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Comparison of early gastrointestinal tract and liver toxicity of the originator iron polymaltose complex (IPC) and an IPC similar preparation in non-anemic rats

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Abstract. Objectives: The originator iron polymaltose complex (Maltofer®, IPC, Vifor International, St. Gallen, Switzerland) has been used for over 30 years to treat iron deficiency anemia. Its physico-chemical properties allow for a controlled release of iron, a property which translates into low toxicity and good gastrointestinal (GI) tolerability of the drug compared to the commonly used ferrous salts. A variety of different iron polymaltose complex similars are commercially available with varying structures and, thus, different efficacy and toxicity compared to IPC. In this study, the median lethal dose, the GI tract and liver toxicity of an IPC similar (Vitalix®, $IPCS_{VITA}$, Laboratorios Roemmers, Buenos Aires, Argentina) were compared with those of IPC in healthy rats. Methods: The median lethal dose of $IPCS_{VITA}$ was determined as the dose required to kill 6 out of 12 rats after 24 h from dosing. To compare the GI and liver toxicities, rats received $IPCS_{VITA}$ or IPC (both 280 mg iron/kg body weight) for 28 days. GI toxicity was assessed macroscopically by scoring lesion severities and microscopically by analyzing the villi/crypt ratio, number of eosinophils/villi and number of Goblet cells/villi. Ferritin was assessed in the small intestine villi and in the liver by immunostaining. Iron deposits in the liver were assessed by Prussian blue staining. Results: Serum iron concentration and transferrin saturation (TSAT) were significantly higher in the $IPCS_{VITA}$ group vs. the IPC and the control groups. Food consumption, body weight, and bowel movement at Day 29 were significantly lower within the $IPCS_{VITA}$ group vs. the IPC or the control groups. The lesion scores in the stomach and in the lower GI tract of the $IPCS_{VITA}$ group were significantly higher than those of the IPC and control groups. The villi/crypt ratio and the number of Goblet cells/villi in the small intestine were significantly lower in $IPCS_{VITA}$-treated animals than in IPC-treated or control animals. The number of eosinophils per villi was significantly increased in the $IPCS_{VITA}$ group vs. IPC and control group. In the lower GI tract, microscopic lesions were observed only in the $IPCS_{VITA}$ group. The amount of ferritin in the small intestine and in the liver was higher in IPC-treated animals vs. $IPCS_{VITA}$-treated or control animals. Conclusions: Higher serum iron and TSAT levels, lesions in the stomach and lower GI tract suggest the presence of weakly bound iron on the surface of the $IPCS_{VITA}$ complex, which has different physico-chemical properties than IPC. The lower levels of iron deposits in the liver suggest that the iron from $IPCS_{VITA}$ is taken up in a less controlled way than from IPC, thus, potentially accumulating in the wrong cellular compartment.

Introduction

Iron deficiency (ID) is the most common and widespread nutritional disorder in the world [1]. Infants, children and females of childbearing age are at the highest risk of ID [1, 2, 3], which is the most frequent cause of anemia in these groups [4]. Iron is an essential component of numerous proteins and enzymes responsible for oxygen transport, cellular respiration, glycolysis, electron transfer, gene regulation, and immune reactions [5]. Thus, in addition to the well-known symptoms of anemia, such as fatigue, reduced immunity and decreased quality of life [1], even in the absence of anemia, iron deficiency can adversely affect cognitive and physical performance [1, 6, 7, 8] as well as the immunological status [9].

Although inadequate dietary iron intake is a frequent cause of iron deficiency, dietary modifications are often insufficient to achieve iron repletion in a timely manner because only a small proportion of the ingested iron is absorbed. Moreover, during periods

of high iron demand, such as pregnancy and rapid growth, dietary iron alone frequently cannot meet the body's increased need [10]. Oral iron is the first choice of treatment, and it is widely administered, with ferrous sulfate, ferrous fumarate, ferrous glycine sulfate and ferrous gluconate being the most commonly prescribed ferrous salts [11]. However, high doses of ferrous compounds can lead to gastrointestinal (GI) intolerance and, thus, poor compliance [12]. The rapid release of iron from ferrous compounds and its uncontrolled uptake can saturate iron transport mechanisms generating non-transferrin bound iron (NTBI) [13, 14]. NTBI is taken up in an uncontrolled way by various tissues and, within the cells, can catalyze the formation of reactive oxygen species (ROS) [15] and, thus, lead to oxidation of proteins, lipids and DNA [16].

The originator iron polymaltose complex (Maltofer®, IPC) is a stable complex made of a polynuclear iron(III)-hydroxide core surrounded by polymaltose ligands. Unlike ferrous compounds, IPC permits controlled, active absorption of iron [17], avoiding an increase in NTBI [13] and lipid peroxidation [16]. As a result, significantly lower rates of adverse events are observed compared to ferrous sulfate [18, 19, 20, 21].

