Cardiovascular surgery and organ damage: Time to reconsider the role of hemolysis

Iris C. Vermeulen Windsant, MD,[a,b,d] Sebastiaan J. Hanssen, MD,[a,c,d] Wim A. Buurman, PhD,[a,b] and Michael J. Jacobs, MD, PhD[a,c,d]

Cardiovascular surgery with cardiopulmonary bypass is associated with postoperative organ injury, which severely affects patient morbidity and mortality. Multiple cardiopulmonary bypass–related mechanisms have been linked to the development of tissue damage, including hypoperfusion, ischemia–reperfusion, and induction of a proinflammatory response. Hemolysis, resulting in increased plasma free hemoglobin concentrations, is generally considered an inevitable but relatively harmless side effect of cardiopulmonary bypass. Recently, however, evidence has been mounting that plasma free hemoglobin scavenges intravascular nitric oxide, thereby attenuating its bioavailability. Any significant reduction in nitric oxide, the most important endogenous vasodilator, impairs tissue perfusion and induces organ injury development. Moreover, urinary free hemoglobin contributes to renal damage, specifically by catalysis of reactive oxygen species formation. In this review, the effects of increased free hemoglobin levels on nitric oxide metabolism are discussed. In addition, we review the role of free hemoglobin in organ injury development, potential sources of free hemoglobin during cardiovascular surgery, and therapeutic options to attenuate the consequences of hemolysis. We propose that hemolysis is more than an innocent bystander effect of cardiopulmonary bypass–assisted surgery. Therapeutic interventions to attenuate the effects of hemolysis seem crucial in the reduction of postoperative morbidity and mortality after cardiovascular surgery.

Cardiovascular surgery with extracorporeal circulation is associated with considerable postoperative morbidity and mortality, especially among patients undergoing complex procedures such as combined coronary artery bypass grafting (CABG) and valve surgery, Bentall procedures, and open repair of thoracic and thoracoabdominal aortic aneurysms. These patients are at high risk for development of such major complications as acute kidney injury,[1-6] pulmonary dysfunction,[7,8] sepsis, and multiple organ failure.[9]

The pathophysiologic mechanisms underlying these complications have been studied extensively in an attempt to develop specific prevention and treatment strategies. The cardiopulmonary bypass (CPB) circuit has been associated with the development of tissue damage as a result of insufficient oxygen delivery through hemodilution,[10] ischemia–reperfusion,[11] and hypoperfusion.[12,13] Cardiotomy suction during CPB has been shown to be a source of lipid microemboli, which form small vascular occlusions in several tissues, including brain, kidney, spleen, and muscle.[14,15] Furthermore, the nonendothelial surface of the CPB system initiates a proinflammatory response that deteriorates cellular function, for instance the function of renal tubular cells.[16,17] Indeed, the use of a mini-CPB system attenuates the release of intestinal and renal tissue damage markers in cardiac surgical patients relative to a normal CPB circuit by reducing both the proinflammatory contact surface area and hemodilution.[18] Similarly, the incidences of liver injury and kidney injury are significantly reduced in patients undergoing CABG without CPB (off-pump CABG) relative to those undergoing on-pump CABG.[19,20] Nevertheless, although off-pump surgery has gained popularity worldwide, CPB-assisted surgery is still widely used. To reduce CPB-related morbidity and mortality, successful efforts have been made to increase CPB biocompatibility and flow performance. Unfortunately, these improvements have not led to a significantly decreased incidence of organ dysfunction after cardiovascular surgery.[21] This relative failure underscores the need for further clarification of underlying pathophysiologic mechanisms of tissue damage and dysfunction in this setting.[21]

A common consequence of CPB is hemolysis, which is generally considered an inevitable but relatively harmless phenomenon. Hemolysis is principally caused by mechanical shear stress within the perfusion circuit and results in the release of hemoglobin into the circulation.[22,23] The role of this cell-free plasma hemoglobin (fHb) in the development of organ injury has gained increasing interest ever since a direct relationship of hemolysis, impaired vascular function, decreased organ perfusion, and organ dysfunction was reproducibly shown in experimental animal models and in chronic hemolytic diseases in human beings.[24,25] Most recently, Meyer and colleagues[26] showed that fHb arising from hemodialysis-induced hemolysis impairs vascular

From the Department of Surgery,[a] NUTRIM School for Nutrition, Toxicology and Metabolism,[b] and Cardiovascular Research Institute Maastricht (CARIM),[c] Maastricht University Medical Center, Maastricht, The Netherlands; and the European Vascular Center Aachen-Maastricht,[d] Department of Vascular Surgery, University Hospital Aachen, Aachen, Germany.

I.C.V.W. and S.J.H. contributed equally to this work.

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Address for reprints: Wim A. Buurman, PhD, Department of Surgery, Maastricht University Medical Center, PO Box 616, Maastricht, The Netherlands (E-mail: w.buurman@maastrichtuniversity.nl).

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Abbreviations and Acronyms

CABG = coronary artery bypass grafting
CO = carbon monoxide
CPB = cardiopulmonary bypass
fHb = cell-free plasma hemoglobin
HO = heme oxygenase
NO = nitric oxide
RBC = red blood cell
SNO-Hb = S-nitrosylated hemoglobin

function in patients, leading to speculation regarding a role for fHb in the development of microcirculatory dysfunction during acute and transient hemolysis.[27] Moreover, the results of Meyer and colleagues[26] substantiate our own recent findings[28] that acute hemolysis during major aortic surgery is independently associated with proximal renal tubular damage and postoperative acute kidney injury. We therefore propose that hemolysis is an important but as yet generally unrecognized contributor to the development of organ injury during surgical procedures associated with hemolysis, such as cardiovascular surgery.

