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THE ROLE OF LABILE IRON IN KIDNEY DISEASE AND TREATMENT WITH CHELATION

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□ *There are two major forms of kidney disease: acute renal failure [also referred to as acute kidney injury (AKI)] and chronic kidney disease (CKD). Acute renal failure is an abrupt loss of kidney function within 48 h, whereas CKD is a loss of kidney function greater than 3 months. There is a large amount of experimental evidence for an increase of labile iron in a wide variety of models of kidney disease. Additionally, iron chelators provide protection, indicating an important role of labile iron in these diseases. These observations suggest that iron chelators may provide a new modality of prevention and treatment of kidney disease.*

Keywords Labile iron, Acute kidney injury (AKI), Chronic kidney disease (CKD), Iron chelator

INTRODUCTION

Iron is the most abundant transitional metal in the body. Critical to iron's importance in biological processes is its ability to cycle reversibly between its ferrous and ferric oxidation states. This precise property, which is essential for its functions, also makes it very dangerous, because free iron can catalyze the formation of free radicals that can damage macromolecular components of the cell. Because of this, the body has evolved many complex iron transport management systems to keep the levels of free iron low.

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Labile iron, also known as catalytic iron, was originally defined as a transitional pool between extracellular and cellular iron and is generally associated with low-molecular-weight chelates. However, the broadest definition of a labile iron pool is that it consists of chemical forms that can participate in redox cycling. This property makes iron potentially hazardous in that it enables it to participate in the generation of powerful oxidant species such as hydroxyl radical (metal-catalyzed Haber–Weiss reaction) and/or reactive iron-oxygen complexes such as ferryl or perferryl ion (1).

Iron also has a major role in the initiation and propagation of lipid peroxidation, by either catalyzing the conversion of primary oxygen radicals to hydroxyl radicals or forming a perferryl ion. In addition, iron can directly catalyze lipid peroxidation, the oxidative reaction of polyunsaturated lipids, by removing hydrogen atoms from polyunsaturated fatty acids in the lipid bilayers of organelle membranes (1).

Although our bodies contain as much as 3–5 g of total iron, the pool of labile iron is estimated to be <70–90 mg. We now recognize that this pool of labile iron is increased in many disease states. Several methods have been described to measure labile iron, also referred to as catalytic iron or toxic iron (1,2). There are two broad lines of evidence for the role of labile iron in disease states: that it is increased in disease states, and that iron chelators provide a protective effect, thus establishing a cause-effect relationship. This has been demonstrated in a variety of disease states including acute and chronic kidney disease (CKD), acute myocardial infarction, and neurodegenerative disorders. Thus, its role in disease processes seems to be a common theme of cellular injury.

The Role of Labile Iron in Acute Kidney Injury

We first focus on the role of labile iron in several causes of acute kidney injury (AKI). The reader is referred to other reviews for a more detailed and comprehensive discussion on the role of oxidants and iron in AKI (3,4). The new suggested definition and the rationale behind it is detailed in the publication resulting from a consensus conference under the auspices of the Acute Kidney Injury Network (AKIN) in which over 20 global societies representing both nephrologists and intensive care physicians participated (5). The definition of AKI is an abrupt (within 48 h) reduction in kidney function currently defined as an absolute increase in serum creatinine of more than or equal to 0.3 mg/dL (≥26.4 mmol/L), a percentage increase in serum creatinine of more than or equal to 50% (1.5-fold from baseline), or a reduction in urine output (documented oliguria of less than 0.5 mL/kg/h for more than 6 h) (5). Several recent studies highlight the clinical importance of AKI. Acute kidney injury is common and occurs in about 7–10% of hospitalized

patients and its overall incidence appears to be increasing. Acute kidney injury has been shown to be an independent risk factor for morbidity and mortality, and even a modest increase (0.3 mg/dL) in serum creatinine is associated with high in-hospital mortality (6). In addition, recent studies indicate that AKI is an important determinant of post-hospital discharge mortality as well as end-stage kidney disease (ESKD). It is estimated that approximately 15% of patients with AKI will progress to ESKD within 3 years.

