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Iron sucrose causes greater proteinuria than ferric gluconate in non-dialysis chronic kidney disease

R Agarwal[1], AR Rizkala[2], MO Kaskas[3], R Minasian[4] and JR Trout[5]

[1]*Department of Medicine, Indiana University School of Medicine and the Richard L Roudebush VA, Medical Center, Indianapolis, Indiana, USA;* [2]*Clinical Affairs, Watson Laboratories, Inc., Morristown, New Jersey, USA;* [3]*Northwest Louisiana Nephrology Associates, Shreveport, Louisiana, USA;* [4]*Glendale Kidney Center, Glendale, California, USA and* [5]*Rutgers University, New Brunswick, New Jersey, USA*

Non-dextran intravenous (i.v.) iron preparations seem to differentially affect proteinuria in patients with chronic kidney disease. To study effects of ferric gluconate and iron sucrose on proteinuria, we conducted a crossover trial in 12 patients with stage 3–4 chronic kidney disease. These patients were randomized to receive the same dose of either drug 1 week apart. Urine samples were obtained immediately before and at frequent intervals after the drug. The urine total protein/creatinine ratio was significantly greater after iron sucrose than ferric gluconate treatment with the effect noted within 15 min post-infusion. Furthermore, when iron sucrose was given first, a significantly greater protein/creatinine ratio was seen subsequently with ferric gluconate than with the reverse order of treatment. The urine albumin/creatinine ratio was also significantly greater with iron sucrose than with ferric gluconate. There was no significant difference, however, between the two i.v. irons in the measured urine *N*-acetyl-*β*-D-glucosaminidase/creatinine ratio. Although our study showed that acutely, iron sucrose increased proteinuria, the long-term effects of repeated i.v. non-dextran iron on kidney function requires further study.

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Correspondence: *R Agarwal, Department of Medicine, Richard L. Roudebush VA Medical Center, 1481 West 10th Street, 111N, Indianapolis, Indiana 46202, USA. E-mail: ragarwal@iupui.edu*

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Anemia of chronic kidney disease (CKD) is frequently complicated by iron deficiency. Iron deficiency in patients with CKD not on hemodialysis may be treated using either oral or intravenous (i.v.) iron.[1,2] However the i.v. route is being frequently utilized. Although the i.v. route offers some advantages including improved adherence to treatment[3] and improved quality of life,[4] the long-term risk of oral compared to i.v. iron have not been studied. Owing to their more favorable short-term side effect profile,[5,6] non-dextran i.v. iron preparations, that is, ferric gluconate and iron sucrose, have largely replaced iron dextrans for use in practice in the United States. However, the long-term side effects of these newer preparations includes the potential for causing nephrotoxicity in clinically relevant concentrations.[7] Studies in animals and human proximal tubular kidney cells in culture have shown nephrotoxicity both due to iron sucrose and ferric gluconate with a strong signal for differential toxicity.[7] In fact, experiments in patients with CKD also demonstrate that these drugs may have differential toxicity. While iron sucrose was associated with greater proteinuria, ferric gluconate was not.[8,9] These are clinically important outcomes and generate the hypothesis that appearance of proteinuria may be linked to accelerated progression to end stage renal disease. If i.v. iron can directly worsen proteinuria, its frequent administration to CKD patients, and even peritoneal dialysis patients, may have dire consequences in the long term. Nevertheless, clinicians may often encounter situations when i.v. iron therapy is the best treatment option, in which case they may wish to use the preparation that results in less proteinuria. Unfortunately, there are no randomized controlled trials comparing ferric gluconate to iron sucrose head-to-head.

