Physicochemical and toxicological characterization of a new generic iron sucrose preparation

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- Toxicity

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Abstract

Intravenous iron preparations are key components in the management of anaemia of various etiologies. These iron-carbohydrate complexes permit safe systemic delivery of iron, whilst protecting from the potential toxic effects of oversaturation. This in turn permits efficient haematopoiesis following erythropoietin administration. Since the rate of release of iron is dependent upon the structure of this iron-carbohydrate complex, it is essential to ensure that an intravenous iron preparation is well characterized and its properties documented. This report describes physicochemical and toxicological studies into a new iron sucrose generic preparation, "Iron Sucrose Azad (ISA)", using the original iron sucrose product as reference. It could be demonstrated that the specifications and physicochemical characteristics of ISA reflect those of the reference product. Furthermore, in a rat model previously shown to identify possible toxicological effects of "unsimilar" iron sucrose preparations, ISA was found to have the same properties as the reference product, with both being well tolerated.

1. Introduction

Iron is present in haemoglobin, which is essential for the transport and utilisation of oxygen. Absolute or functional iron deficiency is a common problem in chronic renal disease which results in iron-deficient erythropoiesis and a reduction in available haemoglobin [1]. Indeed, lack of available iron is the most common reason for diminished response to erythropoietin in patients with renal failure and adequate iron replacement can enhance the response to erythropoietin by ensuring an optimal iron supply to the bone marrow [2]. Although oral iron preparations are used as first-line treatment, their effectiveness in more severe cases of anaemia is limited by factors such as poor absorption, lack of tolerance/compliance and the lengthy time required to replete iron stores. Parenteral (intravenous, i. v.) iron preparations circumvent such problems and are therefore used in the treatment of anaemia of predialysis and dialysis patients receiving erythropoietin, pregnancy and post partum anaemia, chronic heart failure and inflammatory bowel diseases.

I. v. iron preparations are colloids that consist of iron-carbohydrate nanoparticles. At the centre of each particle is an iron-oxyhydroxide core. This core is surrounded by a shell of carbohydrate that stabilizes the iron oxyhydroxide, slows the release of bioactive iron, and maintains the resulting particles in colloidal solution. I. v. iron preparations used in the clinic comprise three classes: iron dextran, iron gluconate and iron sucrose, which share the same core, but differ from each other by the identity and size of the surrounding carbohydrate, leading to pharmacologic and biologic differences, such as pharmacokinetic profile, rate of iron release and maximum tolerated dose. Iron gluconate and iron sucrose have more favourable safety profiles than iron dextran, having a lower incidence of anaphylactoid-type reactions [3].

The bulk of iron delivered during i. v. iron administration passes into phagocytes of the reticuloendothelial system (RES) where the iron is released from the complex and is either stored bound to ferritin or taken up extracellularly by transferrin and subsequently deposited in the marrow and taken up by erythroid precursors

[4]. However, a minor amount of iron is likely to bypass the RES, instead being liberated in the plasma where it binds to circulating transferrin. I. v. iron preparations have been shown to liberate iron that binds to and saturates transferrin *in vitro* [5]. Since a major biologic function of iron, aside from its incorporation into haem, is the participation in a variety of oxidation-reduction reactions, the potential exists that following administration of i. v. iron preparations, iron overload caused by rapid release or over-dose could catalyse the formation of reactive species, inducing cell and tissue damage with resultant complications [6, 7].

The reference product has been well characterized in terms of physicochemical structure and behaviour, including rate of iron release [4, 8], and clinical experience has shown the product to be well tolerated [9]. The use of i. v. iron preparations such as iron sucrose has been demonstrated to allow the reduction of erythropoietin administration, thereby decreasing treatment costs for patients receiving haemodialysis [10, 11], which could be further reduced by use of generics. However, development of a generic i. v. iron preparation is not trivial, since iron sucrose is a complex rather than a defined chemical entity and therefore a relatively detailed examination is required to ensure that the generic's profile replicates that of the original.