The interfering role of fHb in intravascular nitric oxide (NO) metabolism is believed to play a critical role in the development of microcirculatory impairment, organ damage, and organ dysfunction.[24] In this review we will therefore discuss (1) the effects of fHb on intravascular NO bioavailability, (2) the role of fHb in the induction of microcirculatory dysfunction and organ damage, (3) the potential sources of fHb during cardiovascular surgery, and (4) therapeutic options to attenuate the consequences of hemolysis during CPB-assisted surgery.

INCREASED PLASMA FREE HEMOGLOBIN PRODUCED BY HEMOLYSIS REDUCES INTRAVASCULAR NITRIC OXIDE BIOAVAILABILITY

On the intravascular destruction of red blood cells (RBCs), fHb enters the circulation. This fHb either gets bound to haptoglobin or is oxidized to methemoglobin. The hemoglobin–haptoglobin complex is rapidly cleared from the circulation through endocytosis by the surface scavenger receptor CD163, which is expressed on monocytes and tissue macrophages. In this way haptoglobin prevents accumulation of plasma fHb under physiologic circumstances.[29] This effect was illustrated in patients undergoing cardiac surgery in whom intravenous administration of haptoglobin significantly reduced circulating fHb levels.[30] Free heme, another byproduct of hemolysis, is released during oxidation of free hemoglobin and is scavenged by circulating hemopexin. Subsequently, heme oxygenase (HO)-1, activated as a result of reduced microcirculation, degrades heme to carbon monoxide (CO), biliverdin, and iron, mainly in the liver and spleen.[31] This cytoprotective induction of HO-1 by the microvasculature has been shown to modulate inflammation in patients after cardiac surgery with CPB, which may benefit patient recovery postoperatively[32]; furthermore, the induction of HO-1 inhibits vascular inflammation and vasoocclusion in transgenic sickle cell mice.[32,33] Because both haptoglobin and hemopexin are not recycled after clearance of hemoglobin–haptoglobin or heme–hemopexin complexes, excessive RBC lysis rapidly exhausts their storage pools. This results in enhanced levels of fHb and free heme, both harmful products. First of all, free heme is able to react with endogenous hydrogen peroxide, thereby forming toxic free radicals that are involved in the induction of prooxidant damage.[34] Second, oxygenated fHb has been shown to be a potent scavenger of NO, the most important endogenous vasodilator. The fast ($6–8 \times 10^7$ mol/[L · s]) and irreversible reaction of oxygenated fHb with NO results in conversion of fHb to methemoglobin and conversion of NO to nitrate. Circulating fHb is also present in a deoxygenated form. Such deoxygenated fHb also scavenges NO, forming nitrosyl hemoglobin, but this reaction is both slower (10^7 mol/[L · s]) and reversible.[24,29] As a result, hemolysis significantly impairs NO bioavailability, potentially inducing microcirculatory dysfunction.[35,36] In an in vivo canine hemolysis model, fHb-associated NO scavenging has been found to be correlated with systemic vasoconstriction and a reduction in renal function.[25] In patients with chronic high fHb levels as a result of sickle cell disease, forearm blood flow responses were reduced by 80% after infusion of the NO donor sodium nitroprusside relative to patients with below average fHb levels.[24] Third, hemolysis also results in release of arginase 1, an enzyme that converts L-arginine, the substrate for NO synthesis, to ornithine.[37] In this way, hemolysis not only causes scavenging of NO but also theoretically prevents new NO formation. In practice, however, we have shown that arginase 1 release during surgery with CPB does not affect the arginine to ornithine ratio (unpublished data). This implies that arginase levels during this type of surgery are not high enough to affect arginine levels and thus attenuate NO synthesis.[38]

CELL-FREE HEMOGLOBIN CONTRIBUTES TO MICROCIRCULATORY DYSFUNCTION THROUGH NITRIC OXIDE SCAVENGING, POTENTIALLY INDUCING HYPOXIC TISSUE DAMAGE

The role of hemolysis in organ damage development had already been described in the mid 1970s.[24,39] These studies focused on acute kidney injury because glomerularly filtered urinary fHb, rather than plasma fHb, was considered the culprit in organ injury induction. At that time, only the kidney was believed to be at risk for

fHb-induced damage. Two mechanisms were proposed to underlie the association between hemolysis and renal tissue damage development. First, urinary fHb–derived free iron and heme catalyze the generation of reactive oxygen species, which damage the renal tubular epithelium.[40] Indeed, administration of the iron scavenger deferoxamine did attenuate glomerular and tubular dysfunction induced by intravenous fHb administration in rats.[41] Similarly, a reduced intravascular iron scavenging capacity—reflected by low plasma ferritin concentrations—was associated with acute kidney injury after human cardiovascular surgery.[42] Second, intratubular fHb precipitation and heme cast formation in the acidic ultrafiltrate were considered to obstruct the tubular lumen, reducing glomerular filtration.[40,43] Subsequent prevention of cast formation by urinary alkalanization was found to reduce tubular injury and glomerular dysfunction after intravenous fHb infusion in rats.[40]

