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Review

Haptoglobin as a multifaceted therapeutic agent targeting hemolysis-induced multi-organ injury

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ABSTRACT

Haptoglobin (Hp) is an acute-phase glycoprotein and the primary endogenous scavenger of cell-free hemoglobin. By forming stable Hp–hemoglobin complexes cleared by the CD163 receptor, Hp protects tissues from oxidative damage, heme toxicity, and nitric oxide depletion. Beyond scavenging, Hp exhibits anti-inflammatory properties and supports iron homeostasis and vascular integrity. This review summarizes Hp's molecular biology and its therapeutic potential in hemolysis-related conditions, including sepsis, sickle cell disease, acute kidney injury, aneurysmal subarachnoid hemorrhage, and extracorporeal circulation procedures. Experimental studies confirm that Hp supplementation reduces oxidative stress and organ injury, while emerging clinical data suggest benefits in lowering plasma-free hemoglobin. We highlight recent progress in recombinant Hp production, combination therapies, engineered scavenger proteins, and genotype-guided strategies. However, significant challenges remain, including the lack of randomized clinical trials, optimal dosing protocols, genotype variability, and long-term safety data. Overall, Hp represents a promising biologic platform targeting hemolysis-induced pathology. Future research must prioritize large-scale multicenter trials and next-generation scavenger proteins to realize the full therapeutic potential of Hp-based interventions.

1. Introduction

Hemolysis releases intracellular erythrocyte components, particularly hemoglobin (Hb), into the circulation. Although endogenous scavenging systems rapidly remove these products under physiological conditions, excessive or sustained hemolysis can overwhelm these defenses, leading to the accumulation of cell-free Hb (cfHb) and free heme in plasma and tissues.[1] These extracellular hemoproteins contribute to oxidative stress, inflammation, endothelial dysfunction, and multi-organ injury,[2] and are increasingly recognized as important mediators of morbidity in both acute and chronic hemolytic conditions.[3,4] This cfHb toxicity is primarily mediated by nitric oxide (NO) consumption, redox reactions, and the release of free heme.[2] Together, these processes promote vascular dysfunction, oxidative injury, inflammation, and tissue damage, supporting therapeutic strategies that neutralize extracellular Hb and its downstream toxic products.[5–7]

Haptoglobin (Hp) is an endogenous acute-phase glycoprotein and the principal plasma scavenger of cfHb. By binding extracellular Hb and facilitating its controlled clearance, Hp limits Hb-mediated oxidative, inflammatory, and vascular injuries, providing a strong biological basis for its therapeutic development.[8–10] Beyond cfHb scavenging, experimental studies suggest that Hp exerts broader antioxidant, immunomodulatory, vascular-protective, and cytoprotective effects.[1,2] These findings have stimulated interest in Hp-based interventions across a range of hemolysis-associated disorders, although the strength of therapeutic evidence varies substantially among disease settings.[11–13]

Despite these promising findings, clinical translation of Hp-based therapies remains limited. Major challenges include the scarcity of large-scale randomized controlled trials (RCTs), limitations of plasma-derived products, incomplete understanding of genotype-specific pharmacokinetics, and uncertainty regarding optimal dosing.[13] Recombinant and engineered Hp products also face substantial manufacturing and regulatory challenges.[2] This review critically examines Hp as a multi-targeted therapeutic molecule for hemolysis-induced multi-organ damage. We briefly describe the molecular mechanisms of protection and evaluate evidence from pre-clinical and clinical trials. Emerging therapeutic platforms, including recombinant Hp and dual-scavenging ApoHb–Hp complexes, are discussed. Finally, the article outlines the challenges

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and opportunities for advancing these therapies toward clinical application.

2. Methodology

This manuscript presents a narrative review summarizing current knowledge of the biological functions, mechanisms, and therapeutic potential of Hp in hemolysis-induced multi-organ injury. A structured literature search was performed in PubMed/MEDLINE, Scopus, and Web of Science Core Collection for studies published from January 2000 to June 2026. The search strategy combined keywords related to Hp biology, hemolysis, cfHb toxicity, heme-mediated injury, and therapeutic applications, including “haptoglobin”, “Hp”, “haptoglobin therapy”, “hemolysis”, “intravascular hemolysis”, “cell-free hemoglobin”, “heme toxicity”, “haptoglobin–hemoglobin complex”, “CD163”, “heme oxygenase-1”, “oxidative stress”, “endothelial dysfunction”, and “organ injury”, using Boolean operators (AND/OR). Studies were selected based on their relevance to the objectives of this review, including mechanistic studies, experimental models, and clinical investigations evaluating Hp-mediated Hb scavenging, protection against hemolysis-associated toxicity, inflammatory regulation, oxidative stress, endothelial protection, and therapeutic applications. Relevant review articles providing mechanistic and translational perspectives were also considered. Studies unrelated to Hp biology or hemolysis-associated mechanisms, non-English publications, and articles outside the scope of this review were excluded.

3. The biology of Hp and clearance of cfHb

Hp is a plasma glycoprotein synthesized predominantly by the liver and is a major component of the endogenous extracellular Hb-scavenging system. Its biological function is closely linked to its molecular structure, its high-affinity interaction with Hb, and subsequent recognition of Hp–Hb complexes by the macrophage scavenger receptor CD163.[8,9] Hp is composed of two chains, an α-chain and a β-chain, which are connected by disulfide bonds, and has three main phenotypes in humans: Hp1-1, Hp2-1 and Hp2-2 (Fig. 1). The differences in the molecular size, oligomeric structure, antioxidant activity, and Hb binding kinetics of these phenotypes are substantial.[8] Hp1-1 predominantly forms smaller dimers, whereas Hp2-2 forms larger polymeric structures, with Hp2-1 displaying intermediate characteristics.[14]

Hp is biologically important because of its very high affinity for Hb. Erythrocyte lysis releases free Hb, which is quickly bound by circulating Hp, with dissociation constants reaching the femtomolar range, the strongest known protein–protein interaction in human physiology.[15] This interaction prevents Hb dissociation into smaller dimers that might pass through the glomerular filtration barrier (GFB) and cause renal toxicity. Additionally, Hp binding occurs in a less reactive conformation, which inhibits the release of heme and decreases redox cycling reactions that result in the production of reactive oxygen species (ROS).[2]

Hp–Hb complexes are recognized primarily by CD163, a scavenger receptor highly expressed on monocytes and tissue macrophages.[16,17] Ligand binding triggers receptor-mediated endocytosis, followed by endolysosomal processing of the complex. Globin is degraded, heme is subsequently catabolized, and iron is returned to regulated intracellular handling and systemic recycling pathways.[9] Meanwhile, activation of the CD163 pathway induces heme oxygenase-1 (HO-1) and interleukin-10 (IL-10) expression and secretion, along with anti-inflammatory mediators, which support tissue repair and inflammation resolution.[18] Recent structural studies have further clarified the molecular basis of Hp–