IPC has been used for over 30 years to treat iron deficiency anemia [17] and a number of IPC similar preparations (IPCS) are currently available in a variety of different countries [22]. Iron carbohydrate complexes similar to IPC, e.g. iron sucrose, are also used for intravenous (i.v.) treatment of iron deficiency anemia [11]. The active ingredients of all these preparations are complex macromolecules for which the physico-chemical characteristics, and thus their biological properties, largely depend on the manufacturing process [23]. It has recently been shown that variation in the structure of the iron sucrose complex arising from different manufacturing processes can lead to reduced clinical efficacy [24] and higher potential to induce oxidative stress in a non-clinical model [25, 26].

While several studies have reported clinical outcomes using IPCSs in various settings [27, 28, 29, 30, 31, 32, 33], comparisons with IPC are rare [34, 35]. To our knowledge, no study to date has compared the toxicity profile of any IPCS versus the originator. In this study, we used a rat model to compare the GI and liver toxicity of an IPCS commercially available in Argentina (Vitalix®, $IPCS_{VITA}$) with IPC. Part of the data (IPC and control group) were already published in a previous study which compared GI and liver toxicity of ferrous sulfate, iron amino chelate, and IPC [36]. In addition, the physico-chemical properties and median lethal dose of $IPCS_{VITA}$ were determined and compared with those of IPC.

Materials and methods

Physico-chemical analyses of $IPCS_{VITA}$

The physico-chemical analyses were performed on four $IPCS_{VITA}$ formulations (Vitalix® Gotas – Hierro Polimaltosato, lot 00015; Vitalix® Drops (PET bottles), lot 00018; Vitalix® Iron Oral Drops, lots 00112 and 00114; Laboratorios Roemmers S.A.I.C.F., Buenos Aires, Argentina)). Lot 00015 was purchased in 2005, lot 00018 in 2006 and lots 00112 and 00114 in 2011 from the manufacturer. The weight average molecular weight (Mw) was measured by gel filtration chromatography, as described previously [37, 38], and the turbidity point and the rate constant for in vitro degradation were determined according to methods described by Geisser et al. [37] in the Quality Control Laboratory of Vifor (International) Ltd. (St. Gallen, Switzerland).

Animals and treatments

All animal experiments were approved by the Hospital Alemán Ethic Committee and the Teaching and Research Committee and were undertaken according to the NIH Guide for the Care and Use of Laboratory Animals. All experiments were conducted in Sprague-Dawley rats weighing 200 – 220 g (Laboratory of Experimental Medicine, Hospital Alemán, Buenos Aires, Argentina). Rats were housed in a temperature-controlled room (22 ± 2 °C) with free access to tap water and fed standard rat chow (Cooperación, Argentina) ad libitum throughout the study. The experiments were performed with IPC and $IPCS_{VITA}$ products purchased in 2006.

Determination of median lethal dose (LD_{50})

Median lethal dose, i.e., the dose which kills 50% of a population, was determined by administering a single oral dose of IPC or $IPCS_{VITA}$ or an equivalent volume of vehicle (control) to 12 rats each (6 male, 6 female) by stomach tube after an 18-h fast, increasing from a starting dose of 50 mg iron/kg body weight. Food was withheld for another 3 h after dosing. The number of deaths at 24 h after dosing was recorded.

Assessment of early toxicity in GI tract and liver

Animals were randomized to receive IPC (Maltofer®, Vifor International, St. Gallen, Switzerland) or $IPCS_{VITA}$ (Vitalix®, Laboratorios Roemmers, Buenos Aires, Argentina) (n = 12 per group; 6 males, 6 females) for 28 days at a daily oral dose equal to 10% of the established LD_{50} values, i.e. 280 mg iron/kg body weight (bw). IPC and $IPCS_{VITA}$ were given in drinking water. A further group was randomized to tap water (n = 12, control).

In order to administer the right dose of iron, water consumption and body weight were measured daily in all animals. In addition, a daily record of food consumption and number of bowel movements was performed. 24-h urine output was measured by placing the animals in metabolic cages.

All animals were sacrificed on Day 29 by subtotal exsanguination under anesthesia. Prior to sacrifice, blood samples for biochemical analysis were collected from the tail vein after a 14-h fast. Liver, esophagus, stomach and bowel were perfused with ice-cold saline and removed for microscopy and immunohistochemistry.

Biochemical procedures

Hemoglobin (Hb) and hematocrit (Ht) were determined by SYSMEX XT 1800i (Roche Diagnostic GmbH, 68298 Mannheim, Germany). Serum iron, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assessed by colorimetric and UV methods, respectively, using an Auto-analyzer Modular P800 (Roche Diagnostic GmbH) with the correspondent reagents (Roche Diagnostic GmbH). Transferrin saturation (TSAT) was calculated with the following equation: serum iron concentration (g/l)/total iron-binding capacity (g/l) × 100 = TSAT (%) [39, 40].