Since there was plausible evidence suggesting that the two available non-dextran i.v. iron preparations may have different effects on the kidney and on proteinuria,[8,9] we conducted a randomized, controlled, crossover study to compare their effects on proteinuria. The primary objective of this study was to compare the difference between the ferric gluconate and iron sucrose in terms of change in urine total protein to creatinine ratio. Secondary objectives were to compare the two drugs in terms of change in urine albumin

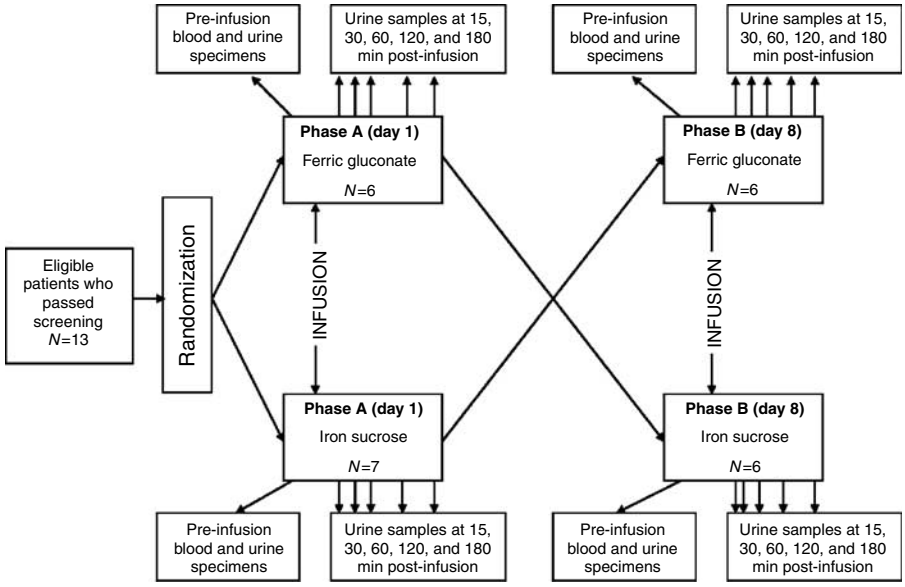

Figure 1 | A schematic of study procedures.

to creatinine ratio and change in urine *N*-acetyl-β-D-glucosaminidase (NAG) to creatinine ratio.

RESULTS

Thirteen patients were randomized into this two-period crossover trial as outlined in Figure 1 and discussed in Methods (see below). One patient, who was randomized to received iron sucrose in dosing phase A and ferric gluconate in dosing phase B, was withdrawn from the study a day after receiving iron sucrose due to hospitalization for a urinary tract infection caused by *Klebsiella* and *Escherichia coli*. Therefore, 12 patients completed the study and were included in the statistical analysis.

Table 1 summarizes the baseline characteristics of the 12 patients who completed the trial, which represents the population of CKD patients, except that there are more women in our sample.

Figure 2 shows the least-square mean change from pre-infusion urine total protein/creatinine ratio for each drug. Overall, iron sucrose resulted in greater urine total protein/creatinine ratio than ferric gluconate ($P<0.001$). On average pre-infusion urine total protein/creatinine ratio were 1.32 (s.d. 2.71) g/g creatinine with ferric gluconate and 1.23 (s.d. 2.53) g/g creatinine with iron sucrose ($P=0.132$). At 180 min post-infusion, urine total protein/creatinine ratios were 1.69 (s.d. 3.20) g/g creatinine and 2.28 (s.d. 3.53) g/g creatinine with ferric gluconate and iron sucrose, respectively ($P=0.002$). As shown in Figure 2, iron sucrose resulted in greater total protein excretion in the urine as early as 15 min post-infusion.

As with all crossover studies, we investigated if the difference between the two i.v. iron preparations in proteinuria response was dependent on the order in which the drugs are given. We found that when ferric gluconate was given at dosing phase A, the difference between the two i.v. iron preparations was larger than when iron sucrose was given at dosing phase A ($P<0.01$). As shown in Figure 3, ferric gluconate resulted in more proteinuria when it was preceded by iron sucrose (solid line) than when it was given first (dashed line), which resulted in this significant period effect ($P<0.01$). Regardless of this phenomenon, iron sucrose always resulted in greater proteinuria than ferric

Table 1 | Baseline characteristics of study completers[a]

