Proteinuria Induced by Parenteral Iron in Chronic Kidney Disease—A Comparative Randomized Controlled Trial

Rajiv Agarwal,*† David J. Leehey,‡§ Scott M. Olsen,1 and Naomi V. DahlII

Summary

Background and objectives Among patients with chronic kidney disease (CKD), differences in proteinuria are seen between intravenous iron preparations after a single dose exposure. This study examined differences in proteinuria between two intravenous iron preparations after multiple doses.

Design, setting, participants, & measurements Patients with iron-deficiency anemia and CKD, stratified by angiotensin converting enzyme inhibitor (ACEI)/angiotensin receptor-blocker (ARB) use, were randomized to iron sucrose or ferric gluconate. Each patient at 12 centers received 100 mg of study drug weekly for 5 weeks. Urine protein/urine creatinine ratio was measured before each dose and frequently thereafter for 3 hours.

Results Postbaseline data were available from 33 patients receiving iron sucrose and 29 patients receiving ferric gluconate. Although neither preparation of intravenous iron increased the predose level of proteinuria, the proteinuric response to intravenous iron was dependent on the type of iron and ACEI/ARB use. Without ACEIs/ARBs, ferric gluconate tended to cause less proteinuria with repeated iron administration; iron sucrose did not mitigate or aggravate proteinuria. Among patients receiving ACEIs/ARBs, in contrast to ferric gluconate, which produced only mild transient proteinuria, iron sucrose produced a consistent and persistent proteinuric response that was on average 78% greater.

Conclusions Although multiple doses of either intravenous iron did not increase basal levels of proteinuria, postdose proteinuria was greater with iron sucrose than with ferric gluconate. These data suggest that nephrotoxicity of iron may depend on type of intravenous iron and on ACEI/ARB use. The long-term effects on kidney function need to be further evaluated.


Introduction

Anemia frequently complicates the course of chronic kidney disease (CKD). Although erythropoietin deficiency is the major cause of anemia, iron deficiency occurs commonly and may evoke poor response to erythropoietin (1). Current guidelines recommend that iron-deficiency anemia among patients with CKD not on hemodialysis may be treated with oral or intravenous (IV) iron (2,3); however, the IV route is being frequently utilized. Although the IV route offers some advantages such as improved adherence to treatment (4,5), concerns have been raised regarding the long-term risk of IV iron (6,7). Because of their more favorable short-term side effect profile (8,9), especially the risk for anaphylaxis, ferric gluconate and iron sucrose have largely replaced iron dextran for use in practice in the United States.

Whereas in the short-term ferric gluconate and iron sucrose have an excellent safety record, in the long-term these drugs may provoke nephrotoxicity (10). In vitro studies using human proximal tubular kidney cells in culture and in vivo studies in mice have shown nephrotoxicity. Although nephrotoxicity is shared by iron sucrose and ferric gluconate, iron sucrose appeared to be more toxic (10). Similar results have been obtained in patients with CKD. Although iron sucrose was associated with worsening of proteinuria, ferric gluconate was not (11,12). In a single dose head-to-head comparison of iron sucrose and ferric gluconate, iron sucrose was found to elicit greater proteinuria (13). Because proteinuria is strongly linked to accelerated progression to ESRD and cardiovascular disease, concerns regarding IV iron have been raised in the long term (6). When oral iron is not a treatment option because it is ineffective or not tolerated, IV iron therapy has to be utilized. In such situations, especially among patients not on dialysis, it may be important to know which drug results in less proteinuria on repeated administration.

The purpose of our study was to answer the question “Which of the two IV irons—iron sucrose or ferric gluconate—results in less proteinuria upon multiple exposure to the drugs?” Accordingly, we conducted a multicenter, randomized controlled parallel group study in patients receiving angiotensin converting enzyme inhib...
itors (ACEIs) and/or angiotensin receptor blockers (ARBs) and those who did not. The primary objective of the study was to assess the change in urine total protein-to-creatinine ratio between ferric gluconate and iron sucrose stratified by ACEI/ARB use.