The discovery of the NO-scavenging properties of circulating plasma fHb by Reiter and coworkers[24] in 2002 provided a complementary explanation for hemolysis-induced organ injury. For the first time, circulating fHb was recognized as a key player in the pathophysiologic mechanisms of complications in patients with chronic hemolytic disorders, such as sickle cell disease and malaria infection.[24] Furthermore, the reported adverse effects associated with administration of a hemoglobin-based oxygen carrier—which basically consisted of fHb—could be explained by intravascular NO scavenging through fHb.[44] The negative effects of increased plasma fHb have been confirmed by many studies since 2002 in both animals and patients. For example, hemolysis induced by water infusion or direct intravascular fHb administration in dogs was associated with a significant increase in plasma NO consumption and with simultaneous enhanced systemic vascular resistance. These effects were attenuated by NO inhalation (which causes conversion of plasma fHb into the less bioactive molecule methemoglobin in the pulmonary circulation), supporting a causal role for NO scavenging by fHb.[25] In human beings, forearm blood flow responses to intra-arterial infusion of sodium nitroprusside, a NO donor, were found to be negatively correlated with plasma fHb levels in patients with sickle cell disease.[24,25]

The potential role of plasma fHb in the development of organ injury is further supported by our observation that plasma fHb levels are significantly associated with renal proximal tubular damage during CPB-assisted major aortic surgery.[26] Moreover, peak plasma fHb levels significantly predict postoperative acute kidney injury. We could not detect fHb in urinary samples during the perioperative period, indicating that urinary fHb is not a major contributor to renal tubular injury development in this setting.[26] Also, forearm blood flow responses after infusion of sodium nitroprusside at the time of peak plasma fHb concentrations are significantly reduced relative to the response measured when fHb-levels are normalized. This observation further underscores a potential causal role of fHb-induced tissue perfusion impairment during surgery (unpublished data). In addition, we have shown that fHb induces intestinal microcirculatory dysfunction and tissue integrity loss in a rat hemolysis model.[45]

SOURCES OF CIRCULATING FREE HEMOGLOBIN DURING CARDIOVASCULAR SURGERY WITH CARDIOPULMONARY BYPASS

Hemolysis can principally be attributed to 3 sources during cardiovascular surgery: the CPB, the cell salvage system, and (massive) RBC transfusion.

Cardiopulmonary Bypass

CPB inflicts sublethal to lethal RBC damage through turbulence and shear stress within the pump, tubes, connectors, cannula, reservoirs, and oxygenator.[23] Blood–air contact, blood–nonendothelial surface contact, wall impact forces, the use of positive and negative pressures to assist venous drainage, and the use of an integrated cardiotomy suction reservoir all contribute to intraoperative hemolysis.[23,46,47] In addition to CPB composition, CPB duration is considered to influence the degree of hemolysis, with longer CPB times resulting in increased lysis of RBCs.[48] As evidence of this phenomenon, we found a positive and statistically significant correlation between total fHb release in the perioperative period and the duration of CPB in a group of 54 patients undergoing CPB-assisted major aortic surgery (unpublished data; Figure 1). In addition to direct RBC lysis, the CPB system induces sublethal RBC injury.[49,50] Such sublethally damaged RBCs have been shown to be more prone to later lysis in vivo.[23,51] Delayed lysis of sublethally damaged RBCs could explain the continuing increase of plasma fHb after the cessation of CPB in these patients (unpublished data; Figure 2).

Cell Salvage

Cell salvage devices are additional sources of fHb.[52,53] The mechanical trauma of washing and the transfusion of damaged autologous shed blood could contribute to increased plasma fHb levels. Although modern cell salvage systems are able to remove the majority of fHb during washing, they do not select between intact RBCs and damaged RBCs, which are prone to later lysis in vivo.[54] In this way, autologous blood transfusion could contribute to increased plasma fHb levels during surgery.

RBC Transfusion

A last potential source of fHb is stored RBC concentrate. Storage of erythrocytes results in irreversible morphologic changes, such as reductions in membrane deformability,

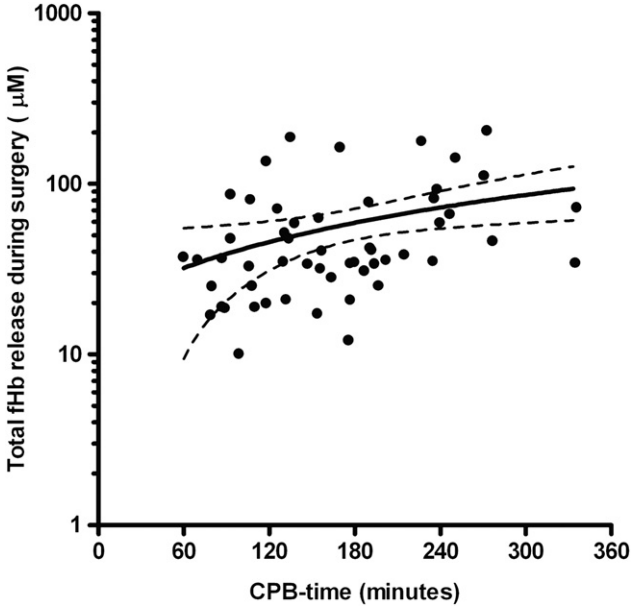

FIGURE 1. Association between cardiopulmonary bypass time and extent of hemolysis. A correlation analysis between cardiopulmonary bypass *(CPB)* time and total free hemoglobin *(fHb)* release (defined as the area under the curve) was performed in 54 patients undergoing open surgical repair of thoracoabdominal aortic aneurysms with cardiopulmonary bypass. A significant correlation (Pearson $r = 0.33$, $P < .05$) was found between cardiopulmonary bypass time and plasma free hemoglobin release (unpublished data).