The Role of Labile Iron in Myoglobinuric Acute Kidney Injury

One of the common causes of AKI is muscle damage, either from crush injury or unaccustomed exercise. We have shown that in this model of injury there is a marked and specific increase in labile iron content (3,4). Paller (7) has also demonstrated that deferoxamine (DFO) treatment was protective in three models of myoglobinuric renal injury, namely hemoglobin (Hb)-induced nephrotoxicity, glycerol-induced acute renal failure, and a combined renal ischemic Hb insult (3,4). Taken together, the histological and functional protective effect of hydroxyl radical scavengers and an iron chelator implicates a role for a hydroxyl radical in glycerol-induced AKI. There is evidence that, in this model of AKI, heme proteins may also play an important role. Baliga et al. (3) have shown that cytochrome P450 may be a significant source of iron in this model of AKI (4).

The Role of Labile Iron in Cisplatin-Induced Acute Kidney Injury

Cisplatin is a widely used antineoplastic agent that has nephrotoxicity as a major side effect. Exposure of kidney cells to cisplatin resulted in a significant increase in labile iron released into the medium (Figure 1, panel A). Concurrent incubation with iron chelators including DFO and 1,10-phenanthroline significantly attenuated cisplatin-induced cytotoxicity as measured by lactate dehydrogenase (LDH) release (Figure 1, panel A). Bleomycin-detectable (iron capable of catalyzing free-radical reactions) was also markedly increased in the kidney of rats treated with cisplatin. Similarly, administration of DFO in rats provided marked functional (as measured by blood urea nitrogen and creatinine) and histological protection against cisplatin-induced acute kidney injury. Baliga et al. (3) and Ueda et al. (4) have also shown that cytochrome P450, a group of heme proteins, may serve as a significant source of catalytic iron in cisplatin-induced nephrotoxicity. Taken together, our data support a critical role for iron in mediating tissue injury via hydroxyl radical (or a similar oxidant) in this model of nephrotoxicity.

