Catalytically active iron and bacterial growth in serum of haemodialysis patients after i.v. iron–saccharate administration

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Abstract

**Background.** I.v. iron is commonly administered to haemodialysis patients suffering from anaemia to improve their response to erythropoietin therapy. It has been unclear whether routinely used doses of i.v. iron preparations could result in iron release into plasma in amounts exceeding the iron binding capacity of transferrin. Here, we have studied the effect of 100 mg of iron saccharate given as an i.v. injection on transferrin saturation and the appearance of potentially harmful catalytically active iron.

**Methods.** We followed serum iron, transferrin and transferrin-saturation before and 5–210 min after administration of iron saccharate in 12 patients on chronic haemodialysis due to end-stage renal disease. We measured catalytically active iron by the bleomycin-detectable iron (BDI) assay and transferrin iron forms by urea gel electrophoresis, and studied iron-dependent growth of Staphylococcus epidermidis inoculated into the serum samples in vitro.

**Results.** The iron saccharate injection resulted in full transferrin saturation and appearance of BDI in the serum in seven out of the 12 patients. BDI appeared more often in patients with a low serum transferrin concentration, but it was not possible to identify patients at risk based on serum transferrin or ferritin level before i.v. iron. The average transferrin saturation and BDI level increased until the end of the follow-up time of 3.5 h. The appearance of BDI resulted in loss of the ability of patient serum to resist the growth of S. epidermidis, which was restored by adding iron-free apotransferrin to the serum. Iron saccharate, added to serum in vitro, released only little iron and promoted only slow bacterial growth, but caused falsely high transferrin saturation by one routinely used serum iron assay.

**Conclusions.** The results indicate that 100 mg of iron saccharate often leads to transferrin oversaturation and the presence of catalytically active iron within 3.5 h after i.v. injection. As catalytically active iron is potentially toxic and may promote bacterial growth, it may be recommendable to use dosage regimens for i.v. iron that would not cause transferrin oversaturation.

**Keywords:** bleomycin-detectable iron; haemodialysis; iron saccharate; Staphylococcus epidermidis; transferrin saturation

Introduction

End-stage renal disease typically results in anaemia, which is primarily due to deficient renal production of erythropoietin [1,2]. Most patients undergoing haemodialysis are therefore treated with recombinant human erythropoietin (rHuEpo). Efficient erythropoiesis during rHuEpo medication requires ample amounts of iron, and it has been shown that iron delivery to the erythroid marrow becomes a restricting factor in the stimulation of erythropoiesis [3,4]. Iron supplementation is therefore routinely used in conjunction with rHuEpo therapy. In patients on haemodialysis oral iron therapy has proved incapable of maintaining iron balance in the long term. I.v. iron administration effectively replenishes iron stores and improves iron delivery and has, therefore, become a recommended therapy for patients on maintenance haemodialysis [5–8].

In normal conditions, practically all iron in plasma is bound to transferrin, which keeps the iron in a catalytically inactive form and prevents iron-catalysed hydroxyl-radical generation [9]. Transferrin-bound iron is also inaccessible to most bacteria [10]. I.v. iron administration raises the concerns that it may result in the oversaturation of transferrin and possibly predispose patients to the harmful effects of catalytically active non-transferrin-bound iron. Such harmful effects are hydroxyl radical-mediated tissue injury [9,11] and bacterial infections [10,12]. Iron preparations currently available for i.v. administration are ferrie–gluconate, iron–dextran and iron–saccharate [13]. Zanen et al.

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showed that i.v. ferric–gluconate administration resulted in transferrin oversaturation, unless it was given as a slow infusion using a low dose [14]. Iron–saccharate and iron–dextran are more stable iron complexes [13]. Sunder-Plassmann and Hörl [15] found that i.v. injections of iron–saccharate at doses of 10–100 mg did not result in oversaturation of transferrin in patients with serum transferrin > 1.8 g/l. Some patients with low serum transferrin had high saturation values during the follow-up time, which was 30 min after iron injection. On the other hand, it has been pointed out that the concept of transferrin oversaturation may be misleading because serum iron assays may measure a fraction of the iron present in the iron–dextran complex after i.v. injection and, thus, give false elevations in calculated transferrin saturation [7].

