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MiniReview

Iron Absorption: Biochemical and Molecular Insights into the Importance of Iron Species for Intestinal Uptake

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Abstract: Recent advances in cloning of proteins involved in intestinal iron absorption can inform design and understanding of therapeutic iron preparations. Redox chemistry of iron is particularly important in iron metabolism, both as a potential source of toxic intermediates and as an essential requirement for efficient iron transport. The initial step in iron absorption (uptake from lumen to mucosa) is particularly important and several pathways involving Fe(III) reduction or transport and Fe(II) transport have been identified. Novel genes associated with iron uptake include Dcytb, a putative iron-regulated reductase and DMT1, a Fe(II) carrier in the brush border membrane. Other mechanisms may also operate, however. We review the recent findings and apply this to understanding the absorption of Fe(III) pharmaceuticals.

The mechanism of absorption of iron in mammals is the subject of continuous investigation which produces controversial results, probably due to the complex chemistry of the element and to the different capabilities that biological membranes have for iron uptake. Iron is widespread in mammalian cells where it catalyses a great number of biochemical reactions.

Iron chemistry and biochemistry

Many of these reactions are related to the chemical nature of the element characterized by two principal oxidation states : *divalent* iron (Fe-II (-d^6)) and *trivalent* iron (Fe-III (-d^5)) and their associated ability to form complexes.

In water and in the absence of oxygen, iron is present in the form of the hexa-aqua complex divalent ion, which is readily oxidised upon increasing oxygen tension to the trivalent aqua complex of similar structure according to the one electron reversible reaction (1), hence becoming source of free radicals

$$Fe(II)_{aq} + O_2 \rightarrow Fe(III)_{aq} + O_2^{-\cdot} \quad (1)$$

The water solubility of the trivalent aqua-complex is a function of pH and rapidly decreases by increasing the pH value from 1 to 9.

Due to the complex hydrolytic reactions of deprotonation of the aqua-complex, oxo-hydroxy species of decreasing solubility are formed in water (fig. 1) (Flynn 1984; Schneider & Schwyn 1987; Cornell *et al.* 1989).

In biological media at low oxygen tension, $Fe(OH_2)_6^{2+}$ is the predominant species, while $Fe(OH_2)_6^{3+}$ is a minor species due to its low solubility (10^{-17} mol/l at pH7).

These chemical characteristics are suggestive, in principle, that iron II derivatives can be taken up more easily than iron III derivatives by cell membranes as a consequence of more favourable solubility properties.

Iron and oxidative damage

Unfortunately, the higher solubility of $Fe(OH_2)_6^{2+}$ (10^{-5} mol/l at pH 7, and 10^{-1} mol/l at lower pH values), accounts for the generation of aggressive reactive oxygen species, through the equations summarised below (fig. 2).

Superoxide anion can be generated enzymatically by the respiratory burst oxidase as a reaction by cells of the inflammatory system against foreign substances or bacteria (step I) (Segal & Abo 1993; Babior 1999). The enzyme responsible is closely regulated so that its activity increases when superoxide is required. Superoxide is also generated by mitochondria during respiration and may have been involved in metabolic control (Droge 2002) whose activities usually increase when a defence mechanism is required. Excess $O_2^{-\cdot}$ production is usually converted by superoxide dismutase to H_2O_2 (step II) which is scavenged by catalase. However, where there is over-production of H_2O_2 or in sites devoid of catalase, the presence of iron ("free iron", (Halliwell & Gutteridge 1986; Gutteridge & Halliwell 1989) is able to transform H_2O_2 to $OH^{\cdot}$ (step III) by the Fenton

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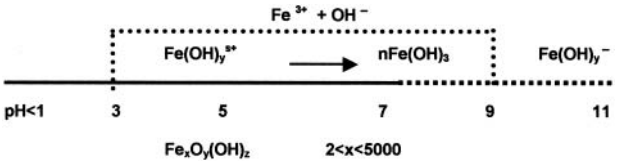

Fig. 1. Hydrolytic reactions of iron as a function of pH. At low pH values iron is present in solution as free ion. Aqua complex oligomers are generated at pH values higher than 2 and polymerisation occurs by futher increasing the pH; x is the estimation of number of iron atoms present in the aquated forms, y and z are the O^- and the $(OH)^-$ in the bridging position of the polynuclear core bonded to x by the relationship 2y-z/p=n. Precipitation of these forms occurs at x values higher than 20.

reaction, which actually involves a far more complex chemistry (Halliwell 1992). This radical is unstable (fig. 2) and reacts rapidly (step IV) with other molecules including proteins or DNA, thus giving rise to tissue damaging events in, for example, inflammation, ischaemia and reperfusion, inflammatory bowel disease, rheumatoid arthritis or cancer (White *et al.* 1985; Ward 1986; Halliwell & Gutteridge 1989; Floyd 1990; Crichton 1991b).

