

Parenteral Iron Compounds: Potent Oxidants but Mainstays of Anemia Management in Chronic Renal Disease

Richard A. Zager

Fred Hutchinson Cancer Research Center and the Department of Medicine, University of Washington, Seattle, Washington

Ferric iron (Fe)-carbohydrate complexes are widely used for treating Fe deficiency in patients who are unable to meet their Fe requirements with oral supplements. Intravenous Fe generally is well tolerated and effective in correcting Fe-deficient states. However, the complexing of Fe to carbohydrate polymers does not block its potent pro-oxidant effects; systemic free radical generation and, possibly, tissue damage may result. The purpose of this review is to (1) underscore the capacity of currently used parenteral Fe formulations to induce oxidative stress, (2) compare the severity of these oxidant reactions with those that result from unshielded Fe salts and with each other, and (3) speculate as to the potential of these agents to induce acute renal cell injury and augment systemic inflammatory responses. The experimental data that are reviewed should not be extrapolated to the clinical setting or be used for clinical decision making. Rather, it is hoped that the information provided herein may have utility for clinical hypothesis generation and, hence, future clinical studies. By so doing, a better understanding of Fe's potential protean effects on patients with renal disease may result.

Clin J Am Soc Nephrol 1: S24-S31, 2006. doi: 10.2215/CJN.01410406

Parenteral iron (Fe) therapy has become a mainstay in the management of anemia in patients with ESRD. The efficacy and short-term safety of parenteral Fe administration has been amply documented. However, Fe also is a potent oxidant that is capable of redox cycling. This results in the production of oxygen-based free radicals that can damage tissue proteins, lipids, and RNA/DNA. Nature has devised highly effective mechanisms to permit Fe to carry out its essential biologic functions (*e.g.*, in mitochondrial electron transport, cytochrome p450 activities, heme O₂ transfer) while at the same time preventing its toxic effects. For example, Fe sequestration within porphyrin rings markedly reduces oxidant stress. As another example, pharmacologic oral Fe therapy does not cause toxicity as a result of Fe transfer to transferrin, which then permits safe systemic delivery. Finally, large amounts of Fe can be stored safely in tissues by transfer to apoferritin/ferritin. The latter serves as a safe reservoir to meet subsequent Fe needs.

Because of poor oral Fe absorption in the setting of ESRD, Fe supplementation now is almost routinely performed by intravenous administration of Fe-carbohydrate (CHO) complexes (dextran, sucrose, or gluconate polymers). Ideally, these CHO carriers should function as molecular "shields," allowing for safe Fe delivery while at the same time preventing Fe-based free radical and cytotoxic reactions. The relative efficacy of these CHO shields has remained an open question. Because of this, our laboratory has addressed this issue in a variety of *in vitro* experiments that have used cultured proximal tubular cells, aortic endothelial cells, and isolated mouse proximal tu-

bule segments. Experimental *in vivo* correlations also have been sought. The purpose of this article is to review some of the resulting information. Multiple biologic end points, used in multiple test systems, have been used because no single marker or test system provides sufficient clarity.

Initial Studies Using Renal Cortical Homogenates as a Target for Lipid Peroxidation

In our initial efforts to define the role of CHO shielding as a determinant of parenteral Fe formulation toxicity (1), we added equal amounts of Fe gluconate (FeG), Fe sucrose (FeS), or Fe dextran (FeD) to renal cortical tissue homogenates. Lipid peroxidative damage was assessed by homogenate malondialdehyde (MDA) content. Figure 1 presents the results that were produced by 200- or 800- $\mu\text{g}/\text{ml}$ concentrations of FeS (Venofer, American Regent, Shirley, NY), FeG (Ferrlecit, Watson Pharmaceuticals, Morristown, NJ), and FeD (InFed, Watson Pharmaceuticals) on homogenate MDA concentrations. Because mitochondrial respiration can be a critical determinant of lipid peroxidation under conditions of oxidative stress (2), the use of tissue homogenates (*i.e.*, with no functional mitochondria) permitted a direct assessment of Fe complex-lipid interactions. Under conditions of equimolar Fe additions, FeS caused far less MDA generation than did FeG or FeD (Figure 1). On the basis of these results, one might predict that FeS would have the least cytotoxic effects of these three test agents. However, this clearly is not the case, a theme that is developed later in this review.

To explore the degree to which CHO shielding might mitigate Fe's lipid peroxidative effects, we next incubated renal cortical homogenates with either FeD (the agent that induced the greatest MDA generation in the above experiments) or an

Address correspondence to: Dr. Richard A. Zager, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N; Room D2-190, Seattle, WA 98109. Phone: 206-667-6222; Fax: 206-667-6519; E-mail: dzager@fhcrc.org

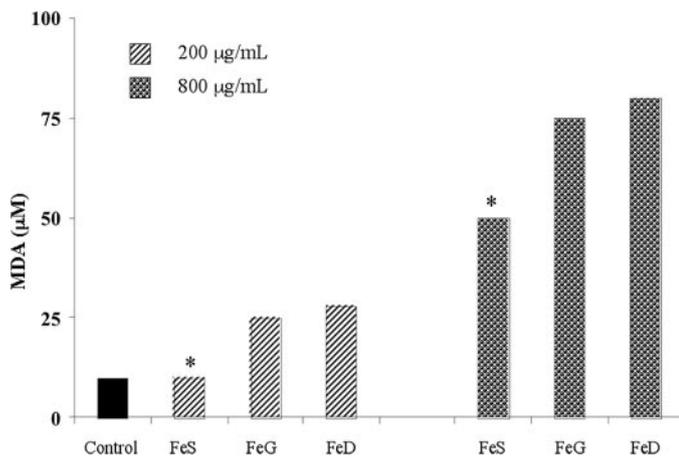


Figure 1. Malondialdehyde (MDA) generation in renal cortical homogenates, as induced by test iron (Fe) preparations. With 200- $\mu\text{g}/\text{ml}$ Fe exposures, Fe sucrose (FeS) failed to increase MDA levels. Conversely, MDA approximately doubled with Fe gluconate (FeG) or Fe dextran (FeD) exposure ($P < 0.01$ versus controls). At the higher Fe concentration (800 $\mu\text{g}/\text{ml}$), all Fe preparations generated MDA, but the amount was significantly less ($*P < 0.01$) with FeS versus FeG or FeD.

