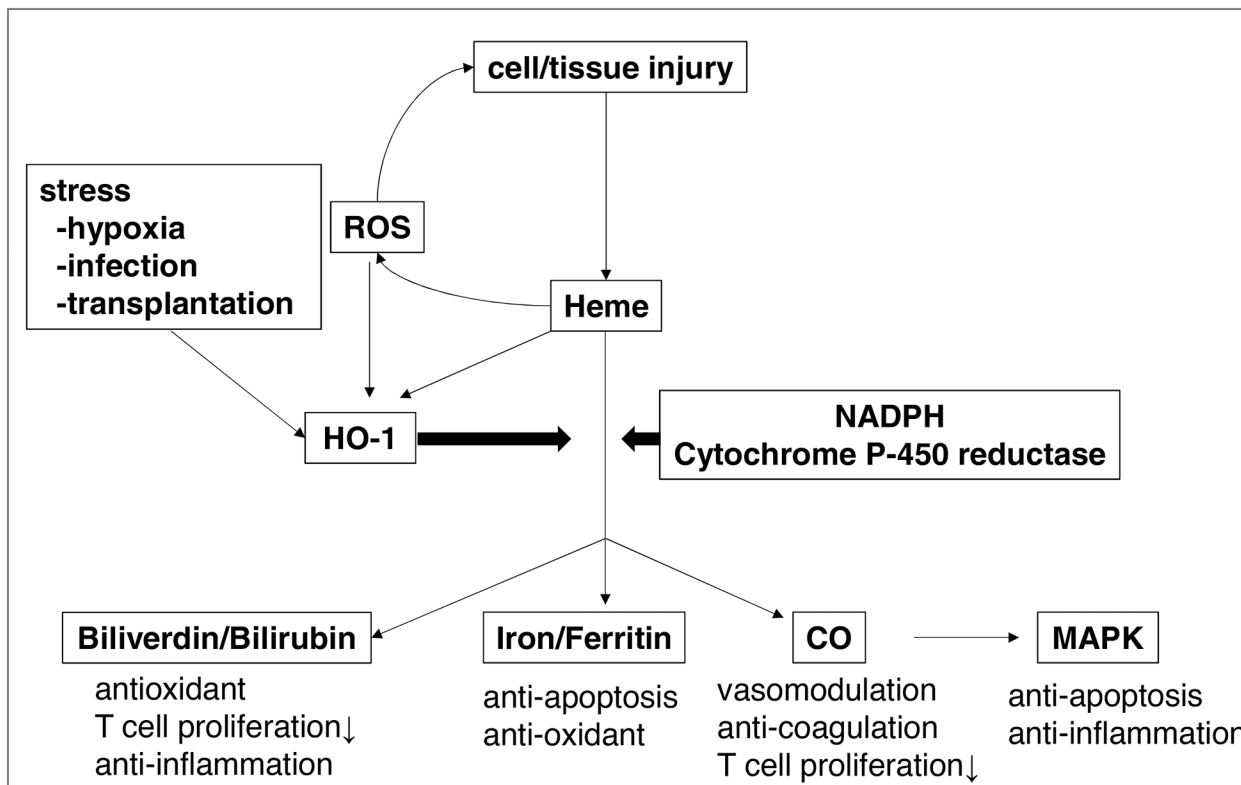


Figure 2:
 The heme degradation cascade. Heme released from damaged cells produces ROS. HO-1, induced by various stimuli such as heme, ROS, hypoxia, infection or transplantation, catabolizes heme into biliverdin, Fe²⁺ and CO. Biliverdin scavenges ROS to exert anti-inflammatory effects. Ferritin provides anti-apoptotic effects via its anti-oxidant function. CO promotes anti-apoptosis and anti-inflammatory effects via MAPK activation, modulates the vascular tone and inhibits platelet aggregation, leading to the microcirculatory maintenance.

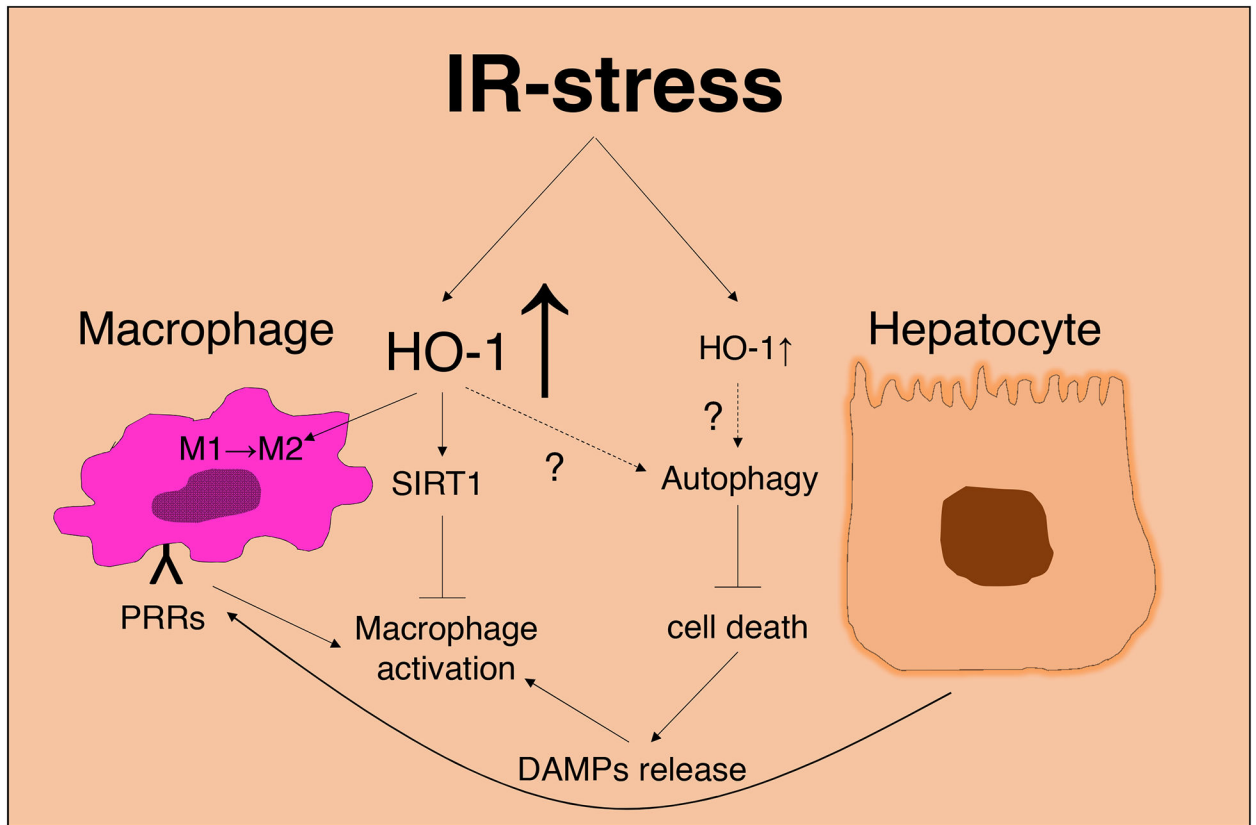


Figure 3:

IR-stress induced HO-1 in liver macrophage and hepatocyte function. Stressed hepatocytes release DAMPs, which activate macrophages through PRRs, leading to pro-inflammatory mediator release. By activating SIRT1, myeloid cell-derived HO-1 may also control macrophage polarization towards the anti-inflammatory phenotype. It remains unknown how macrophage HO-1 regulates this “cytoprotective” autophagy pathway in IR-stressed hepatocytes.