A method for detection of catalytically active non-transferrin-bound iron is based on the formation of a bleomycin–iron complex, which reacts with DNA resulting in its degradation. DNA degradation products can be measured by colorimetry using the thiobarbituric acid reaction [16]. Banyai et al. reported recently that bleomycin-detectable iron (BDI) was present in patients on i.v. iron therapy indicating that i.v. iron therapy may result in potentially toxic plasma concentrations of catalytically active iron [17].

A few recent reports suggest that i.v. iron administration, according to the current treatment regimens, may result in increased susceptibility to bacterial infections. Collins et al. found a 35% increase in the risk of death from infections with the use of i.v. iron–dextran in the analysis of 33 120 haemodialysis patients [18]. In another study from the same group, the use of > 17 vials of i.v. iron–dextran over a 5–6 month period was associated with a 20% increase in mortality from infections [19]. Further, Petruta et al. showed that patients with functional iron deficiency who were given i.v. iron, manifested impairment of neutrophil function [20]. On the other hand, in a recent prospective study of risk factors for bacteremia in 988 chronic haemodialysis patients, Hoen et al. found no association between the risk of infection and the use of i.v. iron [21]. Currently, it is unclear whether certain patterns of i.v. iron dosing are more harmful than others.

In the present study we have investigated the effect of 100 mg of iron–saccharate given as the first dose of i.v. iron supplementation to patients on chronic haemodialysis, on serum-transferrin saturation and possible appearance of catalytically active iron. To measure more accurately the actual transferrin saturation, we used urea gel electrophoresis, which separates the different transferrin iron forms. Appearance of catalytically active iron after i.v. iron injection was investigated by the BDI assay and by studying iron-dependent growth of inoculated *Staphylococcus epidermidis* in the serum samples. It was found that BDI was detectable and bacteria grew in the serum samples of seven out of the 12 patients after iron–saccharate administration.

**Subjects and methods**

**Patients**

Twelve patients on maintenance haemodialysis three times weekly (mean time on dialysis 16 months, range 2 weeks to 5 years) were included in this study. Six patients were men and the mean age was 61 years (range 35–77 years). The aetiology of the renal disease was: IgA nephropathy (n = 3), diabetic nephropathy (n = 3), interstitial nephritis (n = 2), sarcoidosis (n = 1), polycystic kidney disease (n = 1), glomerulosclerosis (n = 1), and unknown (n = 1). I.v. iron supplementation was started on clinical grounds because of an absolute (serum ferritin < 100 μg/l) or functional iron deficiency (serum transferrin saturation < 20%). None of the patients had overt infection before i.v. iron administration, but one patient developed a septic infection caused by *Staphylococcus aureus* on the following day. Six of the 12 patients had serum ferritin < 100 μg/l and the other six 100–400 μg/l. All but one were on rhHuEpo therapy. Haemodialysis and blood sample collection was performed at the haemodialysis unit of the Helsinki University Hospital. The study was approved by the institutional review board of the hospital. Informed consent was obtained from all study patients.

**Iron administration and blood sample collection**

The patients received 100 mg of iron–saccharate i.v. as the first dose of parenteral iron supplementation. The first blood sample (0) was collected from the access cannule at the beginning of the next haemodialysis session. Shortly after starting haemodialysis, a single bolus of 100 mg of iron saccharate (Venofer®, Ferrum, Vifor Inc, Switzerland) was administered within 10–30 min into the venous chamber. The blood samples were obtained from an infusion site of the arterial line at 5, 30, 90, and 210 min after the whole iron–saccharate dose had been given. The last sample was collected 48–72 h after iron administration at the beginning of the next haemodialysis session.