equimolar amount of unshielded Fe^{3+} (FeCl_3) (1). As shown in Figure 2, these results were remarkable in that FeD induced approximately two thirds as much MDA generation as did unshielded FeCl_3 . Similar results were observed with the other Fe-CHO preparations. Therefore, these results strongly suggest that CHO shielding, *per se*, has only a minimal impact on Fe's ability to induce oxidative attack.

Fe Toxicity as Probed with Viable Isolated Mouse Proximal Tubule Segments

To pursue further the issue of potential Fe-CHO complex toxicity, we added equimolar amounts of FeS, FeG, or FeD to

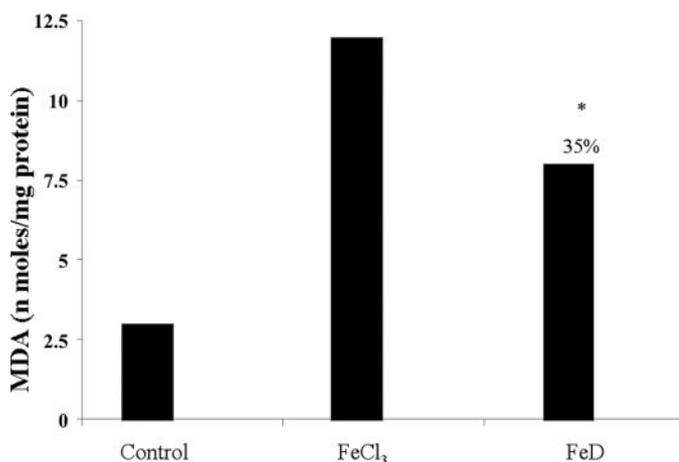


Figure 2. MDA generation in renal cortical homogenates, as induced by either FeCl_3 or FeD. Unshielded FeCl_3 induced marked MDA increases. Despite Fe shielding with dextran (FeD), Fe still caused marked MDA increases (only 35% less than those observed with unshielded FeCl_3). Therefore, CHO shielding seemed to be relatively inefficient in preventing peroxidative damage.

viable proximal tubules that were harvested from normal CD-1 mice (1). The tubules were used simply as a convenient biologic system for assessing cytotoxic potentials, rather than as an index of possible Fe-CHO-induced renal damage. After the tubules were incubated for 60 min under control conditions or with Fe exposure, lethal cell injury and lipid peroxidation were assessed by percentage of tubule lactate dehydrogenase release and by MDA content, respectively. Representative results from these experiments are presented in Figure 3. These were notable in three respects: First, despite that FeS induced the least lipid peroxidation of the three test agents in the renal cortical homogenate experiments (see above), it was the only agent to induce lethal cell injury in these acute toxicity experiments (Figure 3, left). Second, unlike the renal cortical homogenate experiments, FeS induced the same degree of lipid peroxidation/MDA generation in isolated tubules as did FeG and FeD (Figure 3, right). Third, there was no correlation between lethal cell injury (lactate dehydrogenase release) and MDA formation, given that only FeS caused lethal cell injury but evoked no more MDA generation than the other two test agents. These results should not be considered surprising, given our previous observations that degrees of lipid peroxidation and lethal cell injury are not directly linked (3). For example, adding Fe^{2+} and Fe^{3+} salts to isolated tubules each induce marked and equal MDA increases; however, only Fe^{2+} caused tubule cell death (3).

Role of Mitochondria in FeS-Mediated Lipid Peroxidation

The finding that FeS did not cause lipid peroxidation in renal homogenates but did so in isolated tubules raised a compelling hypothesis: That the mechanism by which FeS induces lipid peroxidation is *via* its impact on mitochondrial respiration. In other words, FeS might induce oxidative damage only in the presence of functional mitochondria (present in intact tubules but not in cortical homogenates). To test this hypothesis, we devised a simple experiment: Test whether FeS would induce less lipid peroxidation in isolated tubules that had preexistent

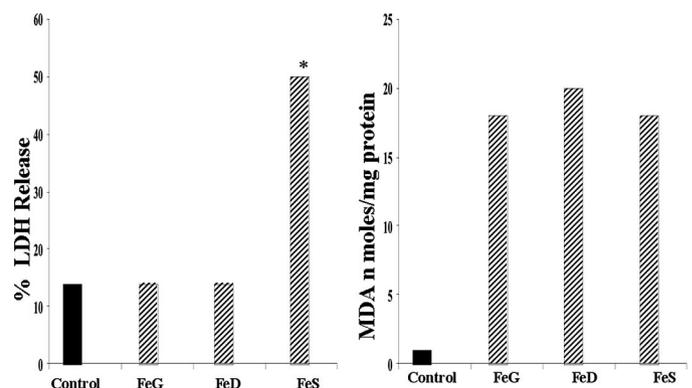


Figure 3. FeG, FeD, and FeS effects on isolated mouse proximal tubule cell viability (percentage of lactate dehydrogenase release [% LDH release]) and on lipid peroxidation (MDA generation). All of the Fe preparations caused marked and comparable MDA increases (right). However, only FeS caused lethal cell injury, as determined by % LDH release (left).

