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Dose-dependent effect of parenteral iron therapy on bleomycin-detectable iron in immune apheresis patients

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Background. Iron deficiency and anemia are commonly encountered in patients with autoimmune diseases undergoing immune apheresis. This makes erythropoietin and iron substitution necessary in most patients. However, intravenous iron therapy may result in an increase of potentially toxic nontransferrin-bound iron.

Methods. We examined the effect of 50 mg or 100 mg of iron (III) sucrose on bleomycin-detectable iron (BDI) in immune apheresis patients. Six patients with autoimmune disorders and normal kidney function were enrolled. Before and after the injection of 50 mg or 100 mg of iron (III) sucrose, BDI was measured in serum samples at five different time points.

Results. There was no BDI traceable before injection of iron (III) sucrose. BDI was present in serum of all patients after the administration of 100 mg of iron (III) sucrose in concentrations up to 0.49 μmol/L. In contrast, only one patient showed BDI at a concentration of 0.16 μmol/L after the administration of 50 mg of iron (III) sucrose.

Conclusion. We conclude that if parenteral iron is administered after apheresis treatment, despite the equal tolerability, use of 50 mg of iron (III) sucrose is superior to 100 mg of iron (III) sucrose in avoiding the formation of potentially toxic nontransferrin-bound iron.

Immune apheresis is an extracorporeal therapy critical in the management of disorders caused by autoantibodies. An apheresis treatment is a modified plasmapheresis that conveys a specific adsorption of human immunoglobulin molecules for efficient clearance of circulating autoantibodies. Thus far, this method has been successfully used in humoral kidney graft rejection, systemic lupus erythematosus, anti-phospholipid antibody syndrome, multifocal motor neuropathy, paraneoplastic pemphigus, Goodpasture syndrome, myasthenia gravis, Basedow-Graves disease, elimination of antibodies against several factors of the hemostasis system, systemic connective tissue disease, and primary vasculitis [1–8].

Iron deficiency and anemia are commonly encountered in patients with autoimmune diseases undergoing immune apheresis, even if the kidney function is normal. Currently, 22 of 24 patients in our apheresis program present with iron deficiency and/or anemia. To date, the underlying mechanisms are not completely understood. Plausible explanations for the anemia include: anemia of chronic disease [9]; the inhibitory effect of immunosuppressive therapy on erythropoiesis [10]; binding of iron to citrate [11] used for anticoagulation during apheresis; or frequent blood sampling. Although immune apheresis was invented to selectively remove antibodies, there is still loss of other plasma proteins with iron-binding capacity. The magnitude of this loss has been stated controversially in the literature. Albumin losses vary from less than 10% to 25% [12–14]. A decrease in other plasma proteins was not detectable, or was 14% per apheresis treatment [12, 14]. This protein loss may also contribute to the iron deficiency observed in patients undergoing immune apheresis treatment.

In order to counteract the iron deficiency leading to anemia and related morbidity, especially cardiovascular complications such as left ventricular hypertrophy [15], routine treatment for patients in the apheresis program at our department includes erythropoietin and intravenous iron substitution. But iron therapy, although indicated, carries potential risks [16]. The first to mention are acute toxic effects such as back pain, nausea, vomiting, hypotension, and allergic or anaphylactic reactions. Anaphylaxis is caused by the dextran component of iron dextran and practically not seen with current iron preparations, iron sucrose, or iron gluconate [17, 18]. In addition, there are studies suggesting a possible relationship between iron

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and infectious [19, 20] or atherosclerotic [21, 22] complications. This is, however, still a controversy because to date epidemiologic studies failed to demonstrate such a correlation [23–25].

It is believed that in vivo iron toxicity appears when transferrin can no longer scavenge the excess of inorganic iron. This occurs in states of iron overload, such as hemochromatosis [26], thalassemia [27], or introduction of large amounts of iron into the circulation, as well as low transferrin levels [28]. All of these factors can lead to a nontransferrin-bound labile iron ion fraction. This labile iron fraction is commonly measured as bleomycin-detectable iron (BDI), and relies on the degradation of DNA by bleomycin in the presence of nontransferrin-bound iron (NTBI) ions. It is conceivable that patients undergoing immune apheresis are at higher risk to be exposed to higher concentrations of labile iron after iron substitutions because apheresis causes a state of hypotransferrinemia resulting from the protein loss. Although harmful effects of NTBI have not been examined in epidemiologic studies and therefore not proven, there are many good reasons to protect patients from exposure to these labile iron ions. Noteworthy are endothelial dysfunction [29], leukocyte dysfunction [30–32], loss of the ability of patient serum to resist the growth of *Staphylococcus epidermidis* [28], or formation of reactive oxygen species [33]. These toxic oxygen radicals lead to depolymerization of polysaccharides, breakages in DNA strands, or lipid peroxidation, with consequential injury and death of the cell [34].

