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FOCUS ISSUE: BIOMARKERS IN CARDIOVASCULAR DISEASE

Novel Biomarkers, Oxidative Stress, and the Role of Labile Iron Toxicity in Cardiopulmonary Bypass-Associated Acute Kidney Injury

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Cardiac surgery-associated acute kidney injury (AKI) is common and carries a poor prognosis. Hemodynamic and inflammatory factors and the release of labile iron, contributing to oxidation from reactive oxygen species are among the major determinants of cardiac surgery-associated AKI. The diagnosis of AKI is typically delayed because of the limitations of currently used clinical biomarkers indicating loss of renal function. However, several novel renal biomarkers, which predict AKI or protection from AKI after cardiopulmonary bypass (CPB), have been identified as early markers of kidney injury. In this state-of-the-art review, the authors analyze the pathophysiological implications of recent findings regarding novel renal biomarkers in relation to CPB-associated AKI. Neutrophil gelatinase–associated lipocalin, liver-type fatty acid-binding protein, and alpha-1 microglobulin predict the development of CPB-associated AKI, while hepcidin isoforms appear to predict protection from it, and these biomarkers are involved in iron metabolism. Neutrophil gelatinase-associated lipocalin participates in local iron transport. Liver-type fatty acid–binding protein and alpha-1 microglobulin function as high-affinity heme-binding proteins in different species, while hepcidin is central to iron sequestration and when increased in the urine appears to *protect* from CPB-associated AKI. Free iron-related, reactive oxygen species–mediated kidney injury appears to be the unifying pathophysiological connection for these biomarkers. Such novel findings on renal tubular biomarkers were further combined with other lines of evidence related to hemolysis during CPB, the associated excess of free heme and iron, knowledge of the effect of free iron on renal tubular cells, and recent trial evidence targeting free iron-mediated mechanisms of AKI. Novel biomarkers point toward free iron-mediated toxicity to be an important mechanism of AKI in patients receiving cardiac surgery with CPB. (J Am Coll Cardiol 2010;55:2024–33) © 2010 by the American College of Cardiology Foundation

Acute kidney injury (AKI) is a common and severe complication in hospitalized patients and is associated with increased morbidity and mortality (1–4). Cardiac disease and cardiac surgery are both common precipitants (5–7). In critically ill patients, after sepsis, cardiac surgery with cardiopulmonary bypass (CPB) is the second most common cause of AKI (8). According to a recently published classification system (9), this condition can be classified as a form of cardiorenal syndrome type 1, a bidirectional condition that reflects an abrupt worsening of renal function secondary to acute cardiac disease or procedures, and vice versa.

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Cardiac surgery-associated AKI is a particular type of type 1 cardiorenal syndrome for which no clear understanding of pathogenesis exists (10) and no proven effective prophylaxis or treatment has yet been established. Furthermore, existing renal markers that confirm loss of renal function in this setting are only very late markers for the diagnosis of AKI. Recently, several novel biomarkers have emerged that show reasonable sensitivity and specificity for the prediction of AKI after CPB (11–13) and for the prediction of protection from CPB-associated AKI (14). Understanding of the physiological roles, and the responses of novel biomarkers to CPB and to interventions offer an opportunity to expand our understanding of the pathogenesis of CPB-associated AKI.

Previous studies have reported injury to red cells and release of free hemoglobin during CPB (15–17). Beside complete red blood cell fragmentation, there can also be sublethal red cell damage, resulting in altered rheological properties. Increased levels of free red blood cell constituents together with an exhaustion of their scavengers, transferrin and haptoglobin, result in a variety of serious clinical sequels, such

as increased systemic vascular resistance, altered coagulation profile, platelet dysfunction, renal tubular damage, and increased mortality (18). Such injury raises concernsthat CPB-associated AKI may be a form of renal sideropathy and that free or inappropriately liganded iron-related toxicity may play a role.

Sources of Evidence

In an attempt to explore whether and to what extent promising novel renal biomarkers point toward common or unifying mechanisms of AKI, we systematically searched the published research for novel renal biomarkers predicting AKI after CPB.

Two investigators (M.H., R.B.) independently searched Medline (via the PubMed interface), Embase, CENTRAL, the reference lists of obtained reports, and congress abstracts (to August 31, 2009) to identify potentially relevant reports or abstracts. We used the following search string: "biomarker" AND "acute kidney injury" OR "acute renal dysfunction" AND "cardiac surgery" OR "cardiopulmonary bypass" OR "coronary revascularization." We selected this type of AKI because patients are relatively homogenous and well characterized, the timing of renal injury is known, and the burden of disease is high (8). We included original studies in humans reporting on biomarkers that were found to be predictive of post-operative renal function after cardiac surgery with the use of CPB (Fig. 1). In a second step of our search, the biomarkers identified were combined with the search string "physiology" OR "pathophysiology" OR "mechanism" exploring what is known about the mechanisms of AKI they may contribute to or protect from.