mitochondrial respiratory blockade (1). Toward this end, isolated tubules were treated with antimycin A (AA) to inhibit mitochondrial electron transport. (Since this produces profound ATP depletion, cell death was prevented in these experiments by adding 2 mM glycine, which blocks the evolution of ATP depletion–induced tubule cell death.) As shown in Figure 4, AA had no independent effect on isolated tubule MDA levels. However, it reduced the extent of FeS-induced MDA production by approximately 50%. Conversely, AA did not alter MDA generation under conditions of FeD treatment (Figure 4). Therefore, these experiments provided the following insights: (1) Mitochondrial respiration helps mediate FeS-induced oxidative stress; (2) the mechanisms of lipid peroxidation induced by the various Fe-CHO complexes differ dramatically (given that mitochondrial participation is present with FeS but not FeD exposure); and (3) because free radicals tend to induce injury at the site of their formation (given their high degree of reactivity), these findings suggested a possible explanation for why FeS but not FeD induced tubular cell death: Perhaps FeS but not FeD, causes mitochondrial injury, ATP depletion, and, hence, a severe energy deficient state.

Impact of Fe Complexes on Mitochondrial ATP Production

To test the above hypothesis, we incubated isolated tubules either under control conditions or with FeS or FeD (1). Cellular energetics were assessed by measuring ATP/ADP ratios (which fall with decreased mitochondrial respiration or, possibly, increased ATP consumption). As shown in Figure 5, control and FeD-exposed tubules maintained normal ATP/ADP ratios (approximately 9:1). However, FeS induced a profound

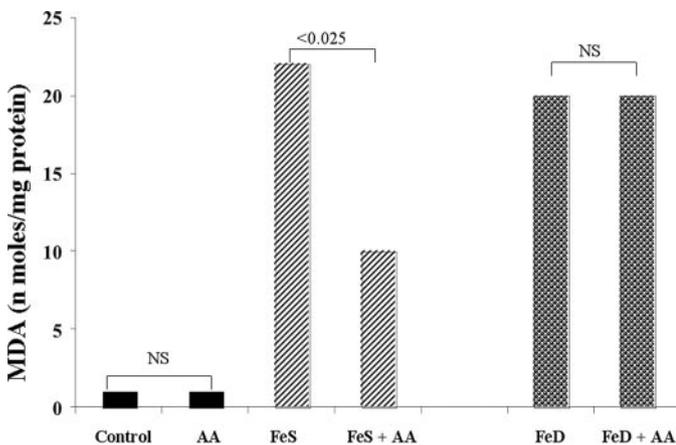


Figure 4. MDA generation in isolated proximal tubules, as induced by either FeS or FeD in the absence or presence of mitochondrial respiratory blockade with antimycin A (AA). AA alone did not alter tubule MDA generation. However, AA approximately halved the extent of MDA generation in tubules that were challenged with FeS. Conversely, AA did not alter MDA generation in tubules that were challenged with FeD. Therefore, these experiments indicated that mitochondrial respiration is involved in the lipid peroxidation that is induced by FeS but not by FeD.

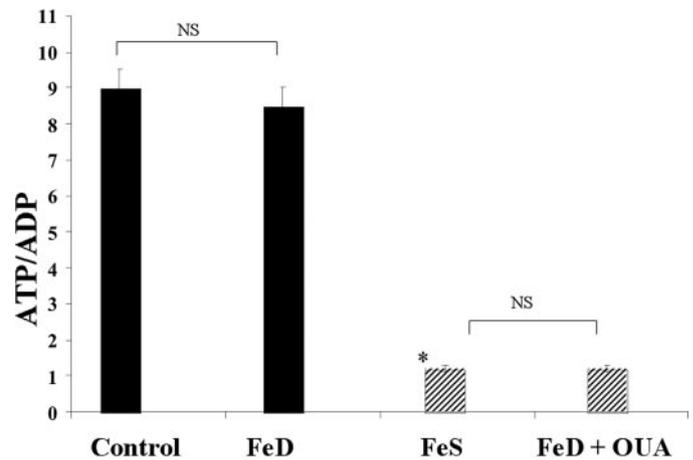


Figure 5. Impact of FeD and FeS on isolated tubule mitochondrial energetics, as assessed by ATP/ADP ratios. FeD did not alter ATP/ADP ratios, indicating a lack of mitochondrial damage. Conversely, FeS dramatically lowered ATP/ADP ratios, an effect that was not blocked by inhibiting NaK ATPase with ouabain (oua). Therefore, these results indicate that FeS but not FeD inhibits mitochondrial ATP production and, thus, likely explains why FeS but not FeD causes tubular cell death.

ATP depletion state (decreased by approximately 90%, resulting in an ATP/ADP ratio of approximately 1). To prove that it was, indeed, decreased mitochondrial ATP production, rather than increased ATP consumption (90% of which is *via* NaK ATPase activity) that caused ATP depletion, NaK ATPase was inhibited by ouabain to ascertain whether ATP preservation would result. However, ouabain did not prevent the FeS-mediated ATP depletion state. Therefore, these results supported the hypothesis raised above: That FeS (but not FeD) causes severe mitochondrial injury, culminating in respiratory blockade and profound ATP depletion. This likely explains why only FeS induced lethal cell injury upon addition to viable proximal tubules.