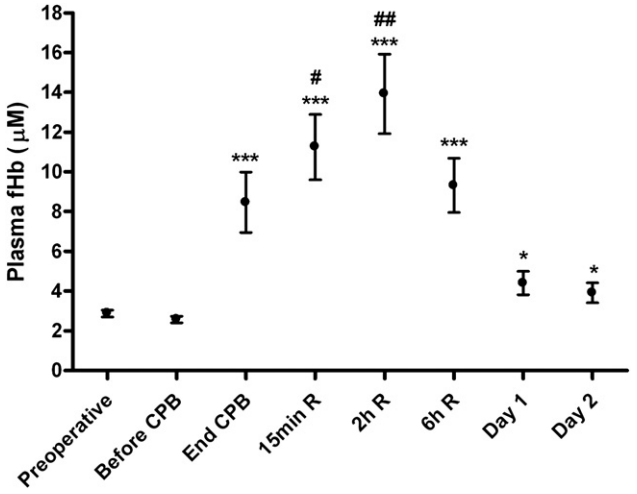

FIGURE 2. Hemolysis during open surgical repair of thoracoabdominal aortic aneurysms with cardiopulmonary bypass *(CPB).* Plasma free hemoglobin *(fHb)* levels increased during surgery and continued to increase significantly in the early postoperative period. This indicates ongoing lysis of red blood cells after cardiopulmonary bypass had stopped. *Asterisk* indicates $P < .05$ versus preoperative level; *triple asterisk* indicates $P < .001$ versus preoperative level; *crosshatch* indicates $P < .05$ versus end-CPB level; *double crosshatch* indicates $P < .01$ versus end-CPB level (unpublished data). *R,* Reperfusion.

oxygen binding, and delivery capacity, in addition to increased adhesiveness, increased aggregability, and accumulation of proinflammatory substances.[55] These changes are considered to underlie the relationship between RBC transfusion and adverse outcome, a phenomenon that has given rise to debate about the pros and cons of allogeneic RBC administration. In addition, the storage duration of blood products appears to be a critical factor in transfusion-related morbidity and mortality. In a study by Kock and colleagues,[55] transfusion of ''old'' blood (stored for >14 days) was associated with a significantly higher mortality among patients undergoing cardiac surgery than was seen with transfusion of RBCs stored for 14 days or less. Transfusion of ''older'' blood was also significantly related to prolonged ventilatory support and increased incidences of renal failure, septicemia or sepsis, and multiple organ failure. In another study, RBC transfusion was the most reliable predictor of adverse outcome in 11,963 patients undergoing isolated CABG, with postoperative morbidity and mortality being dose dependently related to RBC transfusion.[56] We propose that high fHb concentrations caused by RBC lysis in stored blood contribute to posttransfusion morbidity and mortality by inducing microcirculatory dysfunction through NO scavenging. In addition, the shear stress imposed on less viable RBCs within the transfusate could cause additional increases in fHb both during and after infusion. To study the degree of direct hemolysis of packed RBCs, we measured fHb levels by derivate spectrophotometry[57] in samples from 60 randomly collected and transfused packed RBC units (330 mL/U). The levels of fHb in the packed RBC supernatant averaged 36 ± 2 μmol/L (mean ± SEM), indicating severe hemolysis. Moreover, storage durations and fHb levels were significantly correlated (unpublished data, Figure 3). This correlation means that transfusion of especially aged packed RBCs can result in an additional increase in circulating fHb in patients undergoing cardiovascular surgery with CPB. Recently, it was reported that packed RBC transfusion did indeed contribute to systemic fHb levels, with an increase of 7.5μM per transfused unit.[58] Importantly, the supernatant of the packed RBCs, which contains the fHb molecules, was indeed able to consume NO, with a strong correlation between fHb levels and NO consumption.[58] The contributing effect of RBC transfusion to circulating plasma fHb concentrations may thus be considerable. The median transfusion requirement in a large cohort of cardiac surgical patients was found to be 2 packed RBC units,[55] whereas 10 units are required for patients undergoing open repair of thoracoabdominal aortic aneurysms.[28] Transfusion of 2 or 10 packed RBC units could increase plasma fHb levels, with 15 μmol/L and 75 μmol/L, respectively. These levels are in sharp contrast to the plasma fHb levels of 0.1 to 0.2 μmol/L seen in normal healthy volunteers.[24,58] In addition to direct fHb administration through packed RBC