Biomarker Evidence

Neutrophil gelatinase-associated lipocalin (NGAL). Using genomic, transcriptomic, and proteomic screening techniques for novel renal biomarkers (19,20) and innovative research on embryonic tissues (21), NGAL has been recently described as an early, highly sensitive and specific renal biomarker and to be implicated in the differentiation of kidney epithelia. NGAL was nephroprotective when administered simultaneously with renal ischemia-reperfusion (22,23). Kidney epithelia express and excrete massive quantities of NGAL when damaged by ischemia-reperfusion injury, nephrotoxins, and sepsis, as demonstrated initially in rats, mice, and pigs and then in human neonates, children, and adults (11,20,21,24–26).

Abbreviations and Acronyms

AKI = acute kidney injury
α_1MG = alpha-1 microglobulin
AUC = area under the receiver-operating characteristic curve
CPB = cardiopulmonary bypass
L-FABP = liver-type fatty acid-binding protein
NGAL = neutrophil gelatinase-associated lipocalin

In a prospective landmark study of 71 children undergoing CPB, AKI (defined as a 50% increase in serum creatinine) occurred in 28% of the subjects, but the diagnosis using serum creatinine was possible only 1 to 3 days after surgery (11). In marked contrast, NGAL measurements revealed a 10-fold or greater increase in the urine and plasma within 2 to 6 h of surgery in patients who subsequently developed AKI. Both urine and plasma NGAL were independent predictors of AKI, with areas under the receiver-operating characteristic curves (AUCs) of 0.998 for the 2-h urine NGAL measurement and 0.91 for the 2-h plasma NGAL measurement (11). The results of this study were confirmed in several further studies in pediatric cardiac surgery (Table 1) (11,27,28,30,31). In adults, several trials showed NGAL to be of varying value for subsequent AKI, with AUC values ranging from 0.56 to 0.96 (Table 1) (26,27,29,31,33,34). In a recent meta-analysis of diagnostic test studies on the performance of NGAL for AKI after cardiac surgery including 10 studies with 1,204 patients, the mean AUC was 0.78 (range 0.67 to 0.87) (35).

NGAL is a siderophore-binding lipocalin involved in ischemic renal injury and repair processes. In mice and rats, NGAL is expressed at very low levels in neutrophils and stimulated epithelia, including kidney, heart, lung, trachea, liver, colon, stomach, and brain (36). Plasma NGAL in AKI appears to be derived from distal tubular back leakage into the blood and from extrarenal sources as a result of "organ cross-talk" of the injured kidney (37). After glomerular filtration of NGAL, endocytosis via receptors such as megalin receptor (38) and 24p3 receptor into proximal tubules or secretion with the urine may occur.

Urinary NGAL is derived from local synthesis in distal parts of the nephron after injury or by excessively filtered plasma NGAL (20).

Lipocalins are a diverse group of ligand-binding proteins that share a conserved structure including an 8-stranded calyx, or cup-shaped structure, enclosing the ligand binding site.

Siderophores are small, iron-containing molecules produced from bacteria and plants that, through iron transport and supply, are involved in cellular growth and survival. Several hundreds of *microbial* siderophores have been identified (39), with the most common one in medical use, deferoxamine, being such a bacterial product. However, no

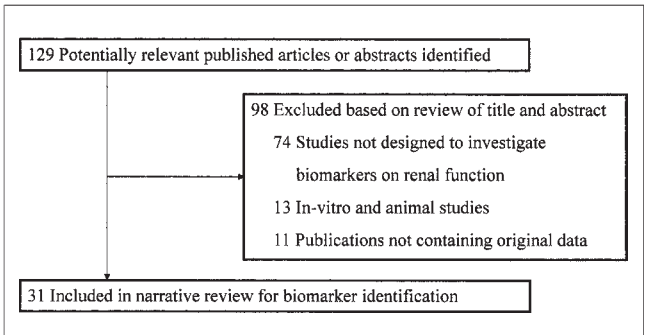

Figure 1 Flow Diagram of the Search Strategy Used to Produce the Review of Published Research

Table 1 Paired Sensitivity and Specificity of Individual Studies for NGAL to Predict AKI After CPB

