Complexity of intravenous iron nanoparticle formulations: implications for bioequivalence evaluation

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Short title: Bioequivalence challenges with intravenous iron

Abstract

Intravenous iron formulations are a class of complex drugs that are commonly used to treat a wide variety of disease states associated with iron deficiency and anemia. Venofer® (iron–sucrose) is one of the most frequently used formulations, with more than 90% of dialysis patients in the United States receiving this formulation. Emerging data from global markets outside the United States, where many iron–sucrose similars or copies are available, have shown that these formulations may have safety and efficacy profiles that differ from the reference listed drug. This may be attributable to uncharacterized differences in physicochemical characteristics and/or differences in labile iron release. As bioequivalence evaluation guidance evolves, clinicians should be educated on these potential clinical issues before a switch to the generic formulation is made in the clinical setting.

Keywords: intravenous iron; nanoparticles; chronic kidney disease, bioequivalence

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/nyas.13461.

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Introduction

Intravenous (IV) iron formulations provide a clinical treatment option for chronic kidney disease (CKD) patients when iron supplementation is required but oral administration is not suitable owing to intolerance or lack of efficacy. IV iron use is increasing worldwide, especially in the CKD population.[1,2] More aggressive IV iron use in the CKD population has been driven by several trials demonstrating adverse safety signals with erythropoiesis-stimulating agents (ESAs). Increased risk of stroke and cardiovascular death and a trend toward higher risk of solid organ cancers were observed in these trials, prompting a product label change by the U.S. Food and Drug Administration (FDA).[3–6] In 2011, the Centers for Medicare and Medicaid Services instituted a partially capitated payment system (i.e., the *bundle*) for dialysis services, which included both ESAs and IV iron, which were previously separately billable.[7] The recombinant ESA therapies are clearly far more expensive than IV iron products, which accelerated a national trend to use larger cumulative doses of IV iron. Doses of ESAs began to decline, and IV iron doses began to rise, even several months in advance of bundle implementation.[2] More than 70% of patients receiving chronic hemodialysis receive IV iron, most frequently the reference listed drug (RLD) Venofer® (iron sucrose).[8] The most common doses administered (19%) range from 1.2–2.5 g of elemental iron annually; however, a nearly equal proportion of patients (15%) receive 4.8 g or more annually.[8] The Dialysis Outcomes Practice Patterns study reported that mean ferritin increased from 640 to 826 ng/mL from prebundle to postbundle (January 2012) and remained stable through December 2013.[8] The percent reported that ferritin > 1200 ng/mL, a biomarker of stored iron, increased from 8.6% to 18%.[9]

Outside the United States, there are a plethora of generic iron–sucrose products (iron–sucrose similar (ISS)) on the global market. When compared to the reference listed drug (RLD) Venofer, several ISS formulations have been shown in translational models to have significantly more tissue iron deposition, induce greater tissue cytokine expression, and cause endothelial dysfunction.[10–12]

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Here, I evaluate IV iron in the context of its complex formulations and biodistribution and the factors that impart challenges in evaluation of bioequivalence.

Complex chemistry and biodistribution of IV iron formulations create inherent challenges for bioequivalence

Early IV iron compounds were formulated as inorganic iron–oxyhydroxide complexes. With little relative protection of the inorganic ferric iron, these formulations were highly toxic, with high incidences of severe hypotension.[13] Current commercially available IV iron formulations consist of an iron–oxyhydroxide core surrounded by carbohydrate shells of various sizes and polysaccharide branch characteristics.[13,14] The size of commercially available IV iron–carbohydrate complexes range from 5 to 100 nm, and thus meet the definition for nanoparticle formulations.[15] The manufacture of iron–carbohydrate complex formulations is highly sensitive to pH, temperature, and other conditions in the manufacturing process. This presents significant challenges to reproducible manufacturing, characterization, and safety of generic or similar IV iron product production.