**Iron and transferrin assays**

Serum iron was measured by two spectrophotometric methods using ferrozine [22] or ferene-S [23] as the chromogenic agents. Serum transferrin was measured by an immunoturbidimetric method. The transferrin saturation value (%) was calculated from the serum iron and transferrin values using the formula: serum iron (μmol/l)/transferrin (g/l) × 3.8. Catalytically active iron in serum was determined using the BDI assay [16] modified for small serum volumes. The reagent solutions except the bleomycin were treated of 10 ml solution of reagent mixture to 7.4. The mixture was incubated at 37°C for 1 h for the reaction of the possible ferrous–bleomycin complexes formed by iron in the sample, which cause DNA degradation. The reaction was stopped by the addition of 50 μl 0.1 mol/l EDTA. Aliquots of 250 μl 1% thioarbituric...
acid (Sigma) and 250 μL 25% HCl were added and the mixture was incubated at 80 °C for 20 min for chromogen formation of the DNA break down products. The samples were cooled to room temperature, 1.5 mL of butanol was added and the chromogen extracted into the organic phase by mixing. The samples were centrifuged for 20 min at 2500 x g to separate the phases. An aliquot (350 μL) of the clear top phase was pipetted into micotiter plates. The absorbance at 350 nm was measured using a microplate reader (TiterTek Multiscan RC, Labsystems, Finland). The samples were measured in parallel with a corresponding blank without the addition of bleomy-
cin. The absorbance value of the blank was reduced from each sample absorbance value. The reagent blank value was reduced from the absorbance values of the standards, and a standard curve between 0.1 and 3 μmol/L was calculated by linear regression from each series.

The iron forms of transferrin were analysed using urea polyacrylamide gel (6% acrylamide gels with 6 M urea) electrophoresis according to Williams et al. [24]. Serum samples containing about 0.15 μg of transferrin were separated at 10 x 10 cm gels. Proteins were electroblotted from the gel onto polyvinylidene fluoride membrane (Immobilon-P, Millipore) in a transfer buffer containing 25 mmol/L Tris, 192 mmol/L glycine and 20% methanol. The membrane was treated with 0.5% Tween in PBS over night. Transferrin bands were visualized by immunostaining using rabbit anti-
human transferrin IgG (Dako A/S, Denmark) as the primary antibody in 1% BSA, 0.05% Tween 20 in PBS for 2 h at room temperature. The blots were washed three times with PBS containing 0.05% Tween 20 and then incubated with the second antibody, anti-rabbit IgG conjugated with alkaline phosphatase (Jackson Immuno Research Laboratories Inc., USA) in 1% BSA, 0.05% Tween 20 in PBS for 1 h at room temperature. Following an additional three washes with 0.05% Tween 20 in PBS, the blots were stained in a solution containing 5-bromo-4-chloro-3-indolyl-phosphate toluidine salt and p-nitro blue tetrazolium dichloride (BCIP/NBT color development solution, Immuno-Blot Alkaline Phosphatase Assay kit, Bio-Rad, USA). The reaction was stopped with 100 mmol/L Na-acetate, pH 5, containing 5 mmol/L Na–EDTA for 5 min. The blots were washed with distilled water and dried.

