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Comparison of the renal, cardiovascular and hepatic toxicity data of original intravenous iron compounds

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Abstract

Background. Intravenous (i.v.) iron is essential for managing haemoglobin levels in haemodialysis patients. However, i.v. iron may cause variable degrees of toxicity. This is mainly related to the pharmacological characteristics of any given i.v. iron compound.

Methods. This blinded study examines the effects of five i.v. iron preparations on haemodynamic and functional parameters. Sixty Sprague-Dawley rats (n=10/group) received high or low molecular weight (HMW/LMW) iron dextran, ferric gluconate (FG), ferric carboxymaltose (FCM), iron sucrose (ISC) or isotonic saline solution (control). Five i.v. doses of iron (40 mg iron/kg) or saline were administered over 4 weeks.

Results. Systolic blood pressure was significantly reduced in the LMW dextran group, whereas serum iron and percentage transferrin saturation were significantly elevated in all treatment groups. Creatinine clearance was reduced and urinary protein excretion increased in the FG group only ($P < 0.01$). Liver enzyme levels in the blood were increased ($P < 0.01$) in the FG and two dextran groups compared with the FCM and ISC groups. Analysis of liver, heart and kidney homogenates showed a significant increase in catalase and malondialdehyde levels in the FG group, and an increase in CuZn-superoxide dismutase and glutathione (GSH) peroxidase activity accompanied with a decrease in the reduced-to-oxidized GSH ratio in the FG and two dextran groups ($P < 0.01$). Tumour necrosis factor alpha and interleukin-6 levels were significantly elevated in liver, heart and kidney samples from the FG and two dextran groups but not the FCM, ISC or control groups.

Conclusions. These findings indicate that FG and HMW/LMW iron dextran have less favourable safety profiles than FCM and ISC in normal rats.

Keywords: anaemia; chronic kidney disease; intravenous iron; oxidative stress; rats

Introduction

Iron deficiency anaemia is a condition present in the majority of chronic kidney disease (CKD) patients receiving haemodialysis [1]. Intravenous (i.v.) iron is essential for the management of haemoglobin (Hb) levels in this patient population, and both the National Kidney Foundation Kidney- Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) [2] and European Best Practice Guidelines [3] recommend use of i.v. iron over oral iron for the treatment and prevention of iron deficiency anaemia in CKD patients. Numerous i.v. iron preparations are utilized in clinical practice including iron dextrans, iron sucrose (ISC) and ferric gluconate (FG) (sodium FG complex in sucrose). Each of these iron preparations has a different molecular weight, unique physiochemical characteristics, degradation kinetics and side-effect profiles [4]. The relative safety of i.v. iron preparations in the management of anaemia in CKD remains unknown since direct head-to-head comparative trials are lacking [5].

All i.v. iron compounds comprise a central core containing polynuclear ferric (i.e. iron[III]) hydroxide iron shielded by a carbohydrate shell [6,7]. The rate at which the complex is eliminated from the circulation varies. In general, the smaller molecular weight compounds have a shorter half-life and release larger amounts of ‘free’ iron into the circulation [8]. The concentration of non-transferrin bound iron (NTBI)/free iron has been shown to correlate with an increase in the incidence of adverse events following i.v. administration of ISC [9]. It has been proposed that NTBI may catalyse a number of reactions that lead to oxidative stress and membrane damage [10]. Thus, the extent of iron release into the circulation determines the maximal single dose for each iron preparation, which must be gauged to avoid the potential saturation of transferrin binding sites [10]. The stability of the iron complex therefore influences the potential toxicity of the i.v. iron preparation [7].

High and low molecular weight (HMW/LMW) iron dextran preparations have been associated with anaphylactic

reactions and dose-related adverse reactions [5,11,12]. As a result, compounds such as ISC [Venofer®, Vifor (International), Switzerland] are considered to have a favourable safety profile since they show no cross-reaction with dextran antibodies, thus making them preferable for the treatment of iron deficiency anaemia in CKD patients [6,11,13]. There is also an absence of dextran in FG [14], and a study by Bailie *et al.* [13] revealed a high incidence of type I adverse events (urticaria, upper airway angiooedema, anaphylactoid reactions and anaphylaxis) in patients receiving iron dextran compared with FG (intermediate reporting rate) and ISC (zero reporting rate). These results further confirm the correlation between the molecular weight and the stability of a compound, i.e. the increased risk of free iron release, which limits total single dosing of low molecular weight preparations. Ferric carboxymaltose (FCM), a new i.v. iron compound that does not contain dextran, has a good safety record and, moreover, appears to meet an unmet clinical need since high doses may be administered in a short time frame [15]. These observations are based on studies involving FCM for the treatment of anaemia that have been published in the last 2 years [16–20].

The present study represents a novel comparative safety study in rats and examines five i.v. iron products (HMW iron dextran, LMW iron dextran, FCM, ISC and FG) in terms of their toxicity profiles and effects on parameters including oxidative stress.

Materials and methods

All experiments were approved by the Hospital Alemán Ethics Committee and the Teaching and Research Committee and performed in accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1985). Thirty male and thirty female 2-month-old Sprague-Dawley rats (Laboratory of Experimental Medicine, Hospital Alemán, Buenos Aires, Argentina) weighing 200–220 g were randomized into six groups with an equal male/female distribution (n=10/group). The control group received isotonic saline solution; group A received FCM (Ferinject®); group B received HMW iron dextran [Dexferrum® (American Regent, Inc., USA)]; group C received LMW iron dextran [INFeD® (Watson Pharmaceuticals, USA)/CosmoFer® (Vitaline Pharma, UK)]; group D received FG [Ferrlecit® (Watson Pharmaceuticals, USA)]; group E received ISC {Venofer® [Vifor (International), Switzerland]}. The investigators were blinded to the treatment group; i.v. iron preparations were labelled A–E in alphabetical order.

Rats were housed in metabolic cages in a temperature-controlled room (22 ± 2°C) subject to 12-h light/dark cycles (07.00–19.00). All animals received free access to tap water and were fed standard rat chow (16–18% protein, Cooperación, Argentina) ad libitum throughout the study. Rats from each experimental group received a single i.v. dose by tail vein injection of the corresponding iron compound (40 mg iron/kg) or control solution (equivalent volume) at the same time every 7 days for 4 weeks (total of five administrations; days 0, 7, 14, 21 and 28) — injections were given by an investigator who was blind to the treatment group. Dose treatments were adjusted each week according to the body weight of each animal.

Blood samples were obtained for biochemical assessment of Hb, serum iron and percentage transferrin saturation (TSAT) 24 h after the first i.v. iron dose and every 7 days for 4 weeks (days 1, 8, 15, 22 and 29). Urine was also collected for 24 h in each experimental group over the same time period, using methods described previously [21]. Rats were sacrificed 24 h after the last i.v. iron dose by subtotal exsanguination under anaesthesia (sodium thiopental 40 mg/kg intraperitoneal) according to institutional guidelines for animal care and use. The liver, heart and kidneys of each rat were perfused with ice-cold saline solution through the abdominal aorta until they were free of blood and then removed for oxidative stress evaluation, microscopy and immunohistochemical study.

