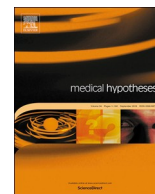




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# Heme oxygenase-1 (HO-1) cytoprotective pathway: A potential treatment strategy against coronavirus disease 2019 (COVID-19)-induced cytokine storm syndrome



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## ABSTRACT

The outbreak of coronavirus disease 2019 (COVID-19) requires urgent need for effective treatment. Severe COVID-19 is characterized by a cytokine storm syndrome with subsequent multiple organ failure (MOF) and acute respiratory distress syndrome (ARDS), which may lead to intensive care unit and increased risk of death.

While awaiting a vaccine, targeting COVID-19-induced cytokine storm syndrome appears currently as the efficient strategy to reduce the mortality of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The stress-responsive enzyme, heme oxygenase-1 (HO-1) is largely known to protect against inflammatory response in animal models. HO-1 is induced by hemin, a well-tolerated molecule, used for decades in the treatment of acute intermittent porphyria. Experimental studies showed that hemin-induced HO-1 mitigates cytokine storm and lung injury in mouse models of sepsis and renal ischemia-reperfusion injury. Furthermore, HO-1 may also control numerous viral infections by inhibiting virus replication.

In this context, we suggest the hypothesis that HO-1 cytoprotective pathway might be a promising target to control SARS-CoV-2 infection and mitigate COVID-19-induced cytokine storm and subsequent ARDS.

## Background

### COVID-19 and cytokine storm

The coronavirus disease 2019 (COVID-19) was first described in Wuhan, China, in December 2019 and the outbreak has rapidly spread across the world. COVID-19 is caused by a novel beta-coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1,2]. Most of COVID-19 cases (about 80%) develop mild symptoms while 5% of infected patients have severe disease characterized by acute respiratory distress syndrome (ARDS) and multiorgan damage [1]. Through the binding of angiotensin-converting enzyme 2 (ACE2) receptor, SARS-CoV-2 targets lung and other organs (e.g., heart, kidney, intestine, brain, liver, and blood vessels), which may lead to subsequent multiple organ failure (MOF) and intensive care unit (ICU) requirement [1,2]. The mortality of ICU patients is mainly due to ARDS and increases to 60% [3,4].

Compelling evidence suggest that cytokine storm syndrome plays a

critical role in severe COVID-19 [5]. Indeed, proinflammatory cytokines/chemokines such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-2, IL-6, IL-7, granulocyte-colony stimulating factor (G-CSF), interferon gamma-induced protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein 1- $\alpha$  (MIP-1 $\alpha$ ) are elevated in plasma of COVID-19 patients, particularly in severe cases [4,6,7]. This cytokine storm may trigger an uncontrolled systemic inflammatory response, which also contributes to ARDS and MOF leading to death [8].

While awaiting a vaccine, targeting COVID-19-induced cytokine storm syndrome appears currently as the efficient strategy to reduce the mortality of SARS-CoV-2 and limit the overload of ICU.

In this context, the use of glucocorticoids as an immunomodulatory therapy remains a current matter of debate. On the one hand, glucocorticoids might exacerbate COVID-19-associated lung injury, but on the other hand, short course of treatment is suggested for moderate-to-severe COVID-19-induced ARDS [5,9].

IL-6 has recently emerged as a key target due to its critical role in

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cytokine storm syndrome and subsequent disease severity [10]. Hence, numbers of clinical trials using tocilizumab (IL-6 receptor blockade) have been approved and in progress (e.g., ChiCTR2000029765, NCT04317092, NCT04346355, NCT04335071).

### Heme oxygenase-1 (HO-1)

Heme oxygenase-1 (HO-1, encoded by *Hmox1*) is a stress-induced enzyme that metabolizes free heme into carbon monoxide, biliverdin, and iron. Through its byproducts, HO-1 exhibits cytoprotective, anti-apoptotic, and immunomodulatory properties that may modulate diseases involving inflammation [11]. HO-1 controls the immune response, for instance, by stimulating the expression of IL-10, the well-known anti-inflammatory cytokine and by enhancing macrophage polarization toward an anti-inflammatory (i.e. M2) phenotype [12–15]. HO-1 also mediates immune response through IRF3 and subsequent IFN $\alpha/\beta$  production, which may induce IL-10 and reduce the production of inflammatory cytokines [16]. Interestingly, HO-1 is induced by hemin (i.e. synthetic heme), a molecule which is well tolerated with low rate of side effects and has been approved by the US Food and Drug Administration for the treatment of acute intermittent porphyria [17,18].

### Hypothesis

We propose an approach to modulate SARS-CoV-2 infection and the subsequent cytokine storm by stimulating an anti-inflammatory pathway. Based on current literature, hemin-induced HO-1 cytoprotective pathway appears as a consistent target to control COVID-19.

