Sodium ferric gluconate causes oxidative stress but not acute renal injury in patients with chronic kidney disease: a pilot study

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Abstract

Background. Intravenous (i.v) iron is widely used to treat anaemia in patients with chronic kidney disease (CKD). Although beneficial and usually well tolerated, concerns have been raised about its ability to cause oxidative stress and renal injury.

Methods. To determine if i.v. iron causes oxidative stress [as assessed by plasma and urine malondialdehyde (MDA)] and/or renal injury (as assessed by urinary albumin, total protein and enzymuria), we conducted a prospective, four-way randomized crossover, blinded end-point trial in eight patients with CKD. Two widely used doses of sodium ferric gluconate (125 mg infused over 1 h and 250 mg infused over 2 h) were given with or without the antioxidant N-acetylcysteine (NAC), resulting in four treatment dose–antioxidant/placebo combinations in each patient. Transferrin saturation was measured with urea polyacrylamide gel electrophoresis, MDA by high performance liquid chromatography, and albuminuria and proteinuria by standard clinical methods. Enzymuria was assessed by measurement of N-acetyl-β-D-glucosaminidase (NAG) excretion by colorimetric assay.

Results. I.v. ferric gluconate infusion at both doses resulted in a marked increase in transferrin saturation and a significant increase in plasma MDA levels. Urinary MDA levels also increased at the higher dose of iron. There was no evidence of acute renal injury, as assessed by albuminuria, proteinuria or enzymuria. Pre-treatment with NAC had no effect on oxidative stress or the above urinary parameters.

Conclusions. I.v. ferric gluconate caused oxidative stress (as reflected by increased MDA), but this was not associated with biochemical manifestations of acute renal injury.

Keywords: anaemia; chronic kidney failure; iron; malondialdehyde; oxidative stress; randomized controlled trial

Introduction

Anaemia commonly accompanies chronic kidney disease (CKD). Its aetiology is often multifactorial, but is usually a result of erythropoietin deficiency and/or insufficient dietary iron absorption [1]. Furthermore, functional iron deficiency may occur in patients treated with recombinant human erythropoetin due to enhanced erythropoiesis that often necessitates therapy with intravenous (i.v.) iron [2]. The correction of anaemia leads to improvement in morbidity, mortality and quality of life [3]; however, potential toxicity due to oxidative stress or direct tissue injury (independent of oxidative stress) is of concern [4,5]. Iron preparations designed for parenteral use have been shown to increase biological markers of oxidative stress in cell cultures [6,7], animal models [7–9], and end-stage renal disease patients on haemodialysis [10–13], which may be ameliorated by antioxidants [13].

Recently, i.v. administration of iron sucrose at FDA-approved doses in patients with CKD not yet on dialysis was reported to result in oxidative stress and transient renal injury [14]. The present study was designed to determine if an alternative i.v. iron preparation, sodium ferric gluconate, is also associated with oxidative stress and/or renal injury in CKD patients. In addition, we wished to determine the effect of pre-treatment with the antioxidant N-acetylcysteine (NAC) in these patients.

Materials and methods

Subjects

Study participants were recruited from the Nephrology Clinic at the Edward Hines Jr VA Medical Center in Hines, IL.
Subjects were required to be at least 18 years of age, have an estimated glomerular filtration rate <60 ml/min by the simplified MDRD formula [15] but not on renal replacement therapy, and have anaemia (haemoglobin <12 g/dl). All patients were determined by their attending nephrologist to require i.v. iron therapy for either absolute or relative iron deficiency [transferrin saturation (TSAT) <20% and/or serum ferritin <100 ng/ml] as determined by the National Kidney Foundation/Kidney Disease Outcome Quality Initiative (NKF-K/DOQI) guidelines [16]. Patients who had evidence of iron overload (serum ferritin >800 ng/ml or TSAT >50%) were excluded. Subjects were excluded if they had demonstrated hypersensitivity to ferric gluconate or NAC, were an organ transplant recipient or received therapy with immunosuppressive agents, used an investigational drug within 1 month prior to the study, or had substantial co-morbid conditions. Patients with anaemia that could require red blood cell transfusion (haemoglobin <7 g/dl and/or evidence of gastrointestinal bleeding) were excluded. Patients were also excluded if renal function was unstable (i.e. variation of serum creatinine of >0.5 mg/dl during the past 3 months) or had received i.v. iron within 3 months of the study. Patients receiving recombinant human erythropoietin were eligible for the study. The protocol was approved by the Human Studies Subcommittee (Institutional Review Board) at Hines VA Hospital, and written informed consent was obtained from each subject prior to study participation.