Blood pressure measurement

Systolic blood pressure (SBP) was measured after i.v. iron treatment by tail-cuff plethysmography every 7 days for 4 weeks. Rats were restrained in a plastic chamber without anaesthesia, and cuff pressure was determined by a Pneumatic Pulse Transducer using a Programmed electrosphygmomanometer PE-300 (Narco Bio-Systems, Austin, TX). Pulses were recorded on a Physiograph MK-IIIS (Narco Bio-Systems, Austin, TX); a minimum of three measurements were taken at each session and SBP was calculated as the average of the three readings.

Biochemical procedures

All animals were subject to 14 h of fasting before blood samples were collected from the tail vein in capillary tubes; Hb concentration, serum iron and percentage TSAT were obtained using traditional methods. Liver enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), were also assessed in the blood samples by standard methods in the same laboratory. Aliquots of urine were assayed for creatinine using the enzymatic ultraviolet method (Randox Laboratories Ltd, Crumlin, Northern Ireland). Creatinine clearance (CrCl) was determined by the standard formula, and urinary protein excretion was determined using the sulphosalicylic acid method.

Evaluation of oxidative stress parameters in liver, heart and kidneys

Samples of the liver, heart and kidneys were homogenized (1:3, w:v) in ice-cold 0.25 M sucrose solution. The 10,000 ×g supernatant was used to determine glutathione (GSH) levels by methods described previously [22,23]. Further samples of the corresponding perfused tissues were homogenized (1:10, w:v) in 0.05 M sodium phosphate buffer solution (pH 7.4) and used for the determination of malondialdehyde to evaluate lipoperoxidation by thiobarbituric acid reactive species (TBARS). The remaining homogenate was centrifuged at 4°C for 15 min at 9500 ×g. The resulting supernatant was used to measure catalase activity. Finally, the remaining tissue samples were homogenized (1:3, w/v) in ice-cold sucrose solution (0.25 M). The supernatant obtained after centrifugation at 105,000 ×g for 90 min was used to measure CuZn-superoxide dismutase (CuZnSOD) and GSH peroxidase (GPx) activity. Enzyme units (U) were defined as the amount of enzyme producing 1 nmol of product or consuming 1 nmol of substrate (catalase) under the standard incubation conditions. Specific activity was expressed as units per milligram protein. One unit of CuZnSOD was defined as the amount of CuZnSOD capable of inhibiting the rate of NADH oxidation measured in the control by 50%.

Light microscopy and immunohistochemical study

Decapsulated kidneys, liver and heart samples were cut longitudinally, fixed in phosphate-buffered 10% formaldehyde (pH 7.2) and embedded in paraffin. Three-micron sections were cut and stained with haematoxylin–eosin (H&E), Masson's trichrome and Prussian blue (iron detection technique). All observations were made using a light microscope Nikon E400 (Nikon Instrument Group, Melville, NY, USA).

Immunolabelling of specimens was carried out using a modified avidin–biotin–peroxidase technique (Vectastain ABC kit, Universal Elite, Vector Laboratories, CA, USA). Following deparaffinization and rehydration, the sections were washed in phosphate-buffered saline (PBS) for 5 min. Quenching of endogenous peroxidase activity was achieved by incubating the sections for 30 min in 1% hydrogen peroxide in methanol. After washing in PBS (pH 7.2) for 20 min, the sections were incubated with blocking serum for a further 20 min. Thereafter, the sections were rinsed in PBS and incubated with biotynilated universal antibody for 30 min. After washing in PBS a final time, the sections were incubated for 40 min with Vectastain Elite ABC reagent (Vector Laboratories, CA, USA) and exposed for 5 min to 0.1% diaminobenzydine (Polyscience, Warrington, PA, USA) and 0.2% hydrogen peroxide in 50 mM Tris buffer (pH 8). Tissue ferritin was quantified using antiferritin monoclonal antibody (Biogen, San Román, CA, USA).