Relative Fe Toxicity: Cultured Proximal Tubule and Endothelial Cell Experiments

To define further the relative degrees of toxicity of clinically used parenteral Fe formulations, we performed additional experiments using two additional biologic systems (*i.e.*, beyond cortical homogenates and isolated mouse tubules): Cultured aortic endothelial cells or cultured proximal tubule (HK-2) cells that were derived from a normal human kidney (1). These cells were incubated with differing dosages of the above three Fe agents. The rationale for studying endothelial cells is that the endothelium is the initial Fe-CHO target after intravenous administration. Because Fe-CHO complexes now are being administered to pre-ESRD patients, the question of whether Fe induces tubular injury is an additional important issue (the stimulus for performing the HK-2 cell experiments). These HK-2 cell and endothelial cell experiments produced results consistent with those derived from the isolated tubule experiments: They confirmed that (1) differential degrees of Fe toxicity exist, depending on the nature of the CHO carrier; (2) the

relative degrees of Fe-CHO cytotoxicity were FeS > FeG > FeD; and (3) cytotoxicity was observed at FeS concentrations (approximately 50 to 100 $\mu\text{g}/\text{ml}$) that are achievable after therapeutic intravenous Fe injection. Representative results are illustrated in Figure 6.

Potential Mechanism for Differential Fe-CHO Toxicities

In an effort to discern why FeS consistently manifested the greatest cytotoxic potential of the three test agents, we questioned whether sucrose, *per se*, rather than Fe was exerting an adverse effect. However, this seemed unlikely given that sucrose addition to either isolated proximal tubules or HK-2 cells induced no cell death. Furthermore, sucrose addition to FeD did not unmask any potential cytotoxic effects. Finally, the addition of an Fe chelator, desferrioxamine, to FeS diminished FeS toxicity, consistent with Fe's, not sucrose's, being the toxic component of Fe-sucrose complexes (1).

A notable observation from the HK-2 cell Fe experiments was that after 24- to 72-h incubations with the various Fe-CHO complexes, the FeS-treated cells were deeply Fe stained, whereas modest (FeG) *versus* no staining (FeD) was seen with these other agents (4) (Figure 7). This suggested to us that the differing toxicologic profiles with these agents might be explained simply by differing degrees of cellular Fe uptake. To test this hypothesis, we subjected FeS-, FeD-, and FeG-exposed HK-2 cells to electron microscopic analysis. As suspected, FeS treatment led to marked cellular Fe loading. Conversely, less Fe uptake was observed with FeG exposure, and no Fe incorpo-

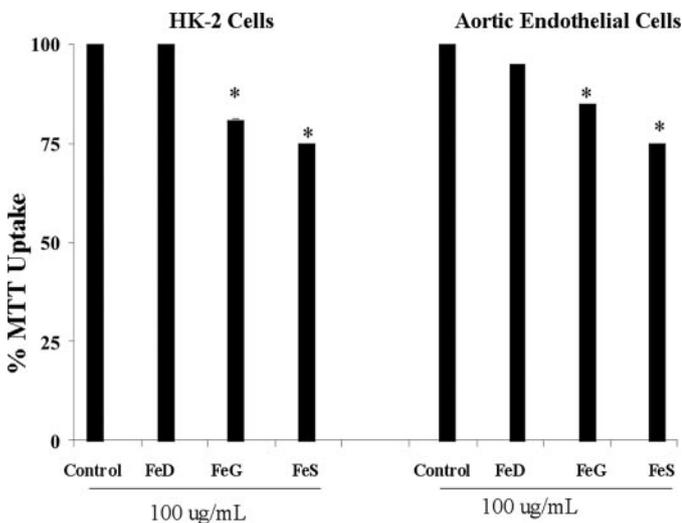


Figure 6. Assessments of Fe toxicity in cultured proximal tubule (HK-2) cells and in aortic endothelial cells. Cell injury was assessed by suppression of cellular/mitochondrial uptake of tetrazolium dye (MTT). FeD caused no significant reduction in MTT uptake in either cell culture systems. Conversely, FeG and FeS each suppressed MTT uptake in both sets of cells ($*P < 0.01$ *versus* controls), with the reductions being significantly greater with FeS *versus* FeG. Therefore, these results were consistent with the rest of the experimental data by suggesting the following rank order of Fe toxicity: FeS > FeG > FeD.



Figure 7. Pellets of HK-2 cells after 24-h incubations with the various test Fe preparations and a new experimental agent Fe oligosaccharide (FeOS). Neither FeD nor FeOS caused HK-2 cell staining/Fe uptake. Of further note, neither FeD nor FeOS induced cell death. Conversely, both FeG and FeS stained HK-2 cells with Fe, a result that was more prominent with FeS. Reproduced from reference (4), with permission.

ration resulted from FeD treatment (representative electron microscopy results appear in Figure 8). As a functional correlate of these studies, cell viability was assessed, and as noted above, the rank order of toxicity exactly paralleled the degree of Fe uptake (FeS \gg FeG \gg FeD). Furthermore, FeS induced far greater mitochondrial cytochrome c release than other Fe compounds, confirming greater mitochondrial toxicity. The reason that FeS gains greater intracellular access than FeG or FeD remains unknown. However, it does not seem to be mediated *via* a sucrose transporter, because adding 10-fold greater sucrose along with FeS failed to decrease FeS's toxic effects.