A common dose of parenteral iron (III) sucrose for therapy of iron deficiency is 100 mg, but other doses are used as well [17, 35–37]. BDI was detected after injection of 100 mg of iron (III) sucrose in healthy individuals [29] or in patients with renal disease [38]. However, it is unknown whether administration of lower doses of iron (III) sucrose has a similar effect in inducing BDI in patients treated for iron deficiency. In this study we examined the effect of 50 mg or 100 mg of intravenous iron (III) sucrose on BDI in patients undergoing immune apheresis treatment.

METHODS

Hypothesis

We hypothesized that intravenous administration of iron (III) sucrose at a dose of 100 mg of elemental iron results in a more pronounced generation of serum BDI compared with injection of 50 mg of iron (III) sucrose.

Participants

We included immune apheresis patients treated at the apheresis unit of the Department of Medicine III at the University of Vienna Medical School. Eligibility criteria for this study were iron deficiency defined as a serum ferritin <18 μg/L in men or <30 μg/L in women, or a transferrin saturation <16%, and normal serum creatinine. The exclusion criteria comprised pregnancy or lactation, hemochromatosis, and hemolytic anemia.

The study was carried out in accordance with the European Union Good Clinical Practice (EU-GCP) guideline, the Austrian Arzneimittelgesetz 1993, and the Declaration of Helsinki (1964), including current revisions. The Ethics Committee of the University of Vienna Medical School approved the study protocol. All patients gave written informed consent before participation.

Study design and interventions

This study was a single-center, controlled trial. Patients were enrolled to receive 50 mg or 100 mg of iron (III) sucrose (Venofer®, Vifor International, St. Gallen, Switzerland) intravenously at the end of two immune apheresis treatments (each treatment consisted of two immune apheresis sessions on two days) that were performed according to the standard procedure at our hospital as previously described [39]. Patients received 50 mg of iron (III) sucrose after the first treatment and 100 mg of iron (III) sucrose after the second treatment.

Blood was drawn before apheresis session on day 1 ("pre-apheresis"), and after the second session on day 2 before the iron (III) sucrose injection ("zero"), as well as 5 minutes, 30 minutes, and 24 hours after iron (III) sucrose injection. Subjects were discharged thereafter. We used washout periods of 2 to 4 weeks between the study days (on average 23 days). The same protocol was repeated with the second treatment.

Outcomes

The primary outcome was defined as the maximal increase of serum BDI after injection of iron (III) sucrose. A less pronounced increase to <33% of BDI after injection of low versus high dose iron (III) sucrose therapy was regarded as a clinically relevant effect.

Secondary outcome variables included serum iron levels, serum transferrin levels, transferrin saturation, serum ferritin levels, and serum albumin concentrations.

Sample size

An increase of nontransferrin bound iron from 0.03 up to 0.29 μmol/L was demonstrated after a single-dose administration of 100 mg of iron (III) sucrose in 12 hemodialysis patients [28]. Based on the assumption that a lower iron (III) sucrose dose would result in a 66% lower level of nontransferrin-bound iron compared with the higher dose of iron (III) sucrose, six individuals were necessary at an alpha of 0.05 and a power of 80%.

Laboratory analyses

Blood chemistry and complete blood counts were determined by standard methods in an International Organization for Standardization (ISO) 9001:2000 certified clinical laboratory at the University Hospital of Vienna. For analysis of BDI serum was kept in plastic, trace element–free tubes at −20°C. In each blood sample BDI as well as serum iron (reference range 40 to 150 μg/dL), ferritin (reference range 10 to 250 μg/L), transferrin (reference range 200 to 360 mg/dL), transferrin saturation (reference range 16% to 45%), and albumin (reference range 35 to 55 g/L) were determined. For serum iron determination the FerroZine method (Boehringer Mannheim, Mannheim, Germany) was used. The test principle is based on the release of Fe^{3+} from transferrin resulting from guanidiumchloride in an environment with a pH of 5.0. Ascorbic acid in the buffer provided reduces this iron to Fe^{2+}, which builds a chromogenic complex with FerroZine reagent, quantified using Hitachi 747 equipment.