First Author (Year) (Ref. #)	Sensitivity	Specificity	AUC
Mishra et al. (2005) (11)*	70.0 (45.7–87.2)	94.1 (82.8–98.5)	0.91 (0.88–0.92)
Mishra et al. (2005) (11)†	100.0 (80.0–100.0)	98.0 (88.2–99.9)	0.99 (0.95–1.00)
Wagener et al. (2006) (27)	68.8 (41.5–87.9)	64.6 (51.7–75.8)	0.73 (0.51–0.97)
Dent et al. (2007) (28)	84.4 (69.9–93.0)	93.6 (85.0–97.6)	0.96 (0.93–0.98)
Wagener et al. (2008) (29)	64.7 (52.1–75.6)	52.0 (46.7–57.2)	0.64 (0.51–0.70)
Bennett et al. (2008) (30)	78.8 (69.2–86.1)	91.8 (83.9–96.1)	0.95 (0.88–0.99)
Xin et al. (2008) (31)	76.9 (46.0–93.8)	70.4 (49.7–85.5)	0.86 (0.78–0.93)
Koyner et al. (2008) (32)*	44.4 (22.4–68.7)	75.9 (62.1–86.1)	0.56 (0.38–0.68)
Koyner et al. (2008) (32)†	66.7 (41.2–85.6)	64.8 (50.6–77.0)	0.68 (0.53–0.80)
Lima et al. (2008) (33)	83.3 (36.5–99.1)	73.9 (58.6–85.3)	0.71 (0.29–0.96)
Tuladhar et al. (2009) (34)*	77.8 (40.2–96.1)	68.3 (51.8–81.4)	0.85 (0.78–0.93)
Tuladhar et al. (2009) (34)†	88.9 (50.7–99.4)	78.1 (62.0–88.9)	0.94 (0.85–0.97)
Haase-Fielitz et al. (2009) (26)	78.3 (55.8–91.7)	77.9 (66.8–86.3)	0.80 (0.67–0.86)
Sample size-weighted mean (AKI: 313/total patients: 1,204)	75.5 (70.2–82.4)	75.1 (65.2–86.3)	0.78 (0.67–0.87)

Values are % (95% confidence interval). *Measured in plasma. †Measured in urine. Modified with permission from Haase et al. (35).
AKI = acute kidney injury; AUC = area under the receiver-operating characteristic curve; CPB = cardiopulmonary bypass; NGAL = neutrophil gelatinase-associated lipocalin.

human siderophore has yet been chemically identified, although siderophore-like activities were detected decades ago (40,41). Under aerobic conditions, ferrous ions will react with oxygen to produce ferric ions. Siderophores can solubilize and sequester iron (mainly ferric iron) such that it can be internalized via suitable transporter molecules within the plasma membrane (42).

A schematic overview of the potential roles of labile iron and the iron metabolism regulators NGAL and hepcidin at renal tubular cells is shown in Figure 2. Siderophore:iron-associated NGAL delivers iron into the cell. After megalin receptor-mediated uptake, NGAL traffics in acidic endosomes, which promote the release and cytoplasmic accumulation of iron, resulting in the regulation of iron-dependent genes (21). Siderophore:iron-free NGAL captures intracellular iron and transports it via a hypothetical intracellular siderophore to the extracellular space (43). Depletion of intracellular iron pools may lead to apoptosis. Hepcidin, a down-regulator of ferroportin (iron efflux channel), contributes to an increase in intracellular iron.

Hepcidin. Using a hypothesis-free analytical approach, Ho et al. (14) investigated proteins detected in urine that reflect underlying tubular injury. They enrolled 44 cardiac surgery patients in a nested cohort study and identified 3 novel biomarkers of renal function after cardiac surgery: NGAL, hepcidin, and alpha-1 microglobulin (α_1MG). Of interest, hepcidin, a central systemic regulator of iron homeostasis, was substantially up-regulated in the urine of patients *not* developing AKI after cardiac surgery (14). In contrast, urine hepcidin has been shown to increase during inflammation and decline as inflammation resolved (44).

Hepcidin is a peptide hormone synthesized in hepatocytes and with lower expression detected in the normal kidney, heart, and brain (45). The human hepcidin gene encodes a precursor protein of 84 amino acids, preprohepcidin (46), which undergoes enzymatic cleavage, resulting in a protein of 64 amino acids, prohepcidin. Hepcidin-25, the biologically active 25-amino acid form, is then produced by post-translational processing. Additional degradation results in the production of 2 isoforms, hepcidin-20 and hepcidin-22 (47). Hepcidin mediates intracellular iron sequestration by binding to the cellular iron export channel ferroportin receptors on hepatocytes, enterocytes, and macrophages, leading to ferroportin endocytosis and degradation, and thereby decreases iron efflux from iron-exporting tissues into plasma. Within the kidney, hepcidin is expressed in the apical tubular epithelium of the thick ascending limb of the loop of Henle, connecting tubules, and cortical collecting duct (48).

