

## Therapeutic Potential of Heme Oxygenase 1 in Ischemia Reperfusion Injury

Tarik Bozoglu<sup>1</sup>, Rabea Hinkel<sup>1,2,3</sup> and Christian Kupatt<sup>1,3\*</sup>

<sup>1</sup>I. Medizinische Klinik, Klinikum Rechts der Isar, TUM, Germany

<sup>2</sup>Institute for Cardiovascular Prevention, LMU Munich, Germany

<sup>3</sup>DZHK (German Center for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany

\*Corresponding author: Christian Kupatt, Medizinische Klinik und Poliklinik, Klinikum Rechts der Isar, TUM Ismaningerstr, 22 81675 Munich, Germany, E-mail: [Christian.kupatt@tum.de](mailto:Christian.kupatt@tum.de)

Received date: June 02, 2016; Accepted date: June 07, 2016; Published date: June 14, 2016

Copyright: © 2016 Bozoglu T, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

### Abstract

Ischemia/reperfusion injury is a reaction occurring after successful treatment of an acute myocardial infarction or in the setting of solid organ transplantation. After successful reperfusion of a previously occluded vessel or a transplanted organ, an additional loss of otherwise vital cells may occur, the so-called lethal ischemia-reperfusion injury. This lethal reperfusion injury is based on rapid tissue oxygenation, leading to a release of reactive oxygen species (ROS) and inducing oxidative stress, endothelial cell activation and inflammation. Experimentally the reperfusion injury can be attenuated via ROS-scavenging, vessel stabilizing and anti-inflammatory interventions. Cytoprotective genes, such as heme oxygenase-1 (HO-1), offer a therapeutic approach to address this problem. Therefore, this review will focus on the beneficial effects of HO-1 in ischemia/reperfusion injury.

**Keywords:** HO-1; I/R injury; Kidney; Heart; Inflammation; Transplantation

### Introduction

With more than 550000 new cases each year only in the US, heart failure is a major component of cardiovascular disease [1]. As cardio protective measures are needed in the face of this epidemic to be utilized in treatment of heart failure and development of novel transplantation strategies; HO-1 offers a promising avenue for this purpose, especially considering the significant body of research evaluating it as a therapeutic target.

Ischemia, literally meaning restriction of blood, induces tissue damage through disruption of aerobic metabolism, inducing cell death via apoptosis and necrosis. Reperfusion of the ischemic tissue, meant to rescue the ischemic tissue, may induce additional damage via endothelial activation, thrombotic and inflammatory cell recruitment as well as ion imbalance and cardiomyocyte damage. The phenomenon is dubbed ischemia/reperfusion (I/R) injury, and has implications in wide-ranging clinical settings, including ischemic heart disease, organ transplantation or even remote organ damage. Molecular mechanisms include oxidative stress, calcium overload and intracellular acidification [2]. In endothelial cells reperfusion injury is characterized by an activation and an inflammatory phenotype. This activation leads to an increased production of reactive oxygen species, induction of vascular permeability and expression of cytokines and leukocyte adhesion factors [3]. Thus, ischemia-reperfusion injury is essentially an inflammatory process, therefore, modulation of inflammatory activation is an attractive therapeutic strategy (Figure 1). For this reason, antioxidant molecules have been intensively studied for potential immunomodulatory effects. Carotenoids, eg., being constituents of a normal diet have been screened for beneficial effects in atherosclerosis and cardiovascular disease, as reviewed by Ciccone

et al. [4]. Plasma carotenoid levels are reduced in various cardiovascular disease stages; but contradictory results are present for the protective effects of carotenoid supplementation.

Since directly targeting inflammatory cells has not proven efficient, due to redundancy of cytokines and adhesion molecules involved, and due to detrimental as well as healing functions of the same cell source in various phases of the inflammatory process, an alternative treatment approach would be cytoprotection in the affected tissue. As shown by the so-called "ischemic preconditioning", tissue subjected to limited I/R is driven to express protective genes that attenuates damage of further ischemic insults [5]. Among these genes, heme oxygenase-1 (HO-1) is particularly promising. Heme oxygenases catabolize free heme into Fe<sup>2+</sup>, CO and biliverdin, which would otherwise act as a damage signal. HO-1 is the inducible isoform; whose function becomes the rate-limiting step of free heme degradation under stress. Although the enzyme and its function has been known for a long time [6], recognition of its cytoprotective effects [7] has a shorter history. The allure of HO-1 as a protective gene is not only caused by its ability to hamper heme accumulation, but individual protective effects of the catalytic products of its enzymatic action, i.e. Fe<sup>2+</sup>, CO and biliverdin.

