Effects of intravenous iron on mononuclear cells during the haemodialysis session

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Abstract

Background. This study analysed, \textit{in vivo} and \textit{in vitro}, the effects of four different intravenous iron preparations (iron gluconate, iron sucrose, iron dextran and ferric carboxymaltose) on activation and damage of mononuclear cells.

Methods. A randomized prospective study was conducted in 10 haemodialysis (HD) patients. Blood samples were collected at baseline (T0); 1 h after starting HD, just before the iron or saline administration (T1); 30 min after the iron or saline infusion (T2) and at the end of HD (T3). In addition, peripheral blood mononuclear cells from 10 healthy individuals and 9 chronic kidney disease Stage-5 (CKD-5) without HD treatment were cultured with the 4 iron preparations.

Results. Iron infusion during the HD session increased the percentage of mononuclear cells with reactive oxygen species (ROS) production, Inter-Cellular Adhesion Molecule-1 (ICAM-1) and apoptosis. There were no significant differences between the four iron preparations. Culture of mononuclear cells from healthy individuals and CKD-5 patients with the different iron preparations resulted in a significant increase in ROS, ICAM-1 and apoptosis as compared with control. In an additional study, the effect of original iron sucrose formulation on mononuclear cells was compared with that of one generic formulation. The generic formulation produced a greater increase in ROS, ICAM-1 and apoptosis than the original iron sucrose.

Conclusions. Our results suggest that intravenous iron has deleterious effects on mononuclear cells. The four iron compounds evaluated produced similar effects on oxidative stress, cell activation and apoptosis. However, the effects of iron compounds with the same formulation were different, thus further investigation may be required to establish the safety of iron preparations that theoretically have the same composition.

Keywords: apoptosis; haemodialysis; ICAM-1; intravenous iron; oxidative stress

Introduction

Decreased erythropoietin production and inadequate iron availability are the chief causes of anaemia in patients with Stage-5 chronic renal failure (CRF) who are treated with haemodialysis (HD) [1–4]. Absolute and functional iron deficiency determines resistance to erythropoietin-stimulating agents (ESAs) [5, 6]. This deficiency is caused primarily by the inhibition of intestinal iron absorption, the increased demands for iron by bone marrow that are associated with ESA use and the frequent blood loss that accompanies HD [7, 8].

To mitigate this deficit, HD patients receive a substantial supplement of exogenous iron each year [7, 9]. Generally, in patients who undergo HD, the administration of oral iron is ineffective, necessitating intravenous therapy to maintain iron stores [10, 11]. Intravenous iron has been demonstrated repeatedly to increase the haemoglobin levels and the dose-sparing effects of ESAs in CRF patients [5, 6, 8, 12]. Numerous intravenous iron preparations are utilized in clinical practice. All iron compounds consist of a ferric hydroxide core surrounded by a protective ‘carbohydrate shell’ that is designed to prevent immediate dissolution of ferric iron [13]. Differences in core size and carbohydrate chemistry determine pharmacological and biological differences that appear to be directly related to molecular weight [14]. The benefits of intravenous iron in the treatment of anaemia in chronic kidney disease (CKD) patients are unquestioned, but it can have potential harmful side effects [15–17]. Recent studies have shown that repeated treatment with certain iron compounds in patients who undergo HD activate immunocompetent cells, effecting oxidative stress and proinflammatory cytokine production [17].

Inflammation and oxidative stress are inextricably linked—their combination promotes endothelial dysfunction, atherosclerosis and infection and, thus, increases the mortality of CKD patients [18–20]. Chronic inflammation is commonly reported in patients with Stage-5 CKD [18] and is characterized by the maintenance of high-serum levels of
inflammatory cytokines and a deficient immune response that is dependent on mononuclear cells [19, 20]. In addition, cytokine activation increases and mitochondrial function becomes impaired, promoting the generation of reactive oxygen species (ROS) and cell death by apoptosis during HD [19, 21].

In a recent study, Kuo et al. [16] reported that repeated doses of intravenous iron sucrose exacerbated oxidative DNA damage in peripheral blood lymphocytes in HD patients. As discussed previously, intravenous iron preparations can enhance the oxidative stress and cellular activation that are induced by dialysis [15, 18], but whether these changes affect cell survival in CKD patients who undergo HD and whether these deleterious effects are common to all available iron preparations for routine clinical use have not been determined.