Gross anatomy of the GI tract

A lesion index for macroscopic analysis of the GI tract was defined as the sum of the erosion length per rat. Lesions were scored for severity as follows: 0 = no lesions; 1 =superficial 1 – 5 hemorrhagic points; 2 = superficial 6 – 10 hemorrhagic points; 3 = sub-mucosal hemorrhagic lesions with small erosions; 4 = severe hemorrhagic lesion and some invasive lesions.

Microscopic analysis of the GI tract and liver

Portions of esophagus, stomach, small intestine, lower GI tract and liver were cut and fixed in phosphate-buffered 10% formaldehyde (pH 7.2) and embedded in paraffin. Three-micron sections were cut and stained with hematoxylin-eosin (H&E). In order to facilitate identification and quantification of Goblet cells (cells that secrete mucus) in the small intestine, Alcian blue staining was performed. All histological evaluations were performed using a light microscope Nikon E400 (Nikon Instrument Group, Melville, New York, USA).

Ferritin immunohistochemistry

Immunolabeling of specimens was carried out by a modified avidin-biotin-peroxidase complex technique Vectastain® ABC kit (Universal Elite, Vector Laboratories, Burlingame, CA, USA). Following deparaffinization and rehydration, the sections were washed in phosphate buffer 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.

Table 1. Physico-chemical characteristics or IPC and $IPCS_{VITA}$.

	IPC [22]	$IPCS_{VITA}$			
Lot	N/A	00015	00018	00112	00114
Mw (kDa)	52.3 [22]	121	123	167	188
Turbidity point (pH)	none	none	none	1.66	1.63
Rate constant of in vitro degradation ($k \times 10^3$/min) at θ = 0.1/0.5/0.8	51/73/118[a]	28/48/75	31/54/81	36/45/56	31/42/54

[a]at θ = 0.1/0.5/0.9; N/A = not available.

After washing them in PBS (pH 7.2) for 20 min, they were incubated with blocking serum for 20 min. Thereafter, the sections were incubated with the primary antibody, rinsed in PBS and incubated with Biotynilated Universal Antibody for 30 min. After washing them in PBS, they 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 with monoclonal antibody against ferritin (Biogen, San Román, CA, USA) at a concentration of 15 mg/ml (dilution 1 : 100, phosphate buffered saline as diluting agent).

Morphometric analysis of the small intestine and liver

Histological sections were studied by 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 evaluated with the observer blind to the animal group, and the data were averaged. Small intestine sections were examined for (a) villi/crypt ratio (b) number of eosinophils per villi (c) number of Goblet cells per villi.

Ferritin immunostaining in the small intestine enterocytes was evaluated by a semi-quantitative score as follows: 0 = no staining; 1 = mild positive staining per villi; 2 = moderate positive staining per villi; 3 = intense positive staining per villi; 4 = very intense positive staining per villi.

Liver sections were assessed for iron deposits, according to positive Prussian blue staining (%/mm^2), and tissue ferritin, according to positive ferritin immunostaining (%/mm^2).

Statistical analysis

Comparisons between groups were carried out using ANOVA for parameters with Gaussian distribution and using Kruskal-Wallis test (nonparametric ANOVA) and Dunn's multiple comparison test for parameters with non-Gaussian distribution (e.g. histological data). All statistical analyses were performed using absolute values and processed through GraphPad Prism, version 2.0 (GraphPad Software Inc., San Diego, CA, USA). Values are expressed as mean (SD). A p-value of < 0.05 was considered significant.

Results

Physico-chemical characterization of $IPCS_{VITA}$

The weight average molecular weight (Mw) of all the IPC batches produced by Vifor (International) Ltd. in 2011 was 53.4 ± 2.7 kDa (mean ± SD). The Mw of each of the four formulations of $IPCS_{VITA}$ was significantly higher than that of IPC, i.e., 121 kDa, 123 kDa, 167 kDa and 188 kDa (Table 1). Turbidity point was detected for two $IPCS_{VITA}$ lots at pH 1.66 and 1.63 (Table 1). Consistent with the larger Mw of all the four $IPCS_{VITA}$ lots, their rate constants for in vitro degradation were lower than that of IPC (Table 1).

LD_{50} values

The LD_{50} values for IPC and $IPCS_{VITA}$ were remarkably high, each exceeding 2,800 mg iron/kg bw. Assessment of higher doses was not possible because additional amounts of iron compound solution exceeded the capacity of the rat stomach.

Table 2. Body weight, food consumption, bowel movement, water consumption and urine output at Day 29. Values are shown as mean (SD).