Transferrin saturation was calculated using the following equation: transferrin saturation (%) = (iron in serum (μg/dL)/transferrin in serum (mg/dL) × 71.

BDI was measured as described by Gutteridge et al with some modifications [40]. Calf thymus DNA (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in water to a concentration of 0.1 mol/L at room temperature, and was then kept at 4°C overnight. Bleomycin sulfate (Lundbeck, Copenhagen, Denmark) was diluted in water to a final concentration of 1.5 U/mL. A Tris-HCl buffer solution with a pH of 7.4 was prepared (Merck, Whitehouse Station, NJ, USA). A stock solution of ascorbic acid (Merck) in water (0.7 g in 10 mL) was freshly prepared before each experiment. Atomic absorption spectroscopy grade iron (III) nitrate in nitric acid (Merck) was prepared and diluted to 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 μmol/L solutions to generate a standard curve. All reagents, except bleomycin sulfate and iron (III) nitrate, were treated overnight with Chelex resin (Bio-Rad, Hercules, CA, USA) (300 mg per 10 mL solution) to remove any possible iron contamination. The following reagents were pipetted into each of the standard or sample tubes in the following order: 0.5 mL DNA (1 mg/mL), 0.05 mL bleomycin (1.5 U/mL), 0.1 mL $MgCl_2$ (50 mmol/L; Merck), 0.05 mL Tris (1 mol/L), 0.1 mL ascorbic acid (0.75 mmol/L), 0.15 mL water, 0.05 mL standard, or sample.

pH was adjusted to 7.4 to 7.8. All tubes were incubated at 37°C for 1 hour and the reaction was terminated with 0.1 mol/L EDTA (Sigma-Aldrich). A half milliliter of HCl 25% and 0.5 mL of thiobarbituric acid (TBA) (1% w/v in 50 mmol/L NaOH; Merck) were added. All tubes were then kept at 80°C for 20 minutes, and cooled down with 3 mL of n-buthyl alcohol (Merck). Finally, tubes were centrifuged for 20 minutes at 2300*g*, and from each tube 0.1 mL was transferred into a 96-well plate (Costar, Cambridge, MA, USA) in duplicates. The chromogenic reaction was measured with a fluorescent reader (CytoFluor Fluorescence Measurement System 2300; Millipore, Billerica, MA, USA) using CytoCalc Software (version 3.0; PerSeptive Biosystems, Framingham, MA, USA). The filters were set to 590 ± 35 nm for emission, and 530 ± 25 nm for excitation. The standard curve showed a linear increase in BDI up to 3 μmol/L and started to come to a plateau at 5 μmol/L.

Table 1. Characteristics of enrolled patients ($N = 6$, mean ± SD)

Age *years*	42.0 ± 7.8
Apheresis therapy prior to the study *months*	7.5 ± 5.4
Apheresis sessions prior to the study *N*	44.5 ± 22.6
Serum creatinine *mg/dL*	0.78 ± 0.07
Weekly s.c. erythropoietin dose *IU*	5833 ± 2500
Hemoglobin at the beginning of the study *g/dL*	10.2 ± 1.6

GFR, glomerular filtration rate; s.c., subcutaneous.

Statistical methods

Chi-square tests and comparison of dependent samples (Student *t* test) for continuous variables were performed using Statistica for Windows 5.1 (Stat Soft, Inc., Tulsa, OK, USA).

Safety

Patients were observed during the entire therapy session by specially trained apheresis nurses. A physician examined each patient at every apheresis session. Side effects and symptoms were documented on a standardized treatment chart.

RESULTS

Patients

Six female patients were eligible and enrolled to the study. Baseline demographic and clinical data of all patients enrolled are shown in Table 1. All had an autoimmune disorder (3 endocrine orbitopathies and 3 myasthenia gravis) that was resistant to conventional therapy. One out of six patients was under immunosuppressive therapy with azathioprine at the time of the study.