Study design

Subjects who satisfied the inclusion and exclusion criteria and signed the consent form were considered to be eligible for the study. All subjects were patients at the Hines VA Hospital and received i.v. iron treatment at the Hines out-patient centre. After obtaining baseline blood samples for measurement of serum iron, total iron binding capacity (TIBC), ferritin, urea nitrogen, creatinine, albumin, lipid profile and a complete blood count, subjects received sodium ferric gluconate (Ferrlecit, Watson Laboratories) 250 mg over 2 h as the initial dose (this is the standard protocol used in the hospital out-patient centre). After the initial dose, they received treatment with Ferrlecit at two different doses (125 mg, the FDA-approved dose, or 250 mg) with or without pre-treatment with NAC. These four iron treatments were administered in random order to each subject at intervals of 1 week. Prior to each iron infusion, patients were given four 15 ml vials containing either NAC 600 mg or sterile water and were instructed to take one vial by mouth every 12 h starting on the morning prior to their scheduled infusion and ending on the evening of the infusion. The patient and study team were blinded as to the contents of the vials.

Thus all subjects received all treatments (in random order) as follows: treatment 1 = Ferrlecit 125 mg i.v. over 1 h plus placebo (sterile water orally given every 12 h beginning 1 day prior to infusion and continuing on the day of infusion for a total of four doses); treatment 2: Ferrlecit 125 mg i.v. over 1 h plus NAC (Mucomyst, Apothecon, Inc., Princeton, NJ) 600 mg orally given every 12 h beginning 1 day prior to infusion and continuing on the day of infusion for a total of four doses; treatment 3: Ferrlecit 250 mg i.v. over 2 h plus placebo as in treatment 1; and treatment 4: Ferrlecit 250 mg i.v. over 2 h plus NAC given as in treatment 2.

During the iron infusions, patients had their vital signs monitored by the nursing staff every 30 min. Each patient was given a monitoring form on which they recorded both the nature and severity of any adverse events. For subjective complaints such as pruritus, headache, nausea, etc. that might occur during the infusion, a symptom severity scaled from 1 to 10 was used by the patient to quantify the level of discomfort and to monitor this discomfort throughout the infusion.

Immediately prior to each of the four ‘treatment’ infusions, blood and urine samples were obtained for measurement of TSAT, plasma and urine MDA, urinary albumin, total protein and N-acetyl-β-D-glucosaminidase (NAG). Blood and urine samples were repeated immediately after the infusion was completed, with the arm not utilized for iron infusion subjected to venipuncture. No subject needed to void urine during the iron infusion, and thus the post-infusion urine sample reflected all urine formed during the time of infusion. Biochemical measurements were performed at the Roudebush VA Medical Center, Indianapolis, IN, by personnel blinded as to the order of the treatment infusions.