In Vivo Correlates of In Vitro Fe-CHO Toxicity

Given the above observations that intravenous Fe-CHO preparations seem to have differing cytotoxic potentials, we next addressed the question of whether *in vivo* renal correlates of that toxicity might exist. Toward this end, mice were treated with high-dosage, short-term FeS, FeD, and FeG, and at varying time points thereafter (up to 1 wk), Fe-induced renal oxidant stress was sought using renal cortical ferritin and heme oxygenase-1 (HO-1) mRNA as biologic readouts (both of which correlate with Fe loading/oxidative stress [5]). FeS and FeG each upregulated renal cortical ferritin and HO-1 mRNA. The

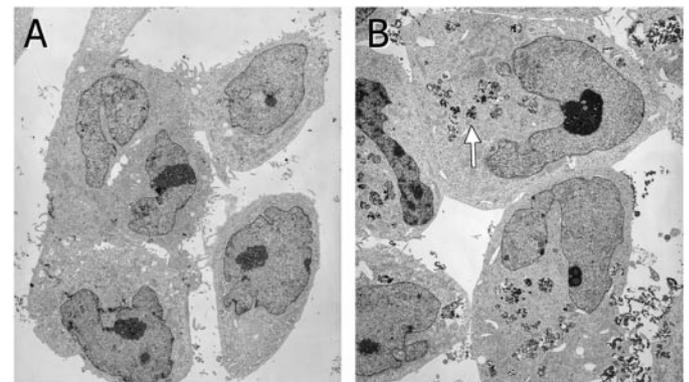


Figure 8. Normal HK-2 cells (A) and HK-2 cells after incubation with FeS (B). FeS uptake was observed, as indicated by its black appearance within intracellular compartments (depicted by the arrow; B). As shown in the bottom right of B, the process of endocytic FeS uptake from the extracellular space can be observed. Reproduced from reference (4), with permission.

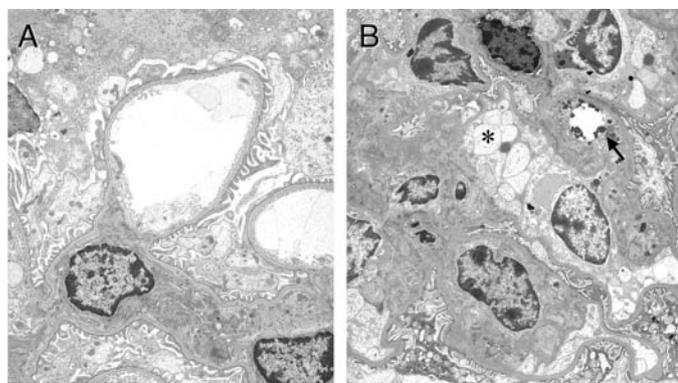


Figure 9. Electron micrograph of normal mouse glomerular capillary loop (A) and a glomerular capillary after intravenous FeS treatment (B). Evidence of FeS-induced endothelial cell swelling is apparent (*). Reproduced from reference (4), with permission.

degree of ferritin increase was significantly greater with FeS versus FeG treatment. Conversely, FeD failed to increase either renal ferritin or HO-1 mRNA. Thus, these observations paralleled the results of the above described *in vitro* experiments (Fe toxicity: FeS > FeG > FeD). To seek again *in vivo*–*in vitro* correlates, we tested whether the differential ferritin/HO-1 results might correlate with degrees of Fe uptake. Renal electron microscopic analyses were performed on Fe-pretreated mice. The dominant findings of these experiments were as follows: (1) The prime site of Fe uptake seemed to be in glomeruli, with accumulation in mesangial cells and, to a lesser extent, in podocytes being observed (Figure 9); (2) the degree of renal Fe uptake correlated with the extent of renal ferritin expression as well as the *in vitro* toxicologic profiles (FeS > FeG; none with FeD); (3) FeS also induced endothelial cell swelling (Figure 10), which was not observed with the other Fe treatments (consistent with the cultured endothelial cell experiments noted above); and (4) although no Fe deposition was observed in proximal tubules, their exposure to Fe clearly occurred. This conclusion was based on observations that proximal tubules that were harvested from FeS-exposed tubules had altered susceptibility to *in vitro* forms of tubular cell damage (5). The clinical relevance of these observations remains to be defined, particularly because the dosages and time course of the Fe treatment protocols that were used in these experiments differ dramatically from those that are used in the clinical arena. However, observations that intravenous FeS can induce transient proteinuria and proximal tubular enzymuria in pre-ESRD patients (4) raises the possibility of potential clinical relevance.

Fe Effects on Renal and Systemic Inflammatory Responses: *Escherichia coli* Sepsis

Systemic inflammation is an important determinant of morbidity and mortality in ESRD patients. Oxidative stress is known to promote inflammatory responses, in part *via* its impact on redox-sensitive transcription factors (*e.g.*, NF- κ B, cFos,

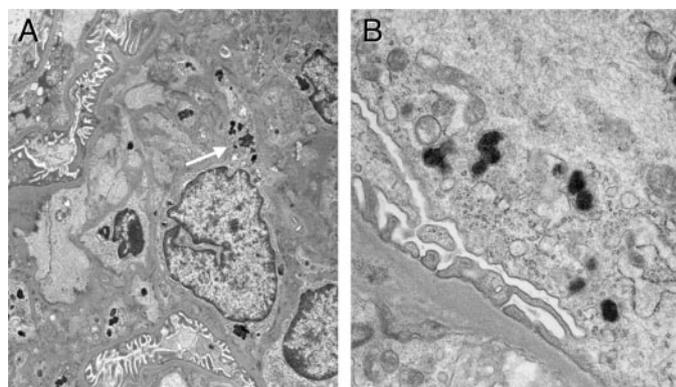


Figure 10. Electron micrograph of mouse glomerulus after intravenous FeS treatment. (A) Fe deposition in the mesangium. (B) Fe deposition within a podocyte. Reproduced from reference (4), with permission.