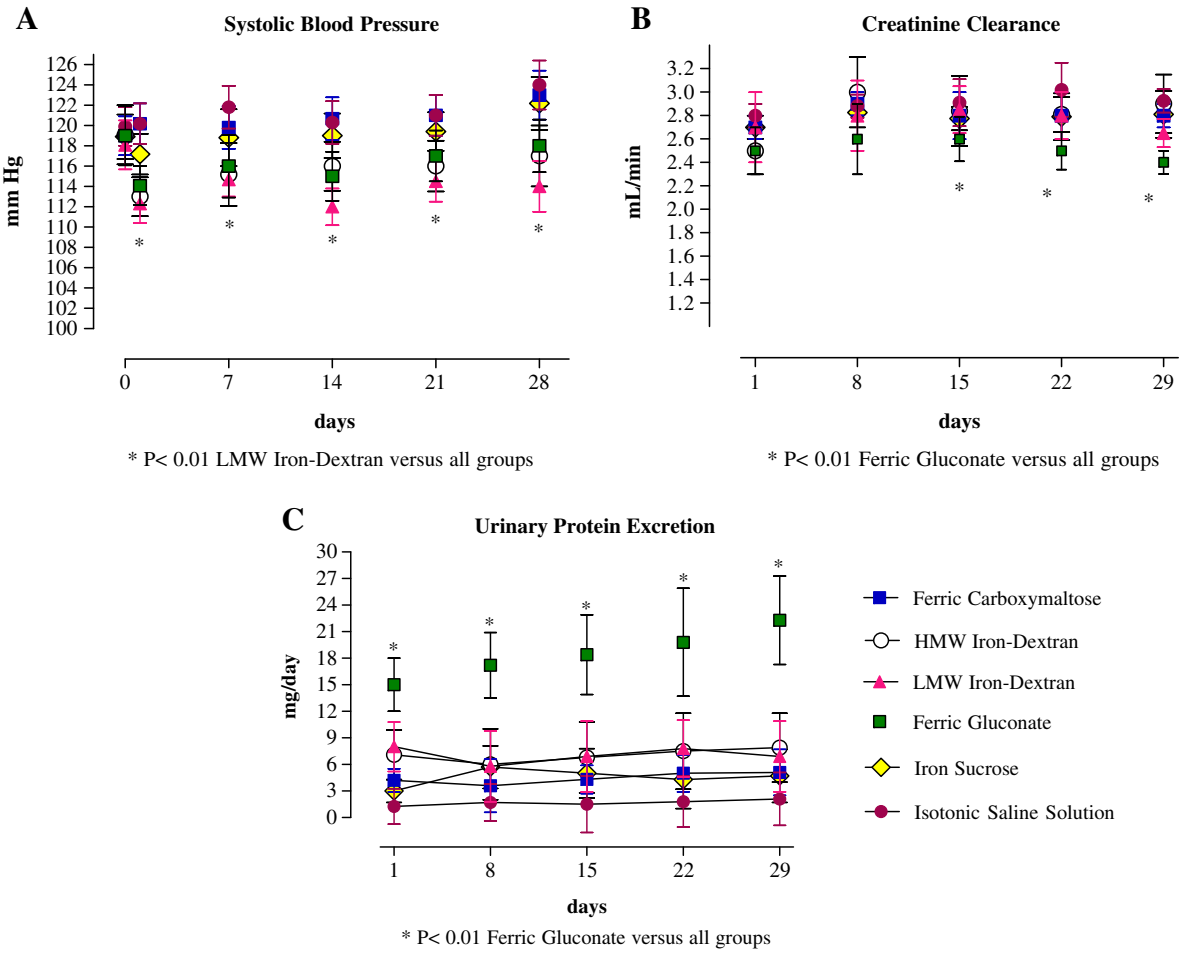

Fig. 1. (**A**) Systolic blood pressure, (**B**) creatinine clearance and (**C**) urinary protein excretion recorded after weekly i.v. administration (40 mg iron/kg or equivalent volume) in the HMW iron dextran, LMW iron dextran, ferric gluconate, ferric carboxymaltose, iron sucrose and control groups over a 4-week time period.

Immunofluorescence

The deparaffinized sections were immersed in 10 mM citrate buffer and heated in a microwave oven at 95°C for 10 min. Sections were then incubated with normal donkey serum for 60 min and subsequently treated with a goat polyclonal antibody [anti-rat interleukin-6 (IL6)] at a dilution of 1:100 (Santa Cruz, San Román, CA, USA). A secondary donkey/anti-goat (IgG-FITC) antibody (Santa Cruz, San Román, CA, USA) was used at a dilution of 1:200 to treat the sections. In order to detect tumour necrosis factor alpha (TNF-α), a goat monoclonal antibody (anti-rat IgG-tetramethyl rhodamine iso-thiocyanate (TRITC)) (Zymed Laboratories, Invitrogen Corporation, Carlsbad, CA, USA) was instead used at a dilution of 1:100 to treat the sections.

Histological sections incubated with PBS rather than the primary antibody were used as negative controls. Specimens were counterstained with 4′,6-diamidino-2-phenylindole to label the total nuclei. Immunostaining was visualized on a Nikon E400 fluorescence microscope equipped with a high pressure mercury lamp and appropriate filter sets. Images were acquired with a digital camera (Nikon Instrument Group, Melville, NY, USA).

Morphometric analysis

Histological sections were studied in each animal with an image analyzer (Image-Pro Plus version 4 for Windows, Media Cybernetics, LP, Silver Spring, MD, USA). Morphological analyses were performed at a magnification of ×100 or ×400 depending on the tissue being evaluated. In all cases, the investigator was blinded to the animal treatment group. In liver, heart and kidney samples, iron deposits, tissue ferritin and inflammatory markers (TNF-α and IL6) were evaluated by the percentage of positive Prussian blue staining, ferritin immunostaining and TNF-α or IL6 immunostaining per square millimetre, respectively, using light microscopy; mean percentage values were calculated for each rat.

Statistical methods

Values were expressed as mean ± SD. All statistical analyses were performed using absolute values and processed through GraphPad Prism version 2.0 (GraphPad Software, Inc. San Diego, CA, USA). For parameters with a Gaussian distribution, comparisons among groups were performed using analysis of variance (ANOVA); for those parameters with a non-Gaussian distribution, comparisons were performed using a Kruskal–Wallis test (non-parametric ANOVA) and a Dunn's multiple comparison test. A value of $P < 0.05$ was considered significant.

Results

Rats from each experimental group received a single i.v. dose of the corresponding iron compound (40 mg iron/kg) every 7 days for 4 weeks (total of five administrations; days 0, 7, 14, 21 and 28). SBP recordings in the LMW iron dextran group were significantly lower than those in the FCM, ISC, FG, HMW iron dextran and control groups throughout the study on days 0, 7, 14, 21 and 28 ($P < 0.01$) (see Figure 1A). Serum iron con-