	Baseline			Day 29		
	IPC	$IPCS_{VITA}$	Control	IPC	$IPCS_{VITA}$	Control
Body weight (g)	210 (8)	212 (6)	211 (10)	328 (5)	309 (7)*	334 (6)
Food consumption (g/d)	17.0 (1.8)	16.8 (1.3)	17.6 (1.2)	19.1 (1.2)	16.9 (0.8)*	19.7 (1.7)
Bowel movement (deposits/d)	19.5 (2.6)	20.0 (2.5)	21.0 (1.6)	22.0 (2.1)	19.0 (1.8)*	22.2 (1.2)
Water consumption (ml/d)	24.0 (3.6)	23.7 (3.1)	24.9 (2.3)	26.0 (3.4)	25.7 (3.0)	26.2 (1.2)
Urine output (ml/d)	21.7 (2.8)	21.5 (3.4)	22.7 (2.6)	23.2 (2.6)	23.2 (2.6)	23.7 (1.7)

*$p < 0.01$ vs. IPC and control.

Table 3. Hematological parameters and liver enzyme activities in IPC, $IPCS_{VITA}$ and control groups on day 29. Values are shown as mean (SD).

	Baseline			Day 29		
	IPC	$IPCS_{VITA}$	Control	IPC	$IPCS_{VITA}$	Control
Hematological parameters						
Hb (g/dl)	15.7 (0.4)	15.6 (0.3)	15.3 (0.3)	15.9 (0.3)	15.8 (0.3)	15.4 (0.2)
Hematocrit (%)	45.4(0.6)	44.9 (0.7)	45.0 (0.5)	46.3 (0.3)	45.4 (0.9)	45.2 (0.7)
Serum iron (µg/dl)	208 (19)	205 (23)	209 (25)	230 (11)	257 (20)*	206 (27)
TSAT (%)	35.2 (3.3)	34.9 (3.8)	34.4 (3.2)	39.0 (3.3)	51.5 (3.1)*	33.7 (2.9)
Liver enzyme activities (IU/l)						
AST	99 (11)	103 (9)	102 (10)	115 (13)	128 (11)	110 (12)
ALT	42 (9)	44 (5)	40 (7)	47 (8)	55 (6)	42 (5)
ALP	529 (27)	537 (30)	526 (34)	542 (30)	570 (39)	531 (43)

TSAT = transferrin saturation; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; *$p < 0.01$ vs. IPC and control.

Body weight, food and water consumption, urine output and bowel movement

Body weight, food consumption and bowel movements were similar in all treatment groups at baseline, but by Day 29 all three parameters were significantly lower in the $IPCS_{VITA}$ group versus both IPC and control group (Table 2). Water consumption and urine output did not differ between groups.

Hematologic values and liver enzyme activities

Mean levels of Hb and Ht were similar in all treatment groups at Day 29. Both serum iron concentration and TSAT, however, were significantly higher in $IPCS_{VITA}$ group versus IPC and control groups (Table 3). Values for serum iron and TSAT were similar in the IPC and control groups at Day 29.

AST, ALT and ALP activities were numerically higher in the $IPCS_{VITA}$ group versus the IPC and control arms at Day 29, but the differences were not statistically significant (Table 3).

Gross anatomy of the GI tract

No macroscopic lesions were observed in the esophagus or small intestine of any group. In the stomach, small sub-mucosal hemorrhagic points were observed only in the $IPCS_{VITA}$ group shown as a higher lesion score versus IPC and control groups (Table 4). In the lower GI tract (colon and rectum), varying degrees of lesions were seen in the $IPCS_{VITA}$ group, ranging from simple mucosal edema and congestion to submucosal hemorrhages, resulting in a higher lesion score versus IPC and control groups (Table 4). The lesion scores in the stomach, small intestine and in the lower GI tract were similar in the IPC and control arms.

Table 4. Macroscopic and microscopic analysis of the GI tract at Day 29. Values are shown as mean (SD).

	IPC	$IPCS_{VITA}$	Control
Macroscopic analysis (lesion score[a])			
Stomach	0.1 (0.4)	0.5 (0.5)*	0.1 (0.4)
Lower gastrointestinal tract	0.1 (0.2)	0.4 (0.4)*	0.1 (0.2)
Microscopic analysis of small intestine			
Villi/crypt ratio	2.1 (0.1)	1.8 (0.1)**	2.3 (0.1)
Goblet cells (n/villi)	10.2 (0.9)	9.0 (0.8)**	11.7 (1.2)
Eosinophils (n/villi)	8.2 (0.9)	13.5 (1.9)*	7.7 (1.2)

[a]0 = no lesions; 1 = superficial 1–5 hemorrhagic points; 2 = superficial 6 – 10 hemorrhagic points; 3 = sub-mucosal hemorrhagic lesions with small erosions; 4 = severe hemorrhagic lesion and some invasive lesions; *p < 0.01 vs. IPC and control; **p < 0.05 vs. IPC and control.

Microscopic analysis of the GI tract

No esophageal lesions were observed in any group. The villi/crypt ratio in the small intestine was significantly lower in the $IPCS_{VITA}$ group versus IPC and control group (Figure 1), as was the number of Goblet cells per villi (Figure 2) (Table 4). The number of eosinophils per villi was also increased in the $IPCS_{VITA}$ arm versus IPC and control arm (Figure 1) (Table 4). In the lower GI tract (colon and rectum), no microscopic lesions were found in the IPC or control groups but varying degrees of congestion in vessels near to the mucosa were seen in rats randomized to $IPCS_{VITA}$.