### HO-1 attenuates ischemia/reperfusion injury in the heart

In a study published by Pachori et al. [8] recombinant adeno-associated virus (rAAV) delivery of HO-1 to rat myocardium; followed by daily repetitive I/R through for five days; HO-1 treatment resulted in reduced wall thinning and better ejection fraction in comparison to LacZ controls, with similar levels of cardiac function to sham operated animals. Apoptosis and superoxide generation was also curbed in HO-1 expressing animals. Liu et al. [9] investigated long term effects of HO-1 rAAV delivery on left ventricular function. Acute I/R injury was induced in rat hearts by left anterior descending artery (LAD) ligation and subsequent reperfusion. In a one-year period, HO-1 treated

animals had twice the survival rate of LacZ controls. Left ventricular (LV) remodeling was alleviated in the treatment group according to echocardiographic and histological analyses, as was the LV function as shown by an invasive pressure-volume analysis in anesthetized animals.

A possible mechanism for HO-1 induced cardioprotection through attenuation of inflammation was suggested by data from the study of Zhao et al. [10] in a mouse model of diabetic cardiomyopathy. Systemic overexpression of HO-1 has decreased cardiac expression of inflammatory cytokines, IL-6 and TNF- $\alpha$ ; whereas transgenic mice with a mutant form of HO-1 displayed increased cytokine expression. Following up on this relation, recently, we have elucidated the inflammatory profile of HO-1 overexpressing cardiac tissue in mice [11]. After ischemia/reperfusion (I/R), we observed in HO-1 knockout mice higher levels of cytotoxic and phagocytic leukocyte recruitment, but not of the regenerative cells. Increase in infarct size and reduced contractile responsiveness upon adrenergic stimulation, as well as more TUNEL positive nuclei, was noted in the knockout hearts. Application of recombinant adeno-associated viral delivery of human HO-1 (rAAV.hHO-1) rescued the phenotype by shifting the immune response from cytotoxic to repair phase, reducing pro-inflammatory neutrophilic and monocytic populations and increasing proangiogenic cells.

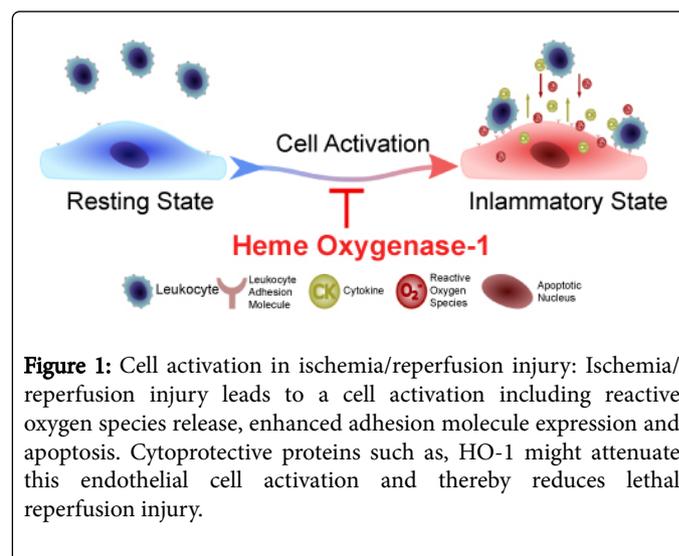
The revelation of inflammatory modulation in HO-1 in knockout mice, concordant with the anti-inflammatory effects of HO-1 overexpression in endothelial cells under flow conditions, led to investigating the cardioprotective potential of HO-1 in a porcine model of acute myocardial infarction. Overexpression of hHO-1 was achieved by generating transgenic pigs ubiquitously expressing hHO-1 as well as by regional application of rAAV.hHO-1. Both methods yielded similar levels of expression as quantified by qPCR. Strikingly, after I/R, transgenic and rAAV treated animals displayed reduced levels of myeloperoxidase expressing cytotoxic neutrophils in the ischemic areas. A similar decrease was also observed in CD14+ monocytes. An inhibitor of HO-1 activity, zinc protoporphyrin (ZnPP) abolished this effect.