Bleomycin-detectable iron

We found no BDI before apheresis or at the time of injection of iron (III) sucrose ("pre-apheresis" and "zero" time points). We detected BDI in concentrations up to 0.49 μmol/L in serum samples of all patients (9 of the 18 measurements) after the injection of 100 mg of iron (III) sucrose (Fig. 1). In contrast, after the injection of 50 mg of iron (III) sucrose, BDI was found only in one patient (1 of

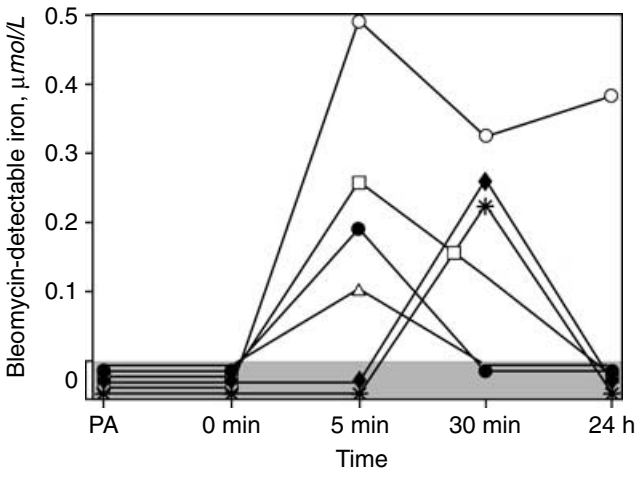

Fig. 1. Course of BDI after injection of 100 mg of iron (III) sucrose in 6 patients [preapheresis (PA), 0 minutes, 5 minutes, 30 minutes, and 24 hours].

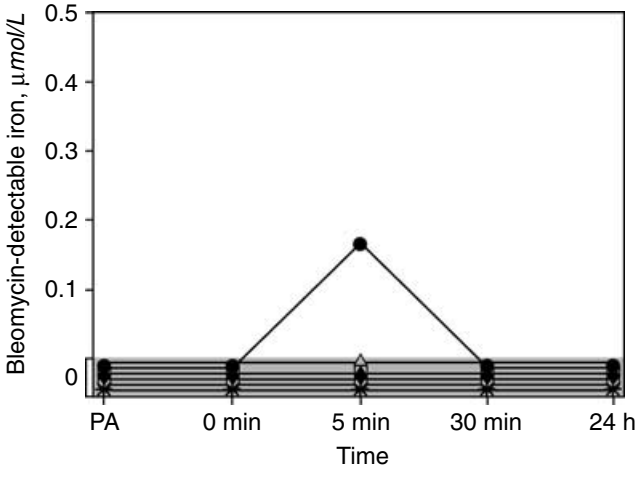

Fig. 2. Course of BDI after injection of 50 mg of iron (III) sucrose in 6 patients [preapheresis (PA), 0 minutes, 5 minutes, 30 minutes, and 24 hours].

the 18 measurements) at a concentration of 0.16 µmol/L (Fig. 2) (χ^2 test: $P = 0.003$).

Serum iron

Serum iron increased in all patients rapidly in the time interval from 5 minutes to 30 minutes (Tables 2 and 3). Although a plateau was reached and a slight decline was observed around 30 minutes, iron levels remained significantly elevated in the interval from 30 minutes to 24 hours. The increase in all time points (5 minutes, 30 minutes, and 24 hours) after injection of iron (III) sucrose was significant when compared to preapheresis values (50 mg group: $P < 0.05$, $P < 0.01$, $P < 0.0001$, respectively, and 100 mg group: $P < 0.005$, $P < 0.0001$, $P < 0.0001$, respectively). There was no difference between preapheresis and zero-time point levels. The increase in serum iron was more prominent after the application of 100 mg of iron (III) sucrose compared to 50 mg of iron (III) sucrose at 5 minutes, at 30 minutes, as well as at 24-hour time points ($P < 0.005$, $P < 0.003$, $P < 0.05$, respectively).

Serum transferrin

Serum transferrin levels decreased immediately after the apheresis treatment and reached the initial level approximately 24 hours later (Tables 2 and 3). The comparison of preapheresis values with 0, 5-minute, 30-minute time points showed a decrease (50 mg group: $P < 0.0001$, $P < 0.0001$, $P < 0.005$, respectively, and 100 mg group: $P < 0.0005$, $P < 0.0005$, $P < 0.001$, respectively). We did not observe a difference between the 50 mg and the 100 mg iron (III) sucrose groups regarding transferrin levels.