Two recent publications [12, 13] studying the effects of relatively high doses of i. v. iron preparations in the rat reported that certain generic iron sucrose products, as opposed to the reference product, showed marked toxicological effects. It was hypothesized that the physicochemical properties of the generic i. v. iron preparations differed to the reference product, and that as such these generics might release free iron at a different rate, leading to liberation of free iron thereby catalyzing the formation of oxidizing species, subsequent inflammation and tissue damage.

This report describes the characterization of a generic iron sucrose preparation, Iron Sucrose Azad (ISA), using the original product as reference. The studies performed involved a detailed analysis of the physico-chemical properties and *in vitro* behaviour of ISA, together with an investigation of the action of ISA when administered in the rat model of Tobbli *et al.* [12, 13]. The results indicate that ISA can be classed as a true generic, being indistinguishable from the reference product in all parameters evaluated.

2. Materials and methods

2.1 Chemical analysis

Chemical analysis according to tests described in the USP Monograph for iron sucrose, which is the specification followed by ISA, was performed by the quality control laboratories of Cilag AG (Schaffhausen, Switzerland).

Molecular weight was determined by gel permeation chromatography (GPC) using an Agilent 1100 HPLC system equipped with a refractive index detector and two columns set up in series (Waters Ultrahydrogel 1000 Å and Waters Ultrahydrogel 120 Å, respectively). A phosphate buffer was used as mobile phase and the system run at a constant temperature of 45 °C. Polysaccharide molecular weight standards in the range of 5000 – 400 000 Daltons were used for calibration.

Turbidity: An aliquot of iron sucrose was dissolved in water. Dilute hydrochloric acid was added dropwise until a slight but steady turbidity developed. The pH of the solution was recorded as the turbidity point of iron sucrose.

Iron(II) content and low molecular weight complexes were determined using the polarographic method outlined in section 2.3. The iron(II) content should be below 0.4 % (w/v) and low molecular weight complexes should be absent.

Atomic Force Microscopy was performed by Solvias AG (Basel, Switzerland), according to a procedure adapted from the literature [14]. A droplet of a dilute solution was allowed to absorb on a freshly cleaved mica surface, rinsed and dried with a stream of nitrogen. The samples were analysed under ambient conditions in tapping mode of operation with a Veeco Multimode III a with Nanoscope software V. 5.12r2 (Santa Barbara, CA, USA) and standard etched single crystalline silicon cantilevers (length 125 µm, resonance frequency about 280 kHz). Statistical analysis was applied to the measured nanoparticles where height and width (at half maximum) were determined (40 – 100 nanoparticles per batch).

2.2 *In vitro* analysis of kinetics of degradation

The *in vitro* kinetics of reduction of Fe(III) to Fe(II) in iron sucrose hydroxide complexes was monitored *in vitro* by detection of the decrease of Fe(III) over time via UV-Vis spectroscopy at 450 nm, according to the method of Erni *et al.* [15]. Upon reduction, the reddish-brown coloured polynuclear hydroxocomplex of ferric hydroxide dissociates to the nearly colourless ferrous hydroxide. The "T75 %" reduction time of three batches of reference product and ISA were determined, this being where 75 % of the colloidal Fe(III) hydroxide is reduced and which is taken as the standard measure of degradation kinetics for the reference product [12], the specification being 450 – 840 s.

2.3 Polarographic analysis

The USP monograph describes a polarographic method for iron sucrose injection using differential pulse (DP) polarography under the following conditions: supplementary electrolyte solution of sodium acetate about 1.1 mol/L, adjusted to pH 7.0 with 0.1 N acetic acid. Sample concentration was 20 – 120 µg/mL of elemental iron in the polarographic cell, with a measuring range of 0 to –1700 mV with a mercury drop electrode being used. The expected signals are iron(III)/iron(II) about -750 ± 50 mV and iron(II)/iron(0) about -1400 ± 50 mV. Low molecular weight complexes are identified as peaks in the region of –100 mV to –300 mV.

The iron(II) content can then be calculated using the formula:

$$C_{Fe(II)} = \left[1 - \frac{2}{R}\right] \cdot C_{Fe(total)}$$

where $C_{Fe(II)}$ is the iron(II) content in %w/v, $C_{Fe(totaI)}$ is the total iron concentration in %w/v and R is the peak response ratio.