N	12
Age (years)	67.3 ± 11.3
Females (*n* (%))	9 (75.0)
Weight (kg)	85.2 ± 19.8
Height (cm)	162.6 ± 10.4
Etiology of chronic kidney disease (*n* (%))	
Diabetes	5 (41.7)
Hypertension	5 (41.7)
Glomerulonephritis	1 (8.3)
Other	1 (8.3)
Estimated glomerular filtration rate (ml/min/1.73 m^2)	29.0 ± 11.2
Patients with diabetes mellitus type I/II/no diabetes mellitus (*n*)	2/7/3
Hemoglobin (g/dl)	11.7 ± 1.0
White blood cells (1000/mcL)	6.6 ± 1.7
Mean corpuscular volume (mcm^3)	92.1 ± 6.6
Mean corpuscular hemoglobin (pg/cell)	29.9 ± 2.5
Blood urea nitrogen (mg/dl)	44.7 ± 16.4
Serum creatinine (mg/dl)	2.2 ± 1.2
Serum albumin (g/dl)	4.2 ± 0.4
C-reactive protein (mg/l)	5.4 ± 5.6
Serum ferritin (ng/ml)	157 ± 134
Transferrin saturation (%)	23.4 ± 10.0
Patients on ACE inhibitors or ARBs (*n* (%))	8 (67%)

ACE, angiotensin converting enzyme, ARB, angiotensin receptor blocker.
[a]Continuous variables are presented in the form of mean ± s.d.

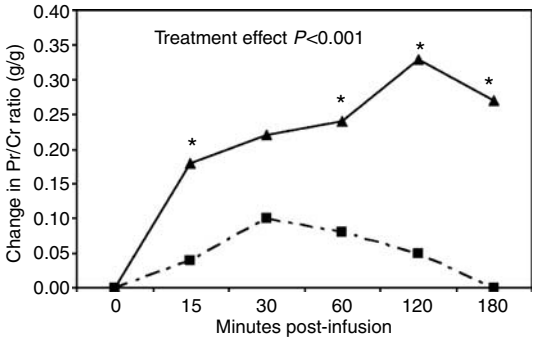

Figure 2 | Change in least-square mean urine total protein/creatinine ratio. Iron sucrose is represented by the solid line (—) and ferric gluconate is represented by the dashed line (– – –). The asterisks (*) denote assessment points were difference between the two treatments is statistically significant ($P<0.05$). Significantly greater total protein was excreted in the urine with iron sucrose as early as 15 min post-infusion. Overall, iron sucrose resulted in greater excretion of protein in the urine than ferric gluconate ($P<0.001$). Pr/Cr, protein/creatinine.

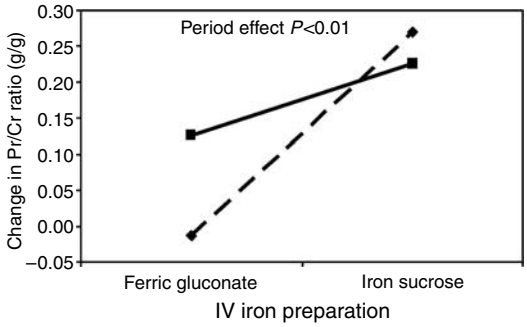

Figure 3 | An illustration of the effect of administration sequence of i.v. iron preparations on the geometric mean of overall change in urine total protein to creatinine ratio from the pre-infusion period. When ferric gluconate is administered first (– – –), the difference between the two treatments is larger than when iron sucrose is administered first (—) ($P<0.01$). Pr/Cr, protein/creatinine.

gluconate. The mean baseline urine data before the first and second infusion were similar.