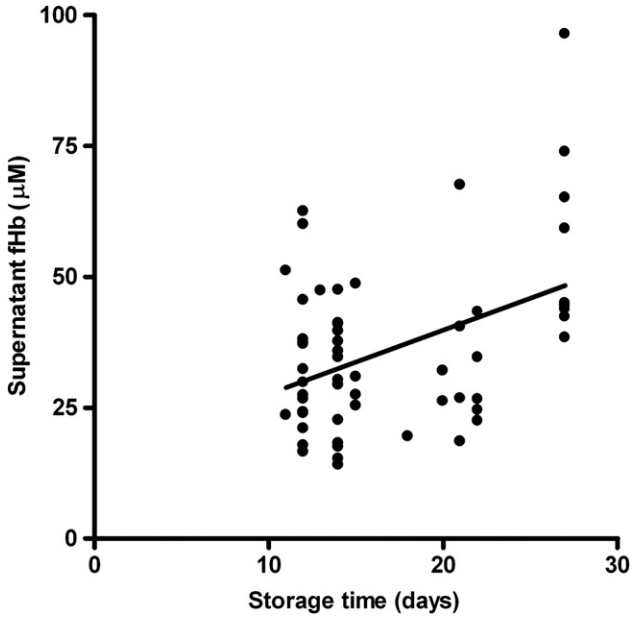

FIGURE 3. Association between storage time and free hemoglobin *(fHb)* levels in packed red blood cell transfusion bags. A significant correlation (Pearson $r = 0.43$, $P < .001$) was found between packed red blood cell storage duration and free hemoglobin concentration in packed red blood cell transfusion bags (n = 60) used for transfusion during cardiovascular surgery. These data indicate that prolonged storage duration results in more red blood cell injury, with concomitantly more free hemoglobin being transfused into the patient (unpublished data).

transfusion, systemic fHb concentrations could additionally increase as a result of delayed lysis of sublethally damaged RBCs. Finally, another consequence of RBC storage is that NO bound to intracellular hemoglobin, known as S-nitrosylated hemoglobin (SNO-Hb), is rapidly depleted. RBCs containing SNO-Hb are believed to contribute to vasodilation under hypoxic conditions through release of NO. Indeed, the capacity of RBCs to induce vasodilation is significantly diminished in parallel with SNO-Hb depletion. In a canine study, reconstitution of SNO-Hb by exposure to aqueous NO restored the vasodilatory capacity of transfused RBCs and improved cardiac blood flow.[59]

THERAPEUTIC OPTIONS TO ATTENUATE THE ADVERSE EFFECTS OF CELL-FREE PLASMA HEMOGLOBIN–MEDIATED NITRIC OXIDE SCAVENGING

The discovery of the NO-scavenging capacity of circulating fHb opened up a new field of study regarding therapeutic options in diseases characterized by chronic hemolysis, such as sickle cell disease and malaria.[60] In this section, we present therapies that we consider to be of value for patients with acute hemolytic episodes, such as occur during cardiovascular surgery (Table 1).

The direct (intravascular) therapeutic use of NO itself is greatly impeded by its extremely short half-life of 0.05 to 1.8 ms in vivo.[61] Therefore either inactivation of fHb or enhancement of the NO-donor pool has the potential to reduce the incidence and severity of complications of hemolytic diseases. We focus mainly on 3 potential therapeutic interventions (Figure 4): NO inhalation, nitrite supplementation, and haptoglobin administration. We consider these therapeutic options to be the most clinically relevant at this time. In addition, we discuss 3 more experimental therapies: arginine and citrulline supplementation, CO inhalation, and endothelin receptor blockade. For a more complete discussion of therapeutic options in chronic hemolytic disease, we refer the reader to articles by Kato and Gladwin[60] and Lundberg and colleagues.[62]

NO Inhalation

Intravascular conversion of plasma fHb into a less bioactive molecule is an interesting option to reduce the adverse consequences of increased fHb concentrations. NO gas inhalation results in pulmonary oxidation of fHb into methemoglobin, which does not scavenge NO, reducing NO consumption in plasma. In a canine hemolysis model, NO inhalation attenuated the pulmonary and systemic vasoconstrictor effects of fHb.[25] In patients with sickle cell disease, NO inhalation of 80 ppm for 1.5 hours reduced pain during vasoocclusive crisis, diminished plasma NO consumption, and increased methemoglobin levels, indicating oxidation of fHb.[24] Moreover, inhalation of NO at 80 ppm for 4 hours in children with sickle cell disease was not associated with any toxic side effects, such as hypotension, clinically significant decreases in oxygenation by pulse oximetry, significant increases in methemoglobin, or toxic concentrations of nitrogen dioxide.[63] Even continuous NO inhalation at 40 ppm for 3.2 days in a patient with sickle cell disease with multiorgan involvement was not associated with adverse side effects and it markedly improved the patient's clinical state.[64] Finally, mixing of NO gas at concentrations as great as 20 ppm with normal ventilation gas showed therapeutic potential in adult patients with cardiac surgery–associated pulmonary hypertension by reducing right ventricular afterload and preventing right ventricular failure.[65,66] In addition to fHb oxidation, NO gas inhalation also results in the formation of relatively stable NO species in the lung. Longer intravascular half-lives of these NO carriers, such as nitrite, enable transport of NO in the blood, mediating extrapulmonary effects of NO gas inhalation.[67,68] In conclusion, we consider NO inhalation to be a promising and potentially easily applicable therapeutic option to attenuate the adverse effects of fHb-mediated NO scavenging during cardiovascular surgery. Ideally, the dose of NO inhalation could even be adjusted according to intraoperative fHb measurements, which are already routinely performed at our institution.

