Pediatr Nephrol (2008) 23:1195–1199
DOI 10.1007/s00467-007-0673-1

EDITORIAL COMMENTARY

# Iron, oxidative stress, and clinical outcomes

**Rajiv Agarwal**

Received: 23 August 2007 / Revised: 4 October 2007 / Accepted: 8 October 2007 / Published online: 21 November 2007


**Abstract** It is well known that iron is pro-oxidant. Chronic kidney disease (CKD) is a pro-oxidant state, and intravenous administration of iron is frequently used to correct anemia. On one hand, there is little doubt that iron causes oxidative stress. On the other, it is far from clear whether oxidative stress, so generated, leads to poor clinical outcomes. Iron has benefits that may be independent of the correction of anemia. Furthermore, concerns surround the use of high doses of erythropoietin in causing excess heart failure and death in patients with CKD. Thus, it would be prudent if iron were to continue to be used judiciously in patients who require erythropoietin. Iron, given orally, would be the preferred first-line agent in patients not on hemodialysis. In patients with sepsis, intravenous treatment with iron should be avoided, because, in animal experiments, intravenous administration of iron can compound the inflammatory response and increase mortality. Clinical trials are needed to ascertain the risk and benefits of the intravenous administration of iron in patients with CKD.

**Keywords** Iron · Oxidative stress · Chronic kidney disease · Cardiovascular disease · Proteinuria · Randomized trial

## Introduction

Iron deficiency is the most important cause of poor response to erythropoiesis-stimulating agents in patients with end-stage renal disease, and its judicious use in pediatric patients for the correction of anemia has been recently reviewed [1]. Extrapolation of data from adults to children is necessary, since few data exist from children. There is little doubt that iron causes oxidative stress in cells and animals. Zager et al. studied the effects of different iron preparations (iron dextran, iron sucrose, iron gluconate and iron oligosaccharide) in mouse proximal tubular segments, cultured human proximal tubular cells and bovine aortic endothelial cells [2]. Each of the iron preparations tested increased oxidative stress. An elegant set of experiments provided the following important observations:

1. The generation of oxidative stress by iron sucrose was dependent on mitochondrial respiration, but not free iron-oxidative stress was not blocked by the iron chelator, desferrioxamine.
2. Cytotoxicity of parenteral iron paralleled the degree of cell iron uptake [3]. Cytotoxicity was protected by reduced glutathione, not by its antioxidant effect, but by providing cellular protection, similar to glycine, in the setting of ATP depletion.
3. The in vitro injury occurred at clinically relevant concentrations of parenterally administered iron [3].
4. Important ultrastructural differences emerged when kidneys were examined by electron microscopy. In clinically relevant concentrations (30 μg/ml), iron sucrose demonstrated greater toxicity to proximal tubules than other iron products did and was accompanied by glomerular iron deposition [3]. In particular, there was greater depletion of ATP and of cytochrome c—markers for toxicity to mitochondria [4]—by iron sucrose than by other iron preparations [3].
5. In vivo, intravenous injection of 2 mg iron sucrose resulted in oxidative stress in renal cortex and heart within 90 min. This was not seen with iron dextran [3]. Endothelial cell damage was seen in vivo with iron sucrose but not with iron dextran [3].

R. Agarwal (✉)
Indiana University School of Medicine,
Richard L. Roudebush VA Medical Center,
1481 W. Tenth Street (111N),
Indianapolis, IN 46202, USA
e-mail: ragarwal@iupui.edu

These experiments demonstrate toxicity to mitochondria, cytotoxicity, and lipid peroxidation with intravenously administered iron compounds and substantial heterogeneity amongst iron preparations and the need for further clinical investigations in patients with chronic kidney disease (CKD).

CKD is a pro-oxidant state, due to a variety of reasons that include inflammation and renin–angiotensin system activation [5]. The evidence for generation of oxidative stress with intravenous iron treatment in healthy volunteers and patients with CKD, including those on dialysis, has accumulated over the past decade. This evidence is summarized in Table 1.