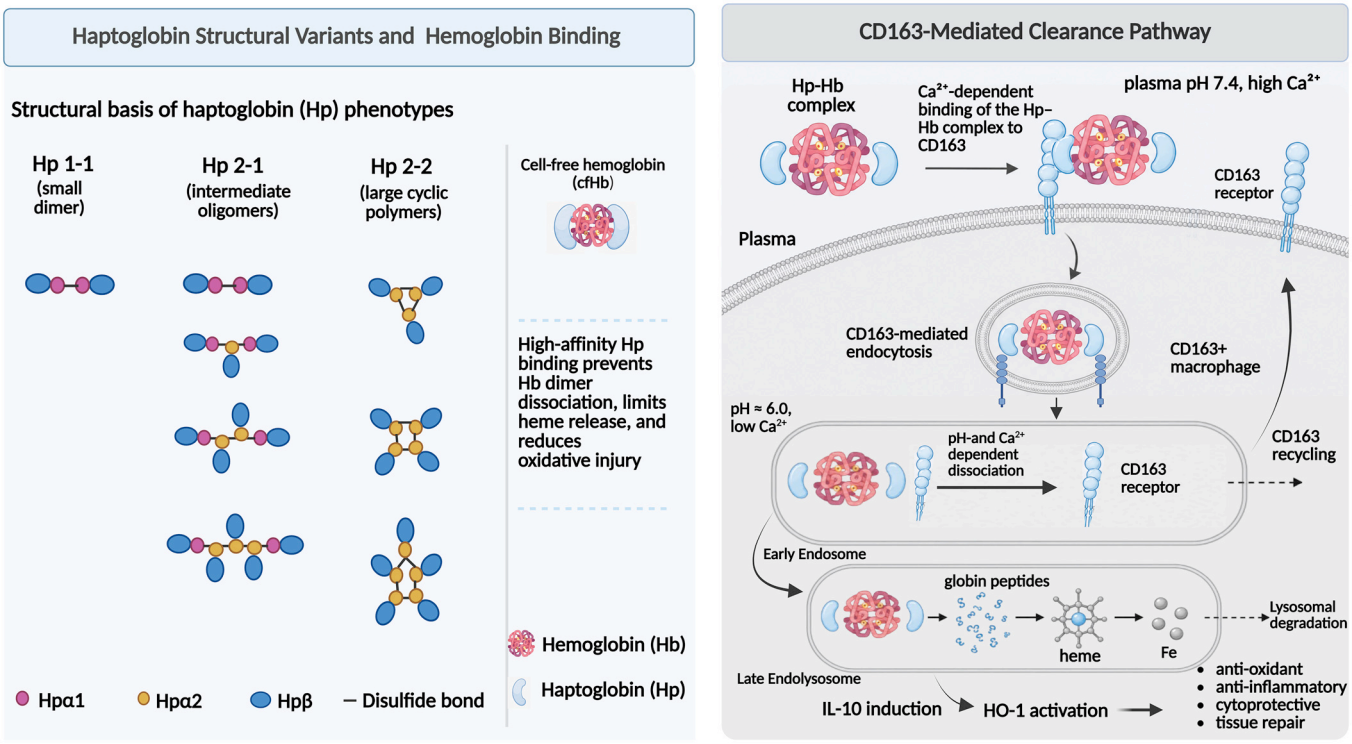


Fig. 1. Structural organization of haptoglobin (Hp) phenotypes and CD163-mediated clearance of cell-free hemoglobin (cfHb). Hp binds cfHb released during intravascular hemolysis and forms stable Hp–Hb complexes that limit hemoglobin (Hb) oxidation and heme release, and prevent glomerular filtration of free Hb. Human Hp occurs as three main phenotypes: Hp1-1, which forms relatively small dimers; Hp2-1, which forms intermediate oligomeric assemblies; and Hp2-2, which forms larger cyclic polymers. These structural differences influence complex size, antioxidant activity, and clearance efficiency. Hp–Hb complexes are recognized by the scavenger receptor CD163 on monocytes and macrophages, thereby initiating receptor-mediated endocytosis and endolysosomal degradation. Within macrophages, the globin peptides are degraded into amino acids, while heme is metabolized by heme oxygenase-1 (HO-1) into biliverdin, carbon monoxide (CO), and ferrous iron. The released iron is subsequently sequestered by ferritin or recycled for erythropoiesis. CD163-mediated uptake of Hp–Hb complexes induces HO-1 expression and promotes anti-inflammatory, antioxidant, and tissue-protective responses.

Hb recognition by CD163. Cryo-electron microscopy revealed that the extracellular region of CD163 engages the Hp–Hb complex through calcium-dependent electrostatic interactions, providing a structural explanation for selective ligand recognition and receptor-mediated trafficking[19,20] (Fig. 1).

When extracellular Hb oxidation and degradation generate free heme, the hemopexin (Hpx) system provides a complementary scavenging pathway. Hpx binds free heme with high affinity and facilitates its uptake, predominantly through hepatic CD91-mediated pathways.[21] Thus, Hp and Hpx form complementary extracellular defense systems targeting Hb and heme, respectively.

4. Protective mechanisms of Hp

Beyond extracellular Hb sequestration and clearance, activation of the Hp–Hb–CD163 axis also influences downstream redox, vascular, and inflammatory responses. These protective effects are discussed in the following section.

4.1. CD163–HO-1-mediated cytoprotective signaling

Following CD163-mediated processing of Hp–Hb complexes, heme catabolism induces the stress-responsive enzyme HO-1. HO-1 converts heme to biliverdin, carbon monoxide (CO), and ferrous iron (Fe^{2+}) by enzymatic activity. Biliverdin is then reduced to bilirubin by biliverdin reductase, and both are potent antioxidants that protect tissues from ROS-induced injury.[22,23] In contrast, low physiological levels of CO exert anti-inflammatory, anti-apoptotic, and vasodilatory activities, mediated by modulation of cellular signaling pathways and by attenuation of overactive inflammatory responses. Free iron is bound safely by ferritin or directed back into well-regulated intracellular pathways, thereby preventing iron-mediated oxidative damage.[24] Activation of the CD163–HO-1 axis not only helps remove extracellular Hb and heme but also triggers an endogenous protective mechanism that repairs tissue, resolves inflammation, maintains vascular homeostasis, and protects against hemolysis-induced damage.[25–27]

4.2. Preservation of NO bioavailability

Cell-free oxyhemoglobin rapidly consumes endothelium-derived NO, forming methemoglobin and biologically inactive nitrate, thereby reducing NO bioavailability [28] (Fig. 2). This reduction in NO bioavailability harms vascular homeostasis and can lead to endothelial dysfunction, vasoconstriction, platelet activation, leukocyte adhesion, inflammation, and microvascular thrombosis. These mechanisms are especially relevant in hemolytic disorders like sickle cell disease (SCD), where plasma cfHb has been shown to inhibit NO-dependent blood flow responses, and in clinical situations involving extracorporeal circulation.[29] The Hp–cfHb complex prevents interaction between free cfHb and endothelial NO, while enhancing macrophage clearance via CD163, resulting in increased NO signaling and maintaining endothelial-dependent vasodilation.[30] Experimental findings suggest that Hp can maintain vascular NO signaling during hemolysis and thus serve as an intrinsic antioxidant system that protects against Hb-mediated vasculopathy. This protective mechanism has clinical implications for chronic and acute hemolytic diseases such as SCD, pulmonary hypertension, extracorporeal membrane oxygenation (ECMO)-related hemolysis, surgery related to cardiopulmonary bypass (CPB), and vascular organ injury.[31]