TABLE 1. Potential therapeutic options to reduce hemolysis-associated morbidity in patients undergoing cardiovascular surgery

Therapy	Main therapeutic mechanisms	Feasibility	References
NO inhalation	Oxidizes and inactivates fHb in pulmonary circulation, reducing NO scavenging Stimulates intrapulmonary formation of NO donors, enhancing NO bioavailability	Already applied in patients with sickle cell disease and those undergoing cardiac surgery Exact dose and duration of inhalation need to be studied	Reiter et al,[24] Minneci et al,[25] Kato et al,[60] Cannon et al[67]
Nitrite supplementation (oral, intravenously, by inhalation)	NO donor during reduction, especially during hypoxia and low pH Mediates cytoprotection through hypoxic vasodilation and decreased formation of reactive oxygen species Oxidizes fHb, thereby reducing NO scavenging	Nitrite successfully used in experimental setting with patients with sickle cell disease Exact dose, duration, and mode of administration need to be assessed	Lundberg et al,[62] Shiva et al,[69] Piknova et al,[71] Minneci et al[76]
Haptoglobin administration	Natural fHb scavenger Accelerates fHb uptake by monocytes and macrophages and accelerates hepatic degradation Limits renal filtration of fHb	Costs currently limit clinical applicability Haptoglobin potentially does not limit NO scavenging by fHb	Tanaka et al,[30] Lim et al,[81] Azarov et al,[82] Boretti et al[83]
Arginine and citrulline supplementation	Enhances substrate delivery for NO formation through nitric oxide synthases	Arginine used experimentally in 10 patients with sickle cell disease Dose and duration of arginine and citrulline administration unknown	Morris et al,[86] Luiking et al[88]
Carbon monoxide inhalation	Regulates vascular tone and induces vasodilation at low doses Reduces proinflammatory response Relatively inert and does not form reactive oxygen species	No human data on applicability in setting of cardiovascular surgery	Belcher et al[33]
Endothelin receptor blockade	Limits endothelin 1–induced vasoconstriction	Only tested in mouse sickle cell disease model	Sabaa et al[90]

NO, Nitric oxide; *fHb*, free hemoglobin.

Nitrite Supplementation

Another promising candidate for therapeutic use is the nitrite anion (NO_2^-), long believed to be merely an inert oxidation product of NO. Recent studies, however, have provided evidence for the existence of multiple nitrite reducing pathways in which nitrite is converted back to NO, making nitrite an important NO donor.[62,69,70] Furthermore, nitrite is able to oxidize fHb in plasma, potentially limiting the capacity of fHb to scavenge NO.[71] The enzymatic and nonenzymatic pathways of nitrite reduction include (free) deoxyhemoglobin and deoxymyoglobin, xanthine oxidoreductase, protons, ascorbate, and polyphenols.[62] The process of NO reduction is most efficient at low Po_2 and low pH and thus occurs preferentially during hypoxia or anoxia.[70,72,73] This is of particular importance because NO synthase activity is greatly limited at low Po_2 values.[74] In adult patients with sickle cell anemia, infusions of 0.4-, 4-, and 40-μmol/L nitrite into the brachial artery led to a dose-dependent increase in forearm blood flow as great as 77%.[75] In a canine hemolysis model, sodium nitrite increased blood flow in a similar way.[76] In addition to promoting vasodilation, nitrite has been shown to exert potent cytoprotective effects in the liver, heart, and brain in several animal models of ischemia–reperfusion.[77] The pathway by which nitrite mediates cytoprotection in this setting is as yet unresolved but is believed to be dual. First, nitrite enables hypoxic vasodilation, as stated previously. Second, nitrite is able to nitrosate complex I of the mitochondrial electron transport chain, inhibiting its activity and decreasing the formation of reactive oxygen species in the reperfusion phase.[69] Considering these properties, it has been suggested that nitrite has therapeutic value in various diseases, such as sickle cell disease, stroke, myocardial infarction, and organ transplantation. Nevertheless, the optimal nitrite dose for