2.4 Toxicological study in the rat

This study was conducted by BSL Bioservice GmbH (Munich, Germany), to comply with OECD Principles of GLP [16] and the German Act on Animal Welfare (Tierschutzgesetz, July 2009),

being authorized under the BSL Bioservice licence for repeat-dose toxicity studies granted by the government of Upper Bavaria, Munich, Germany. Eight-week-old Sprague-Dawley rats (Charles River, Sulzfeld, Germany) weighing 210–240 g (females) and 300–350 g (males) were kept in groups in a temperature and humidity-controlled room (22 ± 3 °C and 55 ± 10 %, respectively), with 12-h light/dark cycles and free access to Altromin 1324 maintenance diet and microbiologically-controlled tap water. After a 5-day acclimatization period, animals were randomised into 3 groups, each comprising 7 rats of either sex (total 14 rats per test group). The control group received isotonic saline solution (B. Braun, Melsungen, Germany; batch 9474A121, expiry Oct 2012), of equal volume (2 ml/kg body weight) to that administered with the test substances (ISA, batch 1070/expiry Aug 2012 or reference product, batch 985000, expiry March 2012). Doses were adjusted according to the body weight of each individual animal, test substances being given at a dose of 40 mg/kg, on day 0 and day 7.

At two time points blood and urine were sampled in all animals in order to perform biochemistry analysis. Blood was sampled on day 1 (24 h ± 15 min after the first administration) and on day 8 (24 h ± 15 min after the second administration).

To collect urine the animals were placed for approximately 20 h in metabolic cages overnight and deprived of food. The exact duration of urine collection as well as the volume collected were recorded. At each time point, blood was collected under anesthesia (isoflurane or ketamine/xylazine) from a named site (jugular vein or abdominal aorta). Blood was sampled in uncoated tubes for measurement of haemoglobin, iron, transferrin saturation (TSAT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and creatinine.

A careful clinical examination was made at least once a day for the period of observation, with special attention to the first 4 h after administration. The animals were observed up to day 8 after the first administration. General clinical observations were made preferably at the same time each day and considering the peak period of anticipated effects after dosing. The health condition of the animals was recorded. General clinical observations included changes in the skin and fur, eyes and mucous membranes. Also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behaviour pattern were examined. Particular attention was directed to observations of tremor, convulsions, salivation, diarrhoea, lethargy, sleep, and coma. Individual reactions of each animal were recorded at each observation time. Toxic response data were recorded by sex and dose level. Nature, severity and duration of clinical observations were described if any.

2.4.1 Biochemical analysis

In blood samples, iron, TSAT, AST, ALT, ALP and creatinine (Synchron C × 5, Beckman, Krefeld, Germany) and hemoglobin (Sysmex Haematology, Norderstedt, Germany) were measured. Measurement of iron and TSAT was performed (non GLP analysis) at the Klinikum Ingolstadt (Ingolstadt, Germany). Urine creatinine and protein were measured using a Synchron C × 5. From the creatinine values measured in serum and urine (Synchron C × 5), the creatinine clearance was calculated. Based on results of a preliminary test (BSL 10-1404) liver samples collected in each animal were evaluated for superoxide dismutase (SOD) using a commercial superoxide dismutase assay kit (Cayman Chemical Company, Tallinn, Estonia). The oxidative stress parameters were normalized to protein.

2.4.2 Histogolgical analysis

Histopathological processing (haematoxylin and eosin (HE) staining and Perl's Prussian blue staining for iron) were performed by the GLP-certified contract laboratory, Propath UK Ltd, Willow Court, Netherwood Road, Hereford, UK. A histopathological evaluation of the sections was then performed by a pathologist, using a semi-quantitative scoring system for the assessment of iron pigment staining. This was based on the number and size of iron deposits that were observed: absent = no deposits detectable; minimal = very few small deposits; mild = some small deposits; moderate = moderate number of deposits of medium size; marked = many large deposits; massive = extensive and large depositions.

2.5 Statistical procedures

Values are given as mean ± SD. Data sets were compared using the unpaired Student's t-test, comparing each treated group with the corresponding control group. Significance was denoted as * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$ by comparison to control values.