Transferrin saturation

Transferrin saturation increased after the iron injection in both the 50 mg and the 100 mg group until 30 minutes and then started to decline, but did not reach the pretreatment values (Tables 2 and 3). The comparison of preapheresis levels with each of the time points 0, 5 minutes, 30 minutes, 24 hours revealed significant increases in each group (50 mg group: $P < 0.005$, $P < 0.0005$, $P < 0.0001$, $P < 0.03$, respectively, and 100 mg group: $P < 0.03$, $P < 0.003$, $P < 0.0005$, $P < 0.03$, respectively). There was no difference in comparison of 50 mg versus 100 mg except at the 30-minute time point ($43.4 \pm 14.0\%$ vs. $64.7 \pm 24.2\%$, $P < 0.01$).

Serum ferritin

Ferritin levels increased only 24 hours after the injection of iron in the 50 mg group from a preapheresis level of 12.2 ± 9.41 µg/L to 53.9 ± 17.0 µg/L ($P < 0.03$), and in the 100 mg group from a preapheresis level of 9.17 ± 6.96 µg/L to 103.5 ± 41.8 µg/L ($P < 0.001$) (Tables 2 and 3). This increase was more pronounced after injection of 100 mg of iron (III) sucrose compared with 50 mg ($P < 0.02$).

Serum albumin

After apheresis treatment, serum albumin levels decreased immediately and reached the initial level approximately 24 hours later (Tables 2 and 3). The comparison of preapheresis values with 0, 5-minute, 30-minute time points showed statistically significant decreases (50 mg group: $P < 0.0001$, $P < 0.0001$, $P < 0.001$, respectively, and 100 mg group: $P < 0.0005$, $P < 0.0005$, $P < 0.0005$, respectively). The decrease in serum albumin level was not different between the 50 mg and the 100 mg iron (III) sucrose groups.

Table 2. Time course of iron metabolism parameters before and after the application of 50 mg of iron (III) sucrose ($N = 6$, mean ± SD)

	Preapheresis	Zero	5 min	30 min	24 h
Serum iron μg/dL	27.3 ± 15.8	52.5 ± 27.6	133.3 ± 32.9[a]	129.7 ± 34.1[b]	110.3 ± 52.8[c]
Transferrin mg/dL	289.3 ± 44.1	195.3 ± 24.8[c]	194.7 ± 32.2[c]	206.2 ± 19.6[d]	291.3 ± 39.5
Transferrin saturation %	7.4 ± 5.4	20.0 ± 12.4[d]	50.9 ± 19.5[e]	45.2 ± 14.4[c]	27.5 ± 13.0[f]
Ferritin μg/L	13.0 ± 10.0	8.7 ± 7.6	8.9 ± 8.3	9.6 ± 9.8	55.9 ± 17.6[f]
Serum albumin g/L	41.6 ± 4.1	34.0 ± 3.4[c]	34.9 ± 3.3[c]	36.9 ± 3.6[g]	45.8 ± 4.6

Preapheresis, 24 hours prior to the immune apheresis session; zero, Immediately after the immune apheresis session and immediately before the iron injection; 5 min, Five minutes after the iron injection; 30 min, Thirty minutes after the iron injection; 24 h, twenty-four hours after the iron injection.
[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.0001$, [d] $P < 0.005$, [e] $P < 0.0005$, [f] $P < 0.03$, [g] $P < 0.001$. All P values are from comparison to preapheresis.

Table 3. Time course of iron metabolism parameters before and after the application of 100 mg of iron (III) sucrose ($N = 6$, mean ± SD)

	Preapheresis	Zero	5 min	30 min	24 h
Serum iron μg/dL	40.3 ± 29.4	63.2 ± 44.2	242.2 ± 161.5[a]	195.2 ± 60.1[b]	203.7 ± 136.2[b]
Transferrin mg/dL	285.0 ± 17.4	208.3 ± 15.0[c]	206.3 ± 17.5[c]	212.3 ± 19.0[d]	297.5 ± 38.1
Transferrin saturation %	10.1 ± 7.5	22.0 ± 16.8[e]	86.3 ± 60.3[f]	66.8 ± 25.7[c]	50.8 ± 35.6[e]
Ferritin μg/L	9.7 ± 7.49	8.1 ± 4.9	8.0 ± 4.9	8.4 ± 4.86	110.6 ± 40.9[d]
Serum albumin g/L	43.3 ± 5.5	34.2 ± 2.4[c]	33.6 ± 2.6[c]	35.2 ± 2.6[c]	43.6 ± 3.6

Preapheresis, 24 hours prior to the immune apheresis session; zero, Immediately after the immune apheresis session and immediately before the iron injection; 5 min, Five minutes after the iron injection; 30 min, Thirty minutes after the iron injection; 24 h, twenty-four hours after the iron injection.
[a] $P < 0.05$, [b] $P < 0.0001$, [c] $P < 0.0005$, [d] $P < 0.001$, [e] $P < 0.03$, [f] $P < 0.003$. All P values are from comparison to preapheresis.