Overall, there is a complex interplay between positive and negative regulation and the distribution of iron caused by changes in hepcidin concentration (49), with, in many cases, the hypoxic response (decreased hepcidin) seeming to dominate the response because of inflammation (increased hepcidin) even when iron levels are high (50,51).

Zhang et al. (52) demonstrated intrarenal expression of hepcidin by infiltrating leukocytes in patients with lupus nephritis, raising the possibility that during renal disease flare, hepcidin is produced within the kidney, rather than simply being filtered. Of interest, urinary hepcidin-20 and hepcidin-25 showed different patterns of expression in relation to injury and repair (52,53). However, the biological roles of these 2 isoforms of hepcidin need to be further investigated. Also, it would be of interest to explore what role genetic variants of siderophores, hepcidin, or ferroportin may play in the regulation of iron homeostasis and AKI.

Alpha-1 microglobulin. Alpha-1 microglobulin is a 26-kDa plasma and tissue glycoprotein and binds heme in

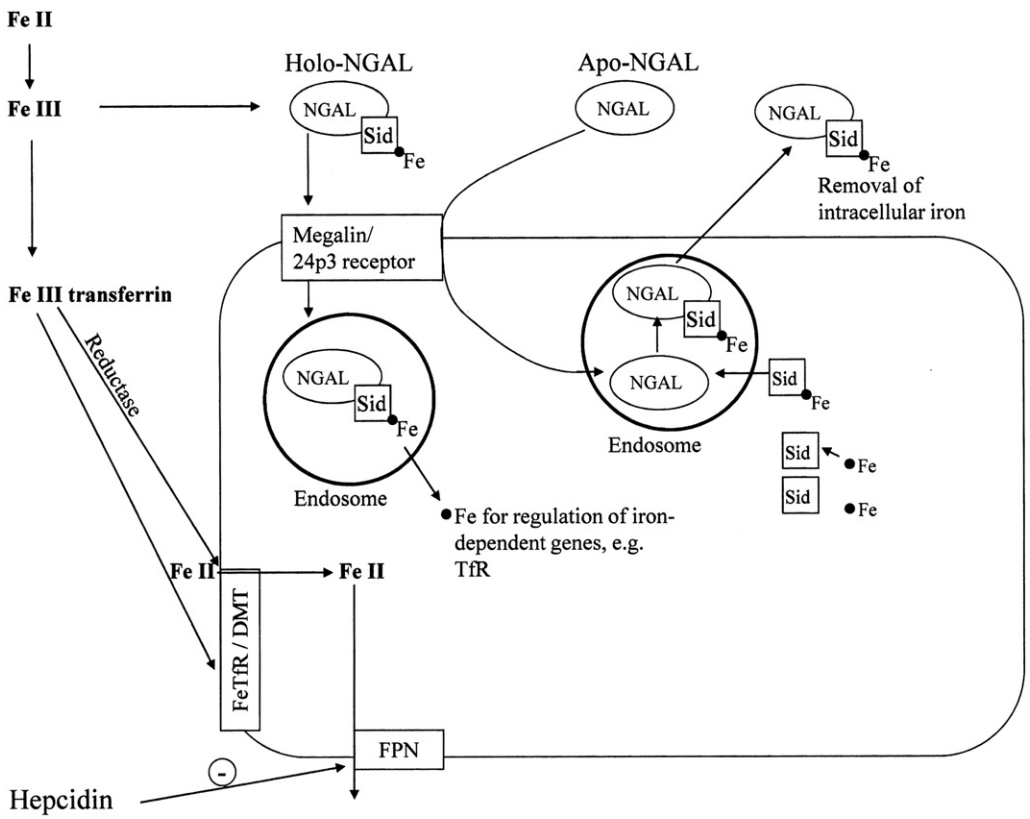

Figure 2 **Schematic Overview of Renal Iron Metabolism**

At the local tissue level, neutrophil gelatinase-associated lipocalin (NGAL) mediates iron trapping in the proximal tubule cell through megalin receptor endocytosis of an NGAL-bacterial siderophore (Sid)-iron complex. Siderophore:iron-associated NGAL (holo-NGAL) delivers iron into the cell. NGAL then traffics in acidic endosomes, which promote the release and cytoplasmic accumulation of iron, resulting in regulation of iron-dependent genes. Siderophore:iron-free NGAL (apo-NGAL) captures intracellular iron and transports it via a hypothetical intracellular siderophore to the extracellular space. The depletion of intracellular iron pools may lead to apoptosis. Hepcidin, a down-regulator of ferroportin (FPN) (iron efflux channel), contributes to an increase in intracellular iron. DMT = divalent metal ion transporter; FeTfR = iron transferrin receptor.