**Materials and Methods**

**Subjects and Protocol**

Eligible patients were at least 18 years old with estimated GFR ≤60 ml/min per 1.73 m² (using the simplified Modification of Diet in Renal Disease equation) and proteinuria (confirmed by positive microalbuminuria dipstick test or recent laboratory test result) who were not on dialysis and not expected to initiate dialysis for at least 6 months. They had to have hemoglobin concentrations ≤12.5 g/dl and either transferrin saturation <25% or serum ferritin <200 ng/ml. The exclusion criteria were known hypersensitivity to either study drug, history of multiple drug allergies, history of organ transplant, use of an investigational drug within 1 month before study, history of alcoholism or active liver disease, hemoglobin <8 g/dl, positive urine pregnancy test or breastfeeding, prior history of IV iron administration within 1 month of the study, serum ferritin >800 ng/ml or transferrin saturation >50%, anemia due to any cause other than iron deficiency in nondialysis CKD, any surgery within 1 month or scheduled to occur during the study period, systemic or urinary tract infection within 1 month, serum albumin <3.0 g/dl, serum sodium <130 mEq/L, symptomatic benign prostatic hyperplasia, or any other bladder obstruction conditions that in the opinion of the investigator would not allow for good urine output.

This study was reviewed and approved by the institutional review boards of the participating clinical sites before enrollment of any participant and was conducted in accordance with the Declaration of Helsinki. It was registered with the National Institutes of Health through the National Library of Medicine at www.clinicaltrials.gov (NCT00534144). Study participants provided informed consent before undergoing any study procedures.

Figure 1 depicts the study procedures. Using a computer-generated randomization code, patients were randomized centrally in a 1:1 ratio to intravenously receive 100 mg weekly for 5 weeks of ferric gluconate (Ferrlecit, Sanofi-Aventis, U.S. LLC, Bridgewater, NJ) or iron sucrose (Venofer, American Regent Laboratories, Inc., Shirley, NY). Randomization was stratified by use or nonuse of ACEIs and ARBs.

At dosing study day 1, blood samples were obtained for complete blood count and serum chemistry assessments before infusion of the iron preparation. Patients drank a volume of water equivalent to 15 ml/kg of total body weight to ensure water diuresis. Immediately after providing a preinfusion urine sample, patients were administered 100 mg of the assigned IV iron preparation over 10 minutes. Postinfusion urine samples were obtained at 15, 30, 60, 120, and 180 minutes after the end of the IV iron infusion. At each assessment point, patients emptied their bladders in a clean container and a sample of that urine was labeled with the time it was obtained. To ensure good urine output needed to obtain the next urine sample, patients drank a volume of water equivalent to the urine volume they had just voided.

Patients returned at 7-day intervals for each of the subsequent doses, which followed the same procedures, and for an end-of-study follow-up visit for laboratory samples.

All urine samples were analyzed for their concentrations of total protein, albumin, and creatinine. Each sample’s total protein-to-creatinine concentration ra-
tio and albumin-to-creatinine concentration ratio were calculated and analyzed. All urine samples were analyzed at the same central laboratory facility. Urine total protein, urine creatinine, and urine albumin concentrations were obtained using colorimetric methodologies and were determined on an Olympus 5400 series analyzer (Olympus Diagnostics, Dallas, TX).

Statistical Analyses

Urine protein-to-creatinine ratios were natural log transformed before analysis. We had specified an ANOVA model with four fixed factors and all of their interactions for the primary statistical analysis. This demonstrated a significant interaction between treatment group and number of doses \((P = 0.0001)\) and among treatment group, number of doses, and ACEI/ARB status \((P = 0.02)\). The ANOVA centers the variables and does not model the covariance structure, which limits the interpretation of the results. A mixed model can account for patient-level variation, repeated measurements, and the covariance structure. Accordingly, in a post hoc analysis, using a linear mixed model, we compared the changes in urine protein-to-urine creatinine ratio between ferric gluconate and iron sucrose groups at baseline and over visits \((14)\). The terms used in the fixed part of the mixed model were urine collection period, visits, ACEI/ARB stratum, iron type, and all interactions including up to four-way interactions. The terms used in the random component of the model were patients and repeated urine collection periods nested within visits. An unstructured covariance matrix was used to create a random coefficient model of patients and repeated urine collection periods. After fitting the mixed model, the changes were computed from the baseline urine protein-to-urine creatinine ratio by using the formula \(100 \times [1 - \exp(\beta)]\), where \(\beta\) was the regression coefficient.

The nominal level of significance was set at a two-sided \(P < 0.05\), and all statistical analyses were performed with Stata version 11 (StataCorp LP, College Station, TX).