Taken together, these findings support the pre-clinical data. Importantly, iron potentiates oxidative stress above and beyond hemodialysis [12, 13]. Although vitamin C has been reported to improve erythropoietin (EPO) hyporesponsiveness [17], co-administration of iron and vitamin C may increase the oxidant potential of intravenous iron (IVIR) [18]; thus, caution is warranted when vitamin C is used in combination with IVIR.

Repeated IVIR exposure may not produce just transient, inconsequential injury. Repetitive acute renal injury may lead to chronic renal failure, as exemplified by acute cellular rejection followed by chronic allograft nephropathy and repeated acute obstruction followed by chronic renal failure. Even if such injury is mild, it can lead to the generation of sodium-sensitive hypertension [19]. Oxidative stress plays an important role in the pathogenesis and progression of renal disease [20, 21]. Data from animals suggest the association of iron exposure, generation of oxidative stress and renal injury [22]. For example, the glycerol model of acute renal failure is an established, well-characterized, model of acute renal failure that involves the iron-dependent, oxidative stress pathway that, with repeated injury at weekly intervals, leads to CKD [23]. Oxidants generated by free iron may be independently associated with proteinuria. For example, hydrogen peroxide, when co-infused with myeloperoxidase, causes profound proteinuria, endothelial cell swelling and effacement of epithelial cell foot processes [24]. The glomerular epithelial cell interacts with the basement membrane via integrin receptors, the distribution of which can be altered by oxidants, perhaps leading to functional changes [25]. Furthermore, oxidative modification of membrane proteins could directly impair the permselectivity of the glomerulus. In animals with the passive Heymann nephritis model of membranous nephropathy, treatment with the iron chelating agent desferrioxamine abrogates proteinuria [26]. Long-term exposure to iron in animals can cause tubulointerstitial fibrosis [27].

Renal biopsies from patients with nephrotic syndrome and/or CKD reveal higher iron accumulation in the proximal tubular lysosomes of patients with nephrotic syndrome than in non-nephrotic patients [28]. The extent of tubular damage correlates with the amount of iron in lysosomes and suggests the association of iron and morphological injury to the kidney [28]. Long-term exposure to high doses of iron sucrose may cause tissue damage and deleterious effects. Case reports from Japan have described toxicity to the proximal tubules, manifested by renal phosphate leakage, calcitriol deficiency and overt osteomalacia after several years of daily exposure to iron sucrose [29]. Epidemiologic data from patients with CKD on hemodialysis suggest the association of increased iron exposure and atherosclerosis, as assessed by carotid intima thickness [15]. Finally, antioxidant therapies in randomized controlled trials, administered to patients on hemodialysis, have been demonstrated to reduce the number of cardiovascular events [30, 31].

Although elevated levels of tissue iron increase the risk for atherosclerosis, perhaps by favoring the formation of pro-atherogenic oxidized low-density lipoprotein (LDL) [15, 32], these conclusions appear to be premature. Apolipoprotein E (ApoE)-deficient mice, a model of atherosclerosis, fed a high iron diet, had twice as much iron in their plasma, a nine-fold increase in bleomycin-detectable free iron in their plasma, and ten-times as much iron in their livers as control mice at 24 weeks [33]. Somewhat surprisingly, this regimen did not exacerbate, but rather reduced the severity of atherosclerosis, by 50%, and it failed to elevate hepatic levels of heme oxygenase mRNA, which is induced by many different oxidative insults in vitro. Moreover, hepatic levels of protein-bound dityrosine and ortho-tyrosine, two markers of metal-catalyzed oxidative damage in vitro, failed to rise in iron-overloaded animals. These observations suggest that elevated serum and tissue levels of iron are not atherogenic in apoE-deficient mice. Moreover, they call into question the hypothesis that elevated levels of tissue iron promote LDL oxidation and promote atherosclerosis in vivo.