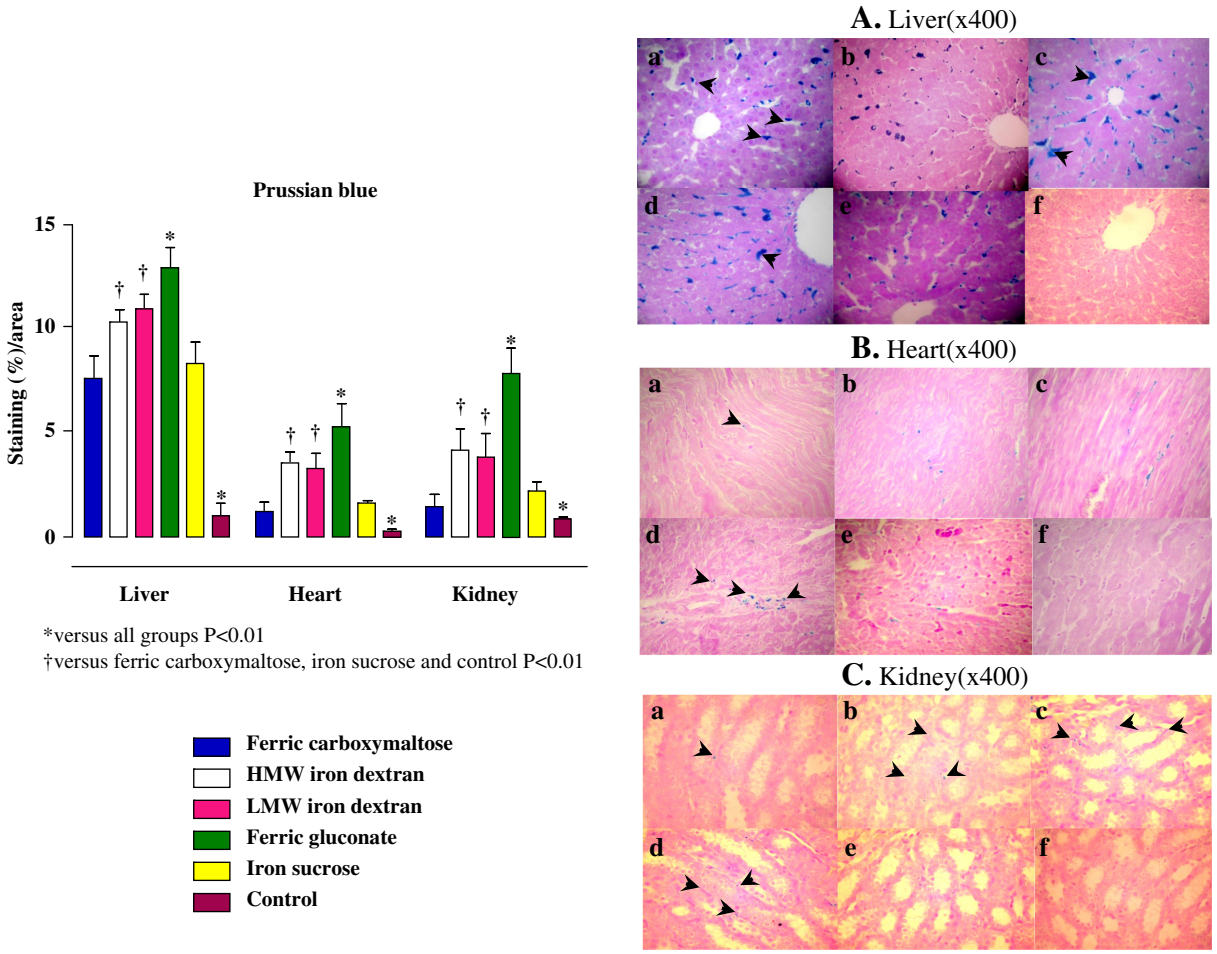

Fig. 2. Bar chart and corresponding micrographs showing Prussian blue staining for iron deposits in (**A**) liver, (**B**) heart and (**C**) kidney samples taken from the HMW iron dextran, LMW iron dextran, ferric gluconate, ferric carboxymaltose, iron sucrose and control groups on day 29.

centration was significantly elevated in all i.v. iron-treated rats compared with control on days 1, 8 and 29 ($P < 0.01$) (see Table 1). Percentage TSAT was also significantly higher in the i.v. iron-treated groups compared with control on days 1, 8 and 29 ($P < 0.01$) (see Table 1). FG caused the greatest increase in percentage TSAT ($P < 0.05$) and serum iron ($P < 0.01$) whilst FCM, ISC and LMW iron dextran produced similar values which exceeded those for HMW iron dextran. No significant differences were observed in Hb concentration between the i.v. iron-treated and control groups throughout the study.

CrCl was significantly reduced in rats treated with FG ($P < 0.01$) compared to the levels in rats treated with FCM, ISC and isotonic saline solution (control) (see Figure 1B). Urinary protein excretion was also significantly increased on days 1, 8, 15, 22 and 29 in the FG group versus all other groups ($P < 0.01$) (see Figure 1C). Liver enzymes (AST, ALT and ALP) were markedly increased ($P < 0.01$) in blood samples from rats receiving HMW and LMW iron dextran and FG on days 1, 8 and 29 compared with FCM, ISC and isotonic saline solution (control) (see Table 1).

Lipoperoxidation was evident in the liver, heart and kidney tissues of FG-treated rats which showed a significant increase in malondialdehyde (TBARS) and catalase at the end of the study on day 29 ($P < 0.01$) compared with all groups (see Table 2). HMW and LMW iron dextran and FG caused a significant increase in CuZnSOD and GPx activity in liver, heart and kidney samples compared with FCM, ISC and isotonic saline solution (control) on day 29 (see Table 2). In contrast, the ratio of reduced-to-oxidized GSH (GSH:GSSG) was significantly reduced on day 29 in liver, heart and kidney samples from the HMW and LMW iron dextran groups and particularly the FG group compared with the FCM, ISC and control groups (see Table 2). Overall, FCM and ISC did not cause a significant increase in oxidative stress parameters in liver, heart and kidney samples compared with isotonic saline solution (control).

Twenty-four hours after the last i.v. iron administration (after 4 weeks/day 29), microscopy studies of the liver showed significantly more positive staining for iron (Prussian blue) in the Kupffer's cells and hepatocytes of rats treated with HMW and LMW iron dextran and FG compared to rats treated with FCM, ISC and isotonic saline solution (control) ($P < 0.01$) (see Figure 2A). However, the area of positive staining for ferritin in the liver was less in HMW and LMW iron dextran and FG-treated rats than in FCM and ISC-treated rats ($P < 0.01$) (see Figure 3A).

