J Am Soc Nephrol 15: S91–S92, 2004

Making Sense: A Scientific Approach to Intravenous Iron Therapy

DAVID B. VAN WYCK,* BO G. DANIELSON,† and GEORGE R. ARONOFF‡
*Department of Medicine and Surgery, University of Arizona College of Medicine, Tucson, Arizona; †Department of Renal Medicine, University Hospital, Uppsala, Sweden; and ‡Department of Medicine; University of Louisville Kidney Disease Program, Louisville, Kentucky

More than 100 yr have passed since parenteral iron was first given to humans (1). Fifty yr ago, carbohydrate was first coupled to iron oxide (2), reducing the fierce toxicity of ferric iron and introducing the era of parenteral therapy with carbohydrate-iron agents (3,4). This is sufficient time to consider what we have learned about the risks and benefits of intravenous (IV) iron therapy; to review what we know and what we don't; and, most important, to develop a comprehensive, unifying view that makes sense of the chemistry, biology, and pharmacology of IV iron agents.

Although treatment of iron deficiency certainly is not confined to patients with kidney disease, the majority of published evidence on IV iron therapy resides in the nephrology literature. Anemia is common among all patients with chronic kidney disease, expected among those with advanced kidney disease, and nearly universal among those who undergo dialysis. Evidence of iron deficiency is currently quite common in patients with chronic kidney disease–associated anemia (5). However, before treatment with erythropoietin receptor agonists (ERA; including epoetin α, epoetin β, and darbepoetin α), it was iron excess, not deficiency, that afflicted most dialysis patients. Because anemia was severe, transfusion dependence was common, and transfusional hemosiderosis resulted.

ERA therapy ended transfusion dependence, unmasked iron loss as the dominant feature of iron balance in hemodialysis patients, converted iron overload to iron deficiency as the prevailing disorder, highlighted the failure of oral iron supplementation to sustain iron sufficiency, and thereby thrust IV iron agents to the forefront of iron replacement. Two additional developments have heightened IV iron use in dialysis patients. The first is evidence that a maintenance IV iron schedule designed to prevent iron deficiency is more effective than a periodic treatment schedule in achieving target hemoglobin and minimizing doses of ERA therapy. The second is acceptance and implementation of anemia management guidelines, including those of the National Kidney Foundation Dialysis Outcomes Quality Initiative (K/DOQI) and European Best Practice Guidelines (EBPG). Publication of the first K/DOQI anemia guidelines in 1997 (6) and the EBPG anemia guidelines in 1999 (7) has been followed by gradual adoption of iron maintenance protocols. IV iron use in the United States has increased every year since 1996. By 2002, the proportion of patients who received IV iron within a single quarter approached 65%, and the average annual IV iron dose for all hemodialysis patients exceeded 2300 mg (8).

Increasing use of IV iron has prompted concerns for the potential hazards of iron therapy and the risks of iron overload and has stimulated a new and welcome wave of inquiry into iron safety. From *in vitro* studies to epidemiologic examination of large dialysis databases, evidence has accumulated rapidly. At the same time, new techniques to examine the structure and chemistry of iron carbohydrate compounds have helped to resolve decades-old controversies about how, for good or for bad, IV iron agents deliver biologically active iron.

A coherent, unifying view of IV iron agents, based soundly on an understanding of structure and chemistry, to encompass *in vitro* findings, explain *in vivo* observations, evaluate risks and benefits, and compare existing IV iron agents is urgently needed. During Renal Week in San Diego, California, in November 2003, Bo Danielson, George Aronoff, and David Van Wyck outlined one such view at a symposium sponsored and organized by the American Society of Nephrology. The current review arises from that collaboration. The groundbreaking work of Mary Cowman and Dina Kudasheva (9,10) on carbohydrate-iron structure and chemistry plays a central role in formulating our review. The findings of these two colloid chemists make possible a remarkable synthesis of the chemistry, biology, and clinical use of IV iron agents.

Our conclusions are reassuring. No IV iron compounds generate detectable free iron. All IV iron agents release biologically available or labile iron. The rate of labile iron release in each agent is inversely related to the size of its iron core. The clinical consequences of labile iron release have little significance at low iron doses but limit the maximum tolerated single dose and rate of infusion of each IV iron agent. All evidence suggests that, in regard to iron release, IV iron use within current guidelines is safe and that K/DOQI limits for iron supplementation (11,12) should continue to be observed.