Iron oxide nanoparticles with magnetic particle cores are well-established magnetic resonance imaging (MRI) agents and have been used safely; however, different carbohydrate shell structures determine the relative uptake by endothelial and lymphatic cells as well as the by the reticuloendothelial system.[15] The clinical use of iron–carbohydrate nanoparticle formulations has not been well studied with regard to potential long-term toxicity beyond immediate labile iron appearance and immunogenicity.[15] Because commercially available IV iron formulations used in CKD meet the criteria for nanoparticles, their pharmacodynamic profile with regard to direct cell uptake and subsequent physiological effects needs to be better characterized for both RLDs and current and future generic formulations.[16,17]

Pharmacokinetic evaluation of IV iron complexes is challenging, unless the compound can be directly measured (ferromagnetic) or is manufactured with a radiolabeled form of iron (^{59}Fe) to distinguish the IV iron formulation from endogenous serum iron. While not well appreciated by clinicians, IV iron formulations exhibit zero-order or capacity-limited metabolism by the

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reticuloendothelial system. This results in longer residence time in plasma with higher administered doses, especially with higher-molecular-weight formulations.[18] This has potentially important implications regarding toxicity profiles, as doses administered beyond the reticuloendothelial system capacity limit will remain circulating in plasma for long periods of time until the concentration falls below the capacity limit, at which time the pharmacokinetic profile will become linear or concentration independent. When the RLD Feraheme® (ferumoxytol) was administered as two 510-mg IV doses 24 h apart to healthy subjects, the metabolism did not appear to become linear until approximately 96 h after the first dose.[18] Hillman *et al.* showed that radiolabeled iron–dextran exhibited capacity-limited metabolism at 500 mg, whereas the 250-mg dose appeared to have a linear pharmacokinetic profile.[19] Ultimately, the complexity of IV iron–carbohydrate complex nanoparticle formulations has important implications with regard to both efficacy and safety in CKD. These agents have not been well studied with regard to comparative biodistribution, metabolic fate, or potential extracellular and intracellular toxicity profiles, and further evaluation of these agents is urgently needed, as long-term clinical use is widespread.

Current regulatory guidance provides some recommendations for physicochemical characterization and pharmacokinetics of these agents. This is especially relevant for abbreviated new drug applications for generic formulations, which necessitate independent clinical and translational studies to elucidate comparative product characteristics.[20] Even slight changes (temperature, pH, polymer content) in the co-precipitation reaction to synthesize iron–carbohydrate nanoparticles can alter the properties of the final product, presenting challenges to reproducible manufacturing of IV iron formulations to be considered for generic approval.[21,22] These formulations have been referred to as similars, as exact copies cannot be formulated.[11,21] Although it has been shown that, if the iron complex is thermodynamically stable, complexes of similar molecular weight can be synthesized using multiple different manufacturing procedures, this may or may not translate to similar disposition *in vivo*.[23,24] Simple fold dilutions in polymer content during iron oxide–dextran co-precipitation have yielded particles with similar hydrodynamic diameters determined by dynamic light scattering; however, the cellular iron uptake and cell viability are markedly different among the particles.[22]

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Several ISSs available outside the United States have been shown to not meet United States Pharmacopeia (USP) Reference Standards.[10,11] Differences in molecular weight, titratable alkalinity, and kinetics of degradation have also been shown between lots of the same generic formulation.[11,24] Toblli *et al.* characterized the physicochemical characteristics of the RLD Venofer and compared these to several of the compounds available and in clinical use in Europe and Asia.[11,25] Notably, only one generic product in these comparative analyses complied with USP criteria. Differences in one or more of the criteria—pH, titratable alkalinity, and turbidity point—were observed in all generic products evaluated. Four of the seven products (57%) evaluated in one study[11] had markedly higher molecular weights measured by gel-permeation chromatography. In animal studies using 40-mg/kg single IV doses, generic iron products have been shown to be associated with higher tissue concentrations of proinflammatory cytokines, higher intracellular antioxidant enzyme activity, adverse effects on the basic metabolic profiles (elevated liver function tests), and kidney dysfunction (elevated serum creatinine and proteinuria).[10,11,25–27] It has been hypothesized that labile iron is principally involved with these observed deleterious effects by generating reactive oxygen species via the Fenton–Haber–Weiss reaction. In a systematic series of experiments, *in vitro* labile iron release profiles were evaluated for six IV iron formulations.[28] The formulations studied included the only approved generic IV iron in the United States (sodium ferric gluconate complex) and the RLD Ferrlecit®. Labile iron release in both saline and rat serum matrices was higher for the RLD versus the generic SFGC, indicative of some formulation variability. To date, no published studies comparing RLDs with generic IV iron formulations have evaluated labile iron release profiles in human subjects.[10,11,25–27] Because generic iron–carbohydrate complex formulations may differ with regard to molecular weight, carbohydrate shell chemistry, shell and particle diameter, and osmolality[13,14] these agents require additional considerations for bioequivalence testing.[21]