Ferritin immunostaining in the small intestine and liver, and Prussian blue staining of iron deposits in the liver

Ferritin immunostaining in the small intestine was increased with both IPC and $IPCS_{VITA}$ compared to control animals, but it was significantly greater with IPC versus $IPCS_{VITA}$ (Figure 3A). In the Kupffer cells of the liver, both the IPC and $IPCS_{VITA}$ groups showed increased Prussian blue staining for iron versus the control group (Figure 3B). Prussian blue staining of iron deposits in the IPC group was significantly greater ($p < 0.01$) than in the $IPCS_{VITA}$ group. Ferritin immunostaining in the liver revealed a marked and significant increase ($p < 0.01$) of ferritin in the IPC versus control group that was also significantly greater ($p < 0.01$) than in the $IPCS_{VITA}$ treatment arm (Figure 3C).

Discussion

A large number of clinical studies with the originator IPC support its efficacy and good tolerability, as well as negligible oxidative stress reactions induced by this oral iron preparation [22]. A number of IPCSs is marketed today in various countries, but only limited clinical data are available to demonstrate their bioequivalence with IPC.

IPC has a narrow, monomodal molecular weight distribution with a weight average molecular weight (Mw) of ~ 52.3 kDa. It has been classified as moderately strong and kinetically semi-robust [41]. Thus, under neutral conditions only limited amounts of iron are released. All four analyzed lots of $IPCS_{VITA}$ presented higher Mw's than that of IPC, highest being 188 kDa and lowest 121 kDa. The analyzed lots were purchased over a range of 6 years (lot 00015 in 2005, lot 00018 in 2006, and lots 00112 and 00114 in 2011). All the physico-chemical parameters of the $IPCS_{VITA}$ products that are currently on the market are distinct from those of IPC.

Complexes with a higher molecular weight will normally also have a slower rate of iron release [37], leading to a lower absorption rate and inferior efficacy compared to IPC, which has similar efficacy to that of ferrous sulfate [22]. Indeed, an iron polymaltose (amylum) complex (Fe-Am) with a weight average molecular weight of 462 kDa and initial degradation kinetics of 9×10^3/min (at $\theta = 0.1$) showed efficacy that was only ~ 6% that of iron sulfate [22]. In this study, the lower values of the rate constants for in vitro degradation of $IPCS_{VITA}$ compared to that of IPC may suggest reduced efficacy of this preparation.

The availability of inorganic iron for absorption in the duodenum and the upper part of jejunum is influenced by intraluminal factors both in the stomach and upper intestine. In healthy adults, the low pH of stomach stabilizes soluble forms of iron potentially available for absorption [42]. The turbidity point gives an indication of the stability of the complex under different pH values as found in the GI tract. IPC does not have a