different species (54). The protein has a heterogeneous yellow-brown chromophore consisting of small, unidentified prosthetic groups localized to a free thiol group and 3 lysyl residues around the entrance to a hydrophobic pocket.

It was recently reported that the lipocalin α_1MG can bind heme and that a C-terminally processed form of α_1MG degrades heme (55). Increased urinary excretion of α_1MG has been shown to indicate proximal tubular injury (56). Alpha-1 microglobulin was markedly increased during the early post-operative phase in patients subsequently developing AKI (14). Urinary excretion of α_1MG had high diagnostic accuracy (AUC = 0.89) in identifying patients developing AKI after pediatric cardiac surgery (n = 365) (13). In critically ill adults, urinary α_1MG had an AUC of 0.86 for the identification of patients requiring renal replacement therapy (57).

Alpha-1 microglobulin contributes to heme degradation by a still unknown mechanism. Heme is highly toxic to renal tissue because it is capable of catalyzing free radical formation and is also a major and readily available source of iron for pathogenic organisms (58).

Fatty acid-binding proteins. Fatty acid-binding proteins are intracellular carrier proteins of 14 kDa with different expression in the kidney. So far, 2 types of fatty acid-binding proteins have been isolated from the human kidney. Liver-type fatty acid-binding protein (L-FABP) is another member of the lipocalin superfamily. It is reabsorbed by the proximal tubule via megalin-dependent endocytosis and is localized in the cytoplasm of proximal renal tubular cells and in the liver and the small intestine. By contrast, heart-type fatty acid-binding protein is localized in the renal distal tubules, heart, small intestine, and skeletal muscles. Both proteins facilitate the transport of intracellular long-chain fatty acids. Fatty acid-binding proteins are endogenous antioxidants by promoting free fatty acid metabolism and by binding long-chain fatty acid oxidation products (59).

Portilla et al. (12) demonstrated that L-FABP predicts the development of AKI in children undergoing cardiac surgery. They found that increases of this biomarker within

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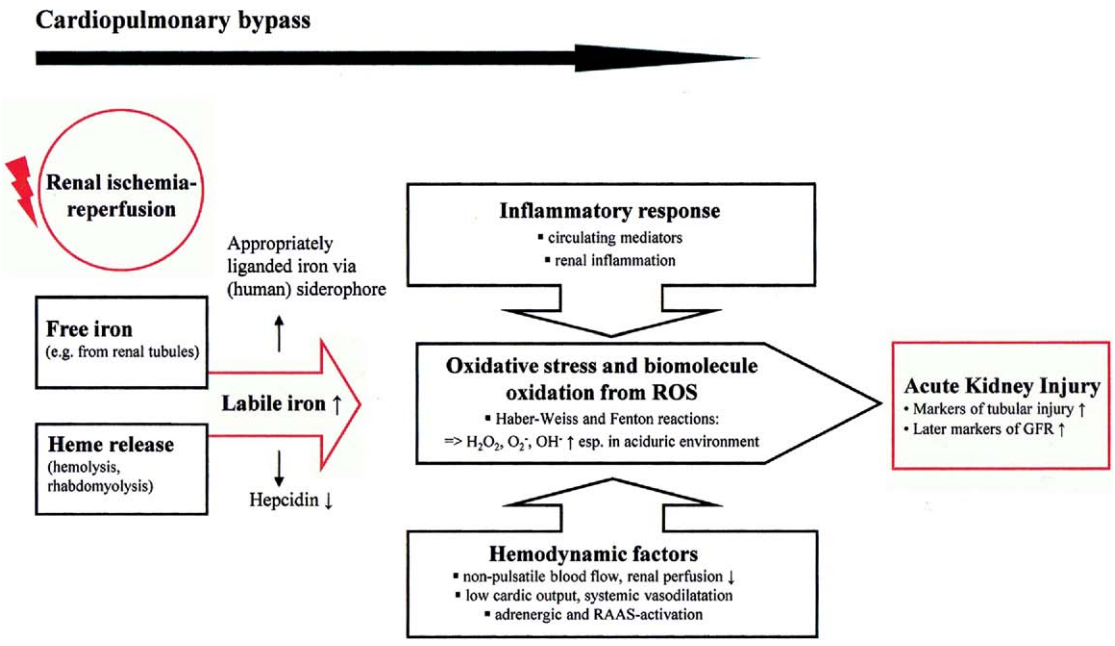