3. Results

The specification for iron sucrose drug product is given by a USP monograph. Table 1 shows the monograph specifications for tests likely to be relevant for safety and efficacy of iron sucrose, compared to the test results for a mean (± SD) of 10 batches of ISA. It can be seen that ISA is within the monograph in all parameters. No test result in any of these batches was outside the USP (individual results not shown), whilst the uniformity of the data indicates a manufacturing process that is robust, a prerequisite for such a product.

Atomic forced microscopy (AFM) was used to image and compare dimensions of ISA and reference product, results being shown in Table 2. The shape of the particles was found to be spheroid, with only very small amounts of aggregated particles. There was a slight but not statistically significant difference between ISA and reference product and their size was in accordance with a detailed analysis of the structure of iron sucrose performed by Kudasheva *et al.* [17] where an average core size of the spherical nanoparticles of the reference product was found to be 3 ± 2 nm.

Table 1: Comparison of USP monograph specifications and ISA test results.

	MW (Dalton)	MN (Dalton)	D (polydispersity)	Turbidity point	Fe(II) content	Low MW complexes
USP	34 000–60 000	NLT 24 000	NMT 1.7	4.4–5.3	NMT 0.4 %	absent
ISA	46 315 ± 1098	36 400 ± 1133	1.28 ± 0.04	4.9 ± 0.1	Not detected	absent

Table 2: Size of iron sucrose particles determined by AFM.

Product	Diameter in nm
Reference product	4.0 ± 2.0
ISA	3.4 ± 1.5

Table 3: Kinetics of degradation.

	Reference product T75 % (s)	ISA T75 % (s)
Batch 1	573 ± 18	588 ± 16
Batch 2	569 ± 18	606 ± 22
Batch 3	571 ± 17	624 ± 11

Since the size of the iron-carbohydrate complex has been shown to be inversely proportional to the rate of iron release *in vitro* [8], it was assessed whether the rates of iron release from ISA and the reference are comparable. The *in vitro* kinetics of degradation of 3 batches of ISA and 3 batches of reference product were analysed, each being measured 10 times. The results in Table 3 show the mean (± SD) of these measurements.

The kinetics of degradation of the reference product batches were slightly faster than those of ISA ($p < 0.05$), although all batches tested were well within the limits for iron sucrose given by the originator [12].

In order to compare the iron(III)/iron(II) reduction potentials of ISA and reference product, 5 batches of ISA and 3 batches of the reference product were compared by polarography. The polarograms and resulting information on the content of low-molecular-weight iron(II) and iron(III) complexes, and on the iron(II) content were compared.

The ISA and reference product samples showed comparable polarograms. In all cases, two peaks corresponding to the iron(III)/iron(II) and the iron(II)/iron(0) redox processes were recorded. Both peaks – especially the iron(III)/iron(II) peak – were relatively broad, which is likely to be caused by the fact that iron sucrose is not a distinct complex with one diffusion constant, but a mixture of different complexes with a molecular-weight and therefore a diffusion-constant distribution. The first peak is broader than the second peak probably because in the first reduction step the iron sucrose complex is destroyed, whilst in the second, only the simple iron(II) ions are reduced. The peak potentials of all ISA as well as the reference product samples were within the USP specifications of –750 ± 50 mV for the iron(III)/iron(II) and of –1400 ± 50 mV for the iron(III)/iron(II) redox process. Representative polarograms are shown in Fig. 1 a (ISA) and Fig. 1 b (reference product), respectively.

None of the samples showed any significant peak potential between –0.3 V (–300 mV) and –0.1V (–100 mV) in the polarogram due to low-molecular-weight iron(II) and iron(III) complexes.

The results for the iron(II) content, effectively being an impurity in the product, are shown in Table 4. The ISA batches showed lower iron(II) contents than the reference product, indicating a marginally higher purity of ISA.

