Intravenous Iron Sucrose Does Not Impair Sonographic Brachial Vasodilation in Peritoneal Dialysis Patients

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Serious concerns have been raised with respect to intravenous (IV) iron as a potential oxidative stress inducer in chronic kidney disease patients. Oxidative stress has been linked to uremia-related inflammation and endothelial dysfunction. Because IV iron promotes oxidative stress and because uremic patients have numerous defects of antioxidant defense unrelated to iron, we hypothesized that IV iron administration might increment oxidative stress and consequently endothelial dysfunction.

We undertook a pilot study of 8 patients from our peritoneal dialysis (PD) program who were in stable clinical condition. We measured high-sensitivity C-reactive protein (hsCRP), von Willebrand factor antigen (vWFa), and fibrinogen in serum, and several sonographic parameters: left ventricular ejection fraction, left ventricular mass index, carotid intima media thickness, and the presence of carotid plaques. We also used a sonographic methodology to measure endothelium-dependent vasodilation (EDV) and endothelium-independent vasodilation (EIV) in the brachial artery. Three hours after IV administration of 200 mg iron sucrose, we repeated the biochemical measurements and the sonographic vasodilation parameter measurements in the brachial artery.

None of the biochemical parameters were modified after administration of IV iron sucrose [hsCRP: <0.5 mg/L (range: <0.5 – 48 mg/L) vs. <0.5 mg/L (range: <0.5 – 37 mg/L), p *= 0.46; vWFa: 192% ± 39% vs. 189% ± 32%;* p *= 0.40; fibrinogen: 449 ± 127 mg/dL vs. 445 ± 128 mg/dL,* p *= 0.80). Furthermore, IV iron stimulus did not affect either EDV (5.8% ± 2.7% vs. 7.8% ± 1.9%,* p *= 0.09) or EIV (15.3% ± 2.9% vs. 21.4% ± 2.2%,* p *= 0.11).*

Our data do not support an acute impact of IV iron in our PD patients with regard to endothelial-related biochemical parameters or sonographic vasodilation of the brachial artery.

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Introduction

Intravenous (IV) iron and erythropoietic agents have both become a mainstay for the treatment of nephrogenic anemia. Nevertheless, serious concerns have been raised about a potential role for IV iron administration in oxidative stress both in patients undergoing chronic dialysis and in those with chronic kidney disease not yet in substitutive treatment (1–3).

After IV administration of iron sucrose, a rapid distribution into plasma binding proteins (primarily apotransferrin, and to a lesser extent, ferritin) occurs. *In vitro* observations suggest that iron dextran, iron gluconate, and iron sucrose may donate iron directly to transferrin, although the degree of donation varies with the agent and the chemical class (iron gluconate > iron sucrose > iron dextran) (4). The alpha elimination phase of an IV dose of iron sucrose is approximately 30 minutes, and its terminal half-life is 5 – 6 hours (4,5). Nevertheless, recent data have shown that parenteral iron formulations contain labile plasma iron, not bound to transferrin, that can potentially catalyze oxygen radical generation. Espósito *et al.* (6) showed that parenteral iron sucrose might induce long-term persistence (more than 2 hours) of low-level labile plasma iron in up to 20% of end-stage renal disease patients. Moreover, Rooyakkers *et al.* (7) showed an steady increment of non transferrin-bound iron levels for up to 4 hours in healthy volunteers.

How iron might participate in atherosclerosis and vascular damage has not been fully elucidated, but several potential mechanisms have been postulated: generation of oxidized low-density lipoprotein cholesterol, endothelial cell dysfunction, arterial smooth muscle proliferation, and ischemia-reperfusion injury (8). Available evidence supports the idea that the iron available to catalyze free radical reactions is more important than overall bodily iron status is (9,10).

Oxidative stress has been found to be involved in uremia-related inflammation and endothelial dysfunction (11), these being the factors that actively promote atherosclerosis (12). *In vivo* studies have shown that an early marker of atherosclerosis such as common carotid artery intima media thickness is associated with the annual IV iron dose administered in hemodialysis patients (13)—presumably among other factors, through an increment in oxidative stress that might promote the atherosclerosis process (8).

A few data in healthy volunteers have shown that IV ferric saccharate transiently diminishes endothelium-dependent vasodilation (EDV) as measured by vascular ultrasound (7), but data on chronic kidney disease patients are scarce. With regard to patients with coronary artery disease, iron chelation with deferoxamine improved endothelium-dependent NO vasodilation, suggesting that iron may contribute to endothelial dysfunction in patients with atherosclerosis (14).