We have performed a randomized prospective study on HD patients who were treated with different intravenous iron. Taking into account that differences in the molecular weight of iron could lead to different results [22], we have chosen different compounds, iron gluconate, iron sucrose, iron dextran and ferric carboxymaltose to determine the effects of these compounds on activation and apoptosis of mononuclear cells during the HD session. In order to clarify that the iron effects are not related to the HD procedure, we have performed an in vitro study incubating mononuclear cells from healthy subjects and CKD patients with the four iron preparations.

Materials and methods

Human subjects

Inclusion criteria were age between 20 and 80 years, HD vintage >12 months, weekly dialysis time >12 h, serum albumin >3.8 g/dL and C-reactive protein within the normal range. The exclusion criteria were diabetes, parathyroid hormone (PTH) >400 pg/mL, ferritin >500 ng/mL, evidence of infection, neoplasia and immune or liver disease. Patients who were taking vitamin D, non-steroidal anti-inflammatory drugs, statins or immunosuppressive drugs were also excluded.

HD patients who fulfilled all the inclusion and exclusion criteria were included in this study (seven females and three men). They were recruited from the HD Unit of Reina Sofia University Hospital (Cordoba, Spain). All patients had been dialysed using helexone membranes (FX80; Fresenius Medical Care, Bad Homburg, Germany) for at least 6 months prior to initiation of the study. The dialysis system was free of bacteria (<10 colony-forming units per millilitre) and bacteriological contaminants (endotoxin levels <0.025 endotoxin units) throughout the entire study.

All patients, except one, had a native arteriovenous fistula. All patients had been treated with darbepoetin (Aranesp®) and iron sucrose (Venofer®) to maintain a target haemoglobin level of 11.5–12.5 g/dL. Median ferritin plasma level was 392 ± 74.3 ng/mL, mean iron saturation ratio was 29.4 ± 6.2 mg/mL and the percentage of hypochromic red cells was 21.1 ± 1.9%. Ten healthy subjects and nine CKD-5 patients not treated with dialysis nor intravenous iron were included in this study. Healthy subjects and CKD-5 patients were matched for age and sex with the HD patients.

Study design

One hour after the start of dialysis, saline (control) or iron was infused for 30 min. The study was conducted with four types of iron compounds. The doses of single iron molecules were ferric gluconate, a 5-mL ampoule of ferrlecit which contains 12.5 mg/mL (62.5 mg/5 mL); iron dextran, a 2-mL ampoule of cosmofer which contains 50 mg/mL (100 mg/2 mL); iron sucrose, a 5-mL ampoule of venofer which contains 20 mg/mL (100 mg/5 mL) and ferric carboxymaltose, a 2-mL ampoule of ferinject which contains 50 mg/mL (100 mg/2 mL). All patients were studied after the infusion of saline. After a 2-week washout period, patients were randomly assigned to receive the different iron compounds. The time frame between administrations of iron molecules was 2 weeks. We collected four samples of peripheral blood from each patient at baseline (T0); 1 h after starting HD, just before the iron or saline administration (T1); 30 min after the iron or saline infusion (T2) and at the end of HD (T3). Neither the patient nor the nurse in charge was aware of the iron preparation administered; only the head nurse knew the solution of iron (diluted in a 100-mL ampoule of saline) that was given to the patient. There was an interval of 2 weeks between the administrations of each iron compound.

We conducted a survey of the patients, which included questions that were related to the clinical tolerance of the four iron compounds and saline (headache, dizziness, cramps, nausea and vomiting). Haemodynamic data were also collected during the dialysis sessions (systolic and diastolic blood pressure, mean arterial pressure and heart rate).

Clinical tolerance and haemodynamic response were similar after administration of the four iron compounds and saline. The clinical relevance of the iron infusion was minimal (data not shown). We did not observe the differences in ferritin and transferrin saturation after the administration of different iron compounds.

The study was approved by the Local Ethic Committee, and signed informed consent was obtained before inclusion in the study.