Correspondence to Dr. David B. Van Wyck, Kidney Health Institute, LLC, 6720 North Nanini Drive, Tucson, AZ 85704-6128. Phone: 520-906-8262; Fax: 520-498-5027; E-mail dvanwyck@sprynet.com

1046-6673/1512-0091
Journal of the American Society of Nephrology

References

1. Stockman R: The treatment of chlorosis by iron and some other

2. Nissim JA: Intravenous administration of iron. *Lancet* 1: 49–51, 1947
3. Fierz F: Contribution concerning the intravenous iron therapy. Investigations with Ferrum-Hausmann. *Praxis* 22: 469–472, 1950
4. Beutler E: The utilization of saccharated Fe^{59} oxide in red cell formation. *J Lab Clin Med* 51: 415–419, 1958
5. Hsu CY, McCulloch CE, Curhan GC: Iron status and hemoglobin level in chronic renal insufficiency. *J Am Soc Nephrol* 13: 2783–2786, 2002
6. Eschbach JW, DeOreo P, Adamson J, Berns J, Biddle G, Comstock T, Jabs K, Lazarus JM, Nissenson AR, Stivelman J, Van Wyck DB, Wish J: NKF-DOQI clinical practice guidelines for the treatment of anemia of chronic renal failure. *Am J Kidney Dis* 30: S192–S240, 1997
7. Barany P, Carrera F, Chanard J, Eckardt KU, Hadjicontantinou V, Leenaerts P, Leunissen ML, Locatelli F, MacLeod A, Rutkowski B, Sanz D, Schaefer RM, Schmieder R, Winearls CG: European best practice guidelines for the management of anaemia in patients with chronic renal failure. *Nephrol Dial Transplant* 14: 1–50, 1999
8. Wish JB, Johnson CA, Frankenfield DL: Intravenous iron use among adult hemodialysis (HD) patients: Results from the 2002 ESRD Clinical Performance (CPM) Project [Abstract]. *J Am Soc Nephrol* 14: 458A, 2003
9. Kudasheva DS, Lai J, Ulman A, Cowman MK: Structure of carbohydrate-bound polynuclear iron oxyhydroxide nanoparticles in parenteral formulations. *J Inorgan Biochem* 2004, in press
10. Kudasheva DS, Cowman MK: Structures of iron-carbohydrate complexes used as parenteral formulations [Abstract]. *J Am Soc Nephrol* 14: 856A, 2003
11. IV. NKF-K/DOQI Clinical practice guidelines for anemia of chronic kidney disease: Update 2000. *Am J Kidney Dis* 37: S182–S238, 2001
12. Van Wyck DB: Lessons from NKF-DOQI: Iron management. *Semin Nephrol* 20: 330–334, 2000

J Am Soc Nephrol 15: S107–S111, 2004

Labile Iron: Manifestations and Clinical Implications

DAVID B. VAN WYCK
Department of Medicine and Surgery, University of Arizona College of Medicine, Tucson, Arizona

As Dr. Danielson discussed in the article "Structure, Chemistry, and Pharmacokinetics of Intravenous Iron Agents" in this supplement, the pharmacokinetics and internal iron disposition of all intravenous (IV) iron agents are characterized by initial clearance from the plasma space into fixed phagocytic cells of the reticuloendothelial system (RES) followed by intracellular liberation of iron from the iron-carbohydrate complex, release of iron from RES cells to circulating transferrin (Tf), and, finally, donation of Tf-bound iron to erythroid precursors in marrow. In the iron-avid patient, utilization of IV iron by this stepwise mechanism is rapid and relatively complete. All IV iron agents, however, show evidence of a second, limited pathway in which iron passes directly from the iron-carbohydrate compound to Tf. Evidence that iron-carbohydrate agents can directly release biologically active iron and bypass the presumed safety of RES uptake has prompted a series of questions with potentially important implications for IV iron administration in patients

Do IV Iron Agents Release Free Iron?

Concern that parenteral iron-carbohydrate compounds release free iron is neither new nor confined to a single iron agent. In the mid-1960s, examination of iron dextran Imferon by polarography and high-voltage electrophoresis suggested that 0.3% of the total iron in the compound consists of ionic iron in the ferrous (Fe^{+2}) state, probably weakly bound to dextran (1). These investigators were the first to predict that a small fraction of weakly bound or labile iron could provoke iron-mediated hypotension if large doses were injected rapidly.