Bacterial growth assay

The growth of bacteria in serum samples in vitro was tested using a multiple drug resistant S. epidermidis strain 16779 isolated from a neutropenic patient with a septic infection at the Department of Bacteriology of HUCH Diagnostics, Helsinki University Hospital. The strain was precultivated in soy broth (bioMérieux, France) overnight to produce the inoculum. The bacteria were harvested by centrifugation, washed and suspended in saline. An iron-poor chemical defined medium at pH 7.4 [25] was used to dilute the serum samples to a final dilution of 1/5. The medium was not able to sustain the growth of the S. epidermidis strain as such. The growth assay was carried out in a total volume of 250 μl in microtiter wells using an initial bacterial density of about 2 x 10^4/mL. Bacterial growth was measured by monitoring optical density with periodic shaking for 24 h at 37 °C in a Bioscreen C analysator (Labsystems, Finland). From the growth curves, the lag time before exponential growth (t_{lag}) and the slope of the logarithmic growth curve during the exponential growth (β) were measured. A growth index was calculated for each sample by dividing the μ/t_{lag} value obtained for the serum sample by the corresponding value obtained from a rich soy broth medium. The growth index was 0 if no growth could be detected within 24 h, and the maximum value was 1.0 corresponding to growth in the soy broth medium.

Other reagents

Ferric nitrotriacetic acid (Sigma Chemical Co., St Louis, MI, USA) was prepared according to Welch and Skinner [26] and a 1-mmol/L solution was used for the in vitro experiments. Apotransferrin with a purity over 98% and less than 1% iron saturation was produced by the Finnish Red Cross Blood Transfusion Service.

Statistics

Correlation coefficient for BDI and bacterial growth index values was calculated with simple linear regression (StatsDirect, version 1.612, Iain E. Buchan).

Results

Serum transferrin and iron parameters after i.v. iron saccharate administration

The average serum transferrin level in the patients was 1.78 g/l (range 0.96–2.52 g/l) and the average transferrin saturation 20% (range 2–45%) before the i.v. iron–saccharate injection (Table 1). After an initial increase to 67%, the average transferrin saturation decreased slightly in the 30 min samples. After this, transferrin saturation continued to rise and the highest average level of 83% was detected in the 210-min samples. At this time point, nine of the 12 patients had transferrin saturation >80%. We used the ferrozine-based iron assay, which showed the lowest interference with iron saccharate (as described later), for the calculation of transferrin saturation.

The calculated transferrin saturation values were compared with the distribution of transferrin iron forms in urea gel electrophoresis. The serum samples taken before i.v. iron administration contained iron-free apotransferrin and monoferric transferrin, which are the main transferrin forms present in normal serum (Fig. 1). The proportions of the monoferric and difer-
ric transferrins increased after i.v. iron administration. Samples with calculated transferrin saturation of >80% contained mainly diferic transferrin in urea gel electrophoresis and no iron-free transferrin. Transferrin saturation values calculated from the ferro-
zine-based iron determinations matched well with the distribution of the different transferrin iron forms in the urea gel electrophoresis (Fig. 1). The calculated transferrin saturation values decreased and non-
saturated iron forms reappeared in the urea gels in the serum samples taken 2–3 days after the i.v. iron injection before the next haemodialysis session.

BDI iron after i.v. iron–saccharate administration

The presence of catalytically active iron in the serum samples was investigated by the BDI method.
Table 1. Iron and transferrin parameters (mean ± SD) in the serum samples of 12 haemodialysis patients before and after i.v. iron–saccharate injection. Samples with BDI levels >0.1 μmol/l are considered positive.

<table>
<thead>
<tr>
<th>Sample time point</th>
<th>S-Transferrin (g/l)</th>
<th>S-Fe (μmol/l)</th>
<th>Transferrin saturation (%)</th>
<th>BDI (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before i.v. iron injection</td>
<td>1.7 ± 0.4</td>
<td>8 ± 4</td>
<td>20 ± 12</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>5 min</td>
<td>1.7 ± 0.4</td>
<td>28 ± 9</td>
<td>67 ± 29</td>
<td>0.14 ± 0.28</td>
</tr>
<tr>
<td>30 min</td>
<td>1.8 ± 0.4</td>
<td>25 ± 10</td>
<td>59 ± 28</td>
<td>0.07 ± 0.11</td>
</tr>
<tr>
<td>90 min</td>
<td>1.8 ± 0.4</td>
<td>28 ± 10</td>
<td>66 ± 27</td>
<td>0.12 ± 0.24</td>
</tr>
<tr>
<td>210 min</td>
<td>1.8 ± 0.4</td>
<td>37 ± 14</td>
<td>83 ± 27</td>
<td>0.29 ± 0.34</td>
</tr>
<tr>
<td>2 to 3 days after i.v. iron</td>
<td>1.8 ± 0.4</td>
<td>10 ± 7</td>
<td>20 ± 16</td>
<td>0.02 ± 0.01b</td>
</tr>
</tbody>
</table>