Figure 4 shows the change from pre-infusion urine albumin/creatinine ratio. Overall, iron sucrose resulted in greater albumin excretion in the urine than ferric gluconate ($P<0.001$). Pre-infusion log urine albumin/creatinine ratios were similar with the two i.v. iron preparations; 0.91 (s.d. 2.0) g/g creatinine with ferric gluconate and 0.97 (s.d. 2.1) g/g creatinine with iron sucrose ($P=0.730$). By 180 min, iron sucrose had resulted in greater log urine albumin/creatinine ratio (1.9 (s.d. 3.1) g/g creatinine) than ferric gluconate (1.3 (s.d. 2.5) g/g creatinine) ($P=0.002$). This trend was also statistically significant at the 30 min assessment point.

As for urine NAG/creatinine ratio, there was no statistically significant difference between the two i.v. iron preparations at any of the assessment points or overall ($P=0.91$). Pre-infusion urine NAG/creatinine ratios were 6.9 (s.d. 3.4) U/g creatinine and 8.9 (s.d. 6.0) U/g creatinine with

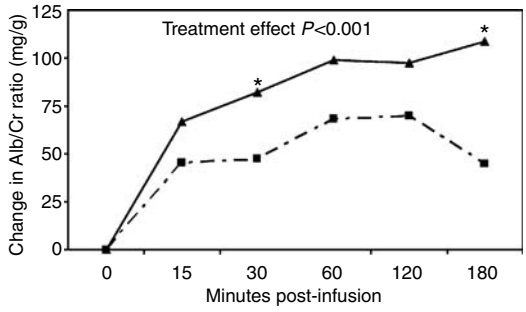

Figure 4 | Change in least-square mean urine albumin (Alb)/creatinine (Cr) ratio. Iron sucrose is represented by the solid line (—) and ferric gluconate is represented by the dashed line (– – –). The asterisks (*) denote assessment points were difference between the two treatments is statistically significant ($P<0.05$). Significantly greater albumin was excreted in the urine with iron sucrose as early as 30 min post-infusion. Overall, iron sucrose resulted in greater excretion of albumin in the urine than ferric gluconate ($P<0.001$).

ferric gluconate and iron sucrose, respectively ($P=0.22$). At the 180 min assessment point, the urine NAG/creatinine ratios were 10.9 (s.d. 7.8) U/g creatinine with ferric gluconate and 20.0 (s.d. 17.1) U/g creatinine with iron sucrose (overall $P=0.91$). Although there were no differences between the i.v. irons in the NAG/creatinine ratio, the mean change in NAG/creatinine ratio after iron infusion was 5.88 U/g (95% confidence interval 1.11–10.65), $P=0.021$. Thus, both i.v. irons increased enzymuria over baseline.

We analyzed whether angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) use was associated with change in albuminuria in response to i.v. iron injection. Pre-infusion level of albuminuria were similar in those using ACE inhibitors or ARBs compared to those not on those agents. Those patients not on the drug experienced greater albuminuria when exposed to iron sucrose (455 mg/g for iron sucrose, 138 mg/g for iron gluconate); albuminuria was similar when exposed to iron sucrose or iron gluconate when patients were on ACE inhibitors or ARBs (133 mg/g for iron sucrose and 143 mg/g for iron gluconate) ($P=0.0006$ for ACE/ARB × treatment group interaction). Similar ACE/ARB × treatment group interaction ($P=0.0003$) was seen in case of protein/creatinine ratio, but no effect ($P>0.20$) was seen for NAG/creatinine ratio. The level of estimated glomerular filtration rate (<30 ($n=8$) or >30 ml/min/1.73 m^2 ($n=4$)) did not influence albuminuria, proteinuria, or enzymuria responses.

From a safety perspective, except for the patient who was hospitalized for urinary tract infection a day after receiving iron sucrose and removed from the study, none of the participants experienced any adverse events.

DISCUSSION

The major finding that emerged from this crossover randomized, controlled trial was that iron sucrose resulted in much greater protein excretion rate compared to iron gluconate in patients with CKD. These findings were

confirmed by albumin/creatine ratio. There was no clinical difference seen for enzymuria. Furthermore, iron sucrose administration conditioned the kidney 1 week later to experience greater injury in response to ferric gluconate than when ferric gluconate was administered first.