Adverse safety signals from *in vitro*, animal, and human studies: RLDs and iron–sucrose similars

The hypothesis for the pathogenesis of acute oxidative stress induced by IV iron formulations is the

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release of labile iron from the iron–carbohydrate structure resulting in transient concentrations of labile plasma iron and induction of the Fenton chemistry and the Haber–Weiss reaction, promoting formation of highly reactive free radicals, such as the hydroxyl radical.[29] Labile plasma iron is the oxidative reactive fraction of non-transferrin-bound iron, iron that is not tightly bound transferrin. Among available RLD IV iron formulations, products with smaller carbohydrate shells are more labile and more likely to release labile iron directly into the plasma (i.e., before metabolism by RES) (Table 1).[30,31] The proposed biologic targets of labile iron–induced oxidative stress include nearly all systemic cellular components, including endothelial cells, myocardium, and liver, as well as low-density-lipoprotein and other plasma proteins. Because of the extremely short half-lives of free radicals and the rapidity of the ensuing oxidative stress reactions produced by labile iron appearance, *in vivo* evaluation of this toxicity profile can only reasonably be accomplished by using biomarkers as surrogates. Recently, a systematic review of widely used biomarkers was conducted to assess oxidative stress in CKD. The authors applied scores for commonly used biomarkers for relationships to other biomarkers and clinical indicators, reliability, and characterization in the CKD literature.[32] Many of the identified "robust" biomarkers have been evaluated in the context of potential IV iron toxicity in CKD (malondialdehyde, protein carbonyl, and F2-isoprostane); however, it should be noted that none of the identified biomarkers have specificity for iron-induced oxidative stress.[33,34] An additional concern regarding appearance of labile plasma iron is the potential for easily accessible iron to impair innate immunity and augment bacterial growth, increasing the risk of infection.[35]

IV iron formulations have clearly been shown to induce oxidative stress, inflammation, and cellular toxicity in cell culture models, animal models, and acutely in human subjects.[10–12,30,34] Differential toxicity profiles have been observed among the available IV iron products *in vitro*, with more labile compounds inducing more toxicity than compounds with larger carbohydrate shells that exhibit better stability.[35,36] In animal models, similar observations have been reported with administration of IV iron compounds inducing labile iron appearance, pro-oxidant cell signaling, tissue inflammation, cellular iron deposition, and cytotoxicity.[10,32,37,38] IV iron has also been associated with immune dysfunction and increased Gram-positive bacteria growth *in vitro*.[11,36,39] In

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some studies, a similar rank order for toxicity (labile products > stable products) has been demonstrated;[35] however, other studies have shown greater cellular iron staining and tissue inflammation with higher-molecular-weight products.[11] In similar rat models, increased tissue oxidative stress has been observed with several ISS (i.e., generic) products compared with the branded product.[10] A caveat to interpretation of these *in vivo* animal model data is the wide variation in doses administered in the experiments (1.4 mg/kg to 500 mg/kg).[10,31,37] While the dose in the rat should be higher on the basis of allometric scaling, the optimal dose to model human IV iron toxicity has not been determined.

Although the biological plausibility and available *in vitro* and animal model data are generally compelling, controversy remains regarding whether iron-induced oxidative stress manifests long-term toxicity, such as cardiovascular disease and infection, in CKD patients. The complex biochemical milieu in CKD, in tandem with the multiple inciting factors for oxidative stress and inflammation, complicates investigation of potential IV iron safety concerns. Epidemiologic analyses conducted with dialysis patient data in the late 1990s demonstrated positive correlations between the number of IV iron vials billed and mortality.[40] Given that the impact of iron-induced oxidative stress on cardiovascular disease likely takes extended periods of time, immediate correlation of iron dose to cardiovascular events is not likely possible. In later analyses with newer data, application of more sophisticated statistical analyses with incorporation of lag times to adjust for time-varying confounders found that the relationship between IV iron and cardiovascular outcomes was not statistically significant.[41] A recent analysis evaluated short-term cardiovascular risk associated with IV iron dosing practices (bolus vs. maintenance and high dose vs. low dose).[42] Large-dose strategies (bolus and high dose) were not associated with increased risk of cardiovascular death, hospitalization for myocardial infarction, hospitalization for stroke, or any composite/combination of any of these three.[42] Evaluating the relationship between iron and infection risk could be more easily evaluable, given that the presumed risk of infection would