Reference values 1.8–3.1 7–30 17–52 <0.10

*a n=11  
*b n=10.

Fig. 1. Transferrin iron forms in the serum of two patients (A and B) receiving 100 mg i.v. iron–saccharate. The 0 min sample was taken before the iron injection. Transferrin iron forms were separated by urea polyacrylamide gel electrophoresis and transferrin bands were visualised by immunoblotting. Apo-Tf, iron free transferrin; FeC-Tf and FeN-Tf; monoferric transferrin with iron in the C and N lobe, respectively; Fe2-Tf, iron saturated transferrin.

According to our validation study, the limit of detection of the assay was 0.1 μmol/l and thus the results <0.1 μmol/l were regarded as negative (L. von Bonsdorff, E. Lindeberg, J. Parkkinen, unpublished results). None of the patients were positive for BDI before i.v. iron–saccharate. The average BDI level increased in parallel with the average transferrin saturation level after i.v. iron–saccharate (Table 1). The proportion of patients having detectable BDI in serum increased correspondingly, and 210 min after i.v. iron saccharate six of the 12 patients were positive for BDI (Fig. 2). Seven of the patients were positive in at least one sample after i.v. iron–saccharate. When compared with transferrin saturation determined by the ferrozine-based iron assay, BDI was positive in 76% (13/17) of the serum samples having transferrin saturation >80% (Fig. 3A).

BDI occurred more often in patients with low serum transferrin (Fig. 3B). Five out of seven patients with serum transferrin <1.8 g/l had one or more positive samples after i.v. iron–saccharate administration. On the other hand, two of the five patients with serum transferrin >1.8 g/l also had BDI in the serum sample taken 210 min after iron–saccharate. Baseline serum ferritin value before the start of i.v. iron supplementation seemed to have less influence on the appearance of BDI. Four of the six patients with serum ferritin <100 μg/l and three of the six patients with ferritin 100–400 μg/l were positive for BDI. Similarly, there was no apparent association between transferrin saturation before i.v. iron–saccharate and the
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Fig. 3. Relationship between BDI, serum transferrin, transferrin saturation and S. epidermidis growth index in the serum samples of the twelve haemodialysis patients receiving 100 mg i.v. iron–saccharate. (A) Relationship of BDI and transferrin saturation. (B) BDI and transferrin. (C) Bacterial growth index and transferrin saturation. (D) BDI and bacterial growth index.

appearance of BDI. Three out of six patients with initial transferrin saturation <20% and four of the six patients with initial transferrin saturation >20% had BDI in serum after iron–saccharate administration.

Bacterial growth in serum after i.v. iron–saccharate administration

The effect of the serum samples on growth of a multiple drug resistant S. epidermidis strain was studied by inoculating the serum samples with the bacteria and monitoring their growth. No growth could be detected in serum samples from healthy donors, nor in any of the patient samples taken before the i.v. iron administration (Fig. 4). This demonstrated the ability of serum to resist the growth of bacteria. In contrast, bacterial growth was observed in the serum of the patients, who had high transferrin saturation values after i.v. iron–saccharate (Fig. 3C). Similarly to BDI, bacterial growth was most frequently observed in the serum samples taken 210 min after iron–saccharate. Bacteria grew in the serum of eight of the 12 patients at this time point. When iron-free apotransferrin was added to the serum samples, which promoted bacterial growth, the ability of the serum to resist the growth was restored (Fig. 4). This confirmed that bacterial

Fig. 4. Bacterial growth curves in serum samples inoculated with S. epidermidis in vitro. The growth was measured as optical density (OD).
growth was critically dependent on the presence of non-transferrin-bound iron in the serum.