### Evidence supporting HO-1 as a potential target

#### *HO-1 as modulator of inflammatory response in animal models*

Sepsis and ischemia-reperfusion injury (IRI) are interesting models to study inflammation. They combine major cell stress, significant burst of free radicals, and strong inflammatory responses comparable to COVID-19-induced cytokine storm, suggesting that findings about these models might be used as potential therapeutic strategy against SARS-CoV-2.

Sepsis is characterized by a systemic inflammatory response syndrome with overexpression of proinflammatory cytokines, which may lead to lethal MOF [19]. In this context, HO-1 has shown protective anti-inflammatory properties [11]. Through down-regulation of proinflammatory cytokines (i.e., IL-1 $\beta$  and TNF- $\alpha$ ), HO-1 induction by using hemin protects mice from lethal endotoxemia and sepsis induced by liposaccharide (LPS) or cecal ligation and puncture [19]. Furthermore, overexpression of HO-1 has also been demonstrated protective against LPS-induced lung injury [11].

Preemptive induction of HO-1 by using hemin is largely known to be an efficient protective strategy against renal IRI in animal models [20,21]. Renal IRI also promotes systemic release of pro-inflammatory cytokines (e.g., IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) that induces a systemic inflammatory response, resulting in proinflammatory cells recruitment and remote organ damage, particularly in lung [22].

Basically, hemin-induced HO-1 improves renal outcomes after renal IRI by decreasing level of various renal proinflammatory cytokines, including IL-1 $\beta$ , IL-6, TNF- $\alpha$ , KC (keratinocyte chemoattractant also called CXCL1, a chemokine involved in neutrophils influx), and MCP-1 [20,22]. Moreover, it has been shown that hemin-induced HO-1 reduces IRI-induced cytokine storm and subsequent lung injury by decreasing plasma level of IL-6 and KC, and lung inflammation (neutrophils influx and lung KC) [22].

### *Resident- and circulating-macrophages are critical for HO-1 anti-inflammatory properties*

Hemin-mediated protection against renal IRI requires specific expression of HO-1 within myeloid cells (i.e., CD11b<sup>+</sup> F4/80<sup>lo</sup> macrophages) [20]. Interestingly, this myeloid cell sub-population was observed in the kidney and spleen, suggesting that protective effects might be provided by both tissue-resident and infiltrating/circulating HO-1<sup>+</sup> myeloid cells [20]. In term of lung injury, resident alveolar macrophages (AMs) prevent lung inflammation and repair tissue damage through several anti-inflammatory mechanisms including HO-1 [23]. Then, *in vitro* hemin significantly induced HO-1 expression in primary rat AMs [23]. Moreover, it was shown that M2 macrophages promote recovery in sepsis-induced lung injury through overexpression of anti-inflammatory cytokines [24]. By inference, tissue-resident AMs expressing HO-1 might explain the mitigation of renal IRI/sepsis-induced lung injury observed with hemin. Altogether, these data show the importance of lung-resident macrophages, which might be targeted by hemin to mitigate local inflammation and subsequent ARDS following cytokine storm.

Otherwise, splenectomy was associated with an exacerbated pro-inflammatory response and lung injury after renal IRI due to decreased splenic IL-10 production, suggesting that circulating macrophages are also involved in the control of lung injury [25]. HO-1<sup>+</sup> spleen myeloid cells might therefore reduce cytokine storm and constitute a reservoir that might be recruited to remote injured lung and dampen subsequent ARDS. Accordingly, these observations suggest that hemin-mediated improvement of lung injury following systemic inflammatory response might also be provided by both tissue-resident and infiltrating/circulating HO-1<sup>+</sup> M2 macrophages.

### *Antiviral effect of HO-1*

A recent study has highlighted the antiviral effect of HO-1 against influenza viruses. Indeed, authors showed that cobalt protoporphyrin (CoPP), a potent HO-1 inducer similar to hemin, inhibits influenza A virus replication through HO-1 interaction with IRF3 and subsequent expression of IFN $\alpha/\beta$  [26]. A same mechanism was found in human respiratory syncytial virus infection with attenuation of viral replication and lung inflammation upon HO-1 induction and expression of IFN $\alpha/\beta$  in the infected lung [27]. Of note, HO-1-mediated type I IFN response may control numerous of other viral infections, such as hepatitis B/C virus, Ebola virus, and human immunodeficiency virus by inhibiting virus replication [26]. By inference, these data suggest that hemin-induced HO-1 may be also used to overcome the outbreak of COVID-19 by inhibiting SARS-CoV-2 replication.