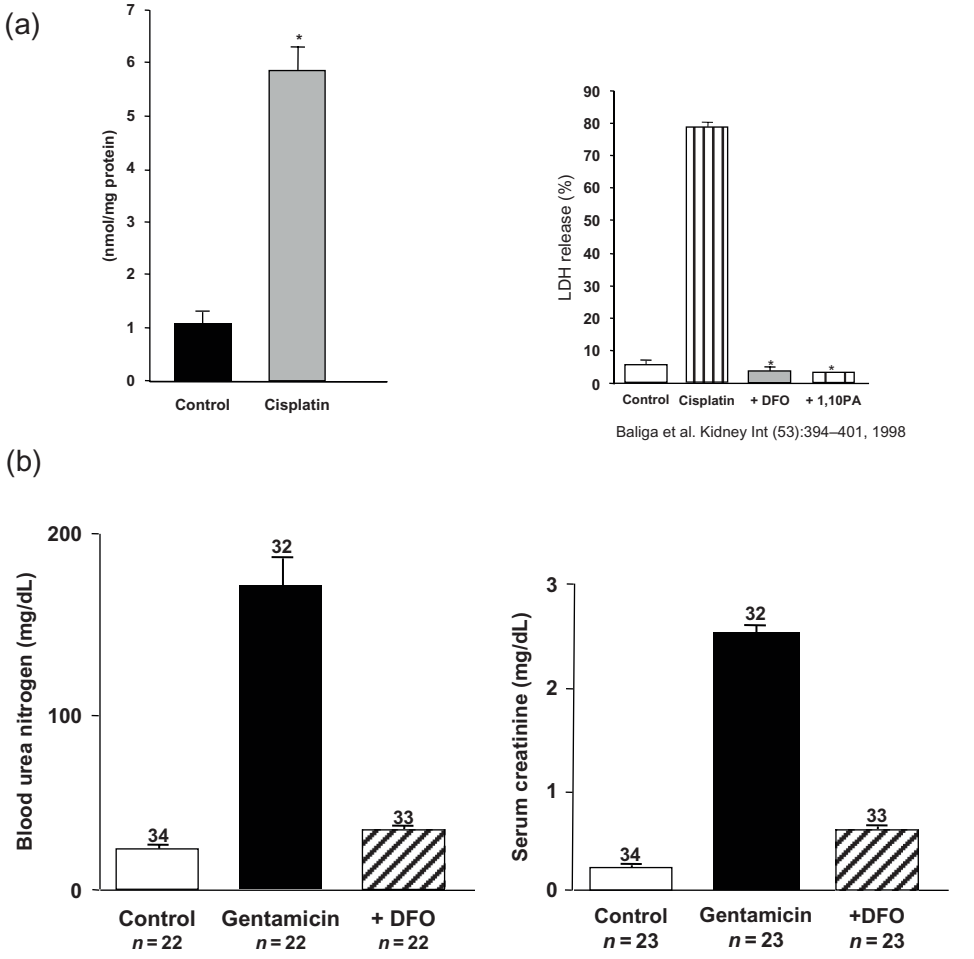

FIGURE 1 (a) Effect of cisplatin on release of catalytic iron in renal tubular epithelial cells and effect of iron chelators on cisplatin-induced cytotoxicity. (b) Effect of iron chelator on gentamicin-induced AKI.

The Role of Labile Iron in Gentamicin Nephrotoxicity

Nephrotoxicity is a major complication of the use of aminoglycoside antibiotics including gentamicin, which are widely used in the treatment of gram-negative infections. We have shown that hydroxyl radical scavengers and iron chelators provide a marked protective effect on renal function in gentamicin-induced acute kidney injury in rats (3,4) (Figure 1, panel B). In addition, histological evidence of damage was markedly reduced by the interventional agents. Additional support for the role of iron-catalyzed free-radical generation has been provided by demonstrating that a gentamicin-induced generation of hydroxyl radicals is reduced by iron chelators in vitro (3,4). We have shown that gentamicin enhances the release of iron from

renal cortical mitochondria, and that it does so through the generation of hydrogen peroxide (3,4). It was shown that the gentamicin-iron complex but not gentamicin itself causes lipid peroxidation in vitro and is a potent catalyst of free-radical formation (3,4).

The Role of Labile Iron in Contrast-Media-Associated Nephrotoxicity

Rajapurkar et al. (8), in a preliminary study, have reported that kidney donors, undergoing either an intravenous pyelogram or a renal arteriogram, have a marked increase in urinary catalytic iron accompanied by evidence of tubular injury, as reflected by an increase in urinary alkaline phosphatase and *N*-acetyl-*β*-glucosaminidase. Although no well-accepted models of contrast-induced AKI exist, an iron chelator has been shown to be protective in one model of multi-insult (contrast- and indomethacin-induced) AKI (9).

The Role of Labile Iron in Chronic Kidney Disease

Chronic kidney disease is a worldwide public health problem that affects approximately 16% of the adult US population and is associated with a high prevalence of cardiovascular disease and high economic cost. The anti-GBM antibody is a well-characterized model of complement- and neutrophil-dependent glomerular injury in which an iron chelator significantly attenuates proteinuria (10). The ability of glomerular cells to generate oxidants suggests that they may be important mediators of glomerular injury in glomerular diseases that lack infiltrating leukocytes. An animal model of minimal change disease is induced by a single intravenous injection of puromycin aminonucleoside.

Bleomycin-detectable iron (iron capable of catalyzing free-radical reactions) was markedly increased in glomeruli from nephrotic rats, and an iron chelator prevented an increase in catalytic iron in glomeruli and provided complete protection against proteinuria, suggesting an important pathogenic role for glomerular catalytic iron in this model (10) (Figure 2, panel A). Shah et al. (10) have recently demonstrated that cytochrome P450 and, more specifically, cytochrome P450 2B1, an isozyme present in the glomerulus, is a source of catalytic iron that participates in glomerular injury in this model.

Passive Heymann nephritis, induced by a single intravenous injection of anti-Fx1A, is a complement-dependent model of glomerular disease that resembles membranous nephropathy in humans. The administration of an iron chelator markedly reduces proteinuria, suggesting the role of labile iron in passive Heymann nephritis (10). It has been shown that feeding an iron-deficient diet provides protection in this model (10).