There was a good correlation ($r=0.87$, CI $0.79–0.92$) between BDI and bacterial growth index values (Fig. 3D). There were only a few samples that promoted slow bacterial growth without containing BDI. The lag phases before bacterial growth were clearly longer in these samples than in the samples, which were positive in the BDI assay. There was only one BDI-positive sample, which did not support bacterial growth.

The ability of bacteria to utilize iron–saccharate as an iron source was studied in vitro in normal serum with increasing levels of added iron–saccharate. No bacterial growth was observed when iron–saccharate was added up to 350 μmol/l calculated as iron concentration. At higher levels, slow bacterial growth was detected but only after a prolonged lag phase. Control samples, in which transferrin had been fully saturated by adding 45 μmol/l of ferric nitrilotriacetic acid, promoted bacterial growth. The results indicated that S. epidermidis cannot effectively utilize iron–saccharate directly as an iron source in serum.

Interference of iron–saccharate with serum iron determination

To find out whether iron–saccharate concentrations, occurring after i.v. injection, could interfere with the commonly used iron methods, we added serum saccharate in vitro to normal serum up to 500 μmol/l as iron concentration. This corresponds to the highest level measured in serum by atom absorption spectrometry 10 min after 100 mg i.v. iron–saccharate [27]. The ferene-S method gave clearly higher results as compared to the ferrozine method, and thus, also higher transferrin saturation values. To examine the actual transferrin saturation, the samples were studied in urea gel electrophoresis (Fig. 5). The intensities of the monoferric and diferric transferrin bands increased somewhat after addition of high iron–saccharate levels (up to 500 μmol/l). In the same samples, the transferrin saturation values calculated from the ferrozine method increased from 30 to 56%, and correlated apparently well with the urea gel results. In contrast, the iron values obtained with the ferene-S method indicated 100% transferrin saturation already when the urea gel still revealed the presence of iron-free apotransferrin in the serum. This demonstrated that the ferene-S method does not give accurate transferrin saturation values in the presence of iron–saccharate at concentrations occurring after i.v. injection.

To further study the stability of iron–saccharate in serum, we incubated the serum samples with added iron–saccharate up to 4 h at 37°C. After the slight initial increase in the intensity of the monoferric or diferric transferrin bands, no further increase in transferrin saturation took place on the basis of urea gel electrophoresis. Neither could catalytically active iron be detected by the BDI assay in any of the samples. These results indicated that iron–saccharate is relatively stable in serum in vitro, and only little iron is liberated from even high concentrations of iron–saccharate during incubation in serum.

Discussion

In the present paper we demonstrate that an i.v. injection of 100 mg iron–saccharate resulted in full transferrin saturation and appearance of catalytically active iron measured as BDI in the serum of seven out of 12 haemodialysis patients. Transferrin saturation and BDI reached the highest level at the end of the 3.5-h follow-up after i.v. iron injection. The appearance of BDI was associated with loss of the ability of the serum to resist the growth of S. epidermidis inoculated in vitro, which was restored by adding iron-free apotransferrin to the serum. This indicated that bacterial growth in the serum was dependent on non-transferrin-bound iron.