Subsequent efforts to identify free, ionic iron in iron-carbohydrate agents have proved unsuccessful. No dialyzable iron has been found in iron dextran (2,3), ferric gluconate (4), or iron sucrose (5). The product package insert for ferric gluconate reports that $<1\%$ of iron in ferric gluconate is dialyzable *in vitro* (6). Neither iron sucrose nor iron dextran release detectable iron to dialysate using high-flux or high-efficiency dialyzers (7).

Evidence for a Labile, Bioactive Iron Fraction

Although there is no convincing evidence of unbound, dialyzable, or free iron in any IV iron agent, all agents show evidence of a labile, biologically active iron fraction. *In vitro* and *in vivo* manifestations of a labile iron fraction in iron-carbohydrate compounds include iron assay interference (agents falsely elevate serum iron results), oversaturation of Tf (true increase in iron available for Tf binding exceeds unbound iron-binding capacity), non–Tf-bound iron (NTBI), direct iron donation to Tf, altered intracellular iron homeostasis, cytotoxicity, neutrophil impairment, bacterial growth enhancement, oxidant stress, or catalytic iron (Table 1).

The results in Table 1 prompt several conclusions. Each manifestation of labile iron is shared by all IV iron agents tested, but not all agents have been tested for each manifestation. Not all attempts to demonstrate labile iron effects have shown positive results, and some positive results more likely are due to tissue iron excess, total iron dose, or underlying disease than to the tested IV iron agent itself.

Serum iron assays falsely detect a portion of iron in iron-carbohydrate compounds as if it were Tf bound. The degree of interference varies by agent class, by agents within the same class, and by assay method. The consequent false elevation of serum iron has confounded assessment of Tf oversaturation after IV iron administration in patients. Of course, assay interference does not exclude a true increase in serum Tf-bound iron. Iron agents convincingly donate iron directly to Tf, and the resulting increase in Tf-bound iron is both theoretically (8) and demonstrably (9) sufficient to saturate Tf fully after rapid IV iron injection.

The relationship among Tf saturation, NTBI, and biologically active iron defies simplicity. Tf oversaturation is not a prerequisite for the appearance of either NTBI or labile iron. Indeed, although both NTBI and biologically active labile iron appear transiently after IV iron administration, each may also arise in patients who do not undergo IV iron therapy, without iron overload, or early after oral iron administration. Neither NTBI nor labile iron has been characterized chemically: NTBI reflects the results of assays for that portion of serum iron that is not bound to Tf, and labile iron is identified only by the biologic activity that it manifests *in vitro* or *in vivo*. Although labile iron may contribute to NTBI, not all NTBI shows evidence of biologic activity, and in some assays, NTBI and labile iron seem to be distinct entities.

It is also apparent that labile iron released from iron-carbohydrate compounds in the extracellular space shows evidence of transport into non-RES cells. Exposure of hepatic parenchymal cells to IV iron agents in tissue culture produces an abrupt increase in the intracellular labile iron pool. The increase in intracellular iron activates key regulatory responses to restore iron homeostasis.

Correspondence to Dr. David B. Van Wyck, Kidney Health Institute, LLC, 6720 North Nanini Drive, Tucson, AZ 85704-6128. Phone: 520-906-8262; Fax: 520-498-5027; E-mail: dvanwyck@sprynet.com

1046-6673/1512-0107
Journal of the American Society of Nephrology

ble 1. Evidence for bioactivity of IV iron agents *in vivo* and *in vitro*: Review of the literature[a]