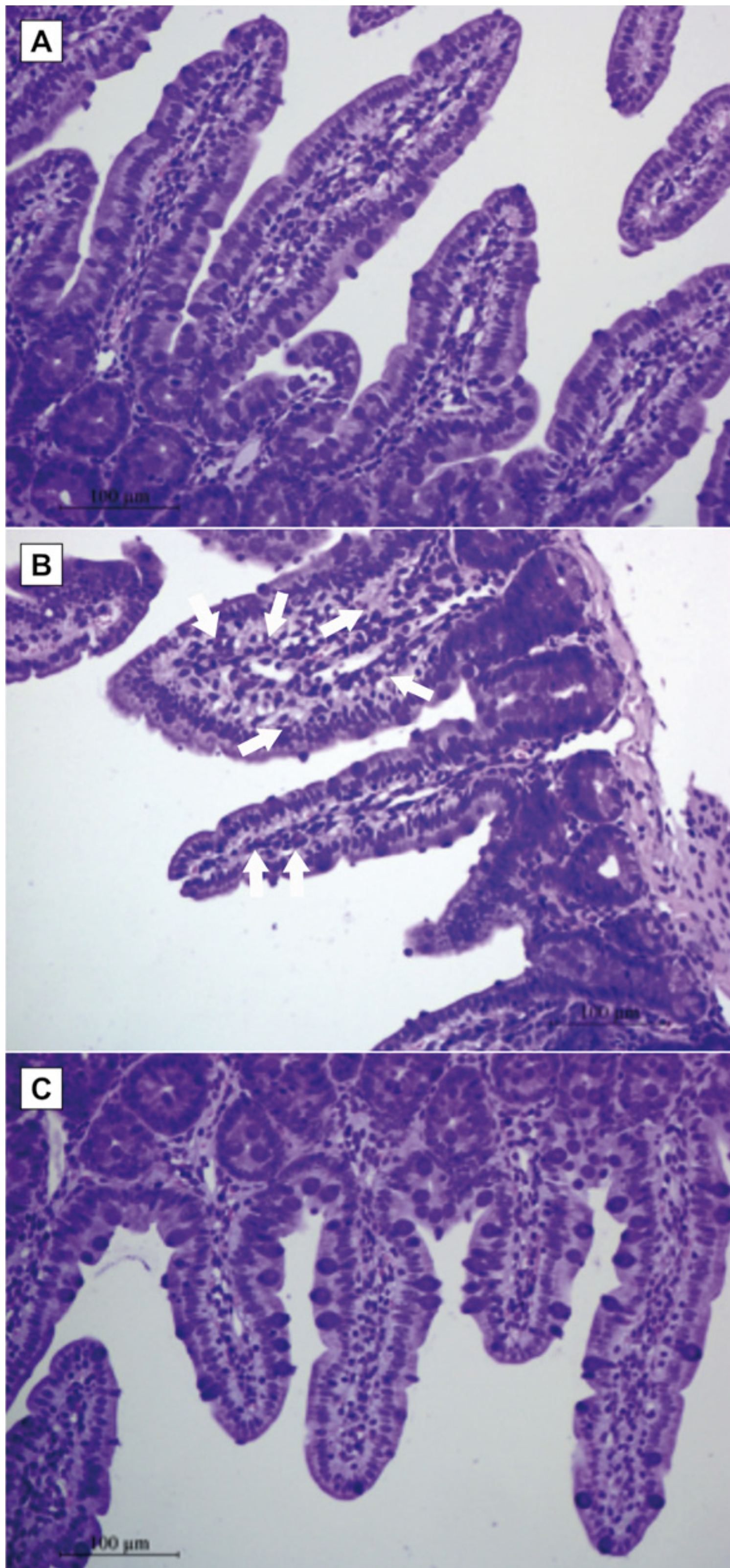

Figure 1. Microscopic analysis of small intestine in (A) IPC, (B) $IPCS_{VITA}$ and (C) control group on Day 29 (H&E, original magnification: × 200). Note edema in villi and a reduced villi/crypt ratio in animals treated with $IPCS_{VITA}$ as well as higher numbers of eosinophils (indicated by arrows) in villi (B). No lesions were observed in the IPC or control groups.

turbidity point, i.e., it remains soluble within the whole range of physiologically relevant pH values [17]. In contrast, two of the four $IPCS_{VITA}$ lots had turbidity points at pH 1.66 and 1.63, suggesting that unlike IPC, these complexes are not stable under GI conditions and may precipitate already in the gastric juice of the stomach if taken on an empty stomach. Thus, they are not likely to reach the same efficacy as that of IPC.

Iron from the food is absorbed in the gut via a pathway closely regulated by the body's iron requirements [43, 44, 45]. However, upon administration of a therapeutic dose of e.g., 100 mg iron as ferrous sulfate, the physiologically controlled pathways can be overridden and ferrous ions are largely taken up by passive diffusion through a paracellular route [43, 44]. Under these conditions, a sharp increase in the serum iron level suggests saturation of the transport mechanism, which leads to a significant increase of NTBI in the blood [13, 14, 43]. NTBI is taken up in an uncontrolled way by various cells, in which it can catalyze ROS formation leading to oxidation of lipids, DNA and proteins [15]. Despite elevated NTBI levels in patients with iron overload disorders have been considered to be responsible for cellular damage [46], the clinical significance of NTBI generated upon administration of standard doses of ferrous sulfate has not been unequivocally demonstrated.

Because iron from IPC is released in a controlled way and taken up in the gut only via an active process, IPC does not lead to an increase in NTBI [13] and does not induce oxidative stress [16]. In this study in non-anemic rats, significantly higher levels of serum iron and TSAT were observed in the $IPCS_{VITA}$ group versus IPC or control groups at Day 29. This finding suggests that under physiologic conditions more iron is released from $IPCS_{VITA}$ than IPC and, thus, that iron from $IPCS_{VITA}$ is taken up in a less controlled manner. Thus, it cannot be excluded that $IPCS_{VITA}$ leads to NTBI and ROS formation, possibly because of the structural differences between IPC and $IPCS_{VITA}$. In this context, it is noteworthy that the levels of the liver enzyme activities AST, ALT and ALP were higher in the $IPCS_{VITA}$ group than in the IPC group indicating possible liver damage in the $IPCS_{VITA}$-treated animals although the