The toxicological study was designed to closely follow the protocol in the publications of Tobbli *et al.* [12, 13], but under highly standardized (GLP) conditions. Since using this protocol toxic effects were clearly apparent

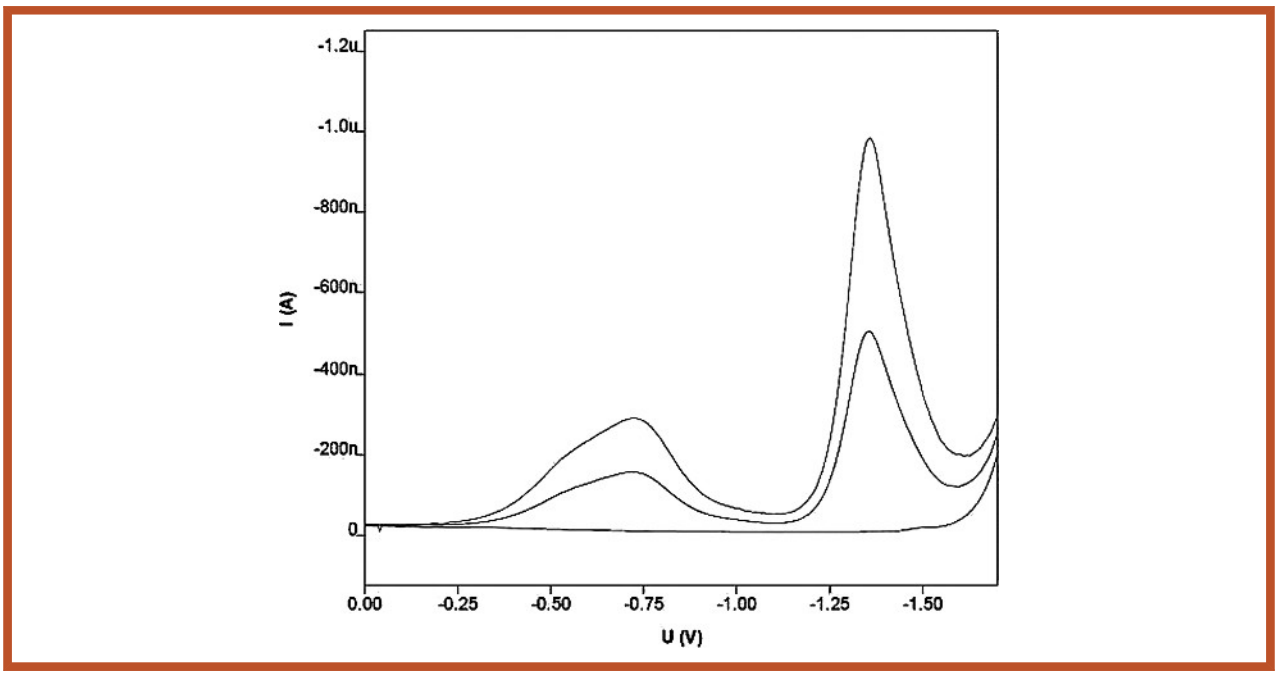

Fig. 1 a: Polarogram of ISA. Top line: iron concentration of 80 µg/mL, middle line: iron concentration of 40 µg/mL, bottom line: blank.

