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Comparison of injectable iron complexes in their ability to iron load tissues and to induce oxidative stress

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Abstract

Iron and copper homeostasis have been studied in various tissues after iron-loading with the polynuclear ferric hydroxide carbohydrate complexes, iron dextran, iron polymaltose, iron sucrose and iron gluconate for four weeks. There were significant increases in the iron content of the different rat tissues compared to controls, with the exception of the brain, which showed no change in its iron content following iron loading. However, the level of iron loading in the different tissues varied according to the preparation administered and only iron dextran was able to significantly increase the iron content of both broncho-alveolar macrophages and heart. The hepatic copper content decreased with iron loading, although this did not reach significance. However the copper content did not alter in the iron loaded broncho-alveolar macrophages. Despite such increases in hepatic iron content, there was little evidence of changes in oxidative stress, the activities of cytosolic (apart from iron dextran) or mitochondrial hepatic superoxide dismutase, SOD, were similar to that of the control rats, confirming the fact that the low reduction potential of these compounds prevents the reduction of the ferric moiety. It was not necessary for macrophages to significantly increase their iron content to initiate changes in $NO^{\cdot}$ release. Iron gluconate and iron sucrose increased $NO^{\cdot}$ release, while iron polymaltose and iron dextran decreased $NO^{\cdot}$ release although only the latter iron preparation significantly increased their iron content. It may be that the speciation of iron within the macrophage is an important determinant in changes in $NO^{\cdot}$ release after *ex vivo* stimulation. We conclude that tissues loaded with iron by such polynuclear iron complexes have variable loading despite the comparable iron dose. However, there was little evidence for participation of the accumulated iron in free radical reactions although there was some evidence for alteration in immune function of broncho-alveolar macrophages.

Introduction

The polynuclear ferric hydroxide complexes constitute a new class of therapeutic agents, mostly injectable, for treatment of iron deficiency anemia, thereby replacing therapy by ferrous salts. Ferrous salts (e.g., ferrous sulphate) have been used as pharmaceutical agent to correct anaemia for many years, however, they have the disadvantage of not being well tolerated and causing serious gastro-intestinal side effects. On the other hand, early studies of parenteral iron compounds such as the ferric iron complex $Fe(NTA)_2$ showed excessive toxicity, particularly to the kidney when administered over a 4-week period. This is, in part, attributable to its reduction potential, +100mV, such that the ferric iron can be reduced by superoxide anion to participate in Fenton chemistry (Geisser 1998; Pierre *et al.* 2002). By contrast, polynuclear ferric hydroxide complexes are relatively inert with a structure relatively similar to that of physiological iron storage protein ferritin. Therefore they have a low reduction potential, less than −324 mV, which would preclude their participation in Fenton reaction (Geisser 1998), even when administered at high doses. Ferric hydroxide polymaltose complexes have different absorption mechanism from ferrous salts when parenterally administered. Initially they are initially taken up by the reticulo-endothelial system probably by phagocytosis and are processed to release iron which will

be either stored in ferritin or released to the circulation ready for transport by transferrin to supply mainly erythroid cell precursors in bone marrow. It was clearly shown that these complexes do not undergo any interaction with food components, such as phytic acid and oxalic acid, that are known to react and to interfere with the iron (II) and (III) salts at both acidic and physiological pH. In addition, they can be administered simultaneously with many drugs like vitamins (A, D3 and E), without impairing their absorption as is often seen with iron II and III salts. These various iron complexes are classified into four groups according to their physico-chemical properties, including molecular mass, kinetics and thermodynamic stability, giving them a variable efficacy in iron store replenishment and thereby in iron deficiency anaemia therapy.

Regarding the selectivity of such iron complexes, they are targeted mainly to the reticulo-endothelial system, such that released iron transits and accumulates in the parenchymal cells (Legssyer *et al.* 2002). Therefore they may represent a useful model for the study of secondary iron overload syndromes, by mimicking the iron loading profile of the RES often seen in such disorders. Indeed, in secondary iron overload syndromes, e.g. thalassemia, repeated blood transfusions results in an iron loading of RES cells due to their role in the processing of effete red blood cells. The RES cells can then release this iron in part as ferritin to parenchymal cells (Kondo *et al.* 1988; Sibille *et al.* 1988). Although such iron accumulation, particularly during the initial stages of iron loading may not be toxic, its rapid reflux to the parenchymal cells might be an important factor in causing tissue iron toxicity. Therefore, the choice of the ideal iron complex for parenteral iron supplementation, by comparing the different iron preparations in their ability to load different tissues principally the RES, in addition to their ability to modulate RES cellular function by modifying their oxidative tone, was the main aim for the current study.