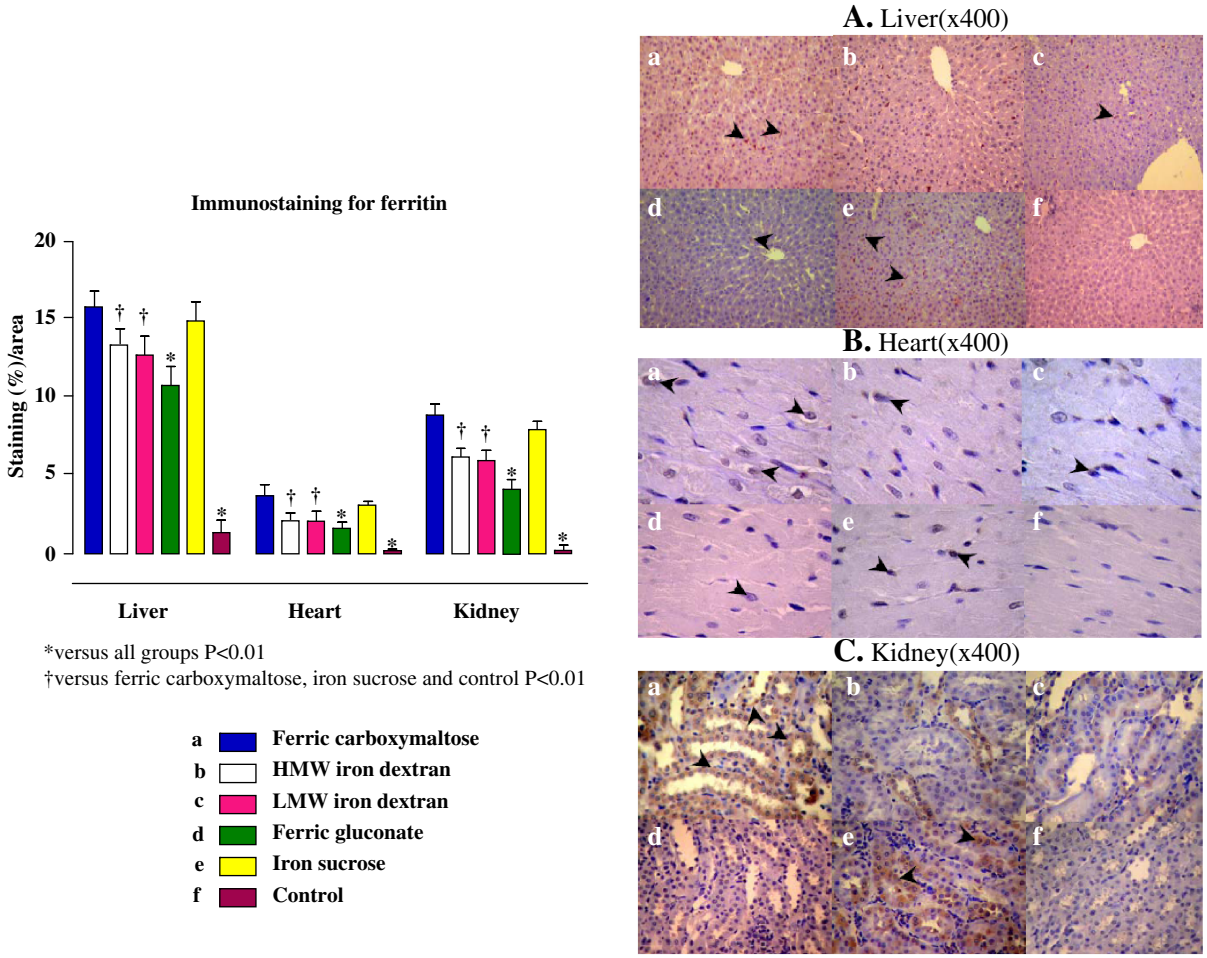

Fig. 3. Bar chart and corresponding micrographs showing ferritin immunostaining for stored iron in (**A**) liver, (**B**) heart and (**C**) kidney samples taken from the HMW iron dextran, LMW iron dextran, ferric gluconate, ferric carboxymaltose, iron sucrose and control groups on day 29.

After 4 weeks (day 29), HMW and LMW iron dextran and FG-treated rats showed significant positive staining for iron (Prussian blue) in the cardiomyocytes and interstitium of heart tissue ($P < 0.01$) (see Figure 2B). In contrast, rats treated with FCM, ISC and isotonic saline solution (control) showed no significant positive staining for iron. Ferritin deposits were conversely smaller in the HMW and LMW iron dextran and FG groups and greater in the FCM and ISC groups (see Figure 3B).

Similar results were observed in the kidneys; at the end of the study (4 weeks/day 29), rats treated with HMW and LMW iron dextran and FG showed a significant positive staining for iron (Prussian blue) in the tubular epithelial cells as well as in the renal interstitium compared to rats treated with FCM, ISC and isotonic saline solution (control) ($P < 0.01$) (see Figure 2C). In parallel with the liver and heart samples, ferritin immunostaining revealed significant renal deposits in the FCM and ISC-treated rats whereas staining was less in the HMW and LMW iron dextran and FG groups (see Figure 3C).

Upon completion of the experiments (4 weeks/day 29), levels of the inflammatory markers TNF-α and IL6 were markedly increased in the liver, heart and kidney samples taken from the HMW and LMW iron dextran groups, and particularly from the FG group compared with the FCM, ISC and control groups ($P < 0.01$) (see Figures 4A–C and 5A–C).

Discussion

Administration of i.v. iron has become an integral part of the approach to optimizing anaemia management in CKD patients, and i.v. iron offers numerous advantages over oral iron supplementation. In clinical practice, i.v. iron treatment has increased dramatically over the last decade but there have been concerns about the side effects associated with certain forms of i.v. iron, particularly iron dextran-based preparations; the different iron dextran preparations have molecular weights between 103,000 and 523,000 Da [7]. Depending on their molecular weight, iron dextran preparations can have a half-life in the blood of up to 3 days [7]. This therapy has the major disadvantage of allergic/anaphylactic reactions to dextran which may be fatal [10,24,25]. As a result, non-dextran containing agents such as ISC, which has a very good safety record, are preferred for the treatment of iron deficiency anaemia in CKD patients [11,13]. Nephrologists utilize a variety of i.v. iron

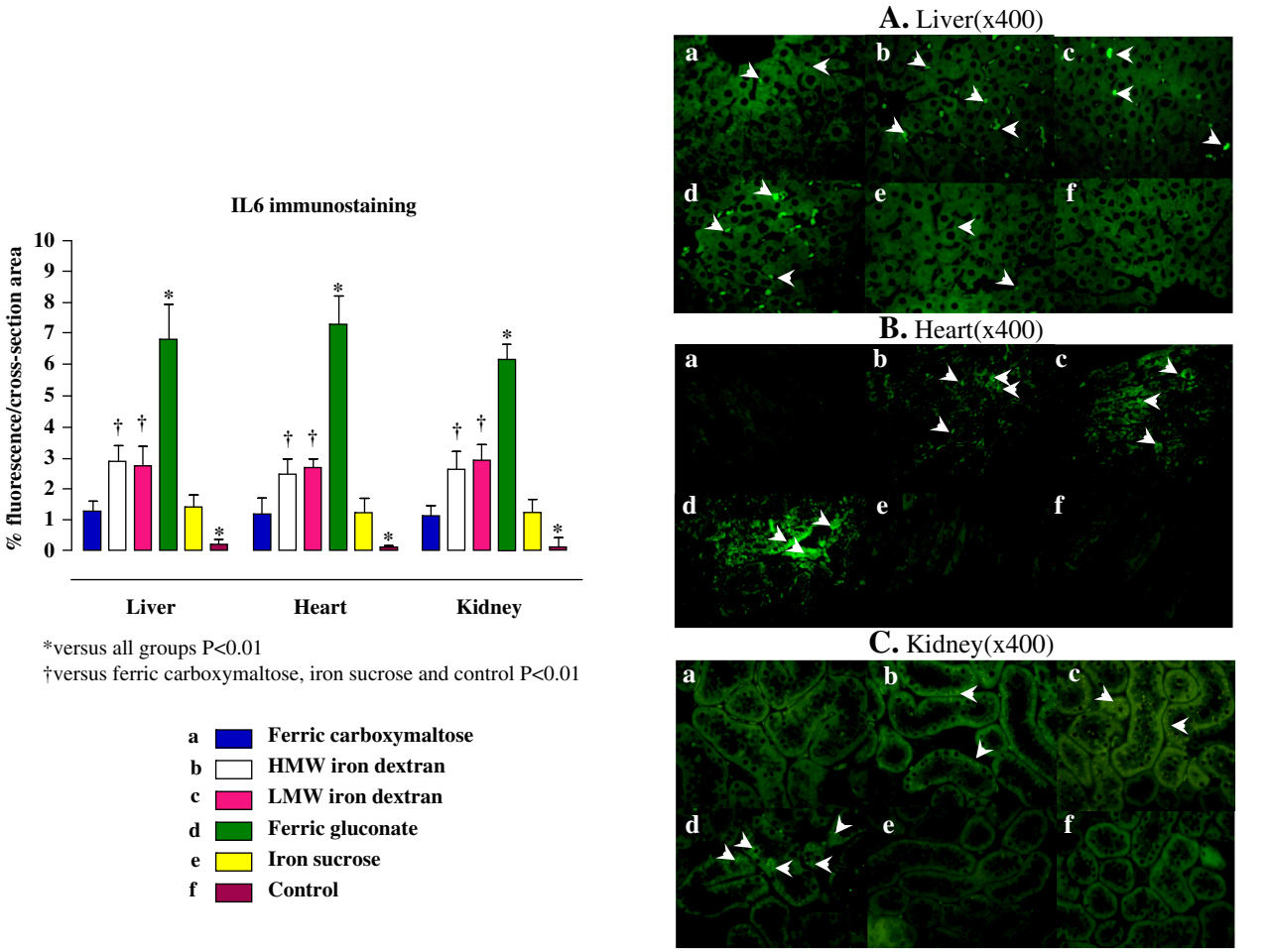

Fig. 4. Bar chart and corresponding micrographs to show IL6 immunostaining in (**A**) liver, (**B**) heart and (**C**) kidney samples taken from the HMW iron dextran, LMW iron dextran, ferric gluconate, ferric carboxymaltose, iron sucrose and control groups on day 29.