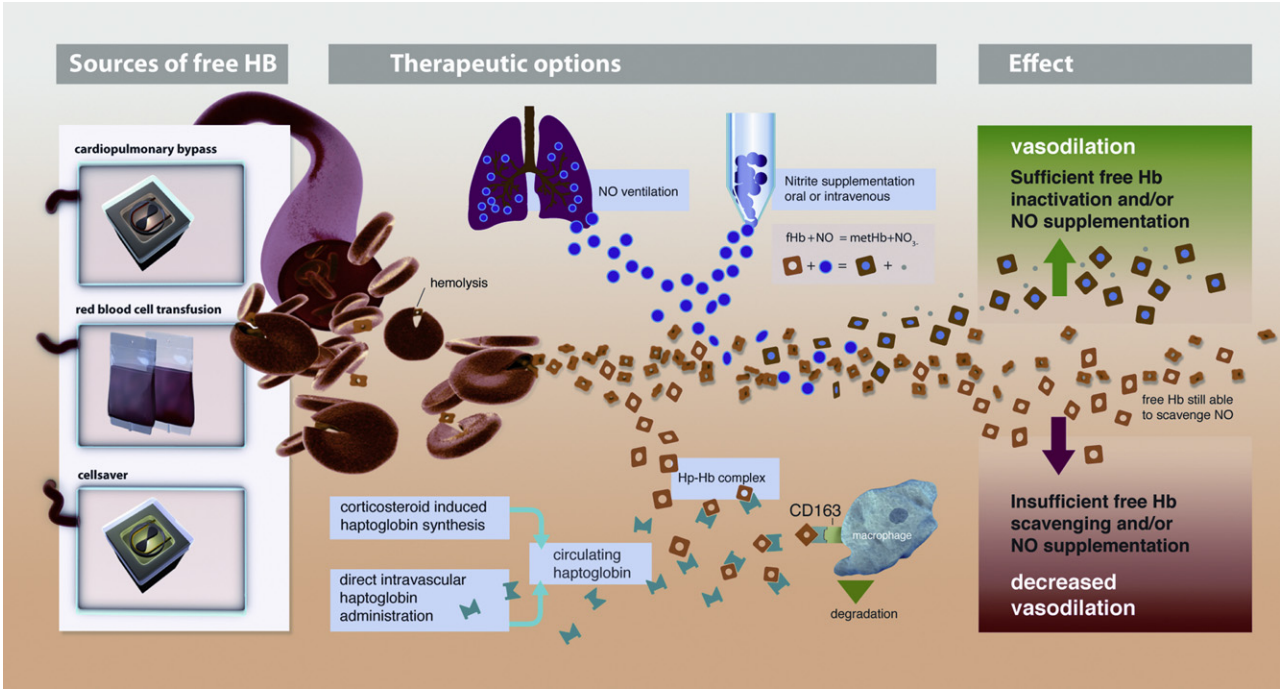

FIGURE 4. Potential sources and effects of plasma free hemoglobin *(HB)* during cardiovascular surgery with cardiopulmonary bypass, and therapeutic options to attenuate hemolysis-induced organ damage. Intravascular hemolysis during cardiovascular surgery can be attributed to the cardiopulmonary bypass, transfusion of red blood cells, and cell salvage use *(left).* Lysis of red blood cells results in increased circulating plasma free hemoglobin *(Hb)* levels *(orange squares).* Under physiologic conditions, free hemoglobin is rapidly cleared by the scavenger haptoglobin. Haptoglobin–hemoglobin *(Hp-Hb)* complexes bind to CD163 expressed by monocytes and macrophages, initiating endocytosis and degradation *(middle, bottom).* Haptoglobin is not recycled, so excessive hemolysis depletes haptoglobin storages rapidly. Nonscavenged free hemoglobin *(fHb)* potently binds circulating nitric oxide *(NO),* thereby limiting its bioavailability. In this way high free hemoglobin levels increase the nitric oxide–scavenging capacity of blood, causing impaired vasodilation as a result of vascular nitric oxide shortage. Decreased vasodilation contributes to impaired tissue perfusion and development of organ damage and organ dysfunction *(right, bottom).* The adverse effects of free hemoglobin may be counteracted by either increasing haptoglobin levels to support free hemoglobin scavenging or enhancing the nitric oxide donor pool to increase nitric oxide bioavailability. Haptoglobin can be administered intravenously, or haptoglobin synthesis may be upregulated through corticosteroid administration *(center, bottom).* The nitric oxide donor pool could be increased by oral or intravenous administration of nitrite, which is oxidized to nitric oxide under low P_{O_2} or low pH. Nitric oxide inhalation inactivates free hemoglobin in the pulmonary circulation (or oxygenator in the cardiopulmonary bypass circuit) by transforming it to bioinactive methemoglobin *(metHb, center, top).* In this way scavenging and inactivation of free hemoglobin and supplementation of nitric oxide prevent the adverse effects of plasma free hemoglobin during cardiovascular surgery *(right, top).*

human systemic administration has not yet been clarified. Interestingly, the effect of nitrite may be greater at low plasma concentrations (<200 nmol/L), being lost at high plasma levels (>1000 μmol/L).[78,79] Furthermore, it has been reported that the reaction between oxygenated fHb (in contrast to deoxygenated fHb) and nitrite could initiate an autocatalytic free radical chain, leading to unwanted oxidative damage.[80] Piknova and colleagues[71] addressed this issue and concluded that free radical formation in plasma during the reaction of pharmacologic doses of nitrite (up to 120 μmol/L) with clinically relevant levels of fHb (30 μmol/L) would be highly unlikely. In conclusion, we consider nitrite to have potential in patients undergoing cardiovascular surgery to prevent and treat hemolysis-associated morbidity. Nevertheless, the optimal dose and application must be studied further.

Haptoglobin Administration

Administration of haptoglobin, which is the physiologic fHb scavenger, appears to be a logical choice for reducing fHb concentrations. Haptoglobin targets fHb for degradation in the liver, monocytes, and macrophages. Furthermore, haptoglobin prevents glomerular filtration of fHb, reducing fHb-induced kidney damage. Increased fHb levels during cardiac surgery have been associated with total depletion of haptoglobin,[30] enhancing the NO-scavenging capacity of plasma. Indeed, haptoglobin knock-out mice were found to be more sensitive to the adverse effects of phenylhydrazine-induced hemolysis.[81] Renal DNA damage was significantly higher and glomerular filtration function (reflected by poorer renal clearance of tritium-labeled inulin) was significantly lower in haptoglobin knock-out mice relative to haptoglobin-positive mice. Interestingly,

administration of vasodilators restored glomerular filtration, implicating renal vasoconstriction as the major contributor to hemolysis-induced acute kidney injury. This supports the mechanism of NO scavenging by plasma fHb. In addition, haptoglobin administration in patients undergoing cardiac surgery with plasma fHb levels surpassing 2.3 μmol/L was associated with a reduction of renal tubular damage.[30] Nevertheless, this positive effect of haptoglobin was attributed to a decrease in urinary fHb levels and a subsequent attenuation of oxidative renal damage.