### *Hemin use in humans*

Hemin was shown to increase efficiently HO-1 protein expression and activity in humans [17]. Currently, hemin is only approved for the treatment of acute intermittent porphyria by the US Food and Drug Administration [18]. Interestingly, hemin is a well-tolerated molecule with low rate of adverse effects, such as headache, fever, and phlebitis at the site of infusion [17,18]. Recently, hemin safely induced HO-1 in renal transplant recipients and further studies are expected to determine the impact of HO-1 expression on clinical outcomes [28].

### Discussion

#### *Dual effect of hemin*

The timing of hemin administration is a critical point to consider in this hypothesis. Indeed, hemin-induced HO-1 protects against renal IRI when hemin is given preemptively to renal insult (i.e. hemin preconditioning) [20,21]. However, hemin does not protect kidney and

even worsened renal insult when acute kidney injury is already established [29]. Hemin may therefore have a dual effect, protective or deleterious, depending on the timing of its administration.

#### *Hemin-induced HO-1 therapy: a polymorphism dependency*

The polymorphism of human *Hmox1* gene should be carefully considered. Indeed, polymorphisms of guanosine thymidine dinucleotide (GT)<sub>n</sub> repeats in the promoter of *Hmox1* are inversely correlated with HO-1 mRNA level and enzyme activity [11]. Individuals carrying the long (L) allele [(GT)<sub>n</sub> ≥ 30] display impaired transcriptional regulation and expression of HO-1 [11]. This genetic variation influences the ability to induce HO-1 and, thereby, hemin treatment efficiency. Of note, HO-1 was found to be elevated in the lungs of patients with ARDS and *Hmox1* promoter polymorphisms also influence the occurrence of ARDS [30,31]. Polymorphisms in HO-1 might be therefore involved in the heterogeneity reported in critically ill COVID-19 patients, and it even more influences susceptibility to various human diseases (e.g., cardiovascular disease, necrotizing acute pancreatitis, chronic obstructive pulmonary disease) [11]. Accordingly, these data provide critical information about eventual pharmacologic targeting of HO-1 in COVID-19<sup>+</sup> patients.

#### *Hypothesis testing*

We would perform a clinical study with hospitalized severe COVID-19<sup>+</sup> patients, which would be randomized into hemin and placebo groups. Patients would be monitored clinically and by usual laboratory tests and plasma cytokines/chemokines/HO-1 measurement. Although it would be practically difficult, we think that polymorphisms in HO-1 should be considered to assess rigorously hemin treatment efficiency. DNA fragments would be extracted from peripheral blood stem cells, and the *Hmox1* locus containing the GT repeat would be amplified by using polymerase chain reaction (PCR).

Based on current knowledge about hemin pharmacology in humans, we propose intravenous dose of 3–4 mg/kg/day (maximum dose of 6 mg/kg/day) similar to that recommended for treating acute intermittent porphyria [17,18]. The duration of the treatment should be considered according to clinical response (e.g. 3–14 days for the treatment of acute intermittent porphyria) [17,18]. Due to its dual effect, hemin should be administered on the onset of respiratory symptoms to prevent ARDS and subsequent overloaded ICU. Hence, we do not recommend hemin use in case of established ARDS because it might worsen the disease based on experimental data [29].

#### **Conclusion**

With respect to current literature, there is a series of compelling evidence indicating a potential role for hemin-induced HO-1 as a treatment strategy against COVID-19-induced cytokine storm syndrome. Conversely to tocilizumab and glucocorticoids, hemin-induced HO-1 is able to mitigate cytokine storm and subsequent ARDS with a deciphered mechanism, by targeting wide range of proinflammatory mediators in animal models of sepsis and IRI. Moreover, due to its antiviral properties, hemin-induced HO-1 might be an interesting target to control the outbreak of COVID-19 by inhibiting SARS-CoV-2 replication.

Obviously, the relevance and translation of animal/*in vitro* findings to humans require further investigations. However, hemin efficiently induces HO-1 in humans and is used safely for decades in the treatment of acute intermittent porphyria and recently in renal transplant [17,18,28]. Due to the low rate of adverse events, hemin appears to be a safer treatment than glucocorticoids. Furthermore, glucocorticoids might exacerbate COVID-19-associated lung injury [5,9]. Hence, hemin-induced HO-1 might be a harmless, novel, and promising approach for controlling SARS-CoV-2 infection and limiting cytokine

storm syndrome with subsequent ARDS following COVID-19.

#### **Contribution**

MR did the literature search and drafted the paper. MR, MP, AVM, and KZB put forward the hypothesis. MP, AVM, and KZB revised the manuscript.

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#### **Declaration of interests**

The authors of this manuscript have no conflict of interest to disclose.

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