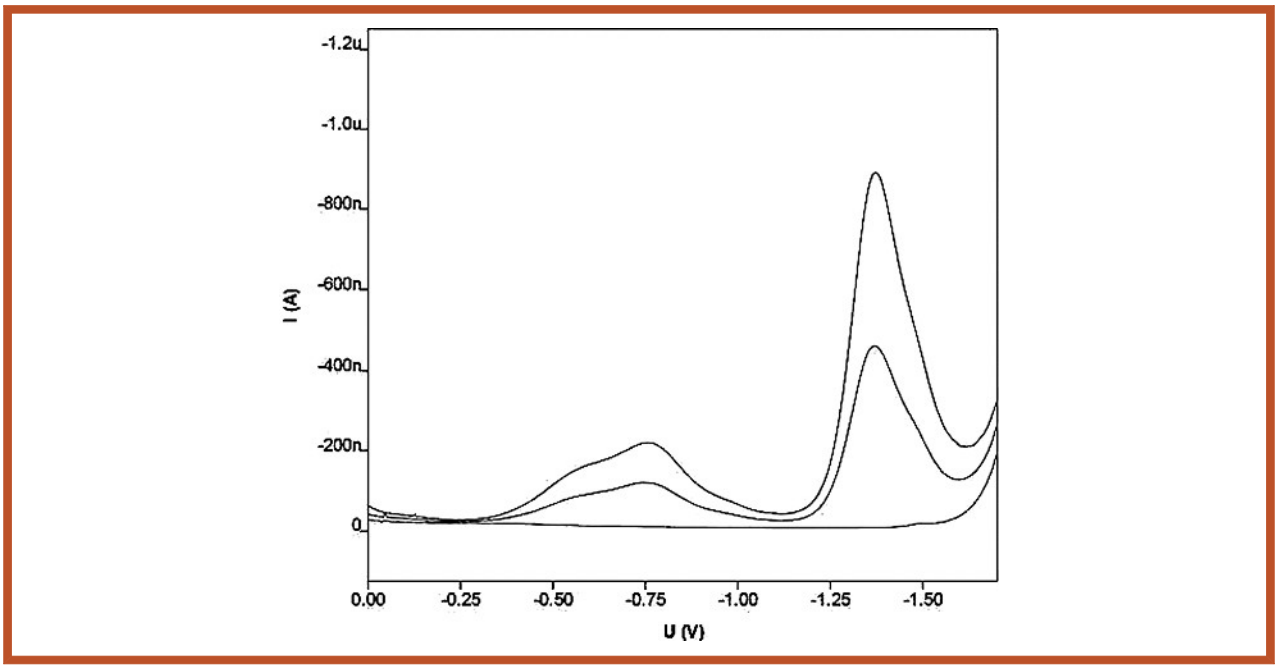

Fig. 1 b: Polarogram of the reference product. Top line: iron concentration of 80 µg/mL, middle line: iron concentration of 40 µg/mL, bottom line: blank.

Table 4: Iron(II) content.

Product	Concentration[1] (µg/mL)	Iron(II) content (%w/v) by peak area[2]
	(20–120 µg/mL)[3]	NMT 0.40[4]
ISA	40	< 0.01[5]
Reference product	40	0.16 %–0.21[6]
ISA	80	< 0.01[5]
Reference product	80	0.07–0.17[6]

[1] Concentration of elemental iron in the polarographic cell (in µg/mL).
[2] All values have been calculated using the peak area under the respective peaks in the polarogram.
[3] Recommended concentration according to the USP monograph for iron sucrose injection.
[4] Acceptance criteria according to the USP monograph for iron sucrose injection.
[5] Five different batches have been tested.
[6] Three different batches have been tested.

after only a single dose and plateaued by day 7 (after 2 intravenous bolus doses of 40 mg/kg), this dosing schedule was used. Following this protocol, multiple test parameters could be assessed: blood biochemistry, markers of organ function and also histopathology, which facilitated detection of any toxicological effects of the administered substances, should these have occurred.

After two intravenous bolus administrations on Day 1 and Day 7 no test product-related adverse clinical effects were seen. Beside slight changes in colour of the liver (brownish) of animals treated with the test products no other macroscopic findings were noted.

Significant increases in blood iron and TSAT levels were observed at both time points, this being the pharmacological action of iron sucrose preparations. Both were more marked at day 1 than day 8. TSAT is a calculated value and levels of over 100 % have been reported in man, following rapid infusion of an i. v. iron preparation [18] being defined as "oversaturation" of the protein. On day 1, but not day 8, a significant increase (around 50 %) in ALT was seen with both reference product and ISA. No other liver enzyme was markedly increased (Table 5). A 20 % increase in blood creatinine was observed for both test products on day 1 (Table 5), which was mirrored by a corresponding decrease in creatine clearance via the urine (Table 6) at this time point. This phenomenon was also seen on day 8, but was considerably less marked.

As can be seen in Table 7, neither the reference product nor ISA influenced SOD levels in the liver. This finding accords with that seen in a preliminary study where both SOD and glutathione peroxidase levels remained unchanged (data not shown).