In vitro studies

Before choosing the dose of iron for the in vitro experiments, a dose–response curve was performed with the different iron compounds. The results varied with the different concentrations of iron. The dose of 200 µg/mL of iron was considered the most appropriate concentration to carry out this study since we observed significant differences in the cell viability >200 µg/mL; we did not find significant differences <50 µg/mL. This study was performed with peripheral blood mononuclear cells (PBMCs) from 10 healthy individuals and 9 CKD-5 patients. PBMCs were isolated by density gradient separation on Ficoll (Ficoll/Hypaque; BioWhittaker Inc., Walkersville, MD). PBMCs (10⁶ cells/mL) were seeded in 24-well culture plates (Falcon; Becton Dickinson and Company, Paramus, NJ) in complete culture medium that contained RPMI 1640, supplemented with 1-glutamine (2 mM), HEPES (20 M), sodium pyruvate (1 mM), streptomycin (50 ng/mL), penicillin (100 U/mL) and 10% fetal bovine serum at 37°C in 5% CO₂/95% air atmosphere (medium and additives were supplied by BioWhittaker).

The cells were cultured for 2, 8 and 24 h with four iron compounds: iron sucrose (Venofer®), low-molecular-weight iron dextran (Cosmofer®), sodium ferric gluconate (Ferrlecitin®) and ferric carboxymaltose (Ferinject®). The dose of iron in all experiments was 200 µg/mL. As a control, cells were cultured with RPMI + phosphate-buffered saline (PBS) (Gibco; Invitrogen, Grand Island, NY). After each incubation time, cell viability was measured using trypan blue (Lonza; BioWhittaker).

Detection of ROS

Cells were incubated for 15 min at 37°C with 2-µM hydroethidine (Sigma; St Louis, MO). Hydroethidine is oxidized by ROS to become hydroethidine. Fluorescence was monitored on a FACS-Calibur flow cytometer (Becton Dickinson) with an excitation wavelength of 488 nm and emission wavelength of 625 nm for superoxide. The results were expressed as percentage of cells with ROS production.

ICAM-1 expression

Cellular activation was measured by ICAM-1 density. Cells were incubated with ICAM-1 against phycoerythrin-conjugated CD54 (Becton Dickinson) for 15 min in the dark at room temperature. After incubation, the cells were washed with PBS and re-suspended in 500 µL Cell Fix. The analysis was performed by flow cytometry.

Apoptosis

Early apoptosis was measured using the Annexin V Apoptosis Detection Kit according to the manufacturer’s instructions (Bender MedSystem, Vienna, Austria). Flow cytometric analysis was performed on a FACSCalibur flow cytometer (Becton Dickinson).

Statistical analysis

A descriptive analysis of the results was performed by calculating mean values ± SDs. Wilcoxon signed-ranks test was used to compare the data at baseline and after administration of saline and iron preparations. Data for
more than two groups were analysed by one-way analysis of variance or Kruskal–Wallis test, as appropriate. Mean values for two groups were compared by Student’s *t*-test for unpaired data. *P* < 0.05 was considered statistically significant.

**Results**

**Basic characteristics**

Demographic and laboratory parameters of HD and CKD-5 patients are shown in Table 1. There were no differences between the groups with respect to these parameters.

**Effects of iron preparations on percentage of cells with ROS production in HD patients**

To determine the effects of the iron compounds on the ROS production in mononuclear cells during the HD session, we conducted a randomized prospective study in 10 HD patients.

At baseline (T0), the percentage of cells with ROS production was similar in the saline (control) (6.5 ± 1.4%) and four iron groups (iron sucrose: 6.4 ± 2.7%; iron dextran: 5.4 ± 2.2%; ferric gluconate: 6.3 ± 2.2% and ferric carboxymaltose: 6.4 ± 2.7%). This percentage increased significantly at the first hour of HD (T1 just before the iron or saline administration); control T1: 12.6 ± 2.8%; iron sucrose T1: 13.5 ± 3.6%; iron dextran T1: 11.5 ± 2.6%; ferric gluconate T1: 12.4 ± 2.23% and ferric carboxymaltose T1: 13.2 ± 3%, compared with baseline (*P* < 0.005, Figure 1). After the saline infusion, the cells with ROS production did not change significantly throughout the study, remaining elevated at T2 (12.8 ± 2.5%) and T3 (12.8 ± 3%) compared with T0 (*P* < 0.05). In contrast, the percentage of mononuclear cells with ROS production increased further 30 min after the iron infusion (iron sucrose T2: 24.1 ± 3.1%; iron dextran T2: 21.7 ± 3.1%; ferric gluconate T2: 23.2 ± 4% and ferric carboxymaltose T2: 20.7 ± 3%) (T2 versus T0 and T1, *P* < 0.001 for the four compounds), decreasing at the end of HD (iron sucrose T3: 13.7 ± 3%; iron dextran T3: 12.6 ± 2.2%; ferric gluconate T3: 13.8 ± 3% and ferric carboxymaltose T3: 12.5 ± 3.1%) (T2 versus T3, *P* < 0.005), although the percentage of cells remained high compared with baseline (T3 versus T0, *P* < 0.005). The percentage of mononuclear cells with ROS production was similar throughout dialysis with the four iron preparations.