treatments in dialysis patients but direct head-to-head trials of the various compounds are lacking [5]. In the present study, iron dextran (LMW and HMW preparations), FCM, ISC and sodium FG — a relatively low molecular weight complex — were compared by their effects on haemodynamic and oxidative stress parameters in rats.

SBP was significantly reduced in the LMW iron dextran group providing an initial indication of the potentially deleterious effects of dextran preparations. Intravenous iron treatment-induced hypotension has been described previously in animal studies and CKD patients [26–28]; moreover, hypotension could be induced by free iron reactions [2]. SBP remained unchanged in the FCM, ISC, HMW iron dextran, FG and control groups throughout the study.

Intravenous iron preparations have differing capacities to saturate transferrin, the iron-transport protein, and generate NTBI. The stability of the iron compounds administered intravenously depends on the exact structure of the complex and in particular the size of the complex and the type of interaction that occur between the polynuclear iron (III)-hydroxide core and the surrounding carbohydrate [29,30]. The weaker or more labile iron complexes liberate iron ions to a larger extent and may saturate transferrin. Thus, the concentration of potentially redox-active NTBI increases [7]. NTBI may catalyse the generation of reactive oxygen species that cause tissue damage and loss of membrane function. Notably, the incidence of adverse events following i.v. iron administration has been shown to correlate with TSAT and the amount of NTBI [9,10]. A moderate increase in serum iron and TSAT was observed following treatment with all of the i.v. iron preparations except FG, for which the increase was significantly larger. This observation suggests that FG is less stable under physiological conditions, as would be expected from its physiochemical properties, in particular the low molecular weight.

Therefore, these findings in our experimental study are in agreement with the clinical experience reported with FG and ISC, in haemodialysis patients [31,32], in which a different outcome was observed when serum markers of iron balance were evaluated. In these studies, even using in both cases a low-dose of maintenance of each i.v. compound, whereas ISC presented a safe iron store profile, therapy with FG was not able to prevent the risk of iron overload in those patients [31,32].

Biochemical analysis revealed that CrCl was significantly reduced in rats treated with FG throughout the study and

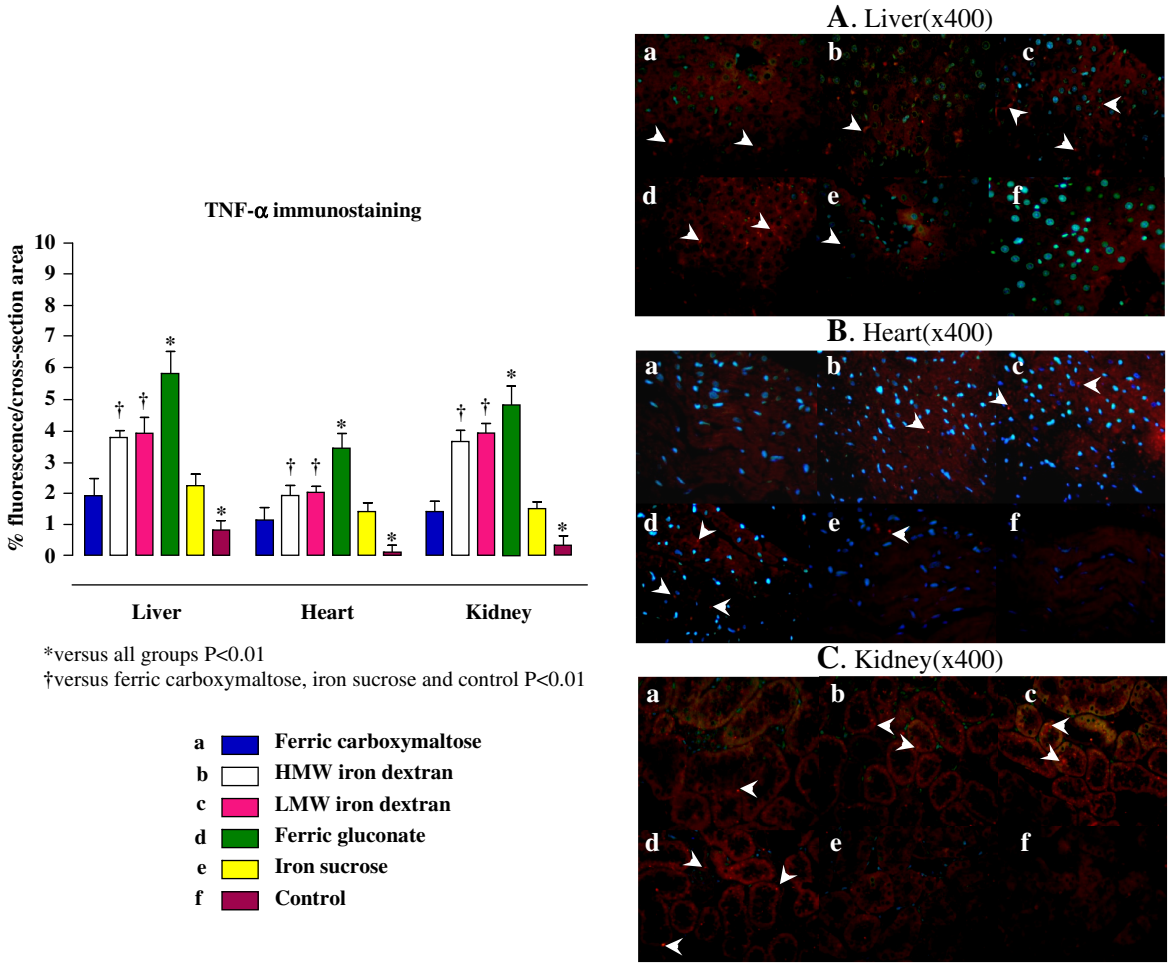

Fig. 5. Bar chart and corresponding micrographs to show TNF-α immunostaining in (**A**) liver, (**B**) heart and (**C**) kidney samples taken from the HMW iron dextran, LMW iron dextran, ferric gluconate, ferric carboxymaltose, iron sucrose and control groups on day 29.

that, furthermore, urinary protein excretion was marked in this group but not the other treatment groups or the control group. These results confirm that treatment with FCM and ISC, as well as HMW and LMW iron dextran, is not associated with renal damage. A significant increase in liver enzymes (AST, ALT and ALP) was recorded in blood samples from rats receiving HMW and LMW iron dextran and FG over 4 weeks compared with FCM, ISC and control. The increased levels of these enzymes are indicative of liver damage and highlight the risk of hepatic injury owing to free iron that accompanies such treatments. These results suggest the relative safety of FCM and ISC.