Although albuminuria is generally considered a better marker of renal injury compared to proteinuria, we selected proteinuria as the primary end point because previous studies have demonstrated that administration of i.v. iron may injure the albumin molecule itself.[10] These studies have demonstrated that the albumin molecule is carbonylated, fragmented and loses immunoreactivity in a time-dependent manner upon administration of i.v. iron. We selected protein/creatinine ratio rather than protein excretion rate as an end point because there is less variability in protein/creatinine ratio compared to protein excretion rate.[11] The greater variability in protein excretion rate from 1 h to the next is presumably due to problems with incomplete bladder emptying.

Zager *et al*. have shown that iron sucrose induces greater cytotoxic effects than ferric gluconate on kidney cells both *in vitro*[12] and *in vivo*,[7] at suprapharmacologic concentrations[12] and at clinically relevant concentrations.[7] They suggested that greater oxidative injury directed specifically at the mitochondria may be the likely mechanism of such damage.[7] Furthermore, they concluded that although the clinical relevance of their findings is not known, caution is warranted when administering these i.v. iron preparations, especially to patients with CKD in whom nephrotoxicity is of greater significance compared to those with normal kidney function or those who are already on dialysis.[7]

Our study confirms and extends the previous observations in patients with CKD. Agarwal *et al.*[8] showed that 100 mg iron sucrose infusion in CKD patients results in renal tubular and glomerular damage as measured by increased proteinuria and enzymuria, which are not attenuated by the administration of the antioxidant *N*-acetylcysteine. The resulting damage resolved within 24 h, but the authors raised important concerns about the effect of repeated administration of iron sucrose and long-term effects on the kidneys. Leehey *et al.*[9] used ferric gluconate at two dosage levels (125 and 250 mg) in patients with CKD and found that although ferric gluconate caused oxidative stress, there was no evidence of acute renal injury.

Taken all together, the *in vitro*,[12] *in vivo*,[7] and clinical evidence[8,9] suggested that iron sucrose may result in greater acute renal injury than ferric gluconate. However, until now, it was difficult to conclusively make this judgment without having studied the effects of both i.v. iron preparations under the same conditions in a randomized, controlled trial. This randomized, controlled, crossover study strongly suggests that the two drugs may have different effects on kidney injury. While it may be that iron sucrose has more 'toxicity' as assessed by proteinuria and albuminuria, both of the iron preparations may exert acute tubular damage, as assessed by increased NAG excretion. These results are supported by the animal studies done by Zager *et al.*[7] in which they demonstrate by electron microscopy that iron sucrose preferentially accumulates in mesangial cells and podocytes compared to iron gluconate. However, both drugs cause some degree of tubular injury in cell cultures.[12] We did not study the mechanism of proteinuria, but it may relate to acute hemodynamic effect on the glomerular circulation or transient cytokine-induced proteinuria. Although, effects of i.v. iron on renal hemodynamics have not been studied, iron sucrose is known to increase blood and urine cytokine concentrations in patients with CKD and this may mediate the proteinuria.[13] On the other hand, hemodynamic effects may play a role. Indeed, in ACE inhibitor/ARB-treated patients, the effect of i.v. iron sucrose was abrogated.

There are some limitations to our study. We did not study repeated administrations of i.v. iron, which is more commonly done in clinical practice. Furthermore, the long-term impact of repeated administrations of i.v. iron on renal function was not studied so we cannot comment on the long-term significance of our findings.

In conclusion, greater protein and albumin excretion induced by iron sucrose suggests potential for harm in the long term on renal function in CKD patients. The conditioning effect of iron sucrose predisposing the kidney to renal injury and the long-term impact of i.v. iron on renal function needs further evaluation.