Compared to the control group, treatment-related histological changes were noted in both treatment groups and were similar in type and degree. Evaluation of H+E-stained slides revealed a minimal increase in the mean severity grade of parenchymal infiltrates with mononuclear cells, in females treated with either reference product or ISA, when compared to the control females or to males of either test group. All other histological findings noted in H+E sections were minimal in degree and considered to represent incidental background changes.

Prussian blue-stained liver slides showed specific staining for iron pigment in hepatic macrophages of all treated rats (Table 8). The zonal distribution of pigment in the liver was predominantly periportal. In addition, in

Table 5: Blood values on Day 1 and Day 8.

	Hb (g/dL)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Creat (µmol/L)	Fe (µg/dL)	Transferrin (g/L)	TSAT (%)
Day 1								
Control	14.5 ± 1.1	44.9 ± 4.7	17.8 ±1.5	171.2 ±80.2	40.2 ± 3.3	182.6 ±104.2	1.20 ± 0.07	108 ± 62
Reference product	15.4 ± 0.6*	49.4 ± 8.0	26.1 ±7.4***	172.9 ±81.9	48.5 ± 4.4***	351.8 ± 60.0***	1.13 ± 0.06*	221 ± 38***
ISA	15.4 ± 0.4**	49.6 ± 8.7	29.4 ± 12.7**	169.9 ± 74.1	48.2 ± 6.4***	314.9** ± 111.7	1.16 ± 0.07	194 ± 71**
Day 8								
Control	14.2 ± 0.5	46.2 ± 7.6	19.8 ± 6.1	151.6 ± 59.2	41.3 ± 5.5	151.8 ± 88.2	1.25 ± 0.1	86.7 ± 51
Reference product	14.05 ± 0.8	52.7 ± 5.6*	20.8 ± 5.0	149.1 ± 53.9	45.6 ± 6.5	270.1 ± 126.6**	1.17 ± 0.12	165 ± 77**
ISA	13.4 ± 1.2*	46.3 ± 6.9	18.2 ± 5.7	142.6 ± 64.2	45.6 ± 4.8*	237.4 ± 124.4	1.16 ± 0.06	145 ± 75*

Significance denoted as *P < 0.05, **P < 0.01 and ***P < 0.001 in comparison to control.

Table 6: Urine values on Day 1 and Day 8.

	Total protein (g/dL)	Creatinine clearance (mL/min)
Day 1		
Control	40.1 ± 17.3	2.11 ± 0.60
Reference product	31.6 ± 22.3	1.68 ± 0.42*
ISA	32.4 ± 19.4	1.59 ± 0.44*
Day 8		
Control	25.5 ± 11.5	2.03 ± 0.65
Reference product	33.4 ± 23.0	1.86 ± 0.63
ISA	35.8 ± 25.2	1.78 ± 0.58

Significance denoted as *P < 0.05 in comparison to control.

Table 7: Liver SOD values on day 8.

	SOD (U/mg)
Control	178.6 ± 28.6
Reference product	169.2 ± 20.1
ISA	185.6 ± 20.0

most treated animals minimal amounts of iron pigment were also found in the hepatocytes. Incidences and severity grades of pigmentation did not indicate any difference between the animals treated with reference product or ISA.

4. Discussion

The aim of these studies was to characterize a new iron sucrose preparation, ISA, in terms of (i) physicochemical properties and (ii) toxicological profile in a rat model reported to be capable of detecting untoward effects of such agents. Results for the chemical analysis of 10 batches of ISA showed that all parameters fully complied with those of the USP monograph for iron sucrose, which had been established to define the chemical nature of the reference product. A more detailed analysis of ISA was performed to ascertain whether the structure and function of ISA and reference product are uniform. Atomic forced microscopy allows the imaging of iron-carbohydrate nanoparticles and direct determination of core size. The results confirmed that ISA and the reference product are of similar size and the data is in line with the literature value for iron sucrose, of 3 ± 2 nm [17]. Polarography data showed that the reduction peak

Table 8: Semi-quantitative scores for iron pigment in the liver.