4.3. Suppression of Hb redox toxicity and oxidative injury

Both cfHb and free heme are powerful catalysts of oxidative injury. Oxidation produces ROS that can cause lipid peroxidation, protein oxidation, mitochondrial dysfunction, and DNA damage.[32] Hp helps to stabilize Hb and to decrease its redox activity by preventing dissociation to highly reactive heme-containing intermediates[33] (Fig. 2). Recent studies have revealed an additional immunoregulatory function of Hp, demonstrating that it can bind and sequester bacterial lipopolysaccharides, thereby reducing their availability for Toll-like receptor 4 (TLR4) activation and delaying nuclear factor kappa B (NF-κB)-driven inflammatory responses in macrophages. This lipopolysaccharide-modulatory capacity suggests a potential role for Hp in regulating excessive inflammatory responses and provides a rationale for investigating Hp-based therapeutic strategies in inflammatory conditions such as sepsis.[34] Thus, Hp reduces oxidative stress by binding free Hb dimers, stabilizing the Hb–Hp complex, and preventing Hb-mediated redox reactions and radical generation associated with heme toxicity.[35]

4.4. Modulation of heme-driven inflammatory signaling

Oxidation of extracellular Hb promotes heme release, and accumulated free heme can activate TLR4-dependent inflammatory signaling in endothelial and immune cells.[36,37] TLR4 activation induces IKK/IκBα signaling and subsequent NF-κB nuclear activation, increasing expression of pro-inflammatory cytokines, including IL-1β and TNF-α. By stabilizing extracellular Hb and limiting heme release, Hp reduces the availability of free heme for TLR4 activation and thereby attenuates NF-κB-driven inflammatory responses [36,37] (Fig. 2).

5. Hemolysis-associated conditions and therapeutic evidence

5.1. Etiologies and clinical settings of hemolysis

Several clinical conditions can initiate or amplify intravascular hemolysis, increasing circulating cfHb and heme. The following subsections summarize the major clinical settings in which hemolysis may provide a rationale for Hp-based intervention, while the downstream organ consequences of hemolysis are discussed separately in Section 5.2.

5.1.1. Sepsis and septic shock

Sepsis and septic shock are associated with intravascular hemolysis and increased circulating cfHb, alongside endothelial dysfunction and microvascular injury. In septic states, cfHb may contribute to NO scavenging, oxidative injury, iron dysregulation, and amplification of inflammatory signaling.[13,38] By binding cfHb and facilitating its macrophage-mediated clearance, Hp may limit vascular exposure to free Hb and reduce its oxidative toxicity.

Preclinical evidence supports this therapeutic concept. In a canine model of *S. aureus* pneumonia-associated septic shock, treatment with human Hp improved hemodynamic parameters, reduced lung injury, and increased survival, with greater benefit observed in animals with higher cfHb levels.[11] These findings suggest that measurable hemolysis may help identify patients most likely to benefit from Hp-based therapy. Hp may also modulate inflammatory responses through the CD163–HO-1 pathway.[9,39] However, the evidence remains predominantly preclinical, and controlled clinical studies are required to establish the therapeutic efficacy of Hp in patients with sepsis.

5.1.2. SCD

SCD is characterized by chronic intravascular hemolysis, recurrent vaso-occlusion, endothelial activation, NO depletion, and progressive organ injury. Persistent cfHb release can overwhelm endogenous Hp, while subsequent heme release further contributes to oxidative and vascular injuries. Chronic hemolysis and cfHb/heme-mediated toxicity have been linked to pulmonary hypertension, nephropathy, cerebrovascular complications, and chronic vasculopathy in SCD.[13,40]

Hp-based therapy is therefore of potential interest because it targets circulating cfHb and may reduce its vascular and oxidative toxicity. Experimental evidence suggests that restoration of endogenous Hb- and heme-scavenging pathways may attenuate vascular dysfunction, inflammation, and organ injury in chronic hemolytic states.[1,13] Combined Hp and Hpx therapy may provide complementary protection, with Hp

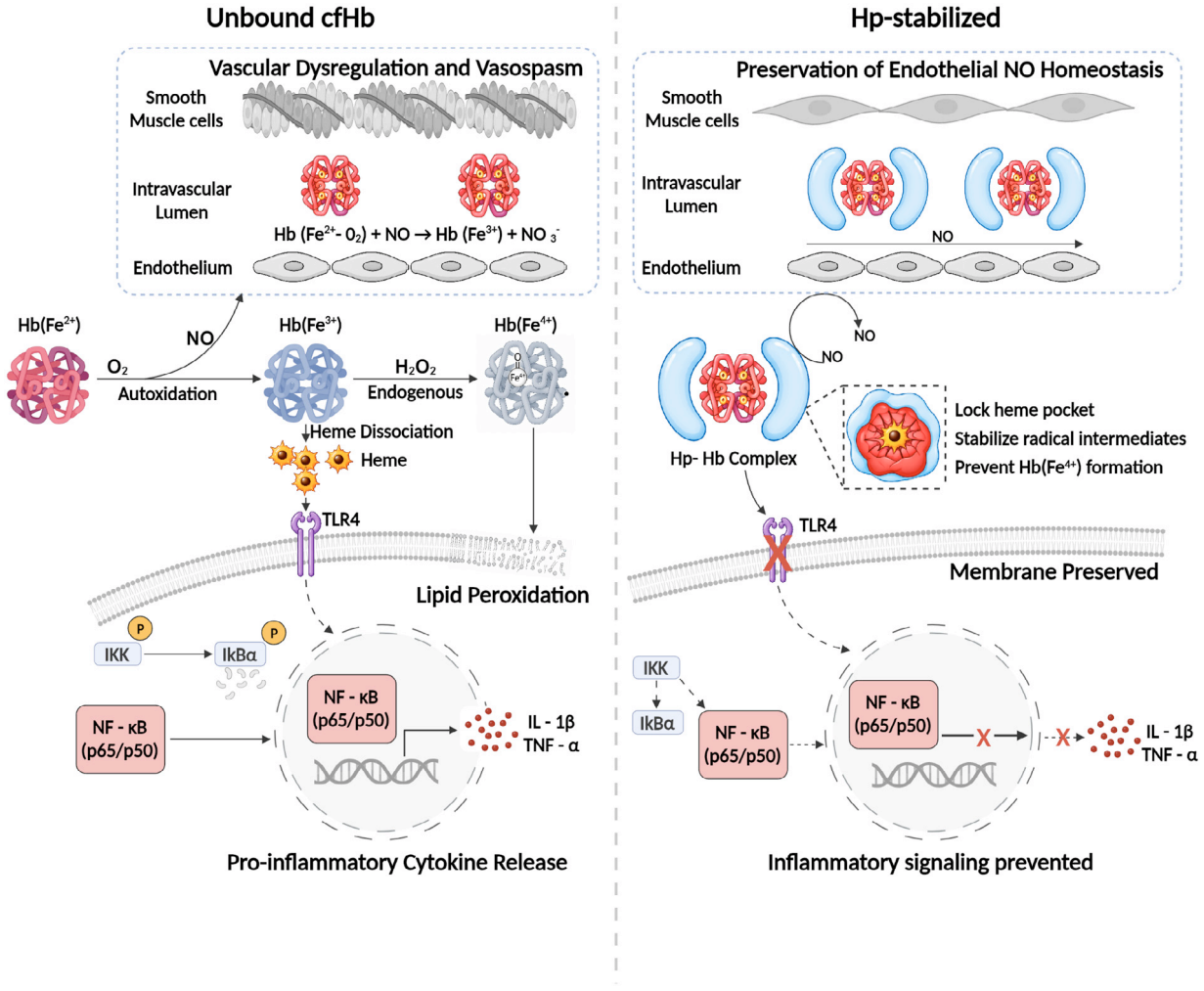


Fig. 2. Hp preserves nitric oxide (NO) bioavailability and vascular homeostasis by limiting cfHb-mediated oxidative and inflammatory injury. Without Hp, cell-free oxyhemoglobin [$Hb(Fe^{2+}-O_2)$] reacts with endothelial NO, generating methemoglobin [$Hb(Fe^{3+})$] and nitrate (NO_3^-), depleting NO, impairing endothelium-dependent vasodilation, and increasing vascular smooth muscle contraction. Oxidized Hb and released heme can also promote oxidative stress, lipid peroxidation, and activation of Toll-like receptor 4 (TLR4)-dependent inflammatory signaling, leading to nuclear factor kappa B (NF-κB) activation and increased expression of pro-inflammatory cytokines. Binding of cfHb by Hp forms stable Hp–Hb complexes that restrict Hb access to the heme pocket, reduce NO scavenging and heme-mediated oxidative reactions, and limit inflammatory pathway activation. This preserves NO signaling, attenuates vascular inflammation, supports vascular smooth muscle relaxation, and maintains vascular homeostasis.