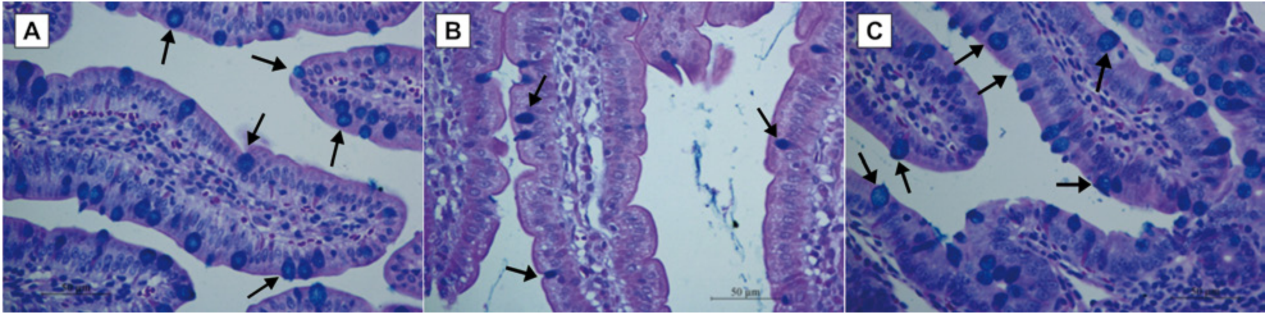

Figure 2. Micrographs of small intestine in (A) IPC, (B) $IPCS_{VITA}$ and (C) control group on Day 29. Note the reduced number of Goblet cells (mucin-secreting cells) in the $IPCS_{VITA}$ group as indicated by arrows (B). (Alcian Blue, original magnification: × 400).

differences were not considered statistically significant.

In animals, oral iron supplementation has been shown to damage the GI tract leading to serious gastric ulcers and erosions [11]. Damage to the gastric epithelium in the stomach and small intestine was present to a variable degree in animals treated with $IPCS_{VITA}$ but not with IPC, as demonstrated by gross anatomy and microscopy. The number of Goblet cells, which secrete lubricating mucous, and the villi/crypt ratio, which is a measure of the normality of intestinal epithelial cell loss and replacement, were both diminished in the $IPCS_{VITA}$ cohort. Interestingly, the higher density of eosinophils in the villi from $IPCS_{VITA}$-treated animals suggests an allergic component to the gastric injury. The lesions in the stomach and lower GI tract, together with higher levels of serum iron and TSAT levels of $IPCS_{VITA}$-treated animals suggest the presence of weakly bound or redox-active iron on the surface of $IPCS_{VITA}$. Even small amounts of such weakly bound or redox-active iron may induce oxidative stress. Similar results were obtained in animals treated with ferrous sulfate or with iron amino chelate, which presented also significant increases in oxidative stress markers [36].

Following uptake of iron in the duodenum via the membrane protein divalent metal transporter 1 (DMT1) [47], iron can be exported through the membrane protein ferroportin [48, 49] into the plasma, or it can be stored within the storage protein ferritin in the enterocytes [47], depending on the current iron requirement of the body. It has been suggested that, because of the physico-chemical properties of IPC, the mechanism of iron absorption and utilization from IPC is similar to that for dietary iron [36, 50]. In this study, we showed that the ferritin deposits were greater within the small intestine of IPC-treated animals versus $IPCS_{VITA}$-treated animals. These results confirm that iron from IPC is taken up exclusively via the active process whereas alternative pathways may be involved in the iron uptake from $IPCS_{VITA}$.

The liver has an extensive capacity for iron storage in the form of ferritin, and is key to effective regulation of iron in the body [51]. In this study, significantly higher levels of ferritin deposits were observed in the liver of IPC-treated animals vs. $IPCS_{VITA}$-treated animals, again consistent with controlled absorption and transport of iron after ingestion of IPC. Moreover, extensive positive staining for iron (Prussian blue) was seen in hepatic Kupffer cells of both IPC and $IPCS_{VITA}$ groups. However, the values for Prussian blue staining were significantly lower in the $IPCS_{VITA}$ treated animals indicating that iron utilization from $IPCS_{VITA}$ is not as controlled as from IPC and iron may end up in the wrong compartment.

Interestingly, to date, two clinical studies have compared IPC versus an IPCS (Hematin, Chalver Laboratorios, Bogotá, Colombia), both over a 3-month period in patients with iron deficiency anemia [34, 35]. One of these trials reported a similar increase in hemoglobin level with the two studied preparations [34]; the second described a lower hematopoietic response in the IPCS cohort [35]. It was also reported that GI adverse events were numerically more frequent in the IPCS-treated patients in both trials [34, 35], consistent with our own findings in this animal model.