The conclusion is that iron is essential for life and should enter in the biological cycle by a regulated balance mechanism which can prevent the formation of iron in a 'free' form (loosely bound iron or excess iron) that may induce damage or originate dysfunctions in the cell.

Iron balance and distribution

In normal conditions the mammals are able to maintain iron homeostasis by controlling absorption from the diet and avoiding iron overload. Molecular aspects of regulation of iron absorption fall outside the scope of the present article and have been reviewed by Fleming & Sly (2002).

Appropriate amounts of iron in the diet (10–15 mg) are taken up in the intestinal mucosa (normally 1 mg/day) and transported by plasma transferrin to the utilization compartment (mainly the red blood cells) and the storage pro-

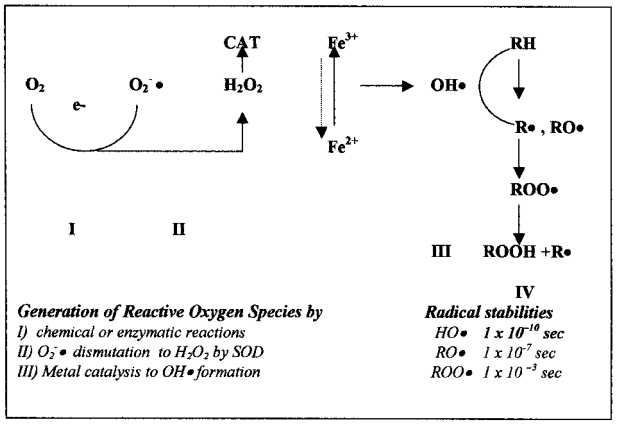

Fig. 2. Mechanisms of generation and decomposition of Oxy-radicals. CAT – catalase, SOD – superoxide dismutase.

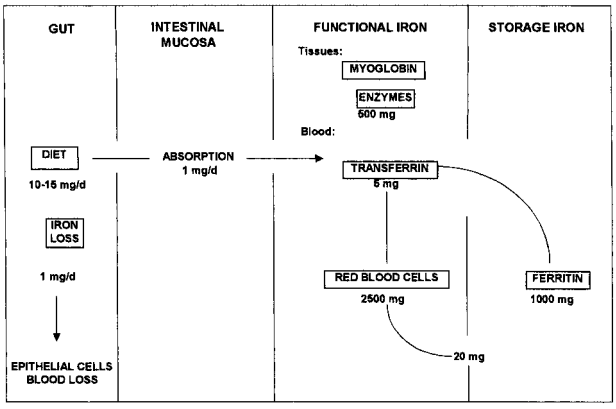

Fig. 3. Iron balance in man.

tein (ferritin) from where it can be mobilised when required (fig. 3). Iron is "recycled" within mammals and any uncontrolled losses from epithelial cells or via blood loss is balanced by the amount of absorbed iron. The daily body turnover is near 1 mg in healthy human beings (but can reach 4–5 mg in menstruating women), whilst the concentration in the body is 40–50 mg/kg distributed as: a) the porphyrin complex of haemoglobin, involved in the functional transport of oxygen (30 mg/kg), b) myoglobin, in muscle cells, involved in oxygen conservation (4–8 mg/kg) and c) haemoproteins (cytochromes, oxidases, peroxidases), sulfoproteins as well as non haem proteins for the catalysis of oxo-reductive reactions (2 mg/kg).

The remaining (10–12 mg/kg) is stored in a form inactive for the free radical generation, namely ferritin, which can accumulate up to 4500 iron atoms within its 24 subunits shell. A small amount of iron circulates in plasma bonded to transferrin, the iron transport protein. These two proteins, and in particular ferritin, are a matter of continuous investigation aimed to define the structure of the iron core, the mode of iron accumulation and the functional role of the proteins (Baker *et al.* 1987; Harrison & Arosio 1996; Yang *et al.* 1998).

Recent reviews (Quian & Tang 1995; Andrews *et al.* 1999a & b; Wessling-Resnick 2000; Rolf & Hediger 2001) deal with the transport mechanism of iron to cells such as reticulocytes, hepatocytes, yeast or cultured cell models. These are able to evaluate the translocation of iron from transferrin to intracellular iron stores and complexes such as ferritin and haem. The present review focusses on studies evaluating the mode of interaction of iron administered in diet or drugs with the enteric mammalian cell membrane that is the first cellular step in iron absorption.