Electrophoretic separation of transferrins

The iron forms of transferrin were separated using a TBE-urea polyacrylamide gel (6% acrylamide gels with 6 M urea) according to Williams et al. [17]. This method separates transferrin into the apotransferrin, monoferric and the diferric forms, according to their electrophoretic mobilities after all serum proteins except β - and γ-globulins are precipitated by 2% 6,9-diamino-2-ethoxyacridine lactate monohydrate (Sigma, St Louis, MO) [18]. Electrophoresis was performed using a Criterion mini-gel system (Biorad, Hercules, CA). Protein bands were visualized through staining with GelCode Blue stain reagent (Pierce, Rockford, IL). Densitometric analysis was performed with a Gel Logic 100 apparatus and 1D image analysis software (Kodak, Rochester, NY). The percent TSAT was calculated as follows: TSAT (%) = [(diferric transferrin + (1/2 x monoferric transferrin(s)))/(apotransferrin + monoferric transferrin(s) + diferric transferrin)]

Plasma and urinary malondialdehyde assay

MDA, a lipid peroxide product, is formed by β-scission of peroxidized polyunsaturated fatty acids and was measured by derivatization with thiobarbituric acid as reported previously [19].

Urine albumin, protein, creatinine and NAG determination

Urinary albumin was determined by immunoturbidimetry and urinary protein by the pyrogallol red-molybdate method. Urine creatinine concentration was determined using an end-point spectrophotometric assay with an alkaline picrate solution (Sigma Diagnostics, St Louis, MO). Urinary NAG was measured by colorimetric assay (Roche Diagnostics Corporation, Indianapolis, IN). Other laboratory assays were performed using standard methods in the hospital laboratory.
Statistical analysis

Comparisons between pre-infusion and post-infusion values for each of the four treatments were made using paired *t*-test. Comparisons of pre-infusion and post-infusion values among the four treatment groups were made using analysis of variance (ANOVA). All *P*-values are two sided and significance set at < 0.05. Data are expressed as mean±SD.

Results

Study participants

Study participation occurred between July 2003 and December 2003. Baseline and clinical characteristics are shown in Table 1. All participants were male with a mean age of 77.5 years (range 73–90) (reflecting the aged veteran population at Hines VA Hospital).

Effect of i.v. ferric gluconate on transferrin saturation

Plasma TSAT increased markedly with iron infusion (Figure 1a), with significant (*P* < 0.01) increases in post-infusion compared with pre-infusion values in all treatment groups. There were ~60 and 95% saturations seen in the 125 and 250 mg infusion groups, respectively, after iron infusion. The difference between the two doses in post-infusion TSAT was statistically significant [four-group comparison by ANOVA, *P* < 0.001; treatment 3 or 4 (250 mg dose iron) vs 1 or 2 (125 mg dose iron), *P* < 0.05].

Effect of i.v. ferric gluconate on generation of oxidative stress and renal injury

Plasma MDA levels increased markedly after iron infusion in all treatment groups (*P* < 0.01) (Figure 1b). The extent of elevation of plasma MDA levels post-infusion was similar among the four groups (*P* > 0.05 by ANOVA), and there was no effect of NAC on the rise of MDA induced by iron administration. Urinary MDA also increased with iron infusion, but only at the 250 mg dose (treatment 3, post- vs pre-infusion, *P* < 0.01), and again NAC had no effect (treatment 4, post- vs pre-infusion, *P* < 0.01) (Figure 1c). The extent of elevation of urinary MDA levels post-infusion was also similar among the four groups (*P* > 0.05 by ANOVA). As shown in Table 2, there was no increase in albuminuria or total proteinuria with iron infusion. NAG excretion appeared to increase slightly at the 250 mg dose, but these changes were not significant [treatments 3 and 4 (250 mg dose iron), post- vs pre-infusion, *P* = 0.12 for each comparison]. Post-infusion NAG excretion was similar among the four groups (*P* > 0.05 by ANOVA).

Safety and adverse events

None of the eight subjects reported any side effects of either NAC (or placebo) or any symptoms during the infusions. We did not observe changes in pulse or blood pressures during the infusions, and no patient suffered hypotension.