scavenging cfHb and Hpx binding free heme. However, direct clinical evidence for Hp therapy in SCD remains limited, and controlled studies are required to determine whether this approach improves clinically relevant vascular and organ outcomes.

5.1.3. Extracorporeal circulation, ECMO, and CPB

CPB and ECMO can cause clinically significant intravascular hemolysis through mechanical shear stress and circuit-related erythrocyte injury. The resulting increase in cfHb has been associated with endothelial dysfunction, microcirculatory impairment, acute kidney injury (AKI), and adverse clinical outcomes.[4,41] These effects provide a biological rationale for Hp supplementation to reduce cfHb-mediated toxicity.

However, recent randomized evidence evaluating pre-emptive Hp treatment in adult cardiac surgery did not demonstrate a clear renal benefit and reported a greater postoperative change in serum creatinine in the Hp group.[42] The differing findings may reflect differences in the clinical contexts in which Hp was used. In the observational cohort, Hp was generally administered at the anesthesiologist's discretion after hemoglobinuria became visible, usually at 2,000 U.[43] In the randomized trial, adults undergoing elective major CPB surgery received a fixed 4,000 U dose pre-emptively when serum-free Hb reached ≥0.05 g/dL within 2 hours of CPB initiation.[42] Because treatment in the observational cohort was clinician-directed, confounding by treatment indication and perioperative care cannot be excluded, whereas randomization reduces these biases but limits inference to the selected population and protocol. Differences in baseline renal risk, hemolysis burden, treatment trigger, dose, and timing may therefore have contributed to the divergent findings.

Clinicians in Japan have used plasma-derived Hp for hemolysis associated with extracorporeal circulation, although its availability and routine use remain limited internationally.[13] Despite the strong biological rationale, broader clinical adoption will require adequately powered randomized trials, standardized dosing, safety monitoring, and validated biomarkers of therapeutic response. Across hemolysis-associated conditions, recent advances have expanded Hp's therapeutic application beyond its physiological role as a Hb-scavenging protein. Preclinical and clinical studies have evaluated plasma-derived Hp, recombinant Hp variants, and engineered Hp-based approaches for limiting cfHb/heme-mediated toxicity and associated organ injury. Representative studies and their principal findings are summarized in Table 1.[44–51]

5.1.4. Trauma- and transfusion-associated hemolysis

Severe traumatic injury can produce endogenous intravascular hemolysis independently of transfusion. Contemporary studies have

Table 1
Summary of representative preclinical and clinical studies evaluating Hp-based therapeutic strategies for hemolysis-associated injury.

Study type	Study model	Intervention	Main findings	Statistical significance	Reference
RCT	Adults undergoing cardiac surgery with CPB	Pre-emptive Hp therapy: 4,000 U at free Hb ≥ 0.05 g/dL versus standard care	Pre-emptive Hp was associated with greater postoperative ΔCr, indicating worsened renal function.	Higher median ΔCr in the Hp group ($P = 0.05$); regression confirmed increased ΔCr ($P = 0.03$).	42
Preclinical	Canine model	Human Hp administration	Improved shock, lung injury, and survival by binding cell-free hemoglobin and reducing iron levels.	Significant improvements across outcomes ($P < 0.05$).	11,44
Preclinical	Rat model	Repeated-dose Hp in hypoxic pulmonary hypertension with chronic Hb exposure	Reduced pulmonary vascular oxidation, inflammation, and remodeling.	Significant effects across several parameters ($P < 0.05$).	45
Preclinical	Mouse model	Hp or Hpx after resuscitation with prolonged-storage RBCs	Prevented acute toxicity, including AKI, by scavenging extracellular Hb and heme.	Reduced kidney injury markers ($P < 0.05$).	46
Preclinical	Mouse model	ApoHb–Hp complex in β-thalassemia mice	Reduced liver and spleen weight, improved liver function and RBC lifespan, lowered serum iron, and mitigated cell-free Hb/heme toxicity.	Reduced iron toxicity and oxidative stress markers ($P < 0.05$).	47
Preclinical	Rat burn model	Hp administration following severe burns	Reduced IFN-γ levels and enhanced clot formation.	Significant effects on IFN-γ and clotting parameters ($P < 0.05$).	48
Observational clinical	Cardiovascular surgery patients	Intraoperative Hp administration	Associated with lower postoperative AKI incidence.	AKI incidence was significantly reduced ($P < 0.001$).	43
Observational clinical	Pediatric cardiac surgery patients	Intraoperative Hp administration	Associated with lower postoperative AKI through plasma-free Hb scavenging.	Significant for selected outcomes; exact *P*-values not reported.	49
Observational clinical	Patients with severe burns	Hp administration	Associated with a lower risk of AKI requiring renal replacement therapy.	Significant association reported; exact *P*-value not provided.	51
Experimental	Guinea pig model	Hp administration during transfusion of stored blood	Attenuated Hb-mediated toxicity by scavenging cell-free Hb.	Significant protective effects reported; exact *P*-value not provided.	50

RCT: randomized controlled trial; CPB: cardiopulmonary bypass; Hpx: hemopexin; ΔCr: change in serum creatinine; AKI: acute kidney injury; RBC: red blood cell; IFN-γ: interferon γ.

demonstrated early elevations of circulating cfHb and heme after trauma, including in pretransfusion samples, with greater hemolytic burden associated with injury severity and adverse clinical outcomes.[52–54] Trauma-related heme release may also contribute to immune dysregulation, while erythrocyte-derived arginase-1 can disturb arginine metabolism and NO homeostasis, providing additional pathways through which endogenous hemolysis may promote vascular dysfunction and organ injury.[52–54]

Transfusion can further increase the hemolytic burden, particularly when stored red blood cells (RBCs) are susceptible to post-transfusion lysis. In experimental models, restoration of endogenous scavenging capacity with Hp or Hpx reduced circulating toxic Hb species, vascular dysfunction, and AKI following transfusion of stored blood.[46] These findings support Hp supplementation as a potential strategy for limiting cfHb-mediated toxicity during trauma resuscitation and massive transfusion.

However, direct clinical evidence for Hp therapy in trauma remains limited. Future studies should determine whether treatment is most effective in patients with measurable elevations in cfHb, depletion of endogenous Hp, or other evidence of substantial hemolytic burden, and should establish the optimal timing and dosing of Hp administration.

The major hemolysis-associated clinical settings and the potential protective effects of Hp therapy are summarized in Fig. 3.