Effect	Iron Agent Class	Author	Model	Note
ron assay interference	Comparative	Seligman *et al.* (10)	*In vitro*	FSGC > ID INFeD. Detects 2–7% INFeD, 7–33% FSGC as serum iron; ascorbate and incubation time increase % detection
	ID	Cox and King (1)	*In vivo*	Detects 0.3% Imferon as ferrous iron
		McIntosh *et al.* (11)	*In vitro*	Detects 1–2.5% Imferon as serum iron by automated method
		Huisman (12)	*In vitro*	Detects 3.1–100% Imferon; diothionate increases detection
	Ferric gluconate	Sekiguchi *et al.* (13)	*In vitro*	Detects 77% as serum iron using constant-voltage coulometry
	Iron sucrose	Kooistra *et al.* (5)	*In vivo*	Detects 57.5% as serum iron
)versaturation of Tf	ID	Milman (14)	*In vivo*	TSAT > 100% 7 d after 500-mg dose. Method not described.
		Jacobs and Alexander (15)	*In vivo*	TSAT = 100% when agent iron concentration = 8400 μg/dl
	Ferric gluconate	Zanen *et al.* (16)	*In vivo*	TSAT > 100% if dose = 125 mg/4 h or 62.5–125 mg/30 min
	Iron sucrose	Parkkinen *et al.* (9)	*In vivo*	TSAT 80–100% in 9/12 HD patients after 100-mg IV push
	Iron saccharate	Sunder-Plassman and Berl (17)	*In vivo*	TSAT > 100% with low TIBC, dose > 50 mg Ferrivenin
ITBI	Iron sucrose	Rooyakers *et al.* (18)	*In vivo*	NTBI not associated with oxidative stress, vasodilation defect
	Oral iron	Breuer *et al.* (19)	*In vivo*	NTBI positive in one patient shortly after taking oral iron
	No iron	Breuer *et al.* (19)	*In vivo*	NTBI positive in 4.3% of control group without iron therapy
		Esposito *et al.* (20)	*In vivo*	Labile plasma iron in dialysis patients if TSAT > 30%
		von Bonsdorff *et al.* (21)	*In vivo*	NTBI in malignancy; BDI present if TSAT > 80%
ron degradation kinetics	Comparative	Geisser *et al.* (22)	*In vitro*	Rate of degradation SFGC > IS > IP > ID in the presence of ascorbate
)irect iron donation to Tf	Comparative	Esposito *et al.* (20)	*In vitro*	SFGC > IS > IP (semiquantitative)
		Van Wyck *et al.* (8)	*In vitro*	SFGC > IS > ID INFed > ID Dexferrum (quantitative)
	ID	Henderson and Hillman (23)	*In vivo*	Imferon
		Kind ((24))	*In vitro*	Imferon
		Jacobs and Alexander (15)	*In vivo*	Imferon
ltered intracellular iron homeostasis	Comparative	Scheiber *et al.* (25)	*In vitro*	Ferritin, DMT-1 expression: FeAC > SFGC > IS > ID INFeD
		Sturm *et al.* (26)	*In vitro*	Labile iron pool, ferritin, DMT-1: FePP ≥ SFGC > IS ≥ ID INFeD
ytotoxicity	Ferric gluconate	Masini *et al.* (27–30)	*In vitro*	Oxidant-induced activation of a specific Ca^{2+} efflux pathway
	Iron sucrose	Zager *et al.* (31)	*In vitro*	IS > SFGC = ID
leutrophil impairment	Comparative	Sengoelge *et al.* (32)	*In vivo*	Compares ferric gluconate with iron saccharate Ferrivenin. Inhibition of PMN migration: iron saccharate > SFGC
	ID	Guo *et al.* (33)	*In vitro*	ID attenuates PMN from HD patients but not controls
	Iron sucrose	Deicher *et al.* (34)	*In vivo*	300-mg IV infusion in PD patients impairs PMN killing capacity
	Iron saccharate	Patruta *et al.* (35)	*In vivo*	10 mg IV after each dialysis in HD patients with TSAT < 20% and ferritin > 650 ng/ml associated with impaired PMN killing capacity

IN, intravenous; HD, hemodialysis; Tf, transferrin; NTBI, non–Tf-bound iron; ID, iron dextran; SFGC, sodium ferric gluconate complex; IS, iron sucrose; IP, iron polymaltose;)I, bleomycin-detectable iron; TSAT, transferrin saturation; TIBC, total iron binding capacity; DMT-1, divalent transporter 1; PMN, polymorphonuclear leukocyte.