**Effects of iron preparations on the ICAM-1 expression in mononuclear cells from HD patients**

At baseline (T0), ICAM-1 expression per cell was similar in the control [171.5 ± 34.6 Mean Fluorescence Intensity (MFI)] and iron groups (iron sucrose: 178.8 ± 30.8 MFI; iron dextran: 173.7 ± 26.4; ferric gluconate: 176.1 ± 24.8 MFI and ferric carboxymaltose 172.5 ± 24.1 MFI). After the first hour of HD, a slight, insignificant increase in ICAM-1 was observed in the five groups. After the saline infusion, ICAM-1 levels were unchanged throughout the dialysis, and there were no significant changes between T2 and T3. Notably, after iron infusion, ICAM-1 rose significantly, independent of the iron preparation (iron sucrose T2: 245.6 ± 35.8 MFI; iron dextran T2: 254.1 ± 28.3 MFI; ferric gluconate T2: 239.8 ± 26.7 MFI and ferric carboxymaltose T2: 236.8 ± 21.7 MFI) (T2 versus T0 and T1, *P* < 0.005, Figure 2), remaining elevated at the end of HD compared with T0 and T1 (*P* < 0.005). There were no significant changes between T2 and T3.

**Effects of iron preparations on the apoptosis in mononuclear cells from HD patients**

At T0, the percentage of apoptotic cells was similar in the control (5.1 ± 1.5%) and four iron groups (iron sucrose: 6.2 ± 2.4%; iron dextran: 5.1 ± 2.2%; ferric gluconate: 5.6 ± 2.2% and ferric carboxymaltose 6.4 ± 2.5%). After 1 h of dialysis, the percentage of apoptotic cells increases significantly in the five groups (control T1: 9.2 ± 3.1%; iron sucrose T1: 13.2 ± 2.8%; iron dextran T1: 10.7 ± 2%; ferric

<table>
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<th>Parameter</th>
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<th>CKD-5 patients</th>
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<tr>
<td>Age (years; mean ± SD; range)</td>
<td>60.2 ± 10.1 (35–80)</td>
<td>58.1 ± 12.3 (38–75)</td>
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<td>Sex (male/female)</td>
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<td>4/5</td>
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<tr>
<td>PTH (pg/mL; mean ± SD; range)</td>
<td>281.7 ± 84.6 (115–384)</td>
<td>303 ± 76.4 (253–353)</td>
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<td>Albumin (g/dL; mean ± SD; range)</td>
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<td>Transferrin saturation (mg/mL; mean ± SD; range)</td>
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<td>24.4 ± 5.6 (21.2–27.9)</td>
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<td>Ferritin (ng/mL; mean ± SD; range)</td>
<td>392 ± 74.3 (276.9–491)</td>
<td>373.3 ± 69.8 (126–512)</td>
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<td>Time on HD (months; mean ± SD; range)</td>
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<td>N.D.</td>
</tr>
<tr>
<td>Renal creatinine clearance (mL/min; mean ± SD; range)</td>
<td>4.2 ± 2.3 (6.8–11.1)</td>
<td>N.D.</td>
</tr>
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*N.D., not determined.*

![Fig. 1. The percentage of cells with ROS production in HD patients with four iron preparations. *P* < 0.005 versus baseline, #P < 0.001 versus T1 and T3. T1: 1 h after starting HD, just before the iron or saline administration; T2: 30 min after iron or saline infusion; T3: at the end of the HD session.](image-url)
gluconate T1: 13.1 ± 2.8% and ferric carboxymaltose T1: 11.9 ± 2.2% (P < 0.005, Figure 3). After saline infusion (T2), the percentage of apoptotic cells was similar to that observed at T1 (T2: 10.3 ± 2.5%), rising slightly but significantly at the end of dialysis T3 (17.1 ± 1.9%) (T3 versus T2, P < 0.005). In contrast, after iron treatment, apoptosis increased significantly in the four groups (iron sucrose T2: 15.8 ± 2.7%; iron dextran T2: 16.5 ± 3.5%; ferric gluconate T2: 18.4 ± 2.1% and ferric carboxymaltose T2: 17.8 ± 3.6%) (T2 versus T1; P < 0.005), climbing further at the end of HD (iron sucrose T3: 24.9 ± 3.3%; iron dextran T3: 22.6 ± 2.9%; ferric gluconate T3: 23.8 ± 3.7% and ferric carboxymaltose T3: 23 ± 3.3%) (T3 versus T1 and T2, P < 0.005). The percentage of apoptotic cells, independently of iron, was similar in all patient groups.