Results

Between October 2007 and November 2008 we enrolled 75 patients from 12 centers. The trial flow is illustrated in Figure 2. Trial participation had excellent retention and adherence to the protocol; 70 participants completed the trial, of which 62 met the criteria for inclusion in the intent-to-treat analysis population \(\left(\text{defined as all patients who received at least one dose of test article with preinfusion and postinfusion efficacy data at 15, 30, and 60 minutes at that visit}\right)\).

Table 1 shows that the baseline characteristics, including baseline urine protein-to-creatinine ratio, were well matched among the intent-to-treat population, except that a statistically significant imbalance was seen between IV irons in sex distribution \((P = 0.041)\) and ESA use \((P = 0.047)\).

First Exposure to IV Iron

On first exposure to IV iron, the proteinuric response was dependent on ACEI use (Figure 3). On average, compared with patients not on ACEIs/ARBs \((n = 17)\), patients receiving ACEIs/ARBs \((n = 45)\) had less proteinuria \((P = 0.027)\), and on average ACEI/ARB users had an 11% lower basal protein-to-creatinine ratio. The proteinuric response to iron was modified by ACEI/ARB use \((P = 0.006 for interaction)\).

After exposure to ferric gluconate, proteinuria worsened in the non-ACEI/ARB stratum \((P = 0.013\) for change; maximal response 48% at penultimate urine collection) but improved among those on treatment with ACEIs/ARBs (Figure 3).

In contrast, after exposure to iron sucrose, proteinuria did not change in the non-ACEI/ARB stratum. On average, compared with ferric gluconate, the mean change in protein-to-creatinine ratio with iron sucrose was 25% lower. Thus, a significant difference \((P = 0.04)\) in proteinuric response between drugs in the non-ACEI/ARB stratum was noted. However, among patients on ACEIs/ARBs, proteinuria worsened with iron sucrose. In contrast to the non-ACEI/ARB stratum, among the ACEI/ARB stratum the mean change in protein-to-creatinine ratio with iron sucrose was 43% greater than ferric gluconate \((95\% \text{ confidence interval 6.9\% to 92.6\%, } P = 0.016)\) (Figure 3).

Second and Subsequent Exposure to IV Iron

Although multiple doses of IV iron did not increase the basal level of proteinuria \((P > 0.5)\), proteinuric response to IV iron was dependent on the type of iron and ACEI/ARB use (Figure 4).
Table 1. Baseline characteristics of the intent-to-treat population

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>21</td>
<td>8</td>
<td>24</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>65.0 ± 11.8</td>
<td>66.7 ± 15.4</td>
<td>68.7 ± 12.7</td>
<td>55.7 ± 21.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>15 (71%)</td>
<td>6 (75%)</td>
<td>11 (46%)</td>
<td>4 (44%)</td>
<td></td>
</tr>
<tr>
<td>Weight, lb</td>
<td>194.1 ± 44.5</td>
<td>177.9 ± 33.5</td>
<td>199.4 ± 61.5</td>
<td>200.8 ± 55.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Height, in.</td>
<td>65.1 ± 3.3</td>
<td>64.6 ± 4.2</td>
<td>66.4 ± 3.6</td>
<td>63.9 ± 4.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>white</td>
<td>10 (48%)</td>
<td>5 (63%)</td>
<td>15 (63%)</td>
<td>5 (56%)</td>
<td></td>
</tr>
<tr>
<td>black or African American</td>
<td>7 (33%)</td>
<td>1 (13%)</td>
<td>7 (29%)</td>
<td>4 (44%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>4 (19%)</td>
<td>2 (25%)</td>
<td>2 (8%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity Hispanic or Latino, n (%)</td>
<td>5 (24%)</td>
<td>4 (50%)</td>
<td>6 (25%)</td>
<td>3 (33%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Etiology of CKD, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>diabetes</td>
<td>12 (57%)</td>
<td>5 (63%)</td>
<td>15 (63%)</td>
<td>1 (11%)</td>
<td></td>
</tr>
<tr>
<td>hypertension</td>
<td>7 (33%)</td>
<td>1 (13%)</td>
<td>5 (21%)</td>
<td>6 (67%)</td>
<td></td>
</tr>
<tr>
<td>GN</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (8%)</td>
<td>1 (11%)</td>
<td></td>
</tr>
<tr>
<td>adult polycystic kidney disease</td>
<td>0 (0%)</td>
<td>1 (13%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>other/unknown</td>
<td>2 (10%)</td>
<td>1 (13%)</td>
<td>2 (8%)</td>
<td>1 (11%)</td>
<td></td>
</tr>
<tr>
<td>Estimated GFR, ml/min per 1.73 m²</td>
<td>34.9 ± 13.6</td>
<td>29.9 ± 13.3</td>
<td>34.7 ± 15.3</td>
<td>37.4 ± 24.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Patients with diabetes mellitus (all type II), n (%)</td>
<td>17 (81%)</td>
<td>6 (75%)</td>
<td>18 (75%)</td>
<td>3 (33%)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Continuous variables are shown as mean ± SD. FG, ferric gluconate; IS, iron sucrose; Chr, reticulocyte hemoglobin content; TIBC, total iron binding capacity; ESA, erythropoiesis stimulating agent.