5.1.5. Subarachnoid hemorrhage

Aneurysmal subarachnoid hemorrhage is a compartmentalized form of hemolytic injury in which erythrocyte lysis releases extracellular Hb into the cerebrospinal fluid (CSF). Because endogenous Hp concentrations in CSF are low, extracellular Hb can persist and contribute to NO depletion, oxidative injury, neuroinflammation, cerebral vasospasm, and delayed cerebral ischemia. In a large-animal model of subarachnoid hemorrhage (SAH), intrathecal Hp administration reduced Hb-induced cerebral vasospasm and preserved NO-dependent vascular function.[12]

Intrathecal or compartment-directed Hp therapy is therefore of interest as a strategy to neutralize extracellular Hb within the subarachnoid space. More recent experimental studies have further supported Hp's ability to reduce brain injury and vasospasm by limiting Hb penetration into brain tissue.[55] These findings provide a strong mechanistic basis for Hp as a potential neuroprotective scavenger therapy. However, uncertainties regarding delivery route, timing, dosing, compartment-specific pharmacology, and potential genotype-dependent responses remain important barriers to clinical translation.[56]

5.2. Consequences of hemolysis

Excess circulating cfHb and heme generated during intravascular hemolysis can produce direct tissue toxicity, with the kidney and vascular system being particularly susceptible to injury.

5.2.1. AKI

The kidney is particularly susceptible to cfHb-mediated toxicity. During substantial intravascular hemolysis, unbound Hb can undergo glomerular filtration and accumulate within renal tubules, promoting oxidative stress, lipid peroxidation, heme–iron toxicity, mitochondrial dysfunction, tubular cast formation, and epithelial injury.[13,57] Hp limits these effects by binding cfHb to form larger Hp–Hb complexes, thereby restricting glomerular filtration of free Hb, reducing redox-active heme exposure, and facilitating clearance through the CD163 pathway.

Experimental and emerging clinical evidence support a potential role for Hp in hemolysis-associated AKI. Elevated plasma-free Hb during CPB has been associated with postoperative AKI,[41] while perioperative Hp administration has been associated with a lower incidence of renal injury in selected cardiovascular surgical patients.[43] Experimental transfusion studies have similarly shown that Hp or Hpx can reduce renal injury following resuscitation with prolonged-storage RBCs.[46] In addi-

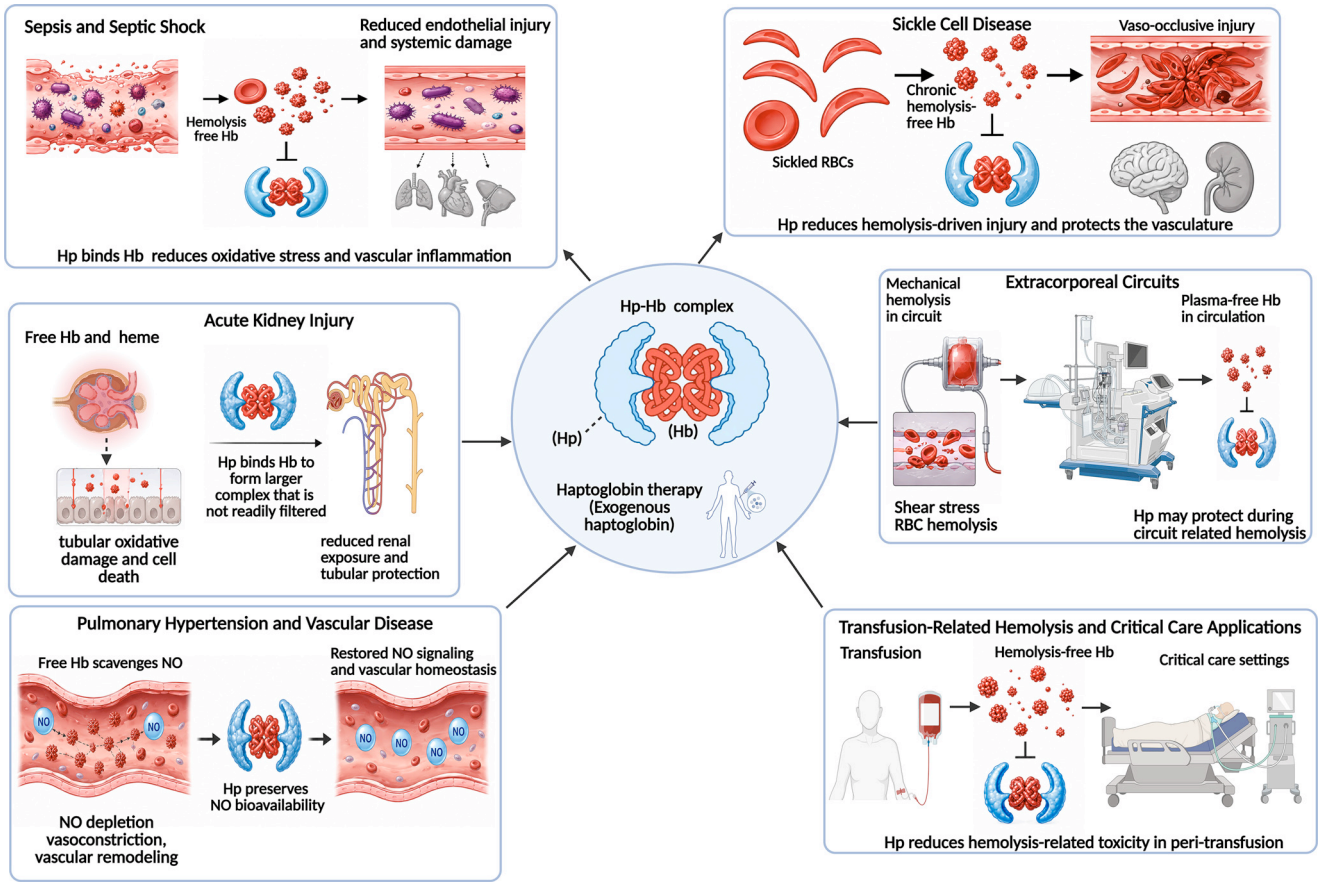


Fig. 3. Potential therapeutic applications of Hp in hemolysis-associated conditions. Intravascular hemolysis releases cfHb, promoting oxidative stress, NO depletion, endothelial dysfunction, inflammation, and organ injury. Hp binds cfHb to form Hp–Hb complexes, reducing Hb-mediated toxicity and enhancing cfHb clearance. These protective effects support the potential use of Hp-based therapies in hemolysis-associated conditions, including sepsis, sickle cell disease, acute kidney injury, extracorporeal circulation, pulmonary hypertension, and transfusion-related hemolysis.

tion, engineered Hpβ-based approaches have shown protection against myoglobin-mediated renal injury in experimental rhabdomyolysis by limiting tubular myoglobin accumulation and promoting clearance.[58]

However, in an RCT of patients undergoing CPB-assisted cardiovascular surgery, pre-emptive Hp administration guided by elevated serum free Hb levels did not clearly reduce postoperative renal dysfunction, suggesting that Hp therapy may require patient selection based on hemolysis severity and renal risk profile.[42] Because a change in serum creatinine is not equivalent to event-defined AKI, this finding should not be interpreted as definitive evidence of an increased AKI incidence. The apparent discrepancy between the observational and randomized studies may reflect differences in patient selection, baseline hemolysis and renal risk, treatment timing and dose, perioperative management, and endpoint definition. Importantly, this clinical inconsistency does not negate the mechanistic and experimental evidence supporting Hp-mediated protection against hemolysis-associated renal injury; rather, it suggests that Hp's therapeutic effect may depend on the clinical context, and Hp-based therapy for AKI should therefore remain investigational, with future studies focusing on patients with substantial hemolysis.