Analysis of cardiac function in hHO-1 overexpressing pig hearts indicated a cardioprotective effect in I/R injury. Structurally, infarct size was abrogated and TUNEL-positive nuclei were found in reduced quantities. Functional preservation was improved in transgenic animals as seen by a preserved left ventricular ejection fraction and reduced end diastolic left ventricular blood pressure. Finally, sonomicrometric measurements showed improved contractile function in the ischemic areas. Reversal of these phenomena by regional application of ZnPP reinforces the causality of the HO-1 treatment and the observed effects.

Evidence of enhanced microvasculature preservation after I/R in HO-1 transgenic pigs was found by PECAM1 staining in infarct border zone. The increase in capillary density was observed in both constitutive transgenic and rAAV.hHO-1 treated animals. As the rAAV serotype 2/9, which we used in our study, has a clear myotropism [12], this effect is mainly dependent on the cardiomyocyte compartment. Indeed, we have shown that in vitro culture of endothelial cells with pre-conditioned media from HO-1 overexpressing myocytes displays improved cell survival after hypoxia and reoxygenation.

Although our study demonstrates the cardioprotective properties of HO-1 against innate immune driven I/R damage, utility of any molecule of therapeutic potential in the context of transplantation

must address the differing inflammatory mechanics of the latter. Innate immunity presents with an early obstacle in a xenotransplantation setting, whereas mitigation of the adaptive immune system is essential for long term survival of both xeno- and allografts. As the ability of HO-1 to abrogate endothelial cell activation has been demonstrated in our study as well as others [13] (Figure 1), it presents a noteworthy target in transplantation technology.



**Figure 1:** Cell activation in ischemia/reperfusion injury: Ischemia/reperfusion injury leads to a cell activation including reactive oxygen species release, enhanced adhesion molecule expression and apoptosis. Cytoprotective proteins such as, HO-1 might attenuate this endothelial cell activation and thereby reduces lethal reperfusion injury.

### Kidney ischemia/reperfusion injury is diminished by HO-1

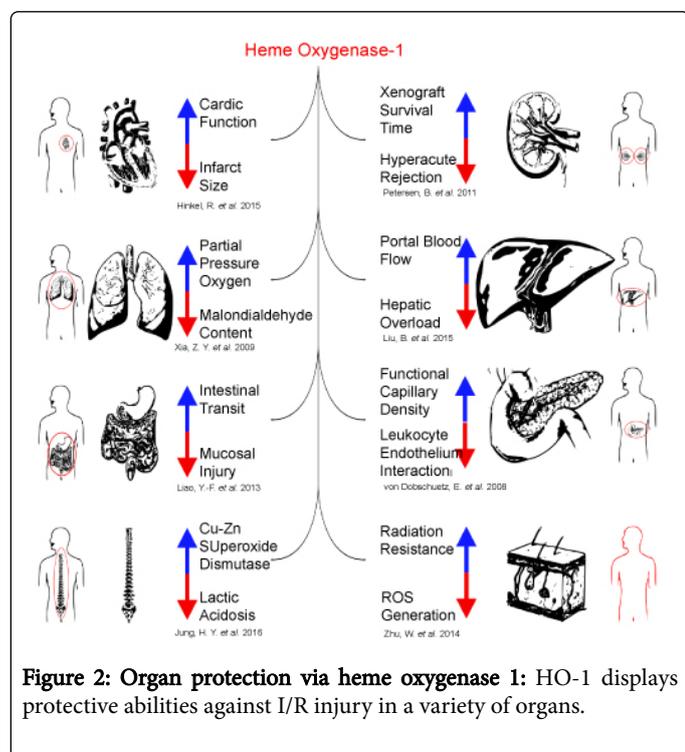
In a complementary study [13] utilizing the same hHO-1 transgenic pig model, ischemia/reperfusion injury following organ transplantation was mirrored. Here, porcine kidneys underwent ex-vivo perfusion with human blood. In wild type kidneys the renal vascular resistance was remarkably increased in contrast to the alloperfused setting. Strikingly, hHO-1 transgenic kidneys mirrored the latter upon perfusion with human blood. Coagulation analyses revealed reduced consumption of anti-thrombin and fibrinogen in transgenic kidneys. Taken together with the reduced activation of HO-1 overexpressing endothelial cells, these results indicate a protective effect of HO-1 on graft endothelium.