In previous studies Ward *et al.* (1998) have shown an inverse relationship between iron and copper homeostasis in iron deficient rat liver. Therefore, we have investigated the ability of these iron complexes to induce a change in copper homeostasis in the different rat tissues, in response to their increasing iron concentration.

Material and methods

Four iron preparations were selected for investigation: iron dextran (Dexferrum), iron polymaltose (Ferrum Hausmann) both of which have degradation kinetics between 15 and 50 (Geisser *et al.* 1992); iron sucrose (Venofer) and iron gluconate (Ferrlecit), with degradation kinetics of between 50 and 100. The four iron complexes have molecular weights of 360 kD, 422 kD, 44 kD and 54 kD, respectively (Geisser *et al.* 1992). Intraperitoneal injections were given to male Wistar rats, 70–80 g, $n = 6$ in each group $3\times$ per week, for a period of 4 weeks (total dose = 110 mg iron). At the end of the loading period, the rats were anesthesised with Nembutal, soduim pentabarbitone, 0.6 mg/kg. Alveolar macrophages (AM) were then recovered by pulmonary lavage with 50 ml sterile phosphate buffered saline (PBS). The liver, heart and spleen were excised and homogenised in sucrose, 250 mM (10% w/v). The cerebellum was dissected from the brain and the cortex dissected from one half.

Liver fractionation

Crude fractions, (supernatant and enriched mitochondrial) were isolated from the liver homogenates after an initial centrifugation, 600 $g \times 10$ min, to remove the nuclear pellet and another centrifugation, 8000 $g \times 10$ min, to precipitate the crude enriched mitochondrial fractions (peroxisomes, mitochondria and lysosomes) and recover the supernatant fraction (cytosol + microsomes).

Primary culture preparation

Primary cultures of alveolar macrophages, at a density of 100,000 cells/ml, were maintained in Dulbecco's medium without red phenol, supplemented with foetal calf serum 10%, together with antibiotics ampicillin/streptomycin. The macrophages were stimulated with lipopolysaccharide, 0.5 μg/ml, and incubated for 40 h at 37 °C, 5% CO2. Nitrite (NO^-) was assayed in the cell-free supernatant of the macrophage primary culture by a spectrophotometric method based on the Griess reaction. The nitrite concentration was deduced from a standard curve in the range 0–50 μM.

The remaining cells were suspended in PBS and were frozen at −20 °C prior to analysis of iron and copper content.

Table 1. Iron content of the different rat tissues after i.p. administration of various polynuclear ferric hydroxide complexes (μg/g tissue).

	Liver	Spleen	Heart
Controls	187.7 ± 15.8	466.4 ± 125	26.8 ± 3.8
Iron gluconate	$995.2 \pm 416.6^{*}$	$1399 \pm 179^{*}$	29.7 ± 2.8
Iron sucrose	$1489.6 \pm 165^{**}$	$1403 \pm 226^{*}$	35.9 ± 7.6
Iron polymaltose	$3684 \pm 896^{**}$	$3550 \pm 1928^{*}$	$53.2 \pm 16.9^{*}$
Iron dextran	$4490 \pm 416^{**}$	$5333 \pm 1117^{**}$	$54.7 \pm 7.6^{*}$

Statistical analyses by ANOVA with Fisher protected t-test.
$^{*}P < 0.05$, $^{**}P < 0.01$ by comparison to control values

Iron and copper analysis

Suitable dilutions of the different samples were prepared in 0.1 M nitric acid and their iron and copper content assayed by electrothermal atomic absorption. Standards in the range 0–50 ng/ml were prepared for iron and copper determination.