5.2.2. Pulmonary hypertension and vascular dysfunction

Hemolysis-associated pulmonary hypertension is driven in part by cfHb-mediated NO scavenging, oxidative vascular injury, endothelial activation, and vascular smooth muscle proliferation. These mechanisms are particularly relevant in SCD and other chronic hemolytic disorders.[13,40] Hp may protect the pulmonary vasculature by reducing cfHb-mediated NO consumption and limiting oxidative remodeling of the vascular wall.

Preclinical evidence supports Hp's potential to attenuate Hb-driven pulmonary vascular oxidative stress, inflammation, and remodeling.[1,13] In chronic hemolytic states, combined targeting of cfHb and free heme may provide broader protection because heme can independently activate endothelial and inflammatory pathways. However, direct clinical evidence remains limited, and future studies should evaluate Hp alone and in combination with Hpx, particularly in patients with high hemolytic burden and reduced endogenous scavenger capacity.

6. Emerging therapeutic strategies

Growing recognition of cfHb and free heme as central mediators of hemolysis-associated organ injury has spurred the development of therapeutic strategies to restore endogenous scavenger systems. Hp-based therapy represents one of the most biologically rational approaches because Hp directly neutralizes cfHb, limits heme release, preserves NO bioavailability, and promotes CD163-mediated macrophage clearance.[9,38] However, therapeutic development is evolving beyond simple plasma-derived Hp replacement toward recombinant proteins, engineered scavenger complexes, combination therapies, and precision medicine approaches.[13]

6.1. Plasma-derived Hp supplementation

The most direct therapeutic strategy is to supplement depleted endogenous Hp using plasma-derived Hp preparations. This approach is based on the observation that pathological hemolysis can rapidly exceed the binding capacity of circulating Hp, leaving cfHb unbound and

capable of inducing oxidative, vascular, renal, pulmonary, and neurological injuries.[1] Exogenous Hp supplementation increases the plasma cfHb-binding capacity and promotes the formation of Hp–Hb complexes, which are subsequently cleared through CD163-expressing monocytes and macrophages.[59]

Plasma-derived Hp has shown beneficial effects in several experimental and clinical contexts, including sepsis, transfusion-related hemolysis, burn injury, CPB, and extracorporeal circulation.[1] In preclinical models, Hp administration reduced Hb-driven oxidative stress, improved vascular function, and attenuated organ injury.[38] In Japan, plasma-derived Hp products have been used clinically for hemolysis associated with extracorporeal circulation and massive transfusion, demonstrating that Hp supplementation is biologically and clinically feasible.[13] Nevertheless, plasma-derived Hp therapy remains limited by supply constraints, batch variability, cost, potential pathogen-transmission concerns, and lack of broad regulatory approval outside selected regions.[13]

6.2. Recombinant Hp

Recombinant Hp is a major emerging strategy intended to overcome the limitations of plasma-derived products. Recombinant production could provide a scalable, standardized, pathogen-free, and genotype-defined therapeutic source.[60] However, Hp is a structurally complex glycoprotein requiring correct folding, disulfide bonding, oligomerization, and glycosylation to maintain biological function.[8] These requirements make recombinant Hp production considerably more challenging than production of many conventional biologics.

A key unresolved question is which Hp phenotype should be used therapeutically. Hp1-1 generally forms smaller dimers and has greater antioxidant and Hb-scavenging efficiency, whereas Hp2-2 forms larger polymers with comparatively reduced protective activity.[1] Recombinant Hp1-1 may therefore be an attractive universal therapeutic candidate. However, researchers must carefully evaluate genotype mismatch, immunogenicity, pharmacokinetic differences, and tissue distribution.[60] Future recombinant Hp development should focus on producing functionally active phenotype-specific variants, defining their Hb-binding kinetics, evaluating CD163-mediated clearance, and testing safety in clinically relevant hemolysis models.

6.3. Hp–Hpx combination therapy

Hemolysis generates two major toxic mediators: cfHb and free heme. Hp primarily neutralizes cfHb, whereas Hpx binds free heme with high affinity and delivers it mainly to hepatic clearance pathways.[1] Because cfHb oxidation and degradation can release heme, targeting only one component of the hemolysis cascade may be insufficient in severe or sustained hemolysis.[38] Therefore, combined Hp–Hpx therapy has emerged as a promising dual-scavenger approach.[13]

Experimental studies have shown that Hp or Hpx therapy can prevent adverse effects of hemolysis after transfusion of prolonged-storage RBCs, including vascular dysfunction, inflammation, and AKI.[46] Combination therapy may be particularly relevant in SCD, severe sepsis, massive transfusion, trauma, ECMO, and SAH, where both cfHb and heme contribute to tissue injury.[1] However, the optimal timing, dosing ratio, sequencing, and patient-selection criteria for Hp–Hpx therapy remain undefined. Future studies should determine whether combined scavenger therapy provides additive or synergistic benefit compared with either protein alone.

6.4. Apohemoglobin–Hp complexes

A more advanced therapeutic concept involves engineered apohemoglobin–haptoglobin (ApoHb–Hp) complexes. ApoHb lacks heme but can bind free heme, while Hp binds Hb. The ApoHb–Hp complex is therefore designed to function as a dual scavenger capable of neutralizing both cfHb and free heme [61] (Fig. 4). This strategy may offer broader protection than Hp alone because it addresses both upstream Hb toxicity and downstream heme-mediated oxidative injury.

Recent studies suggest that ApoHb–Hp complexes can reduce vascular dysfunction and improve microcirculatory outcomes in models of hemolysis and vaso-occlusive injury.[61] This approach is especially relevant to SCD, where both cfHb and heme participate in endothelial activation, NO depletion, leukocyte adhesion, and vaso-occlusion.[62] Despite its promise, ApoHb–Hp therapy remains early in development. Key barriers include protein stability, heme-binding retention, pharmacokinetics, immunogenicity, large-scale manufacturing, and long-term safety.[62] Chemical modification strategies such as PEGylation may improve circulation time and colloidal stability, but they must be optimized carefully to avoid reducing heme-binding activity.

6.5. Engineered scavenger proteins and protein design approaches

Protein engineering offers opportunities to improve the therapeutic properties of Hp and related scavenger systems. Desired improvements include prolonged plasma half-life, enhanced cfHb-binding capacity, reduced immunogenicity, improved tissue penetration, controlled receptor engagement, and resistance to oxidative inactivation.[62] Engineered scavenger proteins may also be designed to combine multiple activities, such as Hb binding, heme binding, antioxidant activity, and macrophage-targeted clearance.[13]

Modular protein platforms have been proposed to develop multifunctional Hb and heme scavengers.[62] Such platforms may allow rational design of biologics tailored to specific hemolytic diseases. For example, an acute high-burden condition such as massive transfusion may require rapid intravascular cfHb neutralization, whereas chronic hemolytic diseases such as SCD may require longer-acting agents with sustained antioxidant and vascular protective effects.[62] Future development should integrate structural biology, glycoengineering, pharmacokinetic modeling, and disease-specific preclinical testing.