ble 1. Continued

Effect	Iron Agent Class	Author	Model	Note
Bacterial growth enhancement	ID	Webster *et al.* (36)	*In vitro*	Mothers given IV iron dextran during pregnancy show lower TIBC; bacteriostasis and opsonization of *E. coli* by serum is also lower.
	Ferric gluconate			No studies
	Iron sucrose	Parkkinen *et al.* (9)	*In vitro*	Serum of patients after IS supported *Staph epidermidis* growth if TSAT > 80%, effect transient
Oxidant stress and catalytic iron	Comparative	Esposito *et al.* (20)	*In vitro*	Labile iron: SFGC > IS > IP
		Zager *et al.* (31)	*In vitro*	Lipid peroxidation: ID > SFGC > IS.
		Legssyer *et al.* (37)	*In vivo*	Macrophage NO_2 in rats after IV iron: SFGC > IS > ID Dexf > IP
	ID	Salahudeen *et al.* (38)	*In vivo*	F2-isoprostanes increase modestly after 700 mg IV over 60 min
	Ferric gluconate	Masini *et al.* (29,30)	*In vitro*	SFGC disrupts rat liver mitochondria by oxidant mechanism
		Michelis *et al.* (39)	*In vivo*	Oxidized proteins rise after 125 mg but not 62.5 mg IV over 1 h
	Iron sucrose	Parkinnen *et al.* (9)	*In vivo*	Transient BDI when TSAT > 80%
		Drueke *et al.* (40)	*In vivo*	Advanced oxidative protein products in HD patients
		Rooyakers *et al.* (18)	*In vivo*	Transient oxygen-radical mediated effect on vasodilation
		Cavdar *et al.* (41)	*In vivo*	No effect at 20 and 100 mg IV on MDA and red cell deformability
		Weiss *et al.* (42)	*In vivo*	100 mg IV every wk decreased oxidant stress, TNF-α; increased IL-4

IN, intravenous; HD, hemodialysis; Tf, transferrin; NTBI, non–Tf-bound iron; ID, iron dextran; SFGC, sodium ferric gluconate complex; IS, iron sucrose; IP, iron polymaltose; DI, bleomycin-detectable iron.

strated after exposure to IV iron agents. However, the concentration of iron agent needed to demonstrate cell toxicity *in vitro* is far higher than can be achieved in patients after IV iron administration.

Relationship between Labile Iron and the Chemistry of IV Iron Agents

Results of comparative studies of labile iron activity associated with IV iron agents consistently show an inverse relationship between labile iron and molecular weight of the iron-carbohydrate compound. Whether the examined manifestation is interference with serum iron assay, rate of iron degradation, direct donation of iron to Tf, generation of oxidant stress, or alteration of intracellular iron homeostasis, the magnitude of the labile iron effect is greatest in iron-carbohydrate compounds of lowest molecular weight and least in those of the highest weight.

Recent imaging and direct measurement of the core radius of iron-carbohydrate compounds provide a potential explanation (43). If, as proposed, labile iron reflects the ionic iron that is first released from IV iron agents, then the point of release likely would be the surface of the iron-oxyhydroxide core. The focus of attention, therefore, should be the total surface area available for iron release.

Because all agents share the same core chemistry, the rate of iron release per unit surface area likely would be similar among agents (differing, perhaps, only by the strength of the carbohydrate ligand-core iron bond). However, for the same total amount of core iron, surface area available for iron release increases dramatically as core radius decreases. In short, a collection of many small spheres exposes a greater total surface area than does a collection of an equal mass of fewer, larger spheres.

That the relationship between surface area and core radius is not linear explains why small core radius differences between agents of small molecular weight are as significant as large core radius differences between agents of high molecular weight. This is simple mathematics. Because surface area is a function of the product of 4π and the square of the radius, Surface area = $4\pi r^2$, and volume is a function of the cube of the radius, Volume = $4/3\pi r^3$, then the ratio of surface area to volume is a function of the product of the constant 3 and reciprocal of the radius: Surface Area:Volume Ratio = $3r^{-1}$.

Thus, as the radius increases, surface area to volume ratio decreases first abruptly, then more gradually (Figure 1). Because large iron-oxyhydroxide cores such as those in iron dextran tend to assume an ellipsoidal (football or cigar-like) rather than spherical shape, the effective core radius is more difficult to estimate, but the same general relationships apply.

Clinical Implications of Labile Iron

Given the reassuring evidence of safety of IV iron in clinical practice, do any of the broad range of findings on labile iron *in vitro* and *in vivo* have implications for IV iron administration in patients? This question returns attention to previous specu-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.