Oxidative stress and ICAM-1 expression in mononuclear cells from healthy subjects and CKD-5 patients cultured with iron compounds

We measured oxidative stress and ICAM-1 expression as indicators of cell activation in mononuclear cells that were cultured with four iron compounds. On the dose-response curve, the maximum activity was observed at 8 h of culture in all experiments (data not shown). In cells cultured without iron, we observed a higher significant percentage of cells with ROS production in CKD-5 (41.3 ± 9.4) as compared with healthy subjects (23.2 ± 7.2; P < 0.005). However, when cells from healthy subjects and CKD-5 cells were cultured with iron compounds, the percentage of cells with ROS production increased significantly in comparison with control (no-iron) (P < 0.003). This increase was higher in cells for CKD-5 (iron sucrose: 67.7 ± 12.1%; iron dextran: 65.9 ± 11.3%; ferric gluconate: 67.2 ± 9.7% and ferric carboxymaltose: 63.1 ± 10.8%) as compared to healthy subjects (iron sucrose: 46.2 ± 7.3%; iron dextran: 48.9 ± 8.1%; ferric gluconate: 46.3 ± 7.9% and ferric carboxymaltose: 46.5 ± 6.6%; P < 0.005) (Figure 4A).

There were no significant differences between the iron preparations. As shown in Figure 4B, the ICAM-1 results were similar to those described for ROS.

Apoptosis of mononuclear cells from healthy and CKD-5 subjects after being cultured with iron compounds

After 8 h of culture with the different iron compounds, the rate of cell apoptosis was measured. When the cells were cultured in the absence of iron, the percentage of apoptotic cells was increased in CKD-5 (9.8 ± 2.1) in comparison to healthy subjects (7.2 ± 2.3; P < 0.005). After adding the four iron preparations, the percentage of apoptotic cells increased significantly in cells from CKD-5 (iron sucrose: 41 ± 4.2%; iron dextran: 37.8 ± 3.6%; ferric gluconate: 41.4 ± 5.1% and ferric carboxymaltose: 39.8 ± 4.8%) as compared to control (iron sucrose: 29.5 ± 3.3%; iron dextran: 26.7 ± 4.1%; ferric gluconate: 30.6 ± 2.2% and ferric carboxymaltose: 30.2 ± 2.7%; P < 0.005). There were no significant differences between the iron preparations (Figure 5).

Effects of original and generic iron sucrose on mononuclear cells

An additional study was performed in 8 of the 10 patients (one patient died and the other received a kidney transplant). A generic compound of iron sucrose was administered into the eight patients following the same infusion protocol. The percentage of cells with ROS production, ICAM-1 expression and apoptosis was significantly increased with generic iron compounds at T2 and T3 in comparison to the original iron sucrose (Table 2). There were no significant differences between T0 and T1.

An in vitro study was also performed to analyse the differences between the sucrose compounds. ICAM-1 (P < 0.002) and ROS (P < 0.03) levels rose higher with the generic compound compared with the original iron sucrose (Figure 6). We also observed a higher percentage of apoptotic cells with the generic iron as compared with the original iron sucrose (P < 0.002) (Figure 6).
Effects of intravenous iron preparations

In this study, we examined the potential effects of various iron compounds in vivo and in vitro on activation and damage in mononuclear cells. We conducted a randomized prospective study in patients who were undergoing HD. The percentage of cells that expressed ROS, ICAM-1 and apoptosis increased significantly after the iron infusion as compared with saline. To confirm the effects that were induced in vivo by these compounds, we developed an in vitro study. After culturing mononuclear cells from healthy individuals and CKD-5 patients with four iron compounds, we noted a significant increase in ROS, ICAM expression and death by apoptosis. Notably, all iron compounds induced activation and damage in mononuclear cells in vitro and in vivo. There were no significant differences between the four iron compounds.