Superoxide dismutase assay

The activity of superoxide dismutase was assayed by the method of Beauchamp and Fridovich (1971), in which xanthine and xanthine oxidase are used to generate superoxide anion, which reduces nitroblue tetrazolium (NBT) to form a purple formazon dye. The SOD in the sample competes with the NBT for superoxide radicals so that the color intensity produced is inversely related to the activity of SOD in the sample. The mitochondrial SOD activity was assayed by substraction from total SOD activity, the SOD activity in the presence of potassium cyanide in the homogenate fraction while cytosolic SOD was assayed in the supernatant fraction. One unit of enzyme activity was equivalent to 50% inhibition of the reaction rate of NBT.

Statistics

All results are presented as mean $\pm$ standard deviation for 6 rats. Statistical significances were calculated by ANOVA with Fisher protected 't'test. Significance denoted as $^{*} = P < 0.05$ and $^{**} = P < 0.01$, by comparison to control values.

Results

Comparison of the iron loading efficacy of the different polynuclear ferric hydroxide complexes in vivo

Parenteral administration of the different ferric complexes during 4 weeks had no effect on the growth and the weight of the rats, which reached 270 g at the end of the experiment. After one month of iron loading with the different iron polynuclear complexes, different rat tissues showed a significant increase in their iron content compared to control rats. However, these different iron preparations gave different iron loading levels which were dependent on the type of the preparation, type I (iron dextran and iron polymaltose) were more efficient in loading different rat tissues than type II (iron sucrose) and III (iron gluconate).

In the livers (Table 1), loading with iron gluconate and iron sucrose increased the iron content by 5- to 7-fold compared to controls, whereas the use of iron dextran and iron polymaltose preparations at the same dose and for the same period increased their hepatic content by approximately 20-fold. Subcellular fractionation of livers by differential centrifugation showed that iron distribution is mainly cytosolic in control homogenates (80%) as well as in those of groups treated with iron sucrose (79%) and iron gluconate (80%).

In parallel, an important increase in iron content in the enriched mitochondrial fraction, presumedly within lysosmes, was evident after administration of both iron dextran (39%) and iron polymaltose (37%) compared to control (28%), iron sucrose (21%) and iron gluconate (25%) (Table 2). Both iron dextran and iron polymaltose increased splenic iron content up to 10-fold compared to controls, while, this content was increased only by 3-fold after iron loading with either iron gluconate or iron sucrose (Table 1).

Table 2. Hepatic iron content (μg/mg protein) and its distribution between cytosolic fraction and mitochondrial pellet (mitochondria, peroxisomes, lysosomes).

	Supernatant fraction	Mitochondrial pellet
Controls	1.52 ± 0.3 (74.5%)	0.52 ± 0.03 (25.5%)
Iron gluconate	9.34 ± 3.0** (76.9%)	2.8 ± 0.55* (23.1%)
Iron sucrose	11.2 ± 1.6** (79.3%)	2.92 ± 0.4* (20.7%)
Iron polymaltose	15.3 ± 4.5** (53.5%)	13.3 ± 2.2** (46.7%)
Iron dextran	19.4 ± 1.8** (55.6%)	15.5 ± 3.4** (44.4%)

Statistical analyses by ANOVA with Fisher protected t-test.
*$P < 0.05$, **$P < 0.01$ by comparison to control values.

Table 3. Hepatic copper content (μg/g tissue) in controls and after administration of the different iron preparations.

	Copper content
Controls	84.7 ± 42.6
Iron gluconate	51.7 ± 5.8
Iron sucrose	76.2 ± 2.3
Iron polymaltose	52.4 ± 3.8
Iron dextran	24.5 ± 4.2*

Statistical analyses by ANOVA with Fisher protected t-test.
*$P < 0.05$ by comparison to control values.

By contrast to these two iron sequestering tissues, the heart showed a low iron loading level and the four iron preparations had only a slight effect in loading this tissue. Only iron dextran and polymaltose increased significantly the iron content in heart muscle up to 2-fold compared to controls (Table 1).

The analysis of the total hepatic copper content show clearly a decrease in the different groups where iron content increased, although there was no significant inverse relationship between iron and copper accumulation in the liver because of the wide variation of results, $P = 0.08$ (Table 3).

Moreover, the copper content in the mitochondrial pellet did not change by comparison to the control while in supernatant, it decreased approximately three fold in the different groups, compared to an increase of 10- to 15-fold in iron content in these fractions compared to controls (Table 4).