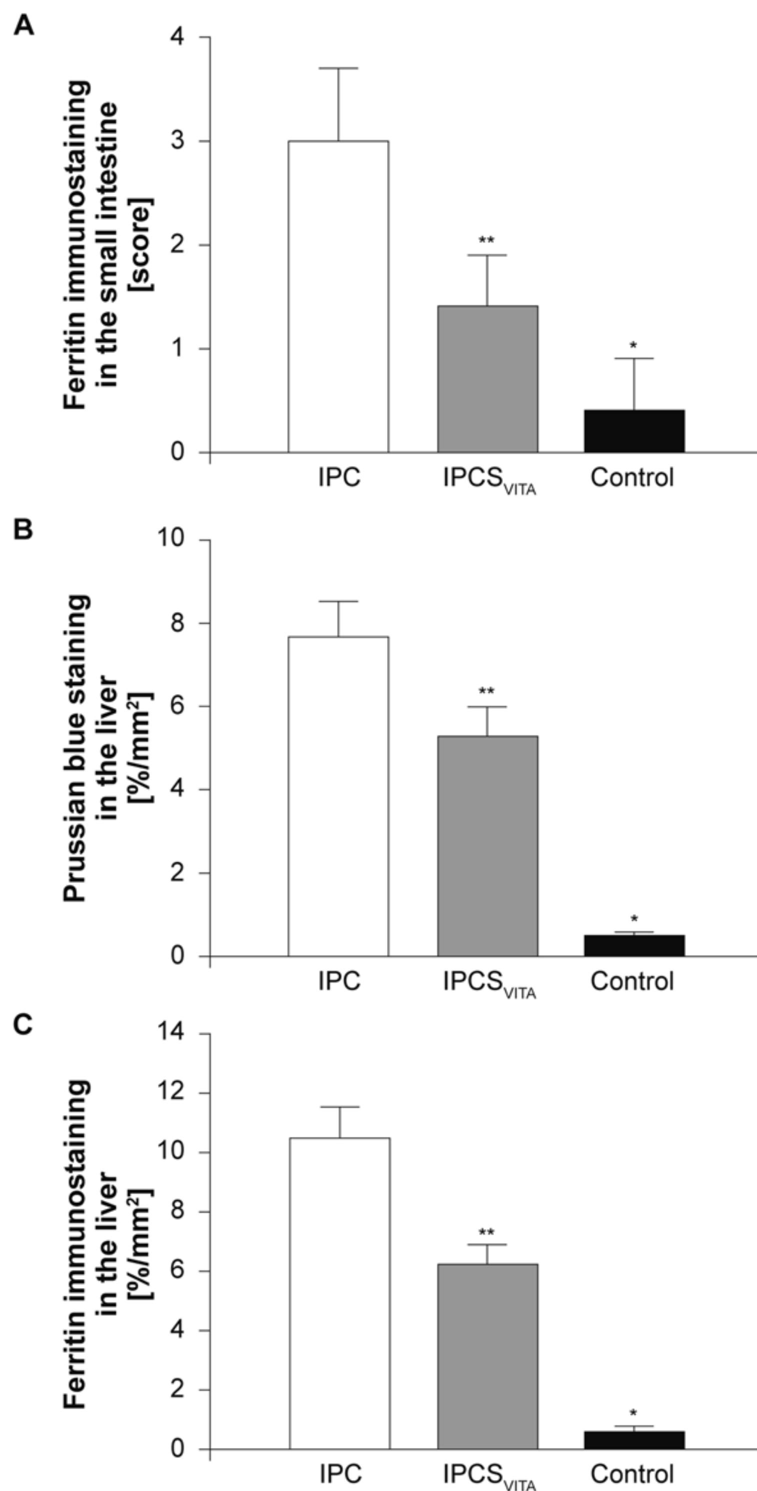

Figure 3. A: Ferritin immunostaining in the small intestine, B: Prussian blue staining of iron deposits in the liver and C: ferritin immunostaining in the liver on Day 29. Values are shown as mean + SD.

Conclusions

The efficacy and safety of iron carbohydrate preparations for the oral treatment of iron deficiency may vary widely, depending on their physico-chemical properties, which are largely defined by the manufacturing process [22, 23]. Although acute toxicity and erythropoietic response were similar for both iron polymaltose complex preparations, significant differences were observed between the originator IPC and the IPC similar $IPCS_{VITA}$ in this series of biochemical, anatomical, microscopic and immunohistochemical investigations in healthy non-anemic rats. This study shows that iron from $IPCS_{VITA}$ may be absorbed in a less controlled way in the gut and thus, besides saturating the iron uptake and transport mechanisms, it may be deposited into the wrong compartment as indicated by lower levels of iron deposits in the liver of $IPCS_{VITA}$- vs. IPC-treated animals.

To our knowledge, this is the first comparison of the toxicological profile of an IPCS versus the originator IPC preparation. Experience from animal studies of intravenous iron sucrose preparations [25, 26] has consistently revealed toxicological variations between the originator and similar preparations, and clinical differences have also been described [24]. The present study showed differences although it was of very short term in animals and, thus, the effects may be of more importance in humans in the scenario of long term ingestion even though the doses involved were higher than in general practice. Although the clinical data comparing oral IPCSs with the originator IPC are still sparse, the available evidence suggests that the differences observed in both the physico-chemical properties and in our preclinical model may be reflected therapeutically, and merit further examination.

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Conflicts of interest

Professor Jorge E. Toblli has received research grants and consultancy fees from Vifor (International) Ltd. The other authors have no conflicts of interest to declare.

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