Figure 3 Renal Free Iron Toxicity and Biomarkers in Cardiac Surgery

Overview of the roles of ischemia and reperfusion during cardiopulmonary bypass and of reactive oxygen species (ROS), poorly liganded iron, and iron metabolism regulators in affecting renal injury. GFR = glomerular filtration rate; RAAS = renin-angiotensin-aldosterone system.

4 h after cardiac surgery anticipated the subsequent development of AKI with an accuracy of 81%. In human L-FABP transgenic mice, urinary L-FABP levels allowed the accurate and earlier detection of both histological and functional insults in ischemia-induced AKI (60). Interestingly, L-FABP is also a high-affinity heme-binding protein (37). Urinary cystatin C (57), interleukin-18 (61,62), and kidney injury molecule-1 (63,64) are other novel tubular biomarkers. How these biomarkers are involved in iron metabolism is currently unknown or has not yet been investigated.

Pathophysiological Aspects of CPB

The pathogenesis of cardiac surgery-associated AKI is complex and multifactorial and includes several injury pathways: ischemia and reperfusion, exogenous and endogenous toxins, inflammation, oxidative stress, and hemodynamic factors (Fig. 3). These mechanisms of injury are likely to be active at different times with different intensities and probably act synergistically (10).

The use of a CPB pump has been associated with an upstream insult such as an elevation in levels of systemic inflammatory factors compared with off-pump operations (65). Oxidative stress is one of the major initiators of myocardial injury during experimental ischemia and reperfusion (66) and is believed to be also an important mechanism of renal injury. Ischemia-reperfusion injury during CPB may further exacerbate oxidoinflammatory stress in the setting of free circulating labile iron. Free labile iron is capable of inducing multiple changes in renal tubular epithelial function, including impaired proliferation (67) and the induction of free radical injuries, such as lipid peroxidation and protein oxidation. The generation of hydroxyl radicals is catalyzed by free iron ions and most active at acid pH (Fig. 4).

CPB creates a hemodynamic state of loss of pulsatile flow and microembolism. Hemodynamic instability may occur during the transition from full hemodynamic support with CPB to full circulation by the patient's own cardiovascular system. A low-cardiac output state contributes to generalized hypoperfusion and renal ischemia.

Length of time on CPB is a well-recognized risk factor for the development of AKI. This association may relate to

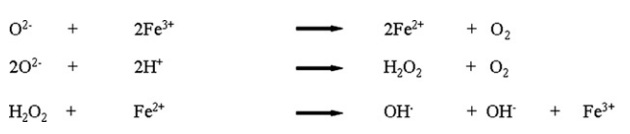

Figure 4 Haber-Weiss and Fenton Reactions

The superoxide-driven Haber-Weiss describes 1 possible mechanism in the generation of hydroxyl radicals, is catalyzed by free iron ions, and is most active at acid pH. Another important reaction of hydrogen peroxide with (free or inappropriately liganded) Fe^{2+} is the Fenton reaction, leading to the very reactive and damaging hydroxyl radical. Reprinted, with permission, from Haase et al. (92).

hemolysis or rhabdomyolysis and the generation of intravascular free hemoglobin and free toxic iron secondary to mechanical trauma to red cells within the bypass system and surgical suction devices (68,69). Pigment nephropathy is known to result from hemoglobinuria and myoglobinuria (15,16,68,70–72). During CPB, plasma-free hemoglobin increased, correlated with early post-operative tubular injury, and was significantly and independently associated with the development of subsequent loss of renal function (73).

The Duration of CPB and the Extent of Kidney Injury

A wide range of causative factors is involved in the release of free hemoglobin or free myoglobin into the serum, including hemolysis from extracorporeal circulation (e.g., CPB), but also mechanical fragmentation of red cells induced by valvular prosthesis, transfusion reactions, or genetic defects predisposing to reduced erythrocyte membrane stability (70). Increased free hemoglobin levels of greater than several-fold above the upper physiological range have been observed during the use of CPB until several hours post-operatively (74). The detrimental effect of CPB on red cell destruction is accentuated by prolongation of CPB time (75,76). Thus, the longer the duration of CPB, the more hemolysis should occur and the more free hemoglobin is likely generated. This may be of importance to the current clinical situation, in which complex surgery of the aortic arch and aortic valve is performed and an increasing number of cardiac surgical centers have implemented time-consuming arterial coronary revascularization, aiming to improve long-term results. Interestingly, there is strong evidence that a longer duration of CPB is independently associated with an increased likelihood of and more severe AKI (77,78). In addition, the use of CPB appears to have a close relation to hemolysis-induced gallstone formation after open cardiac surgery (79).