To test the ability of such iron preparations to cross the blood brain barrier, we analysed the iron concentration in the whole brain and in both cortex or cerebellum. Table 5 shows no significant change in the iron concentration after the administration of the four iron complexes, which remain similar to controls value. Total brain iron content in the different groups was in the range of 0.30 μg/mg protein. Furthermore, the iron concentration in both cortex and cerebellum are quite similar ranging from 0.21 to 0.36 μg/mg of protein.

As model cells for the reticulo-endothelial system, we have investigated the iron loading effect of the four iron preparations in rat broncho-alveolar macrophages. Both iron gluconate and iron sucrose did not alter iron level in these cells compared to the control values (9 ± 1 and 9.5 ± 2.8 vs 8 ± 2.5 ng/μg protein, respectively), whereas, iron polymaltose and iron dextran cause a significant increase in the iron content in comparison to controls (23.5 ± 8.9 and 18 ± 1 vs. 8 ± 2.5 ng/μg protein respectively, $P < 0.05$). (Table 6).

Compared to spleen, the increase in the iron concentration is low, probably due to the fact that these cells do not play an important role in iron sequestration contrary to the splenic macrophages, which are known to be a important mediators of iron homeostasis on account of their ability to phagocyte effete red cells and to release iron in order to supply erythropoietic cells. The copper content of alveolar macrophages after administration of each of these complexes were comparable to controls.

Ability of polynuclear ferric hydroxide complexes to induce oxidative stress

Despite the increase in the total and mitochondrial iron content by these polynuclear iron complexes, there was little alteration in the activity of superoxide dismutase activity (Table 7). No significant change in SOD activity after treatment with the 4 iron preparations was observed compared to controls in the different rat tissues. In iron loaded liver, the mitochon-

Table 4. Sub-cellular content of iron and copper in mitochondrial pellet and supernatant fraction (μ/mg protein).

	Mitochondrial pellet		Supernatant	
	Iron	Copper	Iron	Copper
Controls	0.52 ± 0.03	1.01 ± 0.61	1.52 ± 0.3	3.26 ± 0.32
Iron gluconate	$2.8 \pm 0.55^{*}$	0.87 ± 0.17	$15.3 \pm 4.5^{**}$	$0.93 \pm 0.51^{*}$
Iron sucrose	$2.92 \pm 0.4^{*}$	1.51 ± 0.94	$11.2 \pm 1.6^{**}$	$0.71 \pm 0.28^{*}$
Iron polymaltose	$13.3 \pm 2.2^{**}$	0.99 ± 0.5	$9.34 \pm 3.0^{**}$	$0.69 \pm 0.35^{*}$
Iron dextran	$15.5 \pm 3.4^{**}$	1.07 ± 0.67	$19.4 \pm 1.8^{**}$	$0.85 \pm 0.06^{*}$

Statistical analyses by ANOVA with Fisher protected t-test.
$^{*}P < 0.05$, $^{**}P < 0.01$ by comparison to control values.

Table 5. Iron content (μg/mg protein) of whole brain, cortex and cerebellum after i.p administration of various iron preparations.

	Brain	Cortex	Cerebellum
Controls	0.33 ± 0.07	0.27 ± 0.023	0.33 ± 0.08
Iron gluconate	0.21 ± 0.04	0.21 ± 0.05	0.27 ± 0.03
Iron sucrose	0.35 ± 0.103	0.36 ± 0.091	0.36 ± 0.09
Iron polymaltose	0.26 ± 0.02	0.22 ± 0.04	0.24 ± 0.03
Iron dextran	0.33 ± 0.05	0.31 ± 0.062	0.32 ± 0.07

Values are means $\pm$ SD.

Table 6. Comparison between iron loading in reticulo-endothelial cells, i.e., spleen (μg/g tissue) and alveolar macrophage (ng/μg protein)

	Spleen	Macrophage
Controls	466 ± 125	8.0 ± 2.5
Iron gluconate	$1399 \pm 179^{*}$	9.0 ± 1.0
Iron sucrose	$1403 \pm 226^{*}$	9.5 ± 2.8
Iron polymaltose	$3550 \pm 192.8^{*}$	$23.5 \pm 8.9^{*}$
Iron dextran	$5333 \pm 111.7^{**}$	$18.0 \pm 1.0^{*}$

Statistical analyses by ANOVA with Fisher protected t-test.
$^{*}P < 0.05$, $^{**}P < 0.01$ by comparison to control values.

drial Mn-SOD activity remained similar to the control values, about 12 U/mg of protein, however the cytosolic Cu-Zn SOD showed a small increase in its activity in iron loaded liver by the different iron preparations which correlated with the iron loading profile: Maximal activity was found in the group treated with the iron dextran preparation (25.1 ± 4.8 vs 14 ± 1.2 U/mg, $P < 0.05$).