Because IV iron promotes oxidative stress and because uremic patients have numerous defects of antioxidant defense unrelated to iron (11), we hypothesized that IV iron administration might increment oxidative stress and consequently endothelial dysfunction in such patients. To test this hypothesis, we designed a prospective pilot study in which endothelial function was measured ultrasonographically before and after IV iron administration in patients undergoing chronic peritoneal dialysis (PD).

Patients and methods

Study participants were recruited from the PD unit at our hospital. All had been receiving chronic PD for more than 3 months, were at least 18 years of age, and were clinically stable during the preceding 2 months, having neither experienced an important medical event in that time, nor required hospital admission. Patients using manual and automated techniques were equally eligible for selection, and no subject had a previous history of allergy or was intolerant to parenteral iron. We included 8 patients who met these criteria in the study.

The protocol was approved by the local ethics committee. Written informed consent was obtained from each subject before study entry.

Study design

Study subjects were requested to attend the PD unit early in the morning, in a fasting state from midnight. In addition, they were not allowed to smoke for 12 hours before the study started. At the start of the protocol, a polyethylene catheter was inserted in a peripheral vein of the forearm to obtain blood samples.

Each patient underwent the experimental protocol: A baseline blood sample was drawn to determine routine laboratory parameters. Additionally, high-sensitivity C-reactive protein (hsCRP) was measured to assess inflammatory status, von Willebrand factor antigen (vWFa) was determined as a biochemical parameter of endothelial injury and function, and fibrinogen was measured as a marker of coagulation status. These three parameters were selected because, in each case, available evidence reveals an early increment after an insult (15–17). We used the homeostasis model assessment–insulin resistance index [HOMA-IR: fasting serum glucose (mmol/L) / serum basal insulin (μU/mL) / 22.5] to analyze the percentage of patients who were insulin-resistant (18).

Each patient then received a baseline ultrasonographic heart and carotid artery assessment to define the characteristics of the population. We measured left ventricular ejection fraction [LVEF (%)] and left ventricular mass index [LVMI (g/m^2)]. We also measured the carotid intima media wall thickness [IMT (mm)] and carotid plaques in the right common carotid artery. The study was performed using high-resolution B-mode ultrasound (Sonos 5500: Hewlett Packard, McMinnville, OR, U.S.A.), with a 10-MHz linear transducer. Patients were examined in the supine position with neck extended and chin turned contralateral to the side being examined. Measurement of IMT was assessed in the common carotid artery 1 cm distal to the carotid bifurcation in the posterior wall (19). Scanning involved examination of the right common carotid artery in the longitudinal and transverse planes. All measurements were made manually on digitized still images obtained during ultrasound scanning.

The study of the right brachial artery in a longitudinal section 2 – 12 cm proximal to the antecubital

fossa in supine subjects was performed by B-mode scan using a precision mechanical arm and a 7.5-MHz phased-array transducer on the Sonos 5500. The anterior and posterior intima media interfaces were used to define artery diameter. The diameter was calculated as the average of measurements during 4 cardiac cycles at end diastole.

Diameter of the brachial artery was measured in basal conditions and EDV was analyzed by the percentage of vasodilation obtained after a 5-minute ischemic stimulus, obtained with a cuff in the same arm inflated to a pressure of 50 mmHg above resting systolic blood pressure for 5 minutes. Pressure release resulted in reactive hyperemia, which is the stimulus for flow-mediated EDV. A scan for the brachial artery was performed 1 minute after cuff deflation. If an active arteriovenous fistula was present, the contralateral arm was selected. A resting time of 10 minutes was allowed for recovery of the vessel. Then, the endothelium-independent vasodilatation (EIV) was analyzed by the percentage of vasodilation obtained 4 minutes after 400 μg of sublingual nitroglycerin was given (20).

After that, 200 mg iron sucrose (Venofer: Altana Pharma AG, Konstanz, Germany) diluted in 100 mL NaCl 0.9% was administered over 30 minutes. The patients were then permitted to ingest a small breakfast.

Finally, after a waiting period of 3 hours from the infusion of IV iron, tests for serum hsCRP, vWFa, and fibrinogen, and brachial artery ultrasonographic tests were repeated.

Laboratory analysis of inflammatory and endothelial function markers

Serum hsCRP was measured by turbidimetric immunoassay (Dade Behring, Newark, NJ, U.S.A.). Serum vWFa was measured by an automated latex-enhanced immunoassay (HemosIL: Instrumental Laboratory, Milano, Italy). Serum fibrinogen levels were measured by an *in vitro* test (fibrinogen reagent: Diagnostica Stago, Roche, Mannheim, Germany).