MATERIALS AND METHODS

Eligible patients were at least 18 years old with estimated glomerular filtration rate $\leqslant 60$ ml/min/1.73 m^2 (using the simplified modification of diet in renal disease equation[14]) who were not on dialysis and not expected to initiate dialysis for at least 6 months. They had to have either transferrin saturation $<25\%$ or serum ferritin <200 ng/ml, and hemoglobin $\leqslant 12.5$ g/dl. The exclusion criteria were known hypersensitivity to either study drug, history of multiple drug allergies, history of renal transplant, receiving immunosuppressive therapy, use of an investigational drug within 1 month before study, history of uncontrolled asthma, human immunodeficiency virus, cancer within last 3 years, rheumatoid arthritis, alcoholism or liver disease, hemoglobin <8 g/dl, positive urine pregnancy test or breastfeeding, prior history of i.v. 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 non-dialysis CKD, any surgery within 1 month, 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 condition that, in 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. 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 to receive either ferric gluconate (Ferrlecit®, Watson Laboratories, Inc., Morristown, NJ, USA) 100 mg i.v. at dosing phase A followed

by iron sucrose (Venofer®, American Regent Laboratories, Inc., Shirley, NY, USA) 100 mg i.v. at dosing phase B, or iron sucrose 100 mg i.v. at dosing phase A followed by ferric gluconate 100 mg i.v. at dosing phase B.

At dosing phase A (study day 1), blood samples were obtained for complete blood count and serum chemistry assessments were carried out 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 pre-infusion urine sample, patients were administered 100 mg of the i.v. iron preparation assigned for dosing phase A over 10 min. Post-infusion urine samples were obtained at 15, 30, 60, 120, and 180 min after the end of the i.v. 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 retuned 7 days later (study day 8) to undergo phase B study procedures. Dosing phase B procedures were identical to those of phase A, except that patients received 100 mg of the other i.v. iron preparation over 10 min.

All urine samples were analyzed for their concentrations of total protein, albumin, NAG, and creatinine. Each sample's total protein/creatinine concentration ratio, albumin/creatinine concentration ratio and NAG/creatinine concentration ratio were calculated and analyzed to avoid any dilution effect. All urine samples were analyzed at the same central laboratory facility. Urine total protein, urine creatinine, and urine NAG concentrations were obtained using colorimetric methodologies and were determined on an Olympus 5400 series analyzer (Olympus Diagnostics, Dallas, TX, USA). Urine albumin concentrations were obtained by an immunoturbinimetric methodology using the Roche Cobas Integra analyzer (Roche Diagnostics, Indianapolis, IN, USA).

Statistical analyses

Sample size estimates were not performed since this was a pilot study. The primary outcome end point was the change from pre-infusion to each post-infusion time point (15, 30, 60, 120, and 180 min), for each treatment period, for total urine protein concentration/urine creatinine concentration. The secondary outcome end points were albumin/creatinine concentration ratio and NAG/creatinine concentration ratio. Each of these outcome end points was analyzed using a mixed models analysis of variance (Proc Mixed in SAS®). The model included terms for sequence, patient within sequence, period, study drug, post-infusion time point, and study drug-by-post-infusion time point interaction. Patient was treated as a random effect and all other factors were treated as fixed effects. In mathematical terms, the model can be expressed as follows:

$$Y_{ijklm} = \text{Mean} + S_i + P(S)_{j(i)} + R_k + I_l + T_m + \text{IT}_{lm} + \text{error}_{ijklm}$$

where, S_i represents the Sequence effect; $P(S)_{j(i)}$ represents the patient within sequence effect; R_k represents the period effect; I_l represents the study drug effect; T_m represents the post-infusion time points; IT_{lm} represents the study drug by time point interaction.

It was determined that the distribution of the random error associated with each outcome variable did not follow a normal distribution. As a consequence, all outcome end points were analyzed after performing a log transformation.

ACKNOWLEDGMENTS

This study was sponsored by Watson Laboratories, Inc. The study was designed by RA, ARR, and MOK. The sponsor monitored the clinical sites to ensure proper adherence to good clinical practices and proper collection of study data. MOK and RM recruited patients for the study. Statistical analyses were performed by JRT according to a pre-specified statistical analysis plan. RA interpreted the data and prepared this paper, which was reviewed and commented on by all the authors.

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