In spleen as well as in heart, SOD activity remained constant in the different groups (about 37 U/mg protein in spleen and 4U/mg protein in heart) (Table 7). In brain, SOD activity was invariable after the administration of the different iron complexes, and comparable in both cortex and cerebellum (< 1U/mg protein).

Interestingly, the comparison of SOD activity between the different rat tissues, showed clearly a relationship between the capacity of the tissue to accumulate high amounts of iron and their SOD activity. SOD activity paralleled the iron level in these tissues, such that it is maximal in iron storing organs, e.g., spleen (> 36 U/mg proteins) and liver (> 14 U/mg protein for Cu-Zn SOD and > 11 U/mg protein for Mn SOD) and has a lower activity in the other tissues, e.g., brain (< 1 U/mg protein) and heart (< 4 U/mg protein).

Modulation of nitric oxide release by stimulated AM after iron complexes administration in vivo

Nitric oxide release by AM, showed a significant variation between the different groups (Figure 1). It increased significantly in cells which had been isolated from rats administered either iron sucrose (Venofer) or iron gluconate (Ferrlecit) when compared to control cells ($P < 0.05$). However both iron dextran (Dexferrum) and iron polymaltose (Ferrum-Hausmann) decreased significantly $NO^{\cdot}$ release by more than 2-fold ($P < 0.05$).

Table 7. Superoxide dismutase activity (U/mg protein) in different rat tissues after i.p administration of different iron preparations.

	Controls	IG	IS	IP	ID
Liver					
CuZn SOD	14 ± 1.2	17.5 ± 5.1	22.3 ± 2.1*	21.2 ± 3.7*	25.1 ± 4.8*
Mn SOD	13 ± 1.1	11.1 ± 0.3	11.5 ± 0.85	11.1 ± 0.7	11.4 ± 1.1
Spleen	36.1 ± 9.7	36.2 ± 3.9	38.5 ± 8.2	39.8 ± 12	40.1 ± 2.1
Heart	3.5 ± 1.2	3.2 ± 0.5	3.9 ± 0.5	3.8 ± 1.5	3.7 ± 0.5
Brain	0.63 ± 0.1	0.42 ± 0.1	0.48 ± 0.015	0.4 ± 0.03	0.5 ± 0.03
Cortex	0.63 ± 0.3	0.65 ± 0.2	0.62 ± 0.11	0.5 ± 0.12	0.5 ± 0.06
Cerebellum	0.97 ± 0.18	0.22 ± 0.02	0.8 ± 0.02	0.22 ± 0.08	0.41 ± 0.02

Key: IG–iron gluconate; IS–iron sucrose; IP–iron polymaltose; ID–iron dextran.
Values are means $\pm$ SD. * $P < 0.05$.

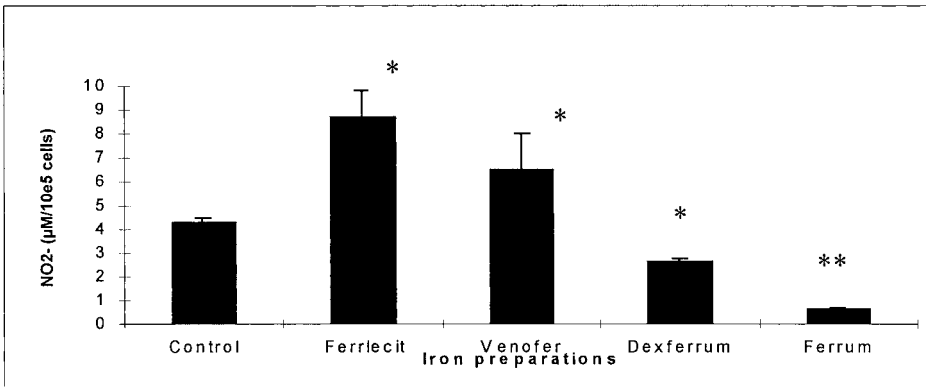

Fig. 1. NO_2- release from alveolar macrophages isolated from control and iron loaded rats with different iron preparations *in vivo*, after *ex vivo* stimulation with LPS (0.5 μg/ml). Cells (100.000) were incubated 40 h at 37 °C, 5% CO_2.