Statistical analysis

Because of the small number of study patients, we performed a nonparametric rank-sum Wilcoxon test to compare parameters before and after the experimental stimulus. Values are expressed as mean ± standard deviation or as median and range if they were not normally distributed. All p values are two sided, with significance set at <0.05. All analyses were performed using SPSS 10.0 for Windows (SPSS, Chicago, IL, U.S.A.).

Results

Table I shows the general characteristics of the 8 patients (6 on continuous cycling PD, 2 on continuous ambulatory PD). The cause of the basal nephropathy was unknown in 3 patients, adult polycystic kidney disease in 3, Henoch–Schönlein purpura in 1, and diabetic nephropathy in 1. Four patients (50%) were receiving an angiotensin converting-enzyme inhibitor or an angiotensin ii receptor blocker at the time of the study, and 7 patients (88%) were also receiving erythropoietic agents.

Table I also shows the main biochemical parameters for our patients. Weekly Kt/V urea was 2.5 ± 0.5; weekly creatinine clearance, 88 ± 34 L/1.73 m^2; and daily normalized equivalent of protein nitrogen appearance, 1.2 ± 0.4 g/kg. Interestingly, apart from the 1 patient who suffered from diabetes mellitus, 5 of the remaining 7 patients (71%) were insulin resistant according to HOMA-IR (>2.5). Ultrasonographic parameters showed a mean LVEF of 64% (range: 34% – 67%), a mean LVMI of 112 ± 26 g/m^2, and a IMT of 0.74 ± 0.16 mm. Four patients (50%) presented carotid atherosclerotic plaques. Five patients (63%) presented target organ damage as measured by an IMT of 0.9 mm or more, the presence of an atherosclerotic plaque, or the finding of left ventricular hypertrophy

TABLE I General characteristics of the patients and baseline biochemical parameters

Patients (n)	8
Age (years)	55.6±18.0
Sex ratio (men:women)	4:4
Diabetes (with/without)	1/7
BMI (kg/m^2)	24.7±5.2
PD duration (months)	18 (3–75)[a]
Average daily UF (mL)	947±282
Daily urinary volume (mL)	519±446
Hemoglobin (g/L)	13.1±1.8
Ferritin (mg/dL)	557±441
Bicarbonate (mmol/L)	26.8±1.9
Calcium (mmol/L)	2.4±0.2
Phosphate (mmol/L)	1.7±0.4
iPTH (pg/mL)	309±149
Lipoprotein a (mg/dL)	28±22
Homocysteine (μmol/L)	20.7±10.0

[a] Median (range).

BMI = body mass index; PD = peritoneal dialysis; UF = ultrafiltration; iPTH = intact parathyroid hormone.

(LVMI ≥ 125 g/m^2 in men or ≥ 110 g/m^2 in women) according to the guidelines from the European Society of Hypertension and the European Society of Cardiology (21). Only 1 patient presented a moderately depressed LVEF because of dilated cardiomyopathy.

Table II shows the results of the biochemical serum endothelial and inflammatory parameters. As can be seen, none of the biochemical parameters changed after the IV iron sucrose stimulus. Furthermore, Figure 1 shows the percentage brachial artery dilation after ischemic stimulus (EDV): 5.8% ± 2.7% baseline as compared with 7.8% ± 1.9% after IV iron administration (p = 0.09). At baseline after nitroglycerin administration, patients showed a percentage brachial artery vasodilation (EIV) of 15.3% ± 2.9%, and after IV iron administration, of 21.4% ± 2.2% (p = 0.14). Consequently, neither EDV nor EIV were observed to be significantly modified after IV iron stimulus in our PD patients. Baseline percentage brachial EDV was significantly lower in the study patients than in a healthy age and sex-matched population from our area (9.2% ± 4.0%, p = 0.02), but data regarding brachial EIV were not significantly different (14.9% ± 6.0%, p = 0.11).

Discussion

Our data do not support an acute effect of IV iron sucrose in our PD patients on either biochemical endothelial-related parameters or vasodilatory brachial artery properties as measured by sonography.

Assessment of vasodilatory properties of arteries is often used as a marker of endothelial function. Brachial artery ultrasound imaging during reactive hyperemia is a clinical method for quantifying endothelium-dependent vasomotion and establishing the presence of endothelial dysfunction (20). Blood vessels dilate in response to shear stress after an ischemic stimulus, principally regulated by release of NO from endothelium. Lowered EDV has previously been described in dialysis patients (22), which accords with the baseline values observed in our patients. Further decrement of EDV after an IV iron stimulus was not observed, a finding that could be brought about by a lack of additional inhibition of NO mediated by iron.