Previous reports have shown that during HD, there is an intra-dialytic activation of cytokines [23–26] and impaired mitochondrial function that promote ROS generation and increase apoptosis in mononuclear cells [27, 28]. The administration of intravenous iron induces severe oxidative stress and can aggravate pre-existing oxidative conditions in HD patients [15, 17, 18], but it is unknown whether these effects are linked to the type of iron compound used. It has been recently reported that high-molecular-weight iron compounds cause less oxidative stress because they theoretically release less free iron [9].

In a recent study in rats, Toblli et al. [29] examined five intravenous iron preparations. They concluded that high- and low-molecular-weight iron dextran and ferric gluconate have deleterious effects on haemodynamic, functional and inflammatory responses, compared with ferric carboxymaltose and iron sucrose—questioning their clinical suitability due to their toxic effects on the liver, heart and kidneys. They confirmed the hypothesis that iron from the preparations is not entirely utilized. In the particular case of hepatic tissue, it is well known that a risk for potential iron overload could be present after continuous intravenous iron therapy. In our study, to prevent patients from having differences in terms of iron deposit in the organs, all patients have been treated with the same compound (iron sucrose) before starting the study.

When iron was administered, we observed a further significant increase in ROS compared with saline, regardless of the iron compound. It is important to highlight that at the end of dialysis ROS activity achieved similar levels as that without iron infusion.

It has been reported that slow intravenous iron administration does not modify oxidative stress or inflammatory markers during HD [30]; although this phenomenon could be caused by the no over-saturation of transferrin and the low level of non-transferrin-bound iron (NTBI), Kooistra et al. [31] demonstrated that there were no differences in serum iron concentration, transferrin saturation or NTBI between the slow and the fast protocol of iron infusion. Due to the controversy about time of infusion, we decided to use the recommended dose for each compound, and the same rate of infusion of 30 min was employed for all iron compounds. Further studies are necessary using different times with the same iron infusions, in order to explore whether an increase in the time of infusion may reduce the peroxidative side effects induced by intravenous iron administration. To evaluate cellular activity, we analysed ICAM-1 expression levels per cell [32]. During HD with saline, ICAM-1 expression did not change, but iron administration induced an increase in cell activation. Although a slight decrease was observed at the end of dialysis, ICAM-1 expression remained elevated compared with baseline. No significant differences were detected between the four iron preparations.

With regard to cell death by apoptosis, we observed an effect that was related to dialysis per se [33, 34]; apoptosis rose significantly after the first hour of dialysis, increasing further at the end of the session. Apoptosis increased significantly after administration of the iron, remaining elevated at the end of HD. Oxidative stress and the induction of apoptosis are linked [35]. Therefore, this marked increase in apoptosis might be promoted initially by the oxidative stress that is caused by HD and subsequently...
results are agreed with some researchers [37–39]. The specific iron compound and the HD session. These are associated with a high percentage of apoptosis, independent of the compound—causing a disparate sequence of events. We noted a higher increase in ROS, ICAM-1 and apoptosis with the generic iron compared with the original iron sucrose. We propose two explanations for these differences: an effect of the vehicle or the 3-dimensional structure of the iron compound. The design of this study does not allow us to address this question. Further studies are needed to investigate the safety of similar iron compounds.

This study has limitations. A potential limitation was the strict inclusion and exclusion criteria that we used in selecting patients (only 10 patients fulfilled all the criteria). All of them were dialysed with ultrapure water, a high-flux membrane and an adequate dose of dialysis, and they were well nourished and not severely inflamed. Further studies will be needed to assess whether these observations can be extrapolated to the general HD population. The data from our ancillary study, comparing the two iron sucrose compounds, should be analysed with caution, because this study was not randomized and we could not perform an in vitro study with the vehicle.

Our results strongly suggest that intravenous iron has deleterious effects on immunocompetent cells. The four iron compounds evaluated produced similar effects on the oxidative stress, cell activation and apoptosis. Although cellular damage is increased after iron administration, previous observational studies have not shown adverse clinical outcomes. Therefore, the clinical relevance of our study should be cautiously analysed. In addition, the effects of iron compounds with the same formula may be different, thus further investigation may be required to establish the safety of iron preparations that theoretically have the same composition.

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Conflict of interest statement. None declared.

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