Oxidative stress in liver mitochondria has been reported in rats subject to acute iron overload with iron dextran [33]. Similarly, in the present study, an increase in the concentration of oxidative stress markers was recorded in response to HMW and LMW iron dextran and FG but not FCM or ISC in liver, heart and kidney samples; elevated malondialdehyde (TBARS) levels and the presence of antioxidant enzymes indicate severe lipoperoxidation. The GSH:GSSG ratio was also significantly reduced in liver, heart and kidney samples from the HMW and LMW iron dextran and FG groups. This symbolizes the consumption of antioxidants under conditions of oxidative stress and thus the reduced defence of cells against further oxidative stress [34,35]. GSH concentration is closely correlated with the degree of renal failure, and reduction of GSH levels may lead to an increase of reactive oxygen species and hence oxidative stress [36]. The results presented in this study confirm that HMW and LMW iron dextran and FG are more toxic than FCM and ISC. FCM is a stable macromolecular complex made of a polynuclear iron (III)-hydroxide core stabilized by carboxymaltose. The stability of the complex is higher than that of the ISC complex and thus the extent of release of ionic iron under physiological conditions is minimal [37]. Single doses of up to 1000 mg iron may be administered in 15 min — a unique property of this preparation — and the results of the present study support the good safety record of FCM [38].

Twenty-four hours after the last i.v. iron administration (day 29), microscopy studies of the liver, heart and kidneys showed significant positive staining for iron (Prussian blue) in HMW and LMW iron dextran and FG-treated rats. This supports the hypothesis that FG releases more iron into the circulation than FCM and ISC, and that the latter compounds are more stable. Moreover, the rapidly released iron ends up in the wrong cellular compartment and may not be completely utilized.

Table 1. Haematology parameters and liver enzymes

Mean ± SD	FCM (n=10)	HMW ID (n=10)	LMW ID (n=10)	FG (n=10)	ISC (n=10)	Control (n=10)
Day 1						
Haemoglobin (g/dL)	15.9 ± 0.2	15.8 ± 0.3	16.0 ± 0.1	16.1 ± 0.2	15.9 ± 0.2	15.8 ± 0.5
Serum iron (μg/dL)	389.0 ± 23.4	345.9 ± 21.8	378.0 ± 30.1	521.0 ± 32.3*	366.0 ± 37.0	300.0 ± 16.3*
TSAT (%)	77.3 ± 4.8	69.2 ± 4.9	78.0 ± 3.8	84.7 ± 5.2*	74.1 ± 5.1	42.8 ± 3.4*
AST (UI/L)	125.6 ± 12.0	179.8 ± 15.2†	183.1 ± 11.8†	212.0 ± 14.6*	128.6 ± 14.9	120.0 ± 15.1
ALT (UI/L)	68.7 ± 10.1	93.5 ± 10.4†	91.1 ± 6.9†	120.3 ± 15.2*	70.1 ± 8.7	60.0 ± 10.5
ALP (UI/L)	553.6 ± 21.1	643.3 ± 19.9†	650.9 ± 15.8†	729.8 ± 30.6*	564.8 ± 25.5	523.0 ± 29.6
Day 8						
Haemoglobin (g/dL)	16.2 ± 0.1	16.4 ± 0.2	16.3 ± 0.2	16.1 ± 0.1	16.4 ± 0.1	15.9 ± 0.4
Serum iron (μg/dL)	412.9 ± 21.9	378.8 ± 12.8	398.0 ± 15.6	526.3 ± 25.1*	400.0 ± 39.0	290.0 ± 20.2*
TSAT (%)	75.5 ± 6.1	69.8 ± 4.1	79.3 ± 3.9	82.9 ± 5.9*	70.0 ± 6.0	42.2 ± 3.3*
AST (UI/L)	130.6 ± 14.8	173.8 ± 13.3†	179.4 ± 10.6†	199.4 ± 22*	135.7 ± 19	126.8 ± 20
ALT (UI/L)	65.1 ± 7.8	90.8 ± 8.9†	87.7 ± 9.0†	108.9 ± 12.7*	68.8 ± 11.6	58.4 ± 13.9
ALP (UI/L)	549.1 ± 25.3	630.2 ± 15.5†	640.8 ± 21.0†	708.0 ± 29.9*	555.1 ± 26.2	515.0 ± 30.2
Day 29						
Haemoglobin (g/dL)	16.4 ± 0.3	16.5 ± 0.2	16.5 ± 0.1	16.4 ± 0.2	16.5 ± 0.3	16.0 ± 0.5
Serum iron (μg/dL)	408.9 ± 17.7	415.1 ± 19.2	422.9 ± 23.0	540.0 ± 19.1*	396.0 ± 27.0	298.8 ± 15.5*
TSAT (%)	74.9 ± 5.9	72.2 ± 6.1	75.5 ± 5.5	83.2 ± 6*	72.9 ± 4	43.9 ± 2.9*
AST (UI/L)	132.0 ± 11.9	161.0 ± 16.0†	172.0 ± 13.3†	195.0 ± 12.1*	138.1 ± 22.3	128.8 ± 18.1
ALT (UI/L)	52.8 ± 11.2	85.8 ± 8†	82.9 ± 5.8†	100.5 ± 11.9*	58.1 ± 9.0	53.9 ± 15.2
ALP (UI/L)	542.9 ± 31.0	633.8 ± 19.4†	645.1 ± 16.9†	694.4 ± 23.8*	540.2 ± 33.2	518.6 ± 22.7

*Versus all groups $P < 0.01$.
†Versus ferric carboxymaltose, iron sucrose and control $P < 0.01$.
FCM: Ferric carboxymaltose; HMW ID: high molecular weight iron dextran; LMW ID: low molecular weight iron dextran; FG: ferric gluconate; ISC: iron sucrose; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase.

The rapid release of iron may also induce extensive lipoperoxidation, leading to secondary cell damage; interestingly, an increased accumulation of iron in rats has been linked to brain ageing and age-related diseases such as Parkinson's disease [39]. The staining observed in the HMW and LMW iron dextran groups further indicates non-metabolized iron.