Side effects of intravenous iron therapy

Both dosages of iron (III) sucrose (50 and 100 mg) were injected within 5 minutes because it was reported to be safe [41]. None of the possible acute side effects of intravenous iron (III) sucrose [42] were encountered in any of the patients during or after the drug administration.

DISCUSSION

We showed that parenteral administration of 100 mg of iron (III) sucrose resulted in an increase of nontransferrin-bound iron, measured as bleomycin-detectable iron, whereas 50 mg of iron (III) sucrose had no major effect on BDI in immune apheresis patients.

Bleomycin is a glycopeptide that binds to DNA and causes single- and double-strand breakages. In addition, degradation of deoxyribose occurs. One of the final metabolites of this degradation is malondialdehyde, which reacts with thiobarbituric acid (TBA) to give a pink chromogen. Thus, the TBA test can be used to follow DNA degradation by bleomycin. The DNA degradation has an absolute requirement for certain transition metal ions such as iron. Therefore, chromogen development is proportional to iron concentration. This test is established to measure the availability of iron complexes that can catalyze free radical reactions such as formation of OH^- from H_2O_2. One of the key issues is that bleomycin cannot remove iron from its binding proteins (transferrin, ferritin, lactoferrin, hemoglobin, and others) in a pH range of 7.4 to 7.8. Bleomycin-detectable iron represents mostly nontransferrin bound, and thus, catalytic iron, with the potential to form free hydroxyl radicals. These are involved in depolymerization of polysaccharides and breakages in DNA strands, which can lead to dysfunction, injury, and death of the cell [34]. Atherogenic factors such as cholesterol oxidation products, oxysterols, or lipid peroxidation were also linked to pro-oxidative properties of iron [34, 43]. An increased abundance of the reactive oxygen species was demonstrated after nutritional iron overload in rats [44]. Appearance of BDI after "oversaturation" of transferrin was shown in various patient groups, including hematologic patients undergoing high-dose chemotherapy or stem cell transplantation, and some patients with septic shock [45–47]. An increase of nontransferrin-bound iron was also reported for hemodialysis patients who received 100 mg of iron (III) sucrose [38].

Although widely discussed and believed, the long-term consequences of iron therapy are an ongoing controversy. Two studies published by Hoen et al [24, 25] failed to demonstrate a correlation between iron therapy and an increased risk for infection. However, at the same time there are numerous in vivo and in vitro data suggesting a role of iron in infectious complications caused by leukocyte dysfunction or iron's effect on bacterial growth [28, 30–32, 48]. It was previously suggested that fever promotes resistance to infection by lowering the concentration of iron in serum [49], but this could be solely caused by a negative acute-phase response. Similarly, there are convincing data both showing no correlation between iron and atherosclerosis [23, 50–53], as well as demonstrating such a link [54–57]. Possible reasons for these conflicting results and potential problems with epidemiologic studies were reviewed previously [21, 22]. It is known that in a multifactorial disease like atherosclerosis it is extremely difficult to demonstrate the importance of a

single risk factor. In the case of iron's role in infection or atherosclerosis, it is even more challenging because of the different forms of iron. There is not a single epidemiologic study examining the role of NTBI in that matter, although the availability of catalytic iron, rather than the overall iron status, might be the factor causing the risk. Two studies with small patient numbers that measured NTBI in serum of patients with iron overload demonstrated oxygen radical stress and endothelial dysfunction [29], as well as a diminished resistance to bacterial growth [28]. Gaenzer et al described endothelial dysfunction leading to an increased intima-media thickness of the carotid artery in hemochromatosis patients [55], while de Valk et al [58] detected NTBI in patients with this disease, supporting the link between NTBI and endothelial dysfunction.

One reason for the lack of large epidemiologic studies so far might be the elaborate methods for detection of NTBI, such as the bleomycin procedure described in this paper. A new method with the potential to overcome this problem has been developed [59]. There are already data from patients with thalassemia demonstrating redox activity of labile iron using this one-step fluorescence based method [60]. Epidemiologic studies incorporating NTBI levels in patients undergoing iron therapy with long follow-up periods would finally provide us with conclusive data about iron's role in infection, atherosclerosis, and mortality.