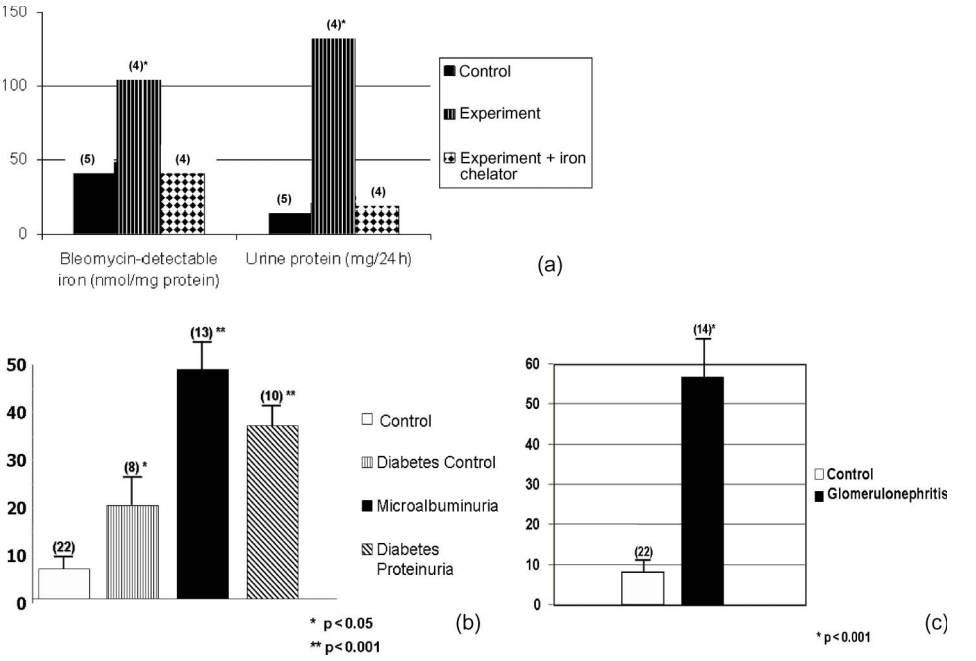

FIGURE 2 (a)Effect of an iron chelator on bleomycin-detectable iron in glomeruli and proteinuria from rats injected with puromycin aminonucleoside. (b) Urinary catalytic iron in patients with diabetic nephropathy. (c) Urinary catalytic iron in patients with biopsy-proven glomerulonephritis.

We will summarize the limited information from human studies that lend support that the mechanisms observed in animal models appear to be applicable to human disease. In preliminary studies, we have compared catalytic iron in subjects with no renal disease or diabetes with patients with diabetes, as shown in Figure 2, panel B. Our data demonstrate that patients with overt diabetes have a marked increase in urinary catalytic iron. Similarly, patients with microalbuminuria also have a marked and highly significant increase in urinary catalytic iron, indicating that urinary catalytic iron is not merely a reflection of albuminuria. Finally, some patients in the diabetic control group who do not have microalbuminuria have high catalytic iron, leading us to postulate that urinary catalytic iron precedes the onset of microalbuminuria and may predict patients at risk for diabetic nephropathy. Interestingly, recent studies have demonstrated that non-transferrin-bound iron levels are frequently increased in diabetes and have been implicated in a few studies with the vascular complications of diabetes (10). Most importantly, in preliminary studies we have shown that treatment with deferiprone (L1) leads to a marked reduction in proteinuria over a 9-month period in patients with diabetic nephropathy (11).

Nankivell et al. (12) have reported increased iron content in patients with CKD. Using the urinary catalytic iron assay described before, we have shown a marked increase in patients with biopsy-proven glomerulonephritis (Figure 2, panel C). There is at least one study in the literature in which the effect of a metal chelator on progressive kidney disease has been examined. Lin et al. (13) have shown that chelation therapy with EDTA in patients with chronic renal insufficiency resulted in a reduced rate of decline in the glomerular filtration rate. The authors attributed the beneficial effect to the chelation of lead, which also participates in the Fenton reaction. However, given the affinity constants for iron and lead, the large experimental evidence for the role of iron in kidney disease, and the demonstrated efficacy of EDTA in enhancing excretion of urinary iron, we believe that the beneficial effects are more likely to be explained by the chelation of iron rather than lead (14). In our preliminary studies, we have also shown that treatment with L1 leads to a reduction in proteinuria in patients with glomerular diseases who were unresponsive to other treatments (15). While these results suggest the role of labile iron, one cannot exclude the possibility that iron chelators have other effects responsible for the observed benefit.

CONCLUSIONS

There is a sufficient body of evidence for the role of labile iron in both AKI and CKD to warrant clinical trials with iron chelators for prevention and treatment of kidney disease. However, one has to be cautious that the animal data, no matter how compelling, may not translate into human experience. Additionally, of the oral chelators available, deferiprone appears to offer the advantage for treating kidney disease because it has no reported nephrotoxicity.

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