Despite controversial reports, serum ferritin remains as a marker of iron status in clinical practice. However, it has been reported that there exists a species-difference in the

Table 2. Oxidative stress evaluation in liver, heart and kidney homogenates recorded after weekly i.v. administration (40 mg iron/kg or equivalent volume)

Mean ± SD	FCM (n=10)	HMW ID (n=10)	LMW ID (n=10)	FG (n=10)	ISC (n=10)	Control (n=10)
a. Liver						
TBARs (mmol MDA/g prot.)	55.8 ± 5.0	52.9 ± 8.1	59.9 ± 7.7	90.4 ± 10.1*	60.2 ± 4.0	58.0 ± 4.6
Catalase (U/mg prot.)	268.9 ± 15.1	288.8 ± 18.9	300.8 ± 23.7	367.0 ± 43.9*	289.0 ± 34.1	276.2 ± 31.1
CuZnSOD (U/mg prot.)	5.9 ± 0.9	7.4 ± 0.9†	7.5 ± 1.2†	10.5 ± 2.1*	6.1 ± 1.2	5.6 ± 1.0
GPx (U/mg protein)	281.0 ± 29.1	361.0 ± 30.2†	382.5 ± 21.2†	696.1 ± 45.0*	294.0 ± 33.2	265.3 ± 23.0
GSH:GSSG ratio	7.3 ± 0.9	5.7 ± 1.1†	6.2 ± 1.0†	3.2 ± 0.9*	7.1 ± 0.8	7.4 ± 0.7
b. Heart						
TBARs (mmol MDA/g prot.)	39.3 ± 6.0	42.9 ± 7.1	43.2 ± 8.0	76.9 ± 6.1*	41.0 ± 7.1	38.2 ± 6.0
Catalase (U/mg prot.)	31.9 ± 3.9	35.3 ± 4.8	36.2 ± 4.4	67.4 ± 12.1*	33.7 ± 8.1	30.0 ± 8.7
CuZnSOD (U/mg prot.)	12.9 ± 1.2	15.8 ± 2.0†	15.9 ± 1.7†	18.8 ± 1.9*	14.1 ± 2.2	12.2 ± 2.1
GPx (U/mg prot.)	170.1 ± 31.2	242.0 ± 38.2†	259.3 ± 21.2†	568.1 ± 36*	175.0 ± 25.4	149.2 ± 28.3
GSH:GSSG ratio	5.9 ± 0.9	4.2 ± 0.4†	3.9 ± 0.5†	2.8 ± 0.5*	5.8 ± 0.8	6.1 ± 0.6
c. Kidney						
TBARs (mmol MDA/g prot.)	67.9 ± 8.9	64.9 ± 10.5	70.3 ± 9.9	87.5 ± 6.4*	72.0 ± 9.2	67.9 ± 8.1
Catalase (U/mg protein)	155.9 ± 11.9	157.8 ± 10.3	165.9 ± 6.9	199.7 ± 21.0*	167.4 ± 15.2	160.0 ± 21.2
CuZnSOD (U/mg protein)	6.4 ± 1.1	7.2 ± 1.2†	7.8 ± 1.4†	9.3 ± 1.7*	6.7 ± 1.3	6.1 ± 1.2
GPx (U/mg protein)	118.5 ± 16.1	198.0 ± 22.3†	208.1 ± 18†	295.3 ± 34.1*	121.1 ± 12.2	103.4 ± 15.0
GSH:GSSG ratio	7.2 ± 0.7	4.9 ± 0.7†	5.1 ± 0.8†	4.1 ± 0.6*	7.1 ± 0.4	7.8 ± 0.5

*Versus all groups $P < 0.01$.
†Versus ferric carboxymaltose, iron sucrose and control $P < 0.01$.
FCM: Ferric carboxymaltose; HMW ID: high molecular weight iron dextran; LMW ID: low molecular weight iron dextran; FG: ferric gluconate; ISC: iron sucrose; TBARS: thiobarbituric acid reactive species catalase; CuZnSOD: CuZn-superoxide dismutase; GPx: glutathione peroxidase; GSH:GSSG: reduced-to-oxidized glutathione; MDA: malondialdehyde.

iron content of serum ferritin. Interestingly, rat serum ferritin contains only a small amount of iron independent of body iron store [40]. Emphasizing this point, in various studies in female and male rats, the iron/protein ratio of tissue ferritin (liver) was found with values around 0.23 [40–42]. On the other hand, the iron/protein ratio of serum ferritin has been found to be 0.02–0.07 in humans [43–45], 0.11 in dogs [46] and 0.20 in bovine foetuses [47]. Taking into account this background, it may be considered that serum ferritin determination is not a suitable marker to evaluate iron metabolism after the administration of i.v. iron in rats. Consequently, in the present study, the assessment of tissue ferritin in different organs, such as liver, heart and kidney, acquires importance for a more accurate evaluation on iron store in tissue.

Noteworthy, tissue ferritin was markedly increased in the FCM and ISC groups relative to the HMW and LMW iron dextran and FG groups. This observation confirms the hypothesis that iron from the latter three 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 i.v. iron therapy. This situation could be relevant in clinical practice, especially in female dialysis patients even with serum ferritin within guideline-recommended range, as it has been reported by Canavese *et al.* [48] using the superconducting quantum interference device. Additionally, although serum ferritin in humans may be a useful tool for assessing iron overload, computed tomography (CT) studies have demonstrated high sensitivity as non-invasive method for this purpose as demonstrated by Cecchin *et al.* [49]. Taken together, these results suggest that HMW and LMW iron dextran and FG are less effective and possibly less safe than FCM and ISC, due to the reduced tissue ferritin and the larger amount of free iron release. Iron circulating in its non-transferrin-bound form can increase the risk of endothelial damage and inflammation in turn predisposing to infection [29]. Accordingly, the pro-inflammatory markers TNF-α and IL6 were significantly increased in liver, heart and kidney samples from the HMW and LMW iron dextran and FG groups.

Conclusion

It may be concluded that HMW and LMW iron dextran and FG have deleterious consequences on haemodynamic, functional and inflammatory responses, compared with FCM and ISC. Differences in the molecular structures and hence stability and reactivity of these compounds may account for the varied safety profiles of the i.v. iron preparations tested. The results confirm that FCM and ISC are less toxic than HMW iron dextran, LMW iron dextran and FG. The results of this study also raise concerns about the suitability of, primarily, HMW, LMW iron dextran and ferric gluconate in clinical practice due to their deleterious effects on the liver, heart and kidneys.

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Circulating pro-inflammatory CD4posCD28null T cells are independently associated with cardiovascular disease in ESRD patients

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Abstract

Background. Cytomegalovirus seropositivity is associated with an increased risk for cardiovascular disease in end-stage renal disease (ESRD) patients. Circulating pro-inflammatory CD4posCD28null T cells are expanded in cytomegalovirus-seropositive ESRD patients and potentially could mediate atherosclerotic plaque instability and rupture.