The role of intravenously administered iron in patients not on hemodialysis is uncertain. A review of the published literature shows that there have been five randomized, controlled, clinical trials comparing orally administered iron with intravenously given iron in CKD patients not on dialysis. Stoves et al. randomly allocated 45 patients to treatment with either ferrous sulfate, 200 mg taken orally three times daily (t.i.d.), or iron sucrose, 300 mg given intravenously over 2 h once a month, and followed them for an average of 5.2 months. Iron sucrose was not superior to ferrous sulfate in improving hemoglobin (Hgb) or decreasing the doses of erythropoietin [34]. Aggarwal et al. randomly allocated 40 erythropoietin-treated patients to receive either ferrous sulfate, 200 mg orally t.i.d., or iron dextran, 100 mg intravenously twice a month, and followed them for up to 3 months [35]. The investigators found that

**Table 1** Examples of studies on humans, demonstrating oxidative stress from the intravenous (*i.v.*) administration of iron (*HPLC* high-performance liquid chromatography, MDA malondialdehyde)

| Author, year | Patients | Type of study | Intervention | Measurement | Results | Reference no. |
|---|---|---|---|---|---|---|
| Rooyakkers et al., 2002 | Healthy volunteers, *n*=20 | Interventional, observational | i.v. ferric saccharate 100 mg | Endothelium-dependent vasodilation at baseline, 10 min and 240 min. Non-transferrin bound iron and in vivo radical formation | Increased generation of free radicals and impairment of endothelium-dependent vasodilation | [6] |
| Agarwal et al., 2004–2006 | CKD, *n*=20 | Randomized controlled trial, parallel group | infusion of 100 mg iron sucrose on two occasions 1 week apart with or without *n*-acetyl cysteine | MDA in urine and plasma, transferrin saturation, and serial urine protein to creatinine ratios at timed intervals | Increase in MDA within 15-30 min and proteinuria with i.v. iron sucrose. Iron infusion led to increase in monocyte chemoattractant protein-1 accumulation and oxidation of urinary albumin | [7–9] |
| Leehey et al., 2005 | CKD, *n*=8 | Four-way, cross-over, randomized trial | i.v. infusion of iron, either 125 mg or 250 mg of ferric gluconate with or without *n*-acetyl cysteine every week | MDA in urine and plasma and spot urine protein to creatinine and urine albumin to creatinine ratio | Ferric gluconate caused oxidative stress but no renal injury | [10] |
| Agarwal et al., 2007 | CKD, *n*=12 | Cross-over randomized trial | i.v. iron sucrose 100 mg or same dose of i.v. ferric gluconate administered 1 week apart in random order | Urine protein/creatinine, albumin/creatinine, enzymuria | i.v. iron sucrose caused greater proteinuria and albuminuria than did ferric gluconate. Enzymuria occurred with either drug in similar amount | [11] |
| Lim et al., 1999 | Hemodialysis, *n*=50 | Interventional, observational | i.v. infusion of 100 mg ferric saccharate | Serum ferritin, lipid peroxides, plasma glutathione peroxidase, erythrocyte superoxide dismutase | Patients with serum ferritin >601 ng/ml had greater increase in plasma lipid peroxides and greatest fall in superoxide dismutase with exposure to i.v. iron | [12] |
| Roob et al., 2000 | Hemodialysis, *n*=22 | Cross-over randomized trial | All received 100 mg i.v. iron sucrose either with or without 1,000 IU of vitamin E | Area under curve of MDA to cholesterol and plasma total peroxides to cholesterol over 180 min after i.v. infusion of iron | Lipid peroxidation was seen with i.v. iron. Vitamin E reduced but did not abolish the generation of oxidative stress | [13] |
| Salahudeen et al., 2001 | Hemodialysis, *n*=22 | Interventional, observational | i.v. infusion of 700 mg iron dextran on a non-dialysis day | Plasma F2-isoprostanes and in esterified lipoproteins 15 min before and 30 min after infusion | Free F2-isoprostanes did not increase, but esterified F2-isoprostanes were increased with i.v. iron | [14] |
| Drueke et al., 2002 | Hemodialysis, *n*=60 | Cross-sectional study | None | Advanced oxidation protein products and carotid intima–media thickness | Iron therapy was associated with advance oxidation protein products and carotid intima–media thickness | [15] |
| Anraku et al., 2004 | Hemodialysis *n*=22 | Randomized controlled trial, parallel group | i.v. saccharated ferric oxide 40 mg every dialysis for 4 weeks; 11 controls, 11 patients | Oxidation of albumin by HPLC Carbonylation of plasma proteins | i.v. iron increased plasma protein carbonyl content mainly by oxidation of albumin | [16] |