6.6. Genotype-guided and precision medicine strategies

Hp polymorphism is a critical consideration for therapeutic development. The major phenotypes, Hp1-1, Hp2-1, and Hp2-2, differ in molecular size, antioxidant efficiency, Hb-binding behavior, and clearance kinetics.[1] These differences may influence both disease susceptibility and response to Hp-based therapy. Patients with the Hp2-2 phenotype may be more vulnerable to oxidative vascular injury and may require different dosing or alternative recombinant formulations.[60] Precision medicine strategies should incorporate Hp genotype, baseline Hp concentration, cfHb level, free heme burden, inflammatory profile, organ-injury biomarkers, and disease context.[13] Hp genotype may help identify patients most likely to benefit from therapy and should be prespecified as a stratification factor in future clinical trials.

6.7. Biomarker-guided therapeutic monitoring

Successful translation of Hp-based therapies will require reliable biomarkers to confirm target engagement and biological response.[13] Useful biomarkers may include plasma cfHb, circulating Hp–Hb complexes, free heme, Hpx levels, soluble CD163, HO-1 induction, ferritin, oxidative stress markers, endothelial injury markers, NO bioavailability, creatinine, cystatin C, and inflammatory cytokines.[9]

Point-of-care assays for cfHb and Hp–Hb complexes may be particularly valuable in acute settings such as CPB, ECMO, trauma, massive transfusion, and septic shock.[13] Omics-based approaches, including proteomics and glycoproteomics, may further refine patient stratification by identifying disease-specific Hp modifications, including altered glycosylation patterns.[62] Biomarker-guided therapy will be essential for moving Hp from a broadly promising biologic toward a clinically precise intervention.

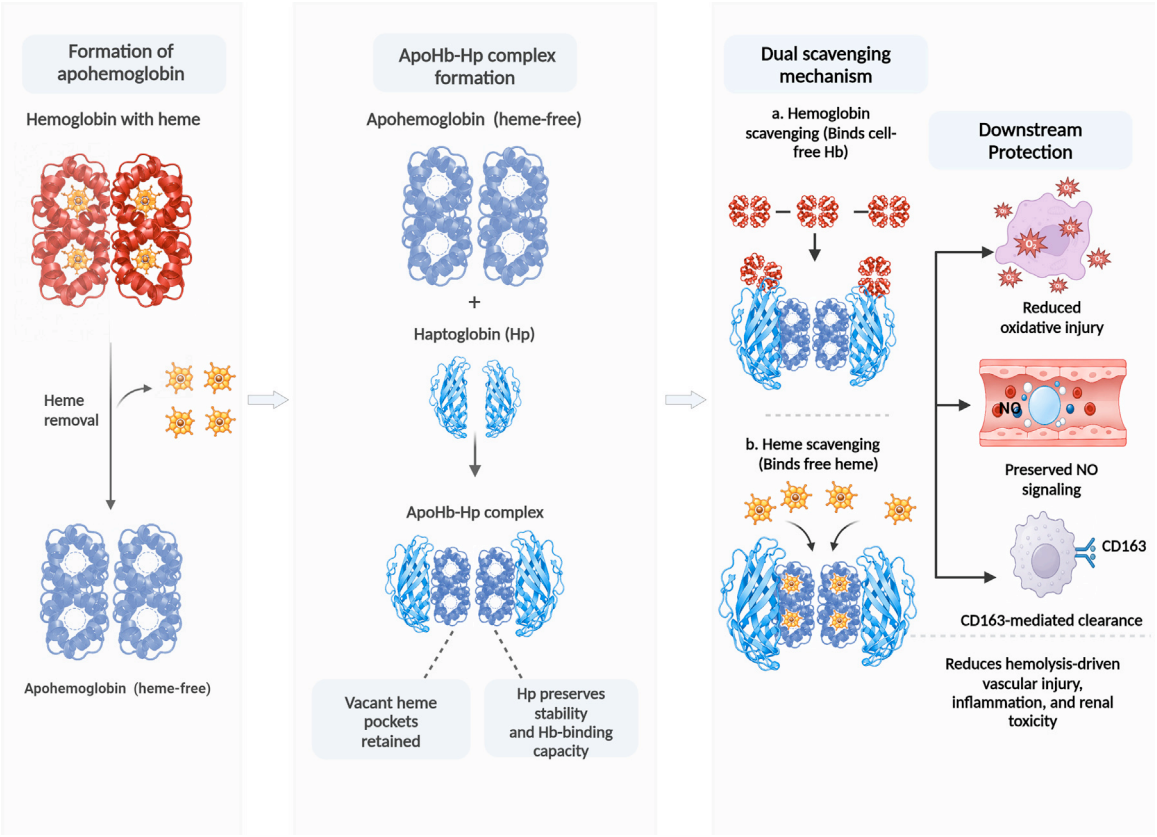


Fig. 4. Proposed mechanism of action of the apohemoglobin–Hp (apoHb–Hp) complex. Removal of heme from native Hb generates apohemoglobin containing vacant heme-binding pockets. Association of apohemoglobin with Hp forms a stable apoHb–Hp complex that combines Hp's Hb-binding properties with apoHb's heme-binding capacity. The complex therefore functions as a dual scavenger: the Hp component sequesters circulating cfHb, whereas the vacant heme pockets of apoHb capture extracellular free heme. By limiting Hb redox activity, heme-mediated oxidative injury, and NO consumption, apoHb–Hp may preserve endothelial function and reduce vascular injury, inflammation, and renal toxicity. The scavenger complex is proposed to undergo CD163-dependent uptake by monocytes and macrophages, followed by intracellular processing and clearance.

6.8. Drug delivery and compartment-specific therapy

The therapeutic route of Hp administration may need to be adapted to disease location. In systemic intravascular hemolysis, intravenous therapy is the most logical approach.[1] However, in compartmental hemolysis, such as aneurysmal subarachnoid hemorrhage, systemic Hp may not achieve adequate concentrations within the CSF because of blood–brain barrier limitations.[13] Intrathecal or localized delivery has therefore been proposed for SAH to neutralize extracellular Hb directly within the subarachnoid space.

Compartment-specific delivery could improve therapeutic efficacy while reducing systemic exposure. However, local administration raises additional safety considerations, including neurotoxicity, immune activation, dosing precision, and clearance from restricted anatomical spaces.[13] Future studies should define optimal delivery routes for neurological, pulmonary, renal, and intravascular hemolytic disorders.

6.9. Translational and regulatory considerations

Despite strong biological rationale, Hp-based therapeutics face several translational barriers. These include manufacturing complexity, product standardization, phenotype selection, dosing uncertainty, safety assessment, cost-effectiveness, and regulatory classification as biologics.[62] Because Hp is an endogenous human protein with disease-modifying potential, regulatory evaluation will require clear evidence of purity, potency, consistency, pharmacokinetics, immunogenicity, and clinical benefit.[13]

Emerging therapeutic strategies for Hp are moving from simple replacement therapy toward engineered, recombinant, combination, and precision-guided approaches.[13] Plasma-derived Hp provides proof of concept, but recombinant Hp, Hp–Hpx combination therapy, ApoHb–Hp complexes, and multifunctional engineered scavengers may offer improved scalability, potency, and disease specificity.[62] Future success will depend on integrating molecular engineering, genotype-guided patient selection, biomarker-based monitoring, and rigorous clinical trial design. Until such evidence is available, Hp-based therapies should be considered promising investigational biologics with substantial translational potential.