The *ex vivo* perfusion setting employed in the study [13] was limited to short term effects, as alloperfusion was terminated at 240 minutes. This duration was more than sufficient as the wild type porcine kidneys perfused with human blood lasted circa one hour. If the complement system is impaired with C1 inhibitor, the survival increased to two hours. In contrast, the hHO-1 transgenic kidneys with complement inhibition could survive up to four hours. Surprisingly, even when the C1 inhibitor was omitted, the HO-1 transgenic kidneys markedly resisted hyperacute rejection, surviving in excess of three hours. Additionally, this resistance was effected despite similar levels of complement activation in wild type and transgenic kidneys.

More recently, the same group developed a pig expressing the protective genes HO-1 and A20 and a knock-out for GGTA1 [14]. In the *ex vivo* perfusion studies, kidneys from transgenic animals displayed low renal vascular resistance and prolonged survival comparable to autologous perfusion. This model may demonstrate feasibility of multitransgenic porcine xenotransplant donors.

## HO-1 improves solid organ ischemia/reperfusion injury

Along with the ischemic heart disease and development of xenotransplants, ischemia/reperfusion injury is also clinically relevant in allografts used in end stage organ diseases. During transplantation, donor organ in anoxic, cold storage conditions is perfused with oxygenated, warm blood of the host, displaying the same activation of endothelium, which contributes to the possibility of rejection. In addition to cardiac and renal tissues as reviewed here, HO-1 has been shown to be protective against I/R injury in the liver [15], gastrointestinal tract [16], spinal cord neurons [17], skin [18], pancreas [19] and lung [20] (Figure 2). With such ubiquitous cytoprotective effects, HO-1 may present with an appealing target in transplant pretreatment. Indeed, Ma et al. [21] demonstrates delivery of recombinant HO-1 with a protein transduction domain to rat heart grafts in cold preservation, which effects increased graft survival time under preservation, as well as reduced I/R injury after transplantation.



## Outlook and Clinical Perspective

Diverse clinical challenges of protection against ischemic heart disease, successful organ transplantation and realization of clinically relevant xenotransplantation share I/R injury as common component, against which heme oxygenase-1 may present as a feasible treatment option. To achieve therapeutic protection, HO-1 application or induction needs to be timely associated to the reperfusion event. Therefore, further investigations are needed to determine if strategies that activate HO-1 have a clinical impact on the event of acute myocardial infarction or solid organ transplantation.

## References

- Roger VL (2013) Epidemiology of Heart Failure. *Circulation Research* 113: 646-659.