the intravenously administered iron dextran was more effective in improving hematological and iron parameters than the orally taken ferrous sulfate was. However, these patients were severely anemic, with a mean Hgb <6.5 g/dl at baseline, which very likely increased their iron requirements. Charytan et al. randomly assigned 96 erythropoietin-treated patients to receive either ferrous sulfate, orally at 325 mg t.i.d. for 29 days, or iron sucrose, 200 mg intravenously given once a week × five doses, and followed them up to 43 days from the onset of treatment [36]. There was no significant difference between the two groups in Hgb levels. Van Wyck et al. randomly selected 188 erythropoietin-treated and non-erythropoietin-treated patients to receive either 1 g of iron sucrose intravenously, in two to five divided doses over a 2-week period, or ferrous sulfate orally, 325 mg t.i.d. for 56 days [37]. The iron sucrose given intravenously was more effective than the ferrous sulfate given orally in improving iron indices at 56 days and in increasing Hgb levels by at least 1 g/dl at any time during the study. Hypotension, nausea, taste disturbances, myalgia, and headaches were associated with i.v. iron sucrose, while constipation, diarrhea, nausea, dyspepsia, and vomiting were associated with the oral ferrous sulfate. Agarwal et al. randomly assigned iron-deficient non-dialysis CKD patients not receiving erythropoietin to receive either ferric gluconate 250 mg intravenously weekly × 4 or ferrous sulfate 325 mg t.i.d. × 42 days [38]. The data from 75 patients were analyzed [i.v. iron $n$=36, per os (p.o.) iron $n$=39]. Change from baseline in Hgb was similar in the two groups [i.v. iron 0.4 g/dl vs p.o. iron 0.2 g/dl, $P$=not significant (NS)]. The most common side effect reported for i.v. iron administration was hypotension, while constipation was more common with iron given orally. Thus, the bulk of the evidence suggests that iron given orally can be first-line therapy for patients with CKD not on dialysis. If this therapy fails, intravenously administered iron can be used, particularly if the patient requires erythropoietin.

The balance of risk and benefit of iron given intravenously to patients with CKD is unclear. Iron has benefits that may be independent of the correction of anemia [39]. Furthermore, given the concerns that surround the use of high doses of erythropoietin, it would be prudent for iron to continue to be used judiciously in patients who require erythropoietin [40]. Whereas there is little doubt that iron causes oxidative stress, it is unclear whether oxidative stress so generated leads to poor clinical outcomes and needs to be evaluated in clinical trials. Till these trials are performed, I feel that there is one clinical situation where special caution should be exercised when one is using any type of iron that is to be given intravenously. In animals with sepsis, Zager et al. have demonstrated that i.v. administration of iron can compound the inflammatory response and increase mortality rates [41]. Thus, in patients with sepsis, i.v. administration of iron (and perhaps even orally given iron) should be avoided.