c-Jun, HIF-1- α , AP-1 [6–9]). Once activated, these molecules recruit a variety of downstream inflammatory mediators, such as TNF- α , cytokine receptors, chemokines, and adhesion molecules (6–9). These considerations led us to consider whether parenteral Fe injection and the resultant oxidative stress have the potential to worsen inflammation. To address this issue initially, we questioned whether responses to endotoxemia/Gram-negative sepsis might be exacerbated by intravenous Fe administration (10). Toward this end, mice received injections of heat-killed (endotoxin-intact) *E. coli*. Nonviable *E. coli* were used to avoid the possibility that intravenous Fe might increase bacterial growth, an additional variable in gauging a sepsis-induced inflammatory response. Four groups of mice were created: Control mice (sham injections), FeS injection alone (2 mg), *E. coli* injection alone, and combined *E. coli* + FeS injection. The following end points were used: (1) Plasma TNF- α levels (as a marker of systemic inflammation), (2) renal and cardiac HO-1 mRNA (reverse transcriptase-PCR; serving as a marker of tissue oxidative stress), (3) renal HO-1 protein levels (Western blots), (4) the severity of endotoxin-induced renal insufficiency, and (5) survival. These studies produced striking evidence of Fe's proinflammatory/pro-oxidant influence. Despite that intravenous Fe had virtually no independent effect on plasma TNF- α levels, it doubled or tripled the TNF- α increases that resulted for *E. coli* injection. FeS's pro-oxidant effects also were indicated by a doubling of baseline cardiac and renal HO-1 mRNA levels, and an exacerbation of HO-1 mRNA/protein increases in response to the *E. coli* challenge. Most dramatic were the survival data: None of the FeS-injected mice or the *E. coli*-injected mice died during the course of the 24-h experiments. However, with combined Fe + *E. coli* injection, an approximate 50% mortality rate was observed. The surviving mice also had worse renal failure, as denoted by blood urea nitrogen concentrations. Therefore, these results indicated that intravenous Fe loading, in the form of Fe-CHO complexes, clearly can exacerbate a systemic inflammatory response (10). In further studies, we demonstrated that Fe's proinflammatory effects are not restricted to endotoxemia, given that it also can

increase TNF- α production in the setting of acute traumatic muscle damage (11).

Comparison of Fe-CHO Complex–Induced Proinflammatory Effects

Given our previous *in vitro* and *in vivo* data suggesting that various Fe formulations exert differing varying of toxicity, we performed additional studies to ascertain whether such differences might extend to the above-noted proinflammatory effects. Toward this end, mice received injections of equimolar amounts of Fe in the form of FeS, FeG, or FeD (11). The mice then were challenged with intravenous endotoxin (instead of heat-killed bacteria as used above) either immediately after Fe injection or after allowing a 24-h post-Fe equilibration period. As shown in Figure 11, left, when no lag time between Fe and LPS injection was allowed, only FeS exacerbated endotoxin-induced TNF- α generation. After a 24-h equilibration period, each of the Fe compounds augmented endotoxin-induced TNF- α production (Figure 11, right). However, FeS seemed to produce the greatest TNF- α increase (approximately five-fold, *versus* three-fold with FeD or FeG pretreatment). These findings underscore that although FeS may exert the greatest acute toxicity, with greater periods of time (presumably allowing for more Fe processing/Fe release), each of these compounds has the potential to mount a proinflammatory effect. Therefore, one cannot conclude simply that one Fe-CHO complex is necessarily “better” or “worse” than another, on the basis of experimental data.

Sites of Fe-Mediated TNF- α Production

Given the systemic nature of intravenous Fe distribution, multiple target organs potentially could contribute to exagger-

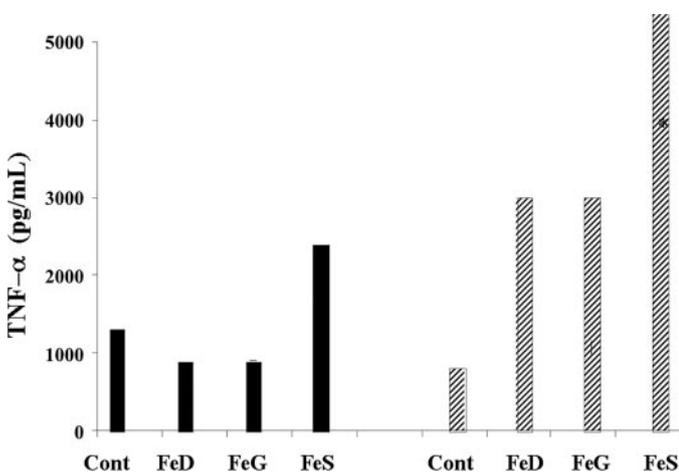


Figure 11. Degrees of TNF- α generation evoked by LPS in the absence or presence of various Fe therapies. The left four bars depict results when LPS and Fe compounds were injected simultaneously. Under these conditions, only FeS exaggerated LPS-induced TNF- α production, as assessed by plasma levels. However, as shown in the right four bars, after a 24-h lag period, each of the Fe compounds potentiated LPS-mediated plasma TNF- α increases. Of note, Fe compounds alone failed to increase TNF- α concentrations (data not shown).

ated inflammatory responses. Therefore, to gain some initial insights into this issue, we quantified sites of TNF- α mRNA generation in multiple organs after Fe with or without endotoxin administration (11). Fe administration alone did not alter TNF- α message levels in kidney, heart, liver, lung, or spleen (the last serving as a repository of immunocompetent cell populations). LPS triggered TNF- α mRNA increases in each of these organs. Fe exaggerated these increases, but this occurred in an organ-specific manner (kidney, heart, and lung but not liver or spleen). Therefore, these results suggested that parenteral Fe can exert divergent effects on stress-induced cytokine production, depending on the target tissue involved. Whether these findings might have an impact on organ function or integrity remains unknown. However, it is of interest that cardiac dysfunction (suppressed Ca²⁺ sensitivity with decreased contractility [12,13]) and lung injury (acute respiratory distress syndrome) are commonplace in sepsis syndrome. This raises the intriguing but highly speculative question of whether tissue Fe loading potentially could worsen clinical sepsis syndrome or outcomes.