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likely be in close proximity to the dose administered when labile iron is presumably present. In a small retrospective study, 132 dialysis patients receiving their first course of IV iron were followed for 1 year after therapy initiation for time to first bacteremia episode.[43] Patients with transferrin saturation values ≥ 20% and ferritin ≥ 100 were defined as iron replete, and this group had 2.5-fold higher risk of bacteremia compared with patients with functional iron deficiency and those who were iron deficient.[43] These data may suggest that iron availability is increased when additional iron is administered to these iron-replete patients, promoting bacterial growth and subsequent bloodstream infections. More recently, a large epidemiologic study examined the risk of infection-related hospitalization with bolus versus maintenance or high versus low IV iron–dosing patterns.[44] Bolus dosing of IV iron was associated with a higher risk of infection-related hospitalization (25 additional events per 1000 patient-years) and increased risk of mortality. Differences in infection rates between iron formulations have been less clear. In two studies evaluating U.S. Renal Data System data, short-term infection risk in hemodialysis patients with sodium ferric gluconate was marginally lower than iron sucrose.[45] In contrast, longer-term infection risk was modestly lower in iron–sucrose–treated hemodialysis patients.[46] Prospective studies are needed to elucidate whether risk and predictors of infection differ among formulations.

In interventional clinical trials and observational reports, when compared to the RLD, different formulations and lots of ISS have been associated with intracellular reactive oxygen species generation, increases in biomarkers of endothelial dysfunction, and adverse drug events, including hypotension and phlebitis.[12,26,47,48] Labile iron release in the immediate postadministration period (directly from the formulation) from RLD iron–carbohydrate complexes has been shown to induce oxidative stress, cytokine activation, and endothelial dysfunction.[12,30,49] Therefore, the biologic plausibility strongly implies that differences in labile iron release are fundamentally responsible for the higher rates of adverse drug events reported with generic iron–sucrose formulations. Table 2 summarizes published studies evaluating RLDs and ISS products across the translational research

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spectrum.

Formulation-based labile iron release is a viable and pragmatic parameter for enhancing BE evaluation of generic IV iron products

As discussed, the biologic plausibility of labile iron being a fundamental cause of adverse drug events (excluding immunogenetic reactions) related to IV iron formulations is strong and supported by translational research evaluating several of the RLD products.[24,48] The higher incidence of hypotension reported with some generic formulations, including different lots of the same formulation, is likely attributable to formulation-based free iron release.[24,48] Thus, labile iron measurement is a both a relevant and practical candidate to further evaluate BE of generic IV iron formulations.[29,49,50] Assessment of labile iron–release profiles extends data provided by physicochemical characterization (PCC) to better understand how the disposition of generic formulation compares to the RLD. As mentioned previously, despite evidence of similar PCC, these complex formulations may behave differently in *in vivo* systems.[51] This underscores the need for a multipronged approach in evaluation of BE among complex drug formulations. Animal studies evaluating generic iron sucrose formulations have evaluated serum iron concentrations and transferrin saturation (TSAT) and found values to be higher in animals receiving the generic formulations versus the RLD.[10,11,25–27] However, TSAT is not a direct measurement of the reactive labile iron species and does not adequately represent the potential for deleterious redox reactions. Although TSAT values greater than 100% strongly infer the presence of labile iron, we and others have shown that labile iron is present at TSAT values less than 100%, limiting the utility of this parameter.[30,52] An optimal approach for BE for generic IV iron formulations would be development of an *in vitro* to *in vivo* correlation (IVIVC) model. A validated IVIVC model would allow *in vitro* labile iron–release kinetics under physiologically relevant conditions to support BE evaluation in addition to rigorous PCC with standards.[50,53] Several assays have been developed and validated to measure labile plasma iron, mainly employing redox-active or chelatable methodologies; however, many of these assays are not viable for i*n vitro* determination of labile iron release from the formulation.[28,29] Jahn *et al*. used the Ferrozine® assay to determine iron

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release among six available IV iron formulations *in vitro* incubated in human serum.[14] Lower-molecular-weight formulations and higher concentrations representing clinically relevant doses were associated with higher concentrations of iron release. However, it should be noted that this assay measures non-transferrin-bound iron (labile reactive iron plus iron weakly bound to other plasma proteins) and may overestimate formulation-based labile iron release.