Free Hemoglobin and Iron Release

CPB exposes blood to nonphysiological surfaces and shear forces that lead to mechanical destruction of red blood cells with release of free hemoglobin into the circulation (80). Free hemoglobin combines with haptoglobin to form a complex, which is carried to the liver, bypassing the kidney, and is metabolized (81).

In the presence of oxidants such as hydrogen peroxide and superoxide, free iron is released from heme molecules into the circulation (82). Heme contains redox-active iron, which is able to participate in organic and inorganic oxygen radical reactions, such as stimulating lipid peroxidation and catalyzing the formation of damaging hydroxyl radicals, with subsequent tissue damage (83). In 1 study, labile iron was released from the injured heart and was a prognostic biomarker of vascular injury (84). Therefore, the main source of labile iron release during syndromes of ischemia-reperfusion should be recognized.

Heme Handling

The cellular content of heme, derived either from the delivery of filtered heme proteins such as hemoglobin and myoglobin or from the breakdown of ubiquitous intracellular heme proteins, is regulated via the heme oxygenase enzyme system. Heme oxygenases catalyze the rate-limiting step in heme degradation, resulting in the formation of iron, carbon monoxide, and biliverdin, which is subsequently converted to bilirubin by biliverdin reductase. Recent attention has focused on the biological effects of products of this enzymatic reaction, which have important antioxidant, anti-inflammatory, and cytoprotective functions (85,86). The stress-response protein heme oxygenase-1 plays an essential role in the prevention of renal injury and has been involved in many clinically relevant disease states, including AKI, as well as others. The beneficial role of heme oxygenase-1 has been implicated in protection from experimental ischemia-reperfusion injury and inflammation or immune dysfunction, and heme oxygenase-1 thus has emerged as a key target molecule with therapeutic implications (87).

Toxicicity of Heme-Carrying and Iron-Carrying Pigments

Hemoglobin has a heme protein chemical core structure. At the center of the heme group is the iron metal ion. Hemoglobin consists of 4 protein chains and 4 heme groups. Given the ability of heme molecules to release free iron, which can act as nephrotoxin, one can assume similar pathogenetic mechanisms in the development of hemoglobinuric and myoglobinuric AKI (68,70).

The association between hemoglobinuria and the development of AKI has been acknowledged in past and current clinical research (15,16,68,71,88–90). Hemoglobin-induced AKI may be a clinically relevant cause for CPB-associated renal injury given a clinical study convincingly linking hemolysis and hemoglobinuria with renal injury (73). It is further conceivable that excess use of red blood cell transfusion is associated with increased incidence of AKI and mortality (91) because of increased iron toxicity. Therefore, it is possible that CPB-induced AKI may be at least in part a renal sideropathy with free iron as the central toxic element.

Labile Iron and Tubulotoxicity

Poorly liganded iron has the potential (82,92) to catalyze the Haber-Weiss and Fenton reactions (Fig. 4), whereby superoxide radical and hydrogen peroxide yield hydroxyl radical (88). It is known that an acid environment typical of tubular urine enhances the formation of reactive hydroxyl radicals, as the Haber-Weiss reaction is pH dependent with a right shift when pH decreases. There is little argument that

hydroxyl radicals are injurious in a wide variety of settings (88,93).

In animal studies, the infusion of free hemoglobin and its conversion to methemoglobin induce renal sideropathy and cause AKI (68,71). Aciduric conditions facilitate this conversion. Tubular obstruction may allow greater time for endocytotic uptake of free hemoglobin into proximal tubular cells, which is associated with proximal tubular cell necrosis (71). The infusion of hemoglobin under alkalinuric conditions causes virtually no renal injury, and urine alkalinization attenuates renal failure in animal model (71,94).