Because iron is a factor implicated in the generation of reactive oxygen species (ROS) and because findings in animal models of chronic renal failure suggest that increment in ROS leads to decreased NO bioavailability and endothelial dysfunction, which might be improved by antioxidant pretreatment (23,24), our findings might be explained by the efficacy of antioxidant systems to counterbalance the pro-oxidant action of parenteral iron.

Previous *in vivo* data reveal that, after administration of 100 mg ferric saccharate, healthy volunteers experienced a transient but significant reduction of flow-mediated dilatation 10 minutes after infusion (7). The discrepancy between that result and the data in our PD patients might be explained by differences in study design: different populations studied, time of

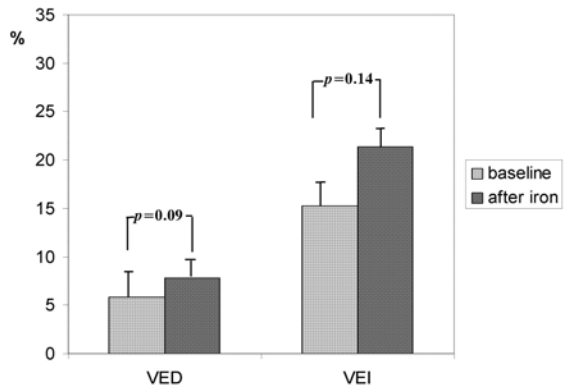

FIGURE 1 Percentage of sonographic brachial artery vasodilation, both endothelium-dependent (VED) and -independent (VEI), at baseline and after intravenous iron sucrose stimulus.

TABLE II Serum biochemical inflammatory and endothelial parameters before and after intravenous (IV) iron sucrose stimulus

Parameter	*Baseline*	*After IV iron sucrose*	p *Value*[a]
C-Reactive protein (mg/L)	<0.5 (<0.5 to 48)[b]	<0.5 (<0.5 to 37)[b]	0.46
von Willebrand factor antigen (%)	192±39	189±32	0.40
Fibrinogen (mg/dL)	449±127	445±128	0.80

[a] Wilcoxon test.
[b] Median (range).

assessment of vessel dilatory properties and biochemical data (3 hours vs. 10 minutes after infusion), or the type and quantity of iron employed (200 mg IV iron sucrose vs. 100 mg ferric saccharate). Nevertheless, a recent placebo-controlled double-blind randomized study undertaken in PD patients who received 300 mg IV iron sucrose did not find impairment of vascular reactivity during the infusion period as measured by strain-gauge plethysmography, although a significant increase was observed in non transferrin-bound and redox-active iron (25). Our findings agree with those data.

In vascular disorders, von Willebrand factor (wWF) is frequently used as an indicator of endothelial cell dysfunction (26,27). Studies undertaken with several platelet secretagogues have shown a optimum vWF release from endothelial cells within 15 minutes and 4 hours (17). Data from dialysis patients showed an increased level of serum vWF (28,29), which agrees with our baseline findings. Administration of IV iron sucrose did not further increment vWFa levels, which does not support a finding of acute endothelial injury. Our study shows a similar result with regard to serum fibrinogen, because the available evidence supports an early rise of vWFa in the hours following an acute clinical event (16). Nevertheless, concerns may arise in our study with respect to an inflammatory marker such as CRP. Although several publications showed an early increase of serum CRP after several stimuli as soon as 4 – 6 hours after the start of the event (30,31) and also as soon as 2 hours in experiments undertaken in mice with injection of leukocyte endogenous mediators (15), the period of 3 hours after the iron stimulus in our study may not have been delayed enough to reveal a modification in serum CRP.

This pilot study has some limitations. First, the small number of patients recruited might have produced a type II statistical error; however, we did not find a trend toward statistical significance. Second, we did not investigate the effect of iron during the infusion nor during the following minutes, and so we cannot discount a transient impairment of vasodilatory vessel properties. Finally, a more delayed action of repeated doses of parenteral iron sucrose on endothelial function remains a possibility.

Conclusions

Our findings do not support an acute effect of IV iron sucrose, in doses currently employed in PD patients, as assessed by short-term biochemical markers of endothelial function and by sonographic brachial artery vasodilatory properties. We cannot rule out a protracted action of repeated doses of IV iron on endothelial function in this population.

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