Discussion

The four therapeutic iron complexes increased the iron content of the different rat tissues investigated, although the degree of iron loading achieved was dependent on the type of iron preparation. Iron dextran and iron polymaltose, which are the most widely used supplements for parenteral iron therapy, gave the highest iron loading level in comparison to iron sucrose and iron gluconate. The explanation for the different iron loading profiles may relate to a variety of factors which include the molecular size as well as the type of the carbohydrate complexing the ferric iron. Both iron dextran and iron polymaltose, which induced comparable iron loading, are type I compounds with a high molecular weight, while iron sucrose and iron gluconate have much lower molecular weight which may favor their renal elimination and thereby may explain their lower efficacy in loading the different tissues (Geisser 1998).

In general, these polymeric iron complexes are presumed to be taken up initially by the reticuloendothelial system, such that it might have been predicted that there would be a pronounced iron loading in both the spleen as well as in the broncho-alveolar macrophages. While splenic iron increased after administration of each of the preparations, only iron dextran and iron polymaltose were able to significantly overload macrophages. Iron dextran and polymaltose have a structural uniformity, and present a slow delivery of iron to endogenous iron-binding proteins such as transferrin by which it is transported to the bone marrow for hemoglobin synthesis. The slow clearance rate of iron derived from the processing of these two iron complexes from the RES may explain their capacity to overload macrophages. Furthermore, since the

iron is released via transferrin, a homeostatic control would be induced in an attempt to reduce the flux of iron across intestinal mucosa. These iron compounds did not increase brain iron content which means that these iron complexes, like iron-loaded transferrin, are unable to permeate the blood brain barrier. This is in contrast to the iron compound ferrocene, which due to its structure is able to permeate the blood brain barrier and increase iron in many brain regions (Ward *et al.* 1995).

The ability of the four iron complexes to induce an oxidative stress in the different tissues was evaluated by measuring the activity of the cytoprotective enzyme, superoxide dismutase. In general, no significant change was seen when compared to control values, with the exception of hepatic Cu-Zn SOD which was slightly induced by the different preparations. The low reduction potential of these compounds ensures that ferric iron cannot be reduced by biological reductants to ferrous iron and thereby to participate in Fenton chemistry. The high levels of iron loading achieved by the different preparations in liver may explain the increase in SOD activity in this tissue which is known to contain high basal levels of antioxidants, such as glutathione. When other iron preparations have been used to iron overload experimental models, e.g., ferrocene, (Ward *et al.* 1991), carbonyl iron and particularly Fe $(NTA)_2$ (Bacon *et al.* 1983), evidence for increased free radical reactions has been found.

The effect of such iron complexes on the functions of immune cells, i.e., macrophages, was investigated specifically by the assay of nitric oxide ($NO^{\cdot}$) release after *ex vivo* stimulation with bacterial lipopolysaccharide. Previous studies have shown that iron loading of these cells with iron dextran significantly decreases their $NO^{\cdot}$ production after stimulation with both LPS/INF-γ, (Zhang *et al.* 1998). Our results confirm this inhibitory effect; and like iron dextran, iron polymaltose was also able to inhibit $NO^{\cdot}$ release, which correlate with its ability to increase the iron content of these cells. The important increase in $NO^{\cdot}$ production by alveolar macrophages that were treated with iron sucrose and iron gluconate remain unexplained. It would appear that iron accumulation in alveolar macrophages is not the only factor involved in altering $NO^{\cdot}$ release, the speciation of iron in the macrophages may influences iNOS expression and thereby $NO^{\cdot}$ release by macrophages.