Table 1. Clinical characteristics of the study subjects

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Race</th>
<th>Aetiology of kidney disease</th>
<th>Hb (g/dl)</th>
<th>Fe (µg/dl)</th>
<th>TIBC (µg/dl)</th>
<th>TSAT (%)</th>
<th>Ferritin (ng/µl)</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>LDL cholesterol (mg/dl)</th>
<th>Albumin (g/dl)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>81</td>
<td>AA</td>
<td>DM, HTN</td>
<td>7.7</td>
<td>52</td>
<td>294</td>
<td>18%</td>
<td>312</td>
<td>85</td>
<td>2.9</td>
<td>81</td>
<td>3.7</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>AA</td>
<td>HTN</td>
<td>11.8</td>
<td>31</td>
<td>196</td>
<td>16%</td>
<td>243</td>
<td>79</td>
<td>3.8</td>
<td>167</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>C</td>
<td>Unknown</td>
<td>9.2</td>
<td>19</td>
<td>301</td>
<td>6%</td>
<td>26</td>
<td>55</td>
<td>2</td>
<td>124</td>
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</tr>
<tr>
<td>4</td>
<td>74</td>
<td>AA</td>
<td>Ischemic</td>
<td>8.7</td>
<td>65</td>
<td>304</td>
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<td>42</td>
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<td>3.5</td>
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<tr>
<td>5</td>
<td>80</td>
<td>C</td>
<td>Amyloidosis</td>
<td>11.6</td>
<td>117</td>
<td>365</td>
<td>32%</td>
<td>23</td>
<td>48</td>
<td>3.7</td>
<td>64</td>
<td>3.7</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>C</td>
<td>IgA nephropathy</td>
<td>9</td>
<td>19</td>
<td>296</td>
<td>6%</td>
<td>28</td>
<td>49</td>
<td>2.4</td>
<td>43</td>
<td>2.4</td>
</tr>
<tr>
<td>7</td>
<td>79</td>
<td>C</td>
<td>DM</td>
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<td>388</td>
<td>19%</td>
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<tr>
<td>8</td>
<td>73</td>
<td>AA</td>
<td>DM, HTN</td>
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<td>53</td>
<td>294</td>
<td>18%</td>
<td>147</td>
<td>109</td>
<td>4.3</td>
<td>151</td>
<td>3.4</td>
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</table>

AA = African-American; C = Caucasian; DM = diabetes mellitus; HTN = hypertension; Hb = haemoglobin; Fe = iron; TIBC = total iron binding capacity; TSAT = transferrin saturation (Fe/TIBC), BUN = blood urea nitrogen.

Table 2. Effect of ferric gluconate infusion on albuminuria, total proteinuria and urinary *N*-acetyl-β-D-glucosaminidase (NAG)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urine albumin/creatinine ratio (mg/mg)</th>
<th>Urine protein/creatinine ratio (mg/mg)</th>
<th>Urine NAG (mU/mg creat)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-infusion</td>
<td>Post-infusion</td>
<td>Pre-infusion</td>
</tr>
<tr>
<td>1</td>
<td>0.13±0.13</td>
<td>0.14±0.11</td>
<td>0.19±0.18</td>
</tr>
<tr>
<td>2</td>
<td>0.11±0.10</td>
<td>0.12±0.08</td>
<td>0.12±0.16</td>
</tr>
<tr>
<td>3</td>
<td>0.11±0.10</td>
<td>0.13±0.13</td>
<td>0.17±0.18</td>
</tr>
<tr>
<td>4</td>
<td>0.11±0.10</td>
<td>0.13±0.11</td>
<td>0.17±0.18</td>
</tr>
</tbody>
</table>

Treatment 1 = 125 mg dose with placebo; treatment 2 = 125 mg with NAC; treatment 3 = 250 mg dose with placebo; treatment 4 = 250 mg dose with NAC. No significant differences among any of the groups were seen. Data are expressed as mean±SD.
Discussion