## References


1. Horl WH (2007) Iron therapy for renal anemia: how much needed, how much harmful? Pediatr Nephrol 22:480–489
2. Zager RA, Johnson AC, Hanson SY, Wasse H (2002) Parenteral iron formulations: a comparative toxicologic analysis and mechanisms of cell injury. Am J Kidney Dis 40:90–103
3. Zager RA, Johnson AC, Hanson SY (2004) Parenteral iron nephrotoxicity: Potential mechanisms and consequences. Kidney Int 66:144–156
4. Zager RA, Johnson AC, Hanson SY (2004) Proximal tubular cytochrome c efflux: determinant, and potential marker, of mitochondrial injury. Kidney Int 65:2123–2134
5. Agarwal R, Campbell RC, Warnock DG (2004) Oxidative stress in hypertension and chronic kidney disease: role of angiotensin II. Semin Nephrol 24:101–114
6. Rooyakkers TM, Stroes ES, Kooistra MP, van Faassen EE, Hider RC, Rabelink TJ, Marx JJ (2002) Ferric saccharate induces oxygen radical stress and endothelial dysfunction in vivo. Eur J Clin Invest 32[Suppl 1]:9–16
7. Agarwal R, Vasavada N, Sachs NG, Chase S (2004) Oxidative stress and renal injury with intravenous iron in patients with chronic kidney disease. Kidney Int 65:2279–2289
8. Agarwal R (2005) On the nature of proteinuria with acute renal injury in patients with chronic kidney disease. Am J Physiol Renal Physiol 288:F265–F271
9. Agarwal R (2006) Proinflammatory effects of iron sucrose in chronic kidney disease. Kidney Int 69:1259–1263
10. Leehey DJ, Palubiak DJ, Chebrolu S, Agarwal R (2005) Sodium ferric gluconate causes oxidative stress but not acute renal injury in patients with chronic kidney disease: a pilot study. Nephrol Dial Transplant 20:135–140
11. Agarwal R, Rizkala AR, Kaskas MO, Minasian R, Trout JR (2007) Iron sucrose causes greater proteinuria than ferric gluconate in non-dialysis chronic kidney disease. Kidney Int 72: 638–642
12. Lim PS, Wei YH, Yu YL, Kho B (1999) Enhanced oxidative stress in haemodialysis patients receiving intravenous iron therapy. Nephrol Dial Transplant 14:2680–2687
13. Roob JM, Khoschsorur G, Tiran A, Horina JH, Holzer H, Winklhofer-Roob BM (2000) Vitamin E attenuates oxidative stress induced by intravenous iron in patients on hemodialysis. J Am Soc Nephrol 11:539–549
14. Salahudeen AK, Oliver B, Bower JD, Roberts LS 2nd (2001) Increase in plasma esterified F2-isoprostanes following intravenous iron infusion in patients on hemodialysis. Kidney Int 60: 1525–1531
15. Drueke T, Witko-Sarsat V, Massy Z, Descamps-Latscha B, Guerin AP, Marchais SJ, Gausson V, London GM (2002) Iron therapy, advanced oxidation protein products, and carotid artery intima-media thickness in end-stage renal disease. Circulation 106:2212–2217
16. Anraku M, Kitamura K, Shinohara A, Adachi M, Suenga A, Maruyama T, Miyanaka K, Miyoshi T, Shiraishi N, Nonoguchi H, Otagiri M, Tomita K (2004) Intravenous iron administration induces oxidation of serum albumin in hemodialysis patients. Kidney Int 66:841–848
17. Attallah N, Osman-Malik Y, Frinak S, Besarab A (2006) Effect of intravenous ascorbic acid in hemodialysis patients with EPO-hyporesponsive anemia and hyperferritinemia. Am J Kidney Dis 47:644–654