Ward *et al.* (1998) showed an inverse interrelationship between hepatic copper and iron in iron deficient rat liver: therefore, in these experiments, we have investigated the copper content in livers with different levels of iron loading. Our results did not show a reciprocal relationship between hepatic copper and iron status over a range of hepatic iron concentrations, although there was a clear trend of decreasing copper concentrations in both the liver and hepatic supernatant fractions which contained microsomes and cytosol. Schonewille *et al.* (1995) reported that high iron intake by goats reduced the mean plasma copper by 18%, caeruloplasmin activity by 13%, and significantly decreased hepatic copper by 27%; in the present studies much higher decreases in hepatic copper were found, between 39% and 72%. Neither the spleen or alveolar macrophages showed any relationship between these two transition metals which indicates that the uptake of iron and possibly copper into these cells i.e. principally by phagocytosis, differs significantly from that of liver.

It has been suggested from studies in yeast that the metabolism of iron and copper are inter-related. In yeast, iron and copper uptake and transfer across the plasma membrane have been elucidated; both iron and copper are reduced prior to uptake by Fre1p and Fre2p, respectively, both of which are membrane associated ferrireductase and have NADPH as co-factor. It is postulated that Fet3p mediates iron transport by acting as a ferroxidase converting ferrous to ferric iron which is then transported by Ftr1p, (Dancis *et al.* 1994; Askwith *et al.* 1984). Fet3p is an integral membrane protein which is a member of the family of blue multicopper oxido-reductase (Stearman *et al.* 1996).

In yeast, a high affinity uptake of Cu^{+}, (after reduction of Cu^{2+} by Fre1p) across the yeast membrane is mediated by Ctr1p and Ctr3p (Dancis *et al.* 1994; Knight *et al.* 1996) in addition to a low affinity system that involves Ctr2p protein (Kampfenkel *et al.* 1995). After copper has entered the cell it is transported to specific sites by copper chaperones (Harrison *et al.* 1999). The 74 residue cytoplasmic protein Atx1p (Lin *et al.* 1997) delivers copper to Ccc2p, the post Golgi vesicular copper transporter which is responsible for copper loading into the apo- Fet3p protein, which therefore, acquires its oxidase activity. In man, loading of isolated hepatocytes with holo-transferrin, results in a decrease in the rate of copper uptake although the levels of copper associated with the cell surface increased (Whitaker & McArdle 1997). The authors speculated that this was due to a lack of reduction of Cu^{2+} to Cu^{+} by the plasma membrane associated ferrireductase activity. Furthermore the authors showed that iron loading of the cells dramatically de-

creases the ferrireductase activity. In man a multitude of genes involved in iron uptake and transport across membranes have been identified, particularly in the intestinal mucosa (Crichton 2002). At the apical membrane of the enterocyte DcytB, a ferrireductase and Nramp2, a proton-coupled divalent cation transporter, which also transport ferrous iron out of the endosomal compartement, seem to represent the major pathway for the uptake of non-haem iron from the gastrointestinal tract. Nramp 2 was suggested to play an important role in the transfer of both iron and copper across the apical and endosomal membranes, this may indicate that in conditions of high iron loading, as reported in this present communication, Nramp2 would be down-regulated thereby diminishing both iron and copper transport. In contrast, in situations of iron deficiency, an up-regulation of this gene in the gut could increase copper transfer resulting in increased copper content in some tissues. One of the two isoforms of Nramp2 mRNA has an IRE in the 3′ position indicating that it could be regulated by IRPs in a similar way to that of transferrin receptor mRNA rather than to that of ferritin (i.e., In the presence of iron the mRNA would be degraded and its translation downregulated). The possible involvement of Nramp2 in copper transport remains to be elucidated. At the basolateral surface of enterocyte, Ireg1 together with hephaestin facilitate the exit of iron and its incorporation into plasma transferrin. Hephaestin is a multicopper ferroxidase, homologous to ceruloplasmin and other multicopper oxidases, which seems to be necessary for iron transport from the intestinal mucosa into the circulation. Vulpe *et al.* (1999), suggested that it was this protein which might be the important link between copper and iron metabolism in mammals.

In conclusion, these results confirm the safety of iron sucrose, which remains the treatment of choice in clinic for iron deficiency anemia, since this compound give a moderate and selective iron loading of the two sequestering iron tissues (liver and spleen), without increasing neither heart nor brain iron content, in addition to its inability to inhibit $NO^{\cdot}$ release by macrophages and thereby to impair their immune response. On the other hand, we may suggest the iron dextran loaded rats as a suitable model for studying secondary iron overload syndromes and the mechanism of iron toxicity related to these disorders, since it was the only compound to significantly load the RES cells with iron.

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