Sodium ferric gluconate has assumed an important role in the management of anaemic patients with CKD in many centres. The side effect profile is very acceptable with the FDA-approved 125 mg dose given over 10 min as a slow i.v. infusion [20]. This dose is very convenient to administer in haemodialysis patients who have readily available i.v. access and are seen thrice weekly in the dialysis unit. However, since most clinicians administer a total dose of 500 mg to 1.0 g or more of i.v. iron to replete iron stores, use of the 125 mg dose in CKD patients not on haemodialysis necessitates repeated visits to the clinic or out-patient infusion centre. Many clinicians therefore use ferric gluconate at higher than recommended doses (e.g. 250 mg) in CKD patients not on dialysis. These doses are generally well tolerated but an increased incidence of side effects may occur [21]. Since major mechanisms of adverse effects with i.v. redox active irons are believed to be generation of oxidative stress and/or direct cellular injury, we believed it was important to determine if the larger dose (i.e. 250 mg) of ferric gluconate was associated with more oxidative stress and/or renal injury than the standard (i.e. 125 mg) dose. The results of this study indicate that administration of i.v. ferric gluconate results in oxidative stress (as determined by evidence of lipid peroxidation) at both the clinically approved 125 mg dose and the frequently utilized 250 mg dose. However, oxidative stress was not associated with evidence of acute renal injury, as there was no effect of ferric gluconate on glomerular permeability (albuminuria and proteinuria) or tubular function (enzymuria). Administration of the antioxidant NAC at the doses utilized in this study did not affect plasma and urinary measurements of lipid peroxidation.

Parenteral iron preparations are clearly effective, but concerns have been raised regarding adverse events and potentially long-term toxicity. In the case of iron dextran, there is a small but serious risk of anaphylaxis, with > 30 deaths reported from use of this agent [22]. Although this potentially fatal adverse effect does not appear to be a problem with newer preparations, such as ferric gluconate and iron sucrose, generation of oxidative stress with i.v. iron preparations may be a universal phenomenon. Increased in vitro superoxide production after infusion of iron sucrose has been noted in normal volunteers [23]. Herrera et al. measured plasma MDA in haemodialysis patients 1 h following iron sucrose administration and found levels to be significantly increased [24]. Lim et al. reported that iron sucrose resulted in significantly elevated levels of plasma lipid peroxide products [25]. Roob et al. found plasma MDA to peak within 30 min after iron sucrose [13]. Tovbin et al. found advanced protein oxidation products to be increased within 3–5 min after iron sucrose [26]. Most recently, Agarwal et al. found that iron sucrose resulted in oxidative stress (elevated plasma and urinary MDA) [14]. Iron dextran was reported to increase the esterified fraction of F2 isoprostanes 60 min after administration [12]. Oxidative stress after administration of ferric gluconate has been reported in one previous study in haemodialysis patients [27]. An increase in carbonyl stress (carbonylated fibrinogen) was noted with the 125 mg but not with the 62.5 mg dose.

Fig. 1. Effect of ferric gluconate infusion on transferrin saturation (TSAT) (a), plasma malondialdehyde (MDA) (b) and urinary MDA (c). Treatment 1 = 125 mg dose with placebo; treatment 2 = 125 mg with N-acetylcysteine (NAC); treatment 3 = 250 mg dose with placebo; treatment 4 = 250 mg dose with NAC. The open bars depict pre-infusion and the closed bars post-infusion values. The asterisks indicate significant differences ($P<0.01$) compared with pre-infusion values. The number symbols (#) indicate significant differences ($P<0.05$) compared with the 125 mg dosage. Data are expressed as mean±SD.
In vitro studies by Zager and associates in mouse proximal tubular segments, murine renal cortical homogenates and cultured human proximal tubular cells have demonstrated oxidative stress, as determined by lipid peroxidation after exposure to iron dextran, iron gluconate, iron sucrose and an iron oligosaccharide [6]. However, iron sucrose was far more cytotoxic than the other compounds tested, as assessed by lactate dehydrogenase release, although some evidence of cellular toxicity was seen at the higher doses of iron gluconate. Cytotoxicity was protected by reduced glutathione (GSH) independent of its antioxidant effect, as MDA generation was not altered. Thus, it is apparent that oxidative stress and cell injury after iron exposure can be dissociated, at least in vitro. In a recent publication from the same group [7] involving in vivo studies in mice, similar amounts of lipid peroxidation (MDA accumulation) were noted in renal cortex with both iron gluconate and iron sucrose, yet histological changes were reported to be more evident with iron sucrose. In these studies, glomerular iron accumulation was observed predominantly in the mesangial and endothelial cells associated with endothelial cell swelling. These renal changes were not seen with iron dextran or the iron oligosaccharide.