Potential Protective Strategies

Ultimately, potential intravenous Fe toxicity could be obviated if new agents were developed to permit effective Fe supplementation *via* the oral route. Until such time, co-administration of antioxidant agents in concert with intravenous Fe might seem worthy of consideration. As an initial probe into the feasibility of this approach, we tested whether intravenous glutathione might mitigate the Fe–endotoxin synergy. However, co-administration of glutathione exacerbated TNF- α production under these experimental conditions (11). Although this result might seem paradoxical, it should be noted that “antioxidants” also can act as “pro-oxidants” by enhancing Fe cycling between the ferrous and ferric forms. For example, myoglobin is far more toxic in the presence of vitamin C (3). These considerations indicate that administration of an antioxidant along with intravenous Fe can lead to unpredictable, if not undesirable, results. As an alternative approach, we tested whether Fe loading *via* the intramuscular route might mitigate Fe’s systemic proinflammatory state (allowing muscle to take to pro-oxidant “hit,” thereby mitigating systemic reactions). This, indeed, seemed to be the case: When FeG was administered intramuscularly, as opposed to intravenously, it no longer potentiated endotoxin’s proinflammatory effects. This was despite that Fe absorption was confirmed by direct histologic analysis at the intramuscular injection site. Clearly, intramuscular Fe administration, although previously given to patients, is no longer an acceptable clinical option. However, these results do illustrate that intravenous administration has potential implications beyond simply Fe loading. Finally, it should be recalled that intramuscular Fe injection was shown previously to induce rhabdomyosarcomas (14,15). This simply underscores that, as currently formulated, Fe-CHO complexes have cytotoxic potentials with possible unique, adverse consequences.

Fe as a Participant in Renal Disease Progression

There is a plethora of experimental data to indicate that renal parenchymal Fe is a critical participant in a host of acute and chronic experimental nephropathies (potentially stemming from direct cytotoxicity, oxidant attack, proinflammatory effects, and induction of TGF- β , which stimulates interstitial fibrosis; *e.g.*, [16–19]). This well-accepted conclusion is based on findings that prevention of intracellular Fe release from binding proteins, chelation of free Fe, or blockade of Fe's pro-oxidant activities with antioxidants can mitigate renal disease onset or progression. As noted above, intravenous Fe clearly gains renal access and can accumulate within renal parenchyma (*e.g.*, mesangial cells and podocytes; Figures 9 and 10). Given that intravenous Fe now is administered widely to pre-ESRD patients, it seems prudent to consider whether this has the potential to hasten renal disease progression. There are a number of theoretical ways in which this might proceed. First, Fe could incite oxidative stress directly. This could be especially relevant given that antioxidant defenses may be reduced in patients with renal disease. Second, Fe may upregulate stress molecules, such as HO-1, which can exert both protective and injury-provoking effects (20). Third, Fe loading potentially might recruit oxidant-sensitive inflammatory molecules within the kidney. To address this last possibility, we recently tested (21) whether intravenous Fe triggers production of monocyte chemoattractant protein (MCP-1; a potent chemokine [22,23]) in kidney or in extrarenal organs and whether such a response might be enhanced in injured kidneys. This, indeed, seemed to be the case. After intravenous FeS injection, marked increases in plasma and renal cortical MCP-1 levels resulted (21). This was accompanied by elevations in renal cortical (as well as extrarenal tissue) MCP-1 mRNA. The ability of proximal tubules to participate directly in this process was confirmed by findings of increased MCP-1 production in cultured renal tubular cells in response to Fe challenges. As in each of our previous studies, FeS had more pronounced effects than did FeD (again consistent with differential Fe impacts depending on the nature of the CHO carrier). Finally, we observed that Fe treatment preferentially increased MCP-1 protein in unilaterally obstructed kidneys, compared with unobstructed contralateral controls. Many of these changes were durable, persisting for at least 1 wk after Fe injection. A critical question remains as to whether the Fe treatment actually accelerates disease progression. This remains a theoretical issue and one that likely can be resolved only in the clinical arena by long-term longitudinal studies.

Conclusions

There is no question that intravenous Fe therapy confers marked benefits to patients with renal disease. This may stem not just from anemia correction but also from repletion of nonheme Fe stores. However, Fe-CHO complexes also have highly potent pro-oxidant, cytotoxic, and proinflammatory effects. When injected intravenously, direct vascular and organ contact with these highly reactive compounds results. This is in distinction to oral Fe administration, which permits more phys-

iological Fe delivery. Experimental studies underscore the potential toxicity of these agents. However, such data cannot/should not be used in the clinical decision-making process. Rather, cell culture and animal studies are viewed best as providing information to stimulate formation of testable clinical hypotheses. The potential impacts of high-dosage intravenous Fe administration on systemic inflammatory responses, atherogenesis (24–27), and rates of renal disease progression seem particularly worthy of analysis. Potential differential toxicities that might exist among current parenteral Fe formulations also might be considered. When dealing with potentially toxic compounds, it seems prudent to consider potential long-term adverse effects, as well as benefits. The renal community's experience with another therapeutic metal, aluminum, serves to underscore this point. Searching for potential adverse effects and efforts to develop new and effective *oral* Fe therapies seem to be worthy pursuits at this time.