		Control		Reference product		ISA	
		Male	Female	Male	Female	Male	Female
Iron pigment macrophages	Mild	0	0	2	0	1	1
	Moderate	0	0	5	7	6	6
	Total	0	0	7	7	7	7
Iron pigment hepatocytes	Minimal	0	0	5	7	6	7

of iron(III) to iron(II) is very similar for both ISA and reference product. Additionally, the data demonstrated the virtual absence of low molecular weight complexes and of iron(II). The polarography data therefore further endorsed the concordance of ISA and reference product. Comparison of ISA with the reference product in terms of rate of iron release from the complex confirmed the behavioural similarity of the products. Although the reference product demonstrated a slightly faster rate of iron release, which might suggest a greater propensity to induce iron overload, this difference was minor and the kinetics of both products were well within the originators specification for this parameter [12].

Having confirmed the essential physicochemical similarity of ISA with the reference product, the generic was then tested in a rat model previously demonstrated to detect toxicological effects of "unsimilar" iron sucrose preparations. This was not a dose-finding study, the aim being to administer a high dose in order to ascertain whether ISA might also show toxicological effects, whilst using the original product as reference. Both ISA and the reference were well tolerated at 40 mg/kg. Changes observed were similar in type and degree for both preparations. Liver ALT was raised, this being more marked on day 1. Although this was not seen by Tobbli *et al.* when using the reference product [12, 13], marked increases in AST and ALT were documented in the sub-acute toxicity studies submitted in the marketing approval application for this product in the United States, where similar doses were used [19]. Both the reference product and ISA caused a slight decrease in creatinine clearance, which again was more marked at day 1. Likewise, an increase of serum urea was reported in [19]. Since the pattern of liver enzyme and creatinine modulation followed that of blood iron and TSAT – being more marked at day 1 – it seems likely that a casual relationship existed between these parameters. Histological findings mirrored those found in [19], iron deposition being found in Kupffer cells and hepatocytes, with few other histological changes taking place. The publications of Tobbli *et al.* [12, 13] indicated that certain generic iron sucrose preparations, but not the reference product, increased parameters of oxidative stress. In this study, no evidence of this effect was seen with either product. This is in line with the report of Legssyer *et al.* [20], where little evidence of changes in oxidative stress were seen despite an increase in hepatic iron content, following administration of iron preparations in the rat. It can be concluded that this relatively high dose caused a (possibly transient) minor impairment of liver and kidney function, the extent of which was indistinguishable between ISA and the reference product. The amount of product administered in the toxicological study was an order of magnitude higher than the maximum recommended therapeutic dose (200 mg) of iron sucrose [21]. In a dose-finding study in man, a 2-h infusion of up to 300 mg iron sucrose was shown to cause no adverse reactions, whilst these did appear at 400 mg and 500 mg [22].

The clinical implications, if any, of potential "free" (non-transferrin bound) iron to catalyse the formation of radicals, thus potentially inducing cell damage, remains to be fully clarified. It has been shown that in haemodyalysis patents appreciable amounts of free iron could already be measured prior to a 60-min infusion of 100 mg iron saccharide, the latter subsequently causing a 5-fold rise in free iron [23]. Similarly, nontransferrin-bound iron was detected following a bolus dose of 100 mg iron sucrose, appearing more often in individuals with low levels of transferrin [24]. However, oxidative stress is likely to be transient, since presence of free iron has been shown to disappear within 1 h due to scavenging by apotransferrin [25]. Therefore, it is suggested that the potential of iron preparations to cause such side-effects is dependent not only upon the physicochemical characteristics of the i. v. iron preparation, but at least as much on other parameters, such as the transferrin status or inflammatory state of the patient. At standard doses of iron sucrose, overloading of the buffering capacity of transferrin and induction of oxidative stress may be of limited significance.

Iron preparations such as iron sucrose confer marked benefits to patients with chronic renal disease and have become a mainstay for enabling effective haematopoiesis in response to administered erythropoietin. Indeed, therapy with iron sucrose has been shown to be associated with a marked reduction in the requirements for erythropoietin to maintain haemoglobin levels [26, 27], thereby resulting in substantial cost savings.

Conflict of Interest

Van Van Khov-Tran and Peter Elford are paid employees of Azad Pharma AG, which owns the rights to ISA. The toxicological study performed by BSL Bioservice GmbH was sponsored by Azad Pharma AG.

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