18. Eiselt J, Racek J, Opatrny K Jr, Trefil L, Stehlík P (2006) The effect of intravenous iron on oxidative stress in hemodialysis patients at various levels of vitamin C. Blood Purif 24:531–537
19. Johnson RJ, Herrera-Acosta J, Schreiner GF, Rodriguez-Iturbe B (2002) Subtle acquired renal injury as a mechanism of salt-sensitive hypertension. N Engl J Med 346:913–923
20. Haugen E, Nath KA (1999) The involvement of oxidative stress in the progression of renal injury. Blood Purif 17:58–65
21. Klahr S (1997) Oxygen radicals and renal diseases. Miner Electrolyte Metab 23:140–143
22. Shah SV (2001) Role of iron in progressive renal disease. Am J Kidney Dis 37:S30–S33
23. Nath KA, Croatt AJ, Haggard JJ, Grande JP (2000) Renal response to repetitive exposure to heme proteins: chronic injury induced by an acute insult. Kidney Int 57:2423–2433
24. Johnson RJ, Couser WG, Chi EY, Adler S, Klebanoff SJ (1987) New mechanism for glomerular injury. Myeloperoxidase-hydrogen peroxide-halide system. J Clin Invest 79:1379–1387
25. Gailit J, Colflesh D, Rabiner I, Simone J, Goligorsky MS (1993) Redistribution and dysfunction of integrins in cultured renal epithelial cells exposed to oxidative stress. Am J Physiol 264:F149–F157
26. Shah SV (1988) Evidence suggesting a role for hydroxyl radical in passive Heymann nephritis in rats. Am J Physiol 254:F337–F344
27. Alfrey AC, Froment DH, Hammond WS (1989) Role of iron in the tubulo-interstitial injury in nephrotoxic serum nephritis. Kidney Int 36:753–759
28. Nankivell BJ, Boadle RA, Harris DC (1992) Iron accumulation in human chronic renal disease. Am J Kidney Dis 20:580–584
29. Sato K, Shiraki M (1998) Saccharated ferric oxide-induced osteomalacia in Japan: iron-induced osteopathy due to nephropathy. Endocr J 45:431–439
30. Boaz M, Smetana S, Weinstein T, Matas Z, Gafter U, Iaina A, Knecht A, Weissgarten Y, Brunner D, Fainaru M, Green MS (2000) Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. Lancet 356:1213–1218
31. Tepel M, van der Giet M, Statz M, Jankowski J, Zidek W (2003) The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a randomized, controlled trial. Circulation 107:992–995
32. Kletzmayr J, Horl WH (2002) Iron overload and cardiovascular complications in dialysis patients. Nephrol Dial Transplant 17 [Suppl 2]:25–29
33. Kirk EA, Heinecke JW, LeBoeuf RC (2001) Iron overload diminishes atherosclerosis in apoE-deficient mice. J Clin Invest 107:1545–1553
34. Stoves J, Inglis H, Newstead CG (2001) A randomized study of oral vs intravenous iron supplementation in patients with progressive renal insufficiency treated with erythropoietin. Nephrol Dial Transplant 16:967–974
35. Aggarwal HK, Nand N, Singh S, Singh M, Hemant, Kaushik G (2003) Comparison of oral versus intravenous iron therapy in predialysis patients of chronic renal failure receiving recombinant human erythropoietin. J Assoc Physicians India 51:170–174
36. Charytan C, Qunibi W, Bailie GR (2005) Comparison of intravenous iron sucrose to oral iron in the treatment of anemic patients with chronic kidney disease not on dialysis. Nephron Clin Pract 100:c55–c62
37. Van Wyck DB, Roppolo M, Martinez CO, Mazey RM, McMurray S; United States Iron Sucrose (Venofer) Clinical Trials Group (2005) A randomized, controlled trial comparing IV iron sucrose to oral iron in anemic patients with nondialysis-dependent CKD. Kidney Int 68:2846–2856
38. Agarwal R, Rizkala AR, Bastani B, Kaskas MO, Leehey DJ, Besarab A (2006) A randomized controlled trial of oral versus intravenous iron in chronic kidney disease. Am J Nephrol 26: 445–454
39. Agarwal R (2007) Nonhematological benefits of iron. Am J Nephrol 27:565–571
40. Singh AK, Szczech L, Tang KL, Barnhart H, Sapp S, Wolfson M, Reddan D; CHOIR Investigators (2006) Correction of anemia with epoetin alfa in chronic kidney disease. N Engl J Med 355: 2085–2098
41. Zager RA, Johnson AC, Hanson SY (2004) Parenteral iron therapy exacerbates experimental sepsis. Kidney Int 65:2108–2112