Even though the potential risks stemming from the use of iron as a therapeutic agent are significant, based on the long-term consequences of anemia, iron therapy is indispensable. It was shown that hemodialysis patients with hematocrit levels over 36% had significantly lower hospitalization rates compared with patients with hematocrit levels between 33% and 36% [61]. Anemia was shown as one of the two major predictors of left ventricular growth, which in turn is a predictor of death and heart failure in patients with renal disease [15]. We counteract iron deficiency and anemia in our apheresis patients with intravenous iron instead of oral substitution, although they are not uremic, and thus, have a normal gastrointestinal iron absorption capacity because it is the most efficient way to fill the iron stores. This is convenient in apheresis patients because of the readily available venous access.

Our results showed that 50 mg and 100 mg of intravenous iron (III) sucrose normalized the ferritin levels after 24 hours of administration. One hundred mg of iron (III) sucrose was more efficient in increasing serum iron levels, filling the iron storages, and producing significantly higher ferritin concentrations than 50 mg of iron (III) sucrose. In all patients the immune apheresis treatment itself caused an immediate decline in serum transferrin and serum albumin levels, as detected directly after apheresis session. However, serum iron and BDI did not increase during apheresis session. When the proteins with iron-binding properties were at their minimum (between zero and 5-minute time points), 50 or 100 mg of iron (III) sucrose was administered. Consequently, transferrin saturation increased rapidly after the slow intravenous injection of iron, and reached the peak levels in the interval between 5 minutes and 30 minutes after the injection. The increase in transferrin saturation was significantly higher after intravenous administration of 100 mg of iron (III) sucrose compared with 50 mg. In parallel to the increase of transferrin saturation, BDI appeared after administration of 100 mg of iron (III) sucrose in all patients at various time points. In contrast, only one patient presented with a BDI level at one time point after injection of 50 mg of iron.

Of interest, no patient experienced adverse side effects, regardless of the dose of iron sucrose or evidence of the presence or absence of NTBI. These findings suggest that at the detected levels, NTBI either lacks clinical significance or poses risks that lack symptoms. If the latter is the case, serum iron and therefore serum transferrin saturation may not be reliable predictors of NTBI early after intravenous iron administration.

The abrupt rise of serum iron after intravenous iron sucrose (Tables 2 and 3) should be interpreted with caution because the presence of intravenous iron agent in serum may falsely elevate the reported serum iron using the ferrozine-ascorbate method [62].

Our studies are the first to demonstrate a dose-response relationship between intravenous iron administration and BDI in patients. BDI is a measure of NTBI. NTBI, in turn, is thought to be one manifestation of the labile iron fraction released from all intravenous iron agents [63]. Quantitative in vitro studies, reported in abstract form [Lewis JM et al, *J Am Soc Nephrol* 14:771A, 2003], suggest that among available intravenous iron agents BDI release follows the sequence ferric gluconate > iron sucrose > iron dextran. Comparative in vivo studies to our knowledge have not been performed.

Our data document that 50 mg of iron (III) sucrose given intravenously does not result in detectable levels of nontransferrin-bound iron in most immune apheresis patients. In contrast, 100 mg of the same iron preparation led to the formation of nontransferrin-bound iron in all patients enrolled in the study. Thus, we suggest avoiding an exposure to high-dose iron to prevent potential long-term risks arising from nontransferrin-bound iron.

It is noteworthy that the iron injection is usually performed immediately after the apheresis treatment. According to our data this is not an optimal time point for application of high dose ($\geq$100 mg) iron therapy because apheresis causes an artifical state of hypotransferrinemia caused by the protein loss. This corresponds to data from hemodialysis patients with a low transferrin level [38]. Therefore, it is not excluded that in these patients the main actor in the generation of BDI is the apheresis therapy and timing of iron injection, rather than the

iron therapy itself. Although it was not the subject of this study and therefore not examined, we suggest that if high doses of iron (III) sucrose are indicated, later time points (e.g., 24 hours after apheresis treatment) should be chosen because serum transferrin and albumin reach their preapheresis levels at this time.

CONCLUSION

In apheresis patients with iron deficiency receiving intravenous iron sucrose, generation of nontransferrin-bound iron in serum is dose-dependent, transient, and related to the reduction in iron-binding capacity caused by apheresis.

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