Physiologically, recycled and absorbed iron is delivered to the main iron-transporting protein in blood, transferrin. Transferrin binds free iron and minimizes its potential toxicity. However, in some cases, the release of free iron can exceed the iron-binding capacity of transferrin. Also, free serum hemoglobin is able to scavenge endothelium-derived nitric oxide, leading to vasoconstriction, decreased blood flow, platelet activation, increased endothelin-1 expression, and AKI (95). Some free hemoglobin will also pass through the glomerulus, will appear in urine, will release free iron (which is involved in the generation of reactive oxygen species), and may cause the occlusion of renal tubules with hemoglobin casts and necrosis of tubular cells (81,96). At this point, all iron-binding antioxidant capacity is lost, and the serum displays pro-oxidant features (97). How often this occurs during CPB is not fully known, but it may be as high as in 25% of cases (98,99). There is also evidence indicating that the generation of reactive oxygen species may contribute to the initiation and maintenance of acute tubular necrosis (100). Oxidative stress has been shown to have a key role in the development of toxic and ischemic AKI. Iron free radicals are considered to be an important cause of renal injury and capable of aggravating tubular damage. They may be derived from intravascular hemolysis in the setting of CPB or released from injured mitochondria in the renal tubule (101,102). Reperfusion injury during CPB may exacerbate further the oxidant stress in the setting of free circulating iron.

In a rat model of gentamicin-induced AKI, free radical damage was mitigated with deferoxamine, an iron chelator (103). Furthermore, decreased serum levels of the iron chelator ferritin are associated with human AKI after CPB (69).

Strategies Targeting Iron Toxicity and Renal Protection

The administration of haptoglobin has been shown to have prophylactic and therapeutic effects on renal injury secondary to hemolysis (16,104,105). Also, iron chelation with deferoxamine has been found to be protective against pigment nephropathy in some animal models (68,71,106). On the basis of these data, clinical trials of deferoxamine are planned to prevent AKI (NCT00870883).

The role of NGAL, as a siderophore-binding agent, is thus consistent with the widespread recognition that iron-induced radical generation is intimately involved in a variety of renal and other diseases (107,108). It is suggested that its main role is in sequestrating iron via a human siderophore to stop inappropriately liganded iron from producing damaging oxygen radicals. Intriguingly, NGAL infusion simultaneous to renal injury prevents ischemic AKI (22).

The beneficial effect of higher tubular pH by urinary alkalinization, achieved for example with the use of sodium bicarbonate infusion, was protective in a rat model of acute renal failure (94). Urinary alkalinization with sodium bicarbonate might protect from the pathophysiological mechanisms causing CPB-associated AKI. There is evidence from a double-blind randomized controlled trial that bicarbonate might attenuate CPB-associated AKI, potentially directly affecting iron-related toxicity, as indicated by a smaller increase in urinary NGAL (109). At neutral or alkaline pH, free ferric ions precipitate as insoluble ferric hydroxide, which is excreted as inert complex in the urine. More alkaline urine reduces the generation of injurious hydroxyl radicals and lipid peroxidation (88,110,111). Bicarbonate directly scavenges hydroxyl ions and, as a not well adsorbable anion compared with chloride, causes more rapid volume excretion and thereby reduces the contact time between injurious radicals and renal tubules.

Once confirmed in large prospective studies, highly predictive renal biomarkers for CPB-related AKI should be used in randomized controlled trials of preventive and therapeutic interventions in cardiorenal syndromes. It is possible that not a single biomarker but rather a combination or a ratio such as the NGAL/hepcidin ratio may further improve diagnostic ability. Finally, given the view that AKI may be a renal sideropathy, future research should be directed toward identifying and characterizing human siderophores and investigating if there are siderophore disorders in cardiorenal syndromes. As suggested, "ironing out" the pathogenesis of CPB-associated AKI (112) or the consequent use of off-pump techniques may be the logical next step for clinical trials in patients at risk.

Conclusions

Animal models as well as human studies have contributed to our knowledge about novel renal biomarkers of AKI and point toward iron-mediated toxicity as a common mechanism of AKI. The lines of evidence supporting this notion include the known effect of CPB on red cells, the associated release of heme and iron, knowledge of the effect of poorly liganded iron on renal tubular cells, information from studies of novel renal biomarkers, and evidence from recent trials targeting free iron-mediated mechanisms of AKI. It is intriguing, in the setting of CPB surgery, to further advance such views and frame a new hypothesis on the role of iron toxicity and siderophores in the pathogenesis of AKI. Finally, we would like to stress that while the pathogenetic role of radical oxygen species in AKI has been previously considered, that of iron as a major contributor and mediator of CPB-associated AKI has

not been generally appreciated. Such appreciation has begun to yield targeted interventions and may open the door to effective preventive or therapeutic strategies.

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