In the non-ACEI/ARB stratum, compared with iron sucrose, ferric gluconate tended to cause less proteinuria with repeated iron administration. Compared with baseline (first-dose) proteinuric response, the reduction in average protein-to-creatinine ratio at sequential visits was 6.5%, 2.5%, 13%, and 31.5%. The last reduction from baseline visit was statistically significant (P = 0.001). In the non-ACEI/ARB stratum, iron sucrose did not mitigate or aggravate proteinuria. The difference between drugs in the non-ACEI/ARB stratum was significant (P = 0.018). In the non-ACEI/ARB stratum, iron sucrose increased the protein-to-creatinine ratio on average by 40.9% compared with the ferric gluconate, (95% confidence interval 2.0% to 94.7%, P = 0.037).

Among patients on ACEIs/ARBs, in contrast to ferric gluconate, which produced transient proteinuria (no more than 10% greater than basal level of proteinuria, P > 0.5 for each visit compared with baseline), iron sucrose produced a proteinuric response that was on average 78% more compared with ferric gluconate. This greater proteinuric response with iron sucrose was consistent (between 16.6% and 23.9% increase), persistent, and significant (P < 0.025 for each comparison).

Results of albumin-to-creatinine ratio after the first dose and repeated doses were similar to that seen with the protein-to-creatinine ratio (Figure 5). Among patients on ACEIs/ARBs, in contrast to ferric gluconate, which produced transient proteinuria (no
more than 10% greater than basal level of proteinuria, P > 0.5 for each visit compared with baseline), iron sucrose produced a proteinuric response that was on average 135% more compared with ferric gluconate. This greater proteinuric response with iron sucrose was consistent (between 13.7% and 30.1% increase), persistent, and significant.

Discussion
The major findings of this study are as follows. Although first exposure to iron gluconate causes proteinuria among patients not receiving concurrent ACEIs/ARBs, repeated administration tends to mitigate this response. Iron sucrose does not cause proteinuria in patients not receiving concurrent ACEIs/ARBs, either on single or repeated administration. Among patients on ACEIs/ARBs, ferric gluconate produces only a transient proteinuria (no more than 10% greater than baseline level of proteinuria); however, compared with ferric gluconate, iron sucrose produces a proteinuric response that was on average 78% more overall. This greater proteinuric response with iron sucrose is consistent and persistent. Multiple doses of IV iron do not increase the basal level of proteinuria, at least in the short term.

This study adds to a growing body of literature that suggests differential toxicity of IV iron drugs. Although it is widely believed that iron causes renal injury via the generation of reactive oxygen species (such as the hydroxyl ion via the Haber–Weiss Fenton reaction) (6), pathways independent of generation of reactive oxygen species are involved (15,16). For example, 100 mg of iron sucrose infusion in CKD patients results in renal glomerular and tubular damage as measured by increased proteinuria and enzymuria, which are not attenuated by the administration of the antioxidant N-acetylcysteine (11). On the other hand, ferric gluconate at two dosage levels (125 and 250 mg) administered to iron-deficient anemic patients with CKD found that although ferric gluconate caused oxidative stress, there was no evidence of acute renal injury (12). In our earlier crossover, randomized, single-dose exposure trial comparing ferric gluconate to iron sucrose, we demonstrated that the urine total protein-to-creatinine ratio was significantly greater after iron sucrose than ferric gluconate treatment with the effect noted within 15 minutes after infusion (13). Furthermore, when iron sucrose was given first, a significantly greater protein-to-creatinine ratio was seen subsequently with ferric gluconate than with the reverse order of treatment. Accordingly, to test the effect of repeated administration of iron on proteinuria, we designed a parallel group trial.