The difficulties in evaluating non-biologic complex drugs, such as IV iron formulations, is appreciated by scientists and regulatory agencies.[53,54] However, most clinicians who use these formulations across a wide spectrum of acute and chronic disease states are not aware of their complicated pharmacokinetic and pharmacodynamic profiles, nor do they appreciate the challenges in BE evaluation of generic formulations. A survey administered to 140 pharmacists in France and Spain was designed to provide insight into the current decision-making process for pharmacists regarding IV iron products in the hospital.[55] Substitution of RLD iron–sucrose for an ISS ranged from 38–47%. However, only 19% and 7% of pharmacists in France and Spain, respectively, thought there were relevant differences between RLD and ISS formulations.

Taken collectively, there is a need for comprehensive clinical and translation investigations of IV iron formulations to mechanistically evaluate and understand the biodistribution, safety, and toxicity profiles of these agents. Such studies would be useful in moving the needle forward on BE evaluation to ensure safe and effective generic IV iron products.

Acknowledgments

Competing interests

The author declares no competing interests.

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Table 1. Comparison of physicochemical characteristics and pharmacokinetics of reference listed drug IV iron formulations

PROPERTIES	Feraheme®	Injectafer®	InFed®	Venofer®	Ferrlecit®
MW (Da)	731,000	150,000	410,000	252,000	200,000
Carbohydrate shell	Polyglucose sorbitol carboxymethylether	Carboxymaltose	Detran polysaccharide	Sucrose	Gluconate, loosely associated sucrose
Median shell/ particle diameter (nm)	26.3	23.1	12.2	8.3	8.6
Relative labile Fe release	+	+	++	+++	+++
Relative stability of elemental Fe within the CHO Shell	High	High	High	Medium	Low
Relative osmolalities	Isotonic	Isotonic	Isotonic	Hypertonic	Hypertonic
Administration (IV push)	30 mg/sec	Bolus push	50 mg (1 mL)/ min	~20 mg/min	12.5 mg/min

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rates					
Half-life (hrs)	~ 15	7–12	5–20	6	~ 1

IV, intravenous; CHO, carbohydrate.

Table 2. Summary of *in vitro*, animal and human subject studies comparing iron–sucrose similars (ISSs) to the RLD Venofer

Author	Formulations studied	Study design	Key findings
In vitro			
Kuo *et al.*[12]	ISS (Nan-Kuang Pharmaceuticals) vs. control	Human aortic endothelial cells (HAECs) and monocytes (U937) incubated with ISS 40–160 μg/mL for 4 hours.	Time-dependent intracellular iron uptake, ROS generation, NADPH oxidase (NOX) activity, VCAM-1, ICAM-1, increased NF-κB in HAECS, and endothelial–monocyte adhesion highest in ISS-treated cells
In vivo animal			
Toblli *et al.*[26]	Venofer, ISS test 1,[a] ISS test 2,[a] control	Single 40 mg/kg IV injection in rats. Serum and tissue samples collected at 24 h, and 7 and 28 days.	Higher tissue iron deposition, antioxidant enzyme, and cytokine generation with both ISS formulations vs. RLD and control observed at 24 h and day 7. No difference at day 28.
Toblli *et al.*[11]	Venofer, six ISS formulations sourced from Pakistan, India, and Taiwan	Rats randomly allocated to receive 40 mg/kg IV of an IV iron formulation or saline at days 0, 7, 14, 21, and 28. Serum collected at day 1, 8, 15, 22, and 29. Tissue samples collected at day 29.	Higher serum iron, TSAT observed at days 1, 8, and 29 with all ISS formulations vs. RLD and control. Higher tissue iron deposition observed with ISS[b] vs. RLD and control; higher tissue cytokines (TNF-α, IL-6) with ISS