Acknowledgments

This work was supported by research grants from the National Institutes of Health (DK38432 and DK54200).

We thank Ali Johnson, Sherry Hanson, and Steve Lund for expert technical assistance during work on the experimental studies that are reviewed herein.

References

1. Zager RA, Johnson AC, Hanson SY, Wasse H: Parenteral iron formulations: A comparative toxicologic analysis and mechanisms of cell injury. *Am J Kidney Dis* 40: 90–103, 2002
2. Zager RA: Mitochondrial free radical production induces lipid peroxidation during myohemoglobinuria. *Kidney Int* 51: 728–738, 1997
3. Zager RA, Foerder CA: Effects of inorganic iron and myoglobin on in vitro proximal tubular lipid peroxidation and cytotoxicity. *J Clin Invest* 89: 989–995, 1992
4. Agarwal R, Vasavada N, Sachs NG, Chase S: Oxidative stress and renal injury with intravenous iron in patients with renal disease. *Kidney Int* 65: 2279–2289, 2004
5. Zager RA, Johnson AC, Hanson SY: Parenteral iron nephrotoxicity: Potential mechanisms and consequences. *Kidney Int* 66: 1441–1456, 2004
6. Haddad JJ: Antioxidant and pro-oxidant mechanisms in the regulation of redox(y)-sensitive transcription factors. *Cell Signal* 14: 879–897, 2002
7. Xiong S, She H, Takeuchi H: Signaling role of intracellular iron in NF-kappa B activation. *J Biol Chem* 278: 17646–17654, 2003
8. Sukamoto H: Iron regulation of hepatic macrophage TNF-alpha expression. *Free Radical Biol Med* 32: 309–313, 2002
9. Rahman I, MacNee W: Regulation of redox glutathione levels and gene transcription in lung inflammation: Therapeutic approaches. *Free Radic Biol Med* 28: 1405–1420, 2003
10. Zager RA, Johnson AC, Hanson SY: Parenteral iron therapy exacerbates experimental sepsis. *Kidney Int* 65: 2108–2112, 2004
11. Zager RA, Johnson ACM, Hanson SY, Lund S: Parenteral iron compounds sensitize mice to injury-initiated TNFalpha mRNA production and TNFalpha release. *Am J Physiol* 288: F290–F297, 2005

12. Tavernier B, Garrigue D, Boulle C, Vallet B, Adnet P: Myofilament calcium sensitivity is decreased in skinned cardiac fibres of endotoxin-treated rabbits. *Cardiovasc Res* 38: 472–479, 1998
13. Tavernier B, Li JM, El-Omar MM, Lanone S, Yang ZK, Trayer IP, Mebazaa A, Shah AM: Cardiac contractile impairment associated with increased phosphorylation of troponin I in endotoxemic rats. *FASEB J* 15: 294–296, 2001
14. Greenberg G: Sarcoma after intramuscular iron injection. *BMJ* 1: 1508–1509, 1976
15. Magnusson G, Flodh H, Malmfors T: Oncological study in rats of Ferastral, an iron-poly-(sorbitol-gluconic acid) complex after intramuscular administration. *Scand J Haematol Suppl* 32: S87–S88, 1977
16. Nath KA, Vercellotti GM, Grande JP: Heme protein-induced chronic renal inflammation: Suppressive effect of induced heme oxygenase-1. *Kidney Int* 59: 106–117, 2001
17. Fernandez-Real JM, Lopez-Bermejo A, Ricart W: Cross-talk between iron metabolism and diabetes. *Diabetes* 51: 2348–2354, 2002
18. Shah SV: The role of reactive oxygen metabolites in glomerular disease. *Annu Rev Physiol* 57: 245–262, 1995
19. Baliga R, Ueda N, Walker PD, Shah SV: Oxidant mechanisms in toxic acute renal failure. *Am J Kidney Dis* 29: 465–477, 1997
20. Platt JL, Nath KA: Heme oxygenase: Protective gene or Trojan horse? *Nat Med* 4: 1364–1365, 1998
21. Zager RA: Parenteral iron treatment induced MCP-1 accumulation in plasma, normal kidneys and in experimental nephropathy. *Kidney Int* 68: 1533–1542, 2005
22. Wada T, Furuichi K, Sakai N: Up-regulation of monocyte chemoattractant protein-1 in tubulointerstitial lesions of human diabetic nephropathy. *Kidney Int* 58: 1492–1499, 2000
23. Viedt C, Orth SR: Monocyte chemoattractant protein-1 (MCP-1) in the kidney: Does it do more than simply attach monocytes? *Nephrol Dial Transplant* 17: 2043–2047, 2002
24. Gackowski D, Kruszewski M, Jawien A: Further evidence that oxidative stress may be a risk factor responsible for the development of atherosclerosis. *Free Radical Biol Med* 31: 542–547, 2001
25. Ponraj D, Makjanic J, Thong PS: The onset of atherosclerotic lesion formation in hypercholesterolemic rabbits is delayed by iron depletion. *FEBS Lett* 459: 218–222, 1999
26. Daugherty A: Atherosclerosis: Cell biology and lipoproteins. *Curr Opin Lipidol* 8: 11–12, 1997
27. Corti MC, Gaziano M, Hennekens CH: Iron status and risk of cardiovascular disease. *Ann Epidemiol* 7: 62–68, 1997