In the aforementioned crossover study, we reported that when patients were not on ACEIs/ARBs, iron sucrose caused greater proteinuria compared with ferric gluconate (13). On the other hand, when patients were on ACEIs/ARBs, ferric gluconate caused as much proteinuria as iron sucrose. There are several reasons for the apparent discrepant observations compared with our earlier crossover study. First, the earlier study only had four patients who were not on ACEIs/ARBs. Second, the level of proteinuria was similar in those on ACEIs/ARBs compared with those who were not. In the study presented here, those who were not on ACEIs/ARBs had greater proteinuria at baseline. Third, findings of the earlier study were confounded by carryover effects due to its crossover design; that is, the proteinuric response to the first dose of iron appeared to affect the response to a subsequent dose of a different preparation. Although albuminuria is generally considered to be a better marker of renal injury compared with proteinuria, we selected proteinuria as the primary end point because previous studies have demonstrated that administration of IV iron may directly alter albumin (17). Upon administration of IV iron, albumin is carbonylated, fragmented, and loses immunoreactivity in a time-dependent manner. We selected the protein-to-creatinine ratio rather than the protein excretion rate as an end point because there is less variability in protein-to-creatinine ratio compared with protein excretion rate (18). The greater variability in protein excretion rate from one hour to the next is presumably due to problems with incomplete bladder emptying. However, results of the time course of the urinary albumin-to-creatinine ratio were qualitatively similar to that seen with the urine protein-to-creatinine ratio. Quantitatively, compared with the urinary protein-to-creatinine ratio, the albumin-to-creatinine increment was greater with iron sucrose. It is increasingly being recognized that albumin hand-
ing is profoundly altered by tubular function. Accordingly, if iron sucrose caused greater renal tubular toxicity, it may account for these findings.

The reason why iron sucrose should be more toxic compared with ferric gluconate is difficult to answer with certainty in human studies. However, several observations made in cell cultures and in animals suggest why this could be so. Comparative toxicology studies of IV irons by Zager and colleagues suggest that iron sucrose causes greater oxidative injury specifically directed to the mitochondria (19). Furthermore, compared with iron gluconate, electron microscopy studies show that iron sucrose preferentially accumulates in mesangial cells and podocytes (10). We did not study the mechanism of proteinuria, but it may relate to acute hemodynamic effects on the glomerular circulation or transient dysfunction of the cubulin-megalin pathway. Although the effects of IV iron on renal hemodynamics have not been studied, iron sucrose is known to increase blood and urine cytokine concentrations in animals (20) and among patients with CKD (21), and this may mediate the proteinuria.

There are some limitations of our study. Patients in this study were not randomly allocated to ACEI/ARB or no-ACEI/ARB therapy. In clinical practice, most CKD patients with proteinuria are treated with ACEIs/ARBs, so we were only able to recruit relatively few patients (17) not receiving ACEI/ARB treatment. Thus, the findings among patients not receiving ACEI/ARB treatment should be interpreted with caution. Although multicenter and randomized, our study was open label and may be subject to bias. More men were randomized to iron sucrose; whether this could have caused differences in proteinuria is unclear. Also, we did not study the long-term effect of IV iron on renal function decline. Thus, the long-term significance of our findings is unclear.

In conclusion, as assessed by proteinuria, important differences in nephrotoxicity between iron sucrose and ferric gluconate have emerged. Although it is encouraging to note that in the short term neither of the two IV irons leads to an increase in basal level of proteinuria, the long-term effect on kidney function still needs to be evaluated. Until more information becomes available, we agree that, among nonhemodialysis patients with CKD, oral iron is a reasonable first-line drug for the management of iron-deficiency anemia.

Acknowledgments
This study was sponsored by Watson Laboratories, Inc. R.A. and N.D. designed the study. The sponsor monitored the clinical sites to ensure proper adherance to good clinical practices and proper collection of study data. R.A. inter-
interpreted the data and prepared this paper, which was reviewed and commented on by all of the authors.

Disclosures
R.A. is a consultant for Watson Pharma and has served on its speaker bureau. D.J.L. has served on the speaker bureau of Watson Pharma. Both S.M.O. and N.V.D. are employees of Watson Laboratories.

References
16. Agarwal R, Warnock D: Issues related to iron replace-


Received: July 12, 2010 Accepted: August 25, 2010

Published online ahead of print. Publication date available at www.cjasn.org.