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			formulations
Kuo *et al.*[12]	ISS (Nan-Kuang Pharmaceuticals) vs. control	Mice (C57BL/6) mice were established in four groups: sham with saline, sham with ISS, uninephrectomy (SNx) with saline, and SNx with iron to evaluate vascular adhesion. A second group of ApoE$^{-/-}$ mice was established in the same four groups to evaluate atherogenesis. Doses of ISS were administered IP at 2 mg/25 g body weight for 5 days.	SNx wild-type mice treated with iron had the highest amount of leukocyte adherence to aortic endothelium compared to sham ± iron. SNx not treated with iron had higher amounts of leukocyte adherence vs. sham ± iron. ROS were highest in SNx + iron mice, as was VCAM-1 and ICAM-1 expression in aortic tissue. ROS generation appeared to be mediated by NOX, confirmed by p22^{phox} expression in the aortic endothelium as well as the medial layer
Toblli et al.[27] 2015	Eight ISS sourced from Europe and Asia vs. three different lots of Venofer and control	Rats were administered 40 mg/kg of Venofer or one of eight ISSs adjusted for weekly body weight and diluted in saline to a final volume of 1 mL at days 0, 7, 14, 21, and 28. Blood samples were collected at 24 h and 29 days. Tissue samples were collected at day 29.	Transferrin saturation at end of study was significantly higher with all ISSs vs Venofer and control. All formulations had significant tissue deposition in liver, heart, and kidney vs. control. One formulation of Venofer showed highest values for ferritin immunostaining in the liver. All ISS formulations had significantly higher IL-6 immunostaining vs. all three lots of Venofer.
Spicher *et al.*[56]	Three approved IV iron formulations (Venofer, Ferrlecit, Ferinject) and one ISS (FerMed, Medice Arzneimittel	ISS and Venofer®were administered at 200 and 400 µg into chicken chorioallantoic	No significant difference in liver or heart tissue deposition was observed between ISS and RLD.

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	Pütter GmbH & Co. KG)	membranes.	
Humans			
Rottembourg et al.[57]	Venofer and ISS (Mylan SAS, Saint Priest, France manufactured by Help SA Pharmaceuticals, Athens, Greece)	Retrospective study of pre–post ISS switch in hemodialysis patients (*n* = 75).	IV iron doses, ESA doses and total drug costs increased and hemoglobin transiently decreased post switch to ISS.
Lee *et al.*[24]	RLD Venoferrum vs. ISS Ferex (SejongPharmas, South Korea)	Retrospective study of postpartum patients who received Venoferrum (200 mg/100 mL NS), ISS (200 mg/100 mL NS), and ISS (200 mg/200 mL NS). *n* = 658.	Adverse events reported were significantly lower with Venoferrum. Injection-site reactions and phlebitis were significantly higher in ISS-treated patients, especially with greater dilution of ISS.
Stein *et al.*[48]	ISS (FerMed, Medice Arzneimittel Pütter GmbH & Co. KG)	Case series of three patients receiving ISS who previously tolerated Venofer® (300 mg/300 mL over 1.5 h).	All three patients experienced adverse drug reactions, including urticaria, headache, and peripheral edema.
Kuo *et al.*[12]	ISS (Nan-Kuang Pharmaceutical) vs. control	CKD stage 5 patients (*n* = 40) were randomly allocated to receive ISS (100 mg/250 mL) or NS 250 mL administered for 60 min postdialysis for 10 weeks. Blood samples were collected every 2 weeks. Healthy subjects (*n* = 20) had blood collected once.	CKD stage 5 subjects receiving ISS had highest ROS production, soluble adhesion molecule concentrations (ICAM-1, VCAM-1), and *ex vivo* monocyte–endothelial adhesion.
Aguera *et al.*[58]	ISS and RLD (manufacturers not supplied)	Prospective study after institutional switch to RLD from ISS (*n* = 342)	Reduced IV iron and ESA doses required during prospective RLD observation period. Hemoglobin remained stable.

[a]Manufacturer not provided. [b]Except ISS_{FERP} (Ferplex®) in liver. VCAM-1, vascular cell adhesion molecule; ICAM-1, intracellular adhesion molecule; NF-κB, nuclear factor κ light-chain enhancer of activated B cells; IP, intraperitoneal; ROS, reactive oxygen species.

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