Of note, in the study by Agarwal and associates [14], administration of iron sucrose at standard clinical doses (100 mg given i.v. over 5 min) resulted in both oxidative stress and evidence of renal injury (increased proteinuria and enzymuria), as might be predicted by the studies of Zager et al. [6, 7]. Likewise, the dissociation between oxidative stress and renal cell injury with ferric gluconate in the present clinical study is consistent with the in vitro and in vivo findings with respect to this iron compound of Zager and colleagues. Differences between iron preparations have been noted by other investigators. Sengeloe et al. demonstrated greater impairment in neutrophil migration when incubated in vitro in iron sucrose compared with sodium ferric gluconate [28].

The regimen of NAC administration utilized in our study was the same regimen that has been reported to be beneficial in preventing oxidative injury due to i.v. contrast [29]. NAC is thought to exert its effect by promoting intracellular GSH synthesis. The failure of this regimen of NAC to prevent oxidative stress could be related to many factors, including inadequate dose or duration of treatment. Of note, in the study of Agarwal and associates [14], the plasma GSSG/GSH ratio was not altered by the same daily dose (1200 mg) of NAC. It is possible that NAC was not protective because it did not sufficiently prevent iron-catalysed hydroxyl radical production, although this is speculative. It is also possible that an alternative antioxidant may have been beneficial, as has been reported previously with vitamin E in haemodialysis patients receiving iron sucrose [13].

A major strength of the above study is the four-way randomized crossover design, in which the order of the experimental treatments was randomized, with end-points determined by personnel blinded as to the treatment group of the patient. The obvious limitation is the small number of patients studied. In this regard, it is possible that the small but non-significant increase in urinary NAG excretion with the 250 mg dose would be statistically significant if enough patients were studied. However, the small magnitude of the effect even if statistically significant in the absence of changes in proteinuria would be of doubtful clinical significance. Pre-infusion values for both oxidative stress parameters and urinary proteinuria/enzymuria were similar for each patient throughout the study period, indicating that any changes seen after infusion of iron were of a transient (i.e. <1 week) nature. However, we cannot be certain as to whether findings would be similar if the iron doses were administered in a different manner (e.g. slow i.v. push rather than by infusion over 1–2 h) or more frequently (e.g. daily). Moreover, it is possible that larger amounts or repeated courses of 1.0 g amounts of ferric gluconate might result in acute renal injury and/or exacerbation of chronic renal injury.

In summary, i.v. sodium ferric gluconate at commonly used clinical doses caused oxidative stress (as reflected by increased lipid peroxidation), but not acute renal injury. Since it appears that there may be differences between different i.v. iron preparations with regard to their ability to induce cell injury, a randomized trial comparing the efficacy and safety of clinically used iron preparations is warranted.

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Conflict of interest statement. D. J. Leehey is a member of the Speaker’s Bureau of Watson Pharmaceuticals. R. Agarwal is a member of the Speaker’s Bureau and a consultant for Watson. The present study was supported by the investigators. No support was requested or received from the pharmaceutical industry including Watson Pharmaceuticals.

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