Haptoglobin administration is an interesting therapeutic option, but its clinical application may be limited. First, it has been recently found that in vitro the hemoglobin–haptoglobin complex still potently scavenges NO at the same rate as fHb. Although binding of fHb to haptoglobin increases the rate of uptake by monocytes and macrophages 2-fold (hereby disabling NO scavenging), it is questionable whether this increased uptake would affect NO bioavailability.[82] Second, the costs involved in retrieving or producing the amounts of haptoglobin necessary for clinical application currently limit widespread implementation. Recently, Boretti and coworkers[83] made use of the fact that the haptoglobin promoter gene contains glucocorticoid-responsive elements. Administration of 4 mg/kg prednisone twice daily for 3 days increased plasma haptoglobin levels 6-fold in dogs.[83] Importantly, the glucocorticoid stimulation of haptoglobin synthesis prevented the fHb-induced increase in mean arterial pressure in dogs after fHb infusion, most probably as a result of scavenging of fHb by haptoglobin.[83] This finding sheds new light on the long-standing debate as to whether corticosteroid administration during cardiac and cardiovascular surgery is useful.[84,85] In summary, haptoglobin could be useful to reduce kidney damage caused by intratubular fHb toxicity. The effect on NO scavenging may, however, be limited.

Other Potential Therapies

We consider NO inhalation and nitrite supplementation to be promising therapeutic interventions, because both can be used successfully in the acute setting of cardiovascular surgery. Notwithstanding, several other therapeutic modalities may be of value for patients at risk for acute hemolytic episodes.

Arginine and citrulline supplementation. The natural nitrogen donor for NO synthesis is L-arginine, and arginine supplementation enhances NO formation. Arginine therapy at a dose of 0.1 g/kg 3 times a day for 5 days in 10 adult patients with sickle cell anemia and pulmonary hypertension resulted in a significant decrease (15.2%) in pulmonary arterial systolic pressures, implicating vasodilation.[86,87]

Another interesting approach would be administration of citrulline, a substrate for de novo arginine synthesis. It has been suggested that in cases of high arginase 1 levels, such as occur during hemolysis, citrulline supplementation might restore the intracellular arginine balance and promote NO production.[88] It remains unknown, however, whether and to what extent arginine or citrulline supplementation is beneficial in patients with acute hemolysis during cardiovascular surgery.

CO inhalation. CO is produced during breakdown of the heme ring of fHb, which is mediated by HO-1. Inhaled CO, at low doses of 250 ppm or less, has been shown to reduce vasoocclusion in a mouse sickle cell model. CO even mimics some of the functions of NO, such as inhibition of platelet aggregation and activation, regulation of vascular tone, and reduction of a proinflammatory response.[33] CO is relatively inert, in contrast to NO, which is able to react with intravascular reactive oxygen species to form the highly reactive peroxynitrite ($ONOO^-$).[89] CO has therefore been proposed to be even more effective in treating hemolytic disease than NO, but this hypothesis has not been substantiated in human studies.

Endothelin receptor blockade. Endothelin 1 is an extremely potent vasoconstrictor, and its secretion is repressed by NO. Diminished NO bioavailability as a result of hemolysis counterbalances this negative feedback, resulting in enhanced endothelin 1 levels and vasoconstriction. A mouse sickle cell model provided evidence for beneficial effects of endothelin receptor blockade on renal blood flow, inflammation, and vascular congestion in the lungs and kidneys.[90] Reduction of inflammation would be an additional positive effect in patients subjected to CPB, which induces a proinflammatory response. Nevertheless, further studies are essential to determine whether short-term endothelin receptor blockage is beneficial in this setting, because it is very different from sickle cell anemia.

In conclusion, there are several promising therapeutic interventions to attenuate the adverse effects of increased plasma fHb levels in patients suffering from acute hemolytic disease, including patients undergoing surgery with CPB, patients with trauma, and patients needing long-term extracorporeal support, for instance extracorporeal membrane oxygenation or hemodialysis.[91,92]

HEMOLYSIS-INDUCED ORGAN INJURY IN CARDIOVASCULAR SURGERY: SUMMARY AND FUTURE PERSPECTIVES

As evaluated in this review, hemolysis during CPB creates a latent adverse effect. The NO-scavenging effect of plasma fHb contributes to the deleterious effects of CPB, such as hypoperfusion and ischemia–reperfusion, thereby further hampering tissue perfusion and resulting in organ injury and dysfunction. These findings shed new light on the pathophysiologic mechanisms and preventive measures of organ injury during on-pump cardiovascular surgery (Figure 4).

CONCLUSIONS

Evidence is mounting that enhanced circulating levels of plasma fHb are detrimental, not only in the setting of chronic hemolytic diseases but also in the acute setting of cardiovascular surgery. The fHb-induced perturbations in microcirculatory blood flow and subsequent hypoperfusion or even ischemic damage, complemented by urinary fHb–induced oxidative stress to renal tissue cells, should be acknowledged as an important risk factor for organ injury development in patients undergoing cardiovascular surgery. Patients undergoing such procedures are at increased risk for development of postoperative organ injury, with correspondingly worse patient outcomes. Circulating fHb appears to be an important determinant in organ injury development, which offers a new therapeutic opportunity to reduce postoperative morbidity and mortality of these patients. Interventional studies with NO inhalation, nitrite supplementation, or haptoglobin administration should be performed to establish the causal links among plasma fHb, NO bioavailability, and organ injury in this particular setting.[25,30,93,94] Furthermore, such interventional studies will provide valuable information for improvement of patient outcome. Finally, the role of fHb in organ injury development is of importance not only for patients undergoing cardiovascular surgery but also for other patient groups at risk for hemolysis, such as patients with trauma, patients undergoing hemodialysis, and patients requiring long-term extracorporeal oxygenation or extracorporeal life-support.

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