- Hausenloy DJ, Yellon DM (2013) Myocardial ischemia-reperfusion injury: a neglected therapeutic target. *The Journal of Clinical Investigation* 123: 92-100.
- Timmers L, Pasterkamp G, de Hoog VC, Arslan F, Appelman Y, et al. (2012) The innate immune response in reperfused myocardium. *Cardiovasc Res* 94: 276-283.
- Ciccone MM, Cortese F, Gesualdo M, Carbonara S, Zito A, et al. (2013) Dietary Intake of Carotenoids and Their Antioxidant and Inflammatory Effects in Cardiovascular Care. *Mediators of Inflammation* 2013: 782137.
- Camara NO, Soares MP (2005) Heme oxygenase-1 (HO-1), a protective gene that prevents chronic graft dysfunction. *Free Radic Biol & Med* 38: 426-435.
- Tenhunen R, Marver HS, Schmid R (1968) Enzymatic Conversion of Heme to Bilirubin by Microsomal Heme Oxygenase. *P Natl Acad Sci USA* 61: 748-755.
- Nath KA, Balla G, Vercellotti GM, Balla J, Jacob HS, et al. (1992) Induction of heme oxygenase is a rapid, protective response in rhabdomyolysis in the rat. *J Clin Invest* 90: 267-270.
- Pachori AS, Melo LG, Zhang L, Solomon SD, Dzau VJ, et al. (2006) Chronic recurrent myocardial ischemic injury is significantly attenuated by pre-emptive adeno-associated virus heme oxygenase-1 gene delivery. *J Am Coll Cardiol* 47: 635-643.
- Liu X, Simpson JA, Brunt KR, Ward CA, Hall SR, et al. (2007) Preemptive heme oxygenase-1 gene delivery reveals reduced mortality and preservation of left ventricular function 1 yr after acute myocardial infarction. *Am J Physiol Heart Circ Physiol* 293: 48-59.
- Zhao Y, Zhang L, Qiao Y, Zhou X, Guodong W, et al. (2013) Heme Oxygenase-1 Prevents Cardiac Dysfunction in Streptozotocin-Diabetic Mice by Reducing Inflammation, Oxidative Stress, Apoptosis and Enhancing Autophagy. *PLoS ONE* 8: e75927.
- Hinkel R, Lange P, Petersen B, Gottlieb E, Ng JK, et al. (2015) Heme Oxygenase-1 Gene Therapy Provides Cardioprotection Via Control of Post-Ischemic Inflammation: An Experimental Study in a Pre-Clinical Pig Model. *J Am Coll Cardiol* 66: 154-165.
- Pleger ST, Shan C, Ksienzyk J, Bekeredian R, Boekstegers P, et al. (2011) Cardiac AAV9-S100A1 gene therapy rescues post-ischemic heart failure in a preclinical large animal model. *Sci Transl Med* 3: 92-64.
- Petersen B, Ramackers W, Lucas-Hahn A, Lemme E, Hassel P, et al. (2011) Transgenic expression of human heme oxygenase-1 in pigs confers resistance against xenograft rejection during ex vivo perfusion of porcine kidneys. *Xenotransplantation* 18: 355-368.
- Ahrens HE, Björn P, Wolf R, Stoyan P, Doris H, et al. (2015) Kidneys From  $\alpha$ 1,3-Galactosyltransferase Knockout/Human Heme Oxygenase-1/ Human A20 Transgenic Pigs Are Protected From Rejection During Ex Vivo Perfusion With Human Blood. *Transplantation Direct* 1: 1-8.
- Liu B, Qian JM (2015) Cytoprotective role of heme oxygenase-1 in liver ischemia reperfusion injury. *Int J Clin Exp Med* 8: 19867-19873.
- Liao YF, Zhu W, Li DP, Zhu X (2013) Heme oxygenase-1 and gut ischemia/reperfusion injury: A short review. *World J Gastroenterol* 19: 3555-3561.
- Jung HY, Kim WD, Yim HS, Yoo DY, Kim JW, et al. (2016) Heme Oxygenase-1 Protects Neurons from Ischemic Damage by Upregulating Expression of Cu,Zn-Superoxide Dismutase, Catalase, and Brain-Derived Neurotrophic Factor in the Rabbit Spinal Cord. *Neurochemical research* 41: 869-879.
- Zhu W, Xu J, Ge Y, Cao H, Ge X, et al. (2014) Epigallocatechin-3-gallate (EGCG) protects skin cells from ionizing radiation via heme oxygenase-1 (HO-1) overexpression. *J Radiat Res* 55: 1056-1065.
- Dobschuetz VE, Schmidt R, Scholtes M, Thomusch O, Schwer CI, et al. (2008) Protective role of heme oxygenase-1 in pancreatic microcirculatory dysfunction after ischemia/reperfusion in rats. *Pancreas* 36: 377-384.
- Xia ZY, Gao J, Ancharaz AK (2009) Protective effect of ischemic postconditioning on lung ischemia-reperfusion injury in rats and the role of heme oxygenase-1. *Chin J Traumatol* 12: 162-166.

21. Ma J, Lau CK, Obed A, Dada A, Doenecke A, et al. (2009) A cell penetrating heme oxygenase protein protects heart graft against ischemia/reperfusion injury. Gene Ther 16: 320-328.