Sunder-Plassmann and HörI [15] have studied transferrin saturation in haemodialysis patients after i.v. iron–saccharate doses of 10–100 mg. They found that the i.v. administration of 100 mg iron–saccharate did not result in transferrin oversaturation if the serum transferrin concentration was $>1.8$ g/l. Two out of the four patients with serum transferrin $<1.8$ g/l had transferrin saturation values $>100$% during the follow-up time, which was 30 min after iron injection [15]. We also found more often high transferrin saturation values and BDI in patients with a low serum transferrin level. However, two of the five patients with a normal transferrin level also had fully saturated transferrin and BDI 3.5 h after i.v. iron–saccharate administration. Other parameters like serum ferritin concentration and transferrin saturation before the parenteral iron injection proved to be less predictive for the appearance of BDI. This suggests that it may not be possible to reliably select the patients beforehand, to
whom 100 mg of iron–saccharate could be administered without the risk of transferrin oversaturation. Banyai et al. [17] reported recently that BDI was detectable in eight out of 10 haemodialysis patients following i.v. injection of 100 mg of iron–saccharate. The time point of BDI positive samples was not reported. After lower iron–saccharate doses (10–50 mg) BDI was detected in only two out of 15 patients. Our results confirm the finding of this earlier report that a considerable portion of patients are positive for BDI following 100 mg i.v. iron–saccharate.

It has previously been shown that serum iron assays may measure a fraction of iron present in the iron–dextran complex [28] and, therefore, give false elevations in transferrin saturation after i.v. iron–dextran administration. We extended these observations to iron saccharate, which was found to interfere with the ferene-S iron method and give falsely high transferrin saturation values within 30 min after i.v. injection. To avoid the interference problem, we studied transferrin saturation by direct assessment of serum transferrin iron forms by urea gel electrophoresis. With this method we could confirm that the ferrozine serum iron assay gave reliable transferrin saturation values even in the presence of high iron saccharate concentrations occurring after an i.v. injection.

The appearance of catalytically active iron after i.v. iron–saccharate was demonstrated with two different methods in the present study. The BDI assay is based on the free radical-induced degradation of DNA in the presence of catalytically active iron and bleomycin [16]. The bacterial growth assay, on the other hand, is based on the concept that S. epidermidis can not utilize transferrin-bound iron for growth [29] and is dependent on the presence of ‘free’ non-transferrin-bound iron. Most of the serum samples which had a calculated transferrin saturation >80% were positive both in the BDI and bacterial growth assay, whereas none of the samples with a saturation level <80% was positive in the BDI assay. In the bacterial growth assay, a few samples with a saturation level <80% sustained slow bacterial growth after a prolonged lag period.

Our results demonstrate a direct mechanism by which iron could predispose haemodialysis patients to bacterial infections. The common causative organisms of bacteraemic infections in these patients include coagulate-negative staphylococci, Gram-negative enteric bacteria and S. aureus [30]. Similarly to S. epidermidis, the growth of Gram-negative enteric bacteria in serum depends on the availability of non-transferrin-bound iron [10,12]. Staphylococcus aureus, on the other hand, can utilize transferrin-bound iron and grow in serum even in the absence of transferrin oversaturation [29]. Another mechanism by which iron can decrease host resistance to infections by various bacteria is impairment of phagocytosis. Iron-induced impairment of neutrophil function has been demonstrated in vitro [31] and in haemodialysis patients after i.v. iron supplementation [20]. The recent epidemiological findings that mortality from infections was higher in haemodialysis patients treated with i.v. iron [18,19], together with the pathophysiological evidence, indicate that i.v. iron dosage regimens which may lead to transferrin oversaturation should be avoided.

In addition to the bacterial growth promoting effect, non-transferrin-bound iron is a powerful catalyst of hydroxyl radical generation [9] and it has been associated with hepatic toxicity [11]. It remains currently open whether short periods with catalytically active iron in circulation could induce cellular toxicity in haemodialysis patients.

Concerning dosage regimens of i.v. iron–saccharate that would not lead to oversaturation of transferrin, apparently doses lower than 100 mg with more frequent administration should be considered. In the assessment of dosage regimens for iron–saccharate, transferrin saturation should be followed at least for 3–4 h after iron injection by using a suitable method for serum iron determination, which does not measure saccharate-complexed iron.

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