7. Limitations of Hp-based therapies

Although progress has been made in understanding the biological role of Hp and experimental and early clinical trials have shown promising results, significant gaps remain that limit translating Hp-based therapies into clinical practice. While there is considerable mechanistic evidence that Hp is a scavenger of cfHb and a modulator of oxidative and inflammatory injury, the amount of evidence to date is still not enough for broad clinical use.[13]

7.1. Limited availability of high-quality clinical trials

The main drawback is the lack of large multicenter RCTs assessing the efficacy and safety of Hp therapy. Most available clinical studies are observational cohorts, retrospective studies, case reports, or small

pilot studies, which reduces the strength of clinical conclusions. In a recent systematic review, Baldetti *et al.* concluded that, across 13 studies involving 677 patients, Hp administration consistently decreased plasma free Hb concentration and may reduce AKI.[63] However, they concluded that evidence remains insufficient to show improvements in mortality or long-term clinical outcomes. Therefore, definitive recommendations on clinical use can not currently be made.[63]

7.2. Heterogeneity of clinical population

Furthermore, existing clinical studies show high heterogeneity across patient groups complicating interpretation. There are a variety of different clinical situations where Hp therapy has been studied: during CPB, ECMO, massive transfusion, sepsis, burns, and trauma, as well as chronic hemolytic disorders.[1,13] These differ significantly in hemolysis, inflammatory load, organ involvement, and disease course. Furthermore, baseline endogenous Hp, circulating cfHb, and oxidative stress markers vary significantly from patient to patient, which may affect treatment responsiveness and make inter-study comparisons more difficult .[13]

7.3. Lack of standardized treatment protocol

Another drawback is the lack of uniform dosage and therapy. Published studies have used various Hp preparations, dosing regimens, routes of administration, and durations. Therefore, the optimum dose needed to neutralize different cfHb concentrations remains unclear. Moreover, the pharmacokinetic and pharmacodynamic properties of Hp are not yet fully described in critically ill patients, especially those with continuous hemolysis, systemic inflammation, and multi-organ dysfunction.[63] Treatment standardization is insufficient to compare study outcomes and develop evidence-based clinical guidelines.

7.4. Inadequate understanding of genotype-specific response

Human Hp is highly genetically polymorphic and has three predominant phenotypes: Hp1-1, Hp2-1, and Hp2-2. These phenotypes differ in molecular structure, anti-oxidant activity, Hb binding, and clearance.[1] Recent findings have demonstrated that Hp genotype is associated with susceptibility to cardiovascular disease, diabetic complications, stroke, and hemolysis-associated organ injury.[64] However, clinical studies to date have not included genotype stratification in study design. This means the therapeutic value of Hp supplementation is unknown in specific Hp phenotypes, while other phenotypes may require phenotype-specific therapies. This knowledge gap must be addressed for future precision medicine strategies.

7.5. Challenges regarding manufacturing and supply

Limited manufacturing capabilities also hamper development of therapeutic options based on Hp. The Hp formulations available today mostly rely on pooled human plasma, which creates dependence on donors, limits scalability, increases batch-to-batch variability, and raises manufacturing costs.[13] Plasma-derived Hp is clinically viable, but routine therapeutic use requires a robust, scalable production system. Recombinant Hp is a promising alternative, but it has proven difficult to produce in the correct form because Hp is a highly glycosylated protein that requires complex post-translational modifications, folding, and oligomerization to be biologically active.[8] These manufacturing challenges limit the availability of pharmaceutical-grade Hp products.

7.6. Insufficient long-term safety data

Safety data from available studies are generally good in the short term, but long-term safety data are not available. Most studies have been conducted in acute treatment settings with short-term follow-up. The consequences of chronic or high doses of Hp adminstration are not well known. Potential concerns include immunogenicity, changes in iron metabolism, excessive macrophage activation, and unexpected effects of chronic modulation of hemoglobin clearance pathways.[9] Chronic hemolytic diseases like SCD will thus need long-term safety studies before Hp can be used to treat them.

7.7. Unavailability of validated biomarkers

Reliable biomarkers for patient selection, treatment monitoring, and dose optimization will be essential to implement Hp-based therapies successfully. Various candidate biomarkers have been suggested, such as plasma-free Hb, Hp–Hb complexes, soluble CD163, free heme, ferritin, and HO-1, but no universal markers exist for routine clinical use.[9,13]

7.8. Gap between experimental model and human disease

A substantial gap remains between preclinical efficacy and clinical application. Experimental models use controlled conditions and relatively uniform disease phenotypes, whereas patients vary in age, comorbidities, genetic background, disease severity, and concurrent treatments. Animal models may therefore fail to capture the full complexity of chronic hemolytic disorders and critical illness.[38] Therefore, therapeutic effects seen in laboratory experiments do not necessarily reflect effects in human populations, thorough translational research is needed.

Current evidence supports the biological rationale and therapeutic potential of Hp in hemolysis-related disorders. However, clinical translation remains limited by the lack of large RCTs, patient and genetic heterogeneity, variable treatment protocols, manufacturing challenges, insufficient long-term safety data, and unvalidated biomarkers. Addressing these gaps will require multidisciplinary translational research and well-designed clinical trials to define Hp's role in precision therapy.[13,63]

8. Conclusion

In recent years, Hp has emerged as a potential therapeutic target for hemolysis-induced organ injury. In addition to its high affinity for cfHb, Hp acts as an important endogenous antioxidant, protects NO bioavailability, and is a potent anti-inflammatory that supports safe Hb clearance via the CD163–HO-1 pathway. In addition to Hb scavenging, recent studies have shown that Hp has broader cytoprotective, immunomodulatory, and vasculoprotective activity that could benefit a wide array of hemolysis-associated diseases.

Experimental studies have demonstrated the therapeutic potential of Hp in several conditions such as sepsis, SCD, AKI, transfusion-related hemolysis, CPB, and SAH. Early clinical studies have also confirmed its effectiveness in lowering circulating free Hb levels and preventing hemolysis-associated complications. However, the lack of large-scale RCTs, differences among treatment methods, genotype-specific differences in response, production difficulties, and limited long-term safety evidence limit clinical translation.

Current progress in recombinant protein engineering, dual scavenger strategies with Hp and Hpx, ApoHb–Hp complexes, biomarker-guided therapy, and precision medicine strategies presents new opportunities to improve the therapeutic potential of Hp. Further studies are needed to confirm these promising approaches through appropriately designed multicenter clinical trials and to incorporate patient stratification by hemolytic burden, biomarker profile, and Hp genotype.

Finally, Hp is a biologically interesting and translationally relevant therapeutic platform directly targeting a key mechanism in the pathology associated with hemolysis. Its full potential as a novel therapeutic intervention for hemolysis-associated diseases will require continued advances in molecular engineering, clinical development, and precision medicine.

Author contribution

Sania Saeed: Writing – original draft, Visualization, Investigation. Jing Wang: Writing – original draft, Investigation. Ming-Ying Gao: Investigation, Writing – review & editing. Naila Aslam Shad: Visualization. Ning Li: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. Yan-Hua Gong: Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of generative AI and AI-assisted technologies use

During the preparation of this manuscript, the authors used Grammarly and QuillBot to assist with grammatical correction, language polishing, paraphrasing, and to improve sentence clarity and the overall academic writing style. Representative prompts and instructions included: "Correct the grammar and improve the academic tone," "Improve the clarity and flow of this paragraph without changing its scientific meaning," and "Paraphrase this sentence while preserving the original meaning."

These tools were not used to generate or analyze research data, interpret results, or formulate scientific conclusions. All revised content was carefully reviewed, verified, and edited by the authors, who take full responsibility for the accuracy, originality, and integrity of the final manuscript.

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Conflict of interest disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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