Iron absorption

Absorption is regulated according to the iron status of the storage proteins. In iron-replete conditions absorption is down-regulated, while in iron-deficient conditions absorption is enhanced. Alterations in iron metabolism that generate a gradual depletion of the element from the storage pro-

teins are associated with different forms of anaemia. Iron deficiency reduces the activity of all the above indicated iron metabolic systems and, in severe cases, also of the iron-dependent enzymes.

Iron deficiency is normally treated with oral iron derivatives capable of being absorbed in the intestine and in particular, as shown in fig. 4, in duodenum, while the stomach and the other parts of the gut are involved to a lesser extent.

The mechanism of absorption of iron either from the diet or from drugs (there is a similarity between these two forms of iron donor) is complex and with multiple processes occurring. Despite the great deal of work reported in the literature on mucosal iron absorption/metabolism (Forth & Rummel 1973; Wollenberg & Rummel 1987; Dietzfelbinger 1987; Bezkorovainy 1989), it has been a matter of debate whether or not iron is absorbed in the ferrous or ferric form, while it was well accepted that the intestinal solubility of the drug plays an important role in promoting iron absorption.

Recent findings derived from the development of molecular genetics (Wood & Hen 1998, and references therein) have helped to elucidate part of the molecular mechanism of iron absorption.

Absorbable iron species

Different forms of iron are absorbable: haem, soluble complexes or salts. These are present in diets or in drugs and may be in the divalent or trivalent state. As far as the mechanism is concerned, the facile oxidation that divalent salts or complexes undergo *in vitro* (reaction 1), also occurs *in vivo*. This has been observed by administering iron as iron sulphate to mice.

Using ferrozine as a reagent to detect the valency state of iron (ferrozine reacts only with divalent iron to form a chromogen), it has been found that divalent iron sulphate

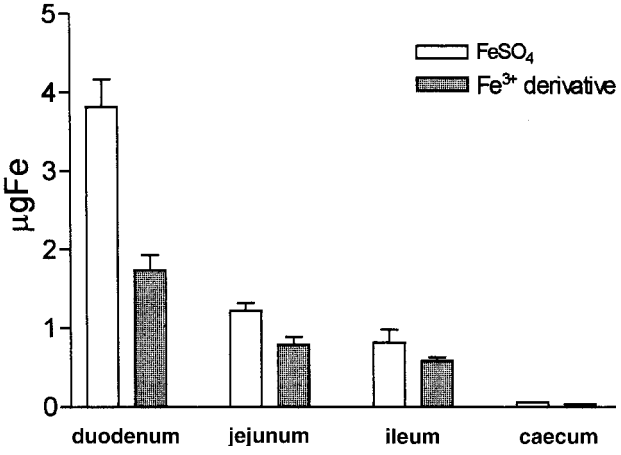

Fig. 4. ^{59} Fe uptake in the mouse 1 hr after oral dosing of 20 µg ^{59}Fe labelled ferrous sulphate (bold) or ferric protein compound into duodenal, jejunal and ileal wall (Simpson *et al.* 1991).

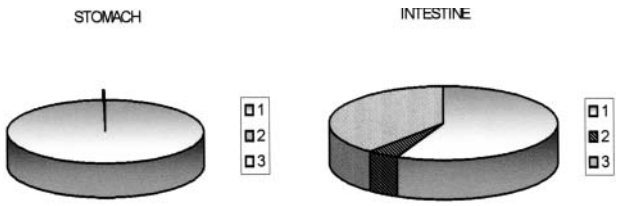

Fig. 5. Distribution of iron species in the stomach and in the intestine after administration of FeII as Fe Sulphate to mice. 1) iron that reacts rapidly with ferrozine (FeII ion); 2) iron slowly reactive (FeII complexes); 3) iron non reactive with ferrozine but ascorbate reducible (FeIII).

is easily oxidised to trivalent when it passes from the stomach to the duodenum of the mice (fig. 5).

Furthermore two species of complex iron are generated: one fast-reduced that represents a divalent iron form that is easy absorbable and a second that is slow-reducible, representing a less absorbable or non-absorbable form.

This is suggestive that even when iron is administered in the divalent state, a great amount of the species present in the duodenum is reducible trivalent iron. Actually food iron is initially released in the lumen mainly as non-haem ferric complexes or as haem. Haem, derived from the digested haemoproteins, is able to interact with the enteric membranes. The finding that a specific receptor for haem has been identified in mucosa (Grasbeck *et al.* 1979) suggests that haem is taken up through receptor-mediated transport. Experimental data indicate that haem absorption is much less responsive to store protein repletion than inorganic iron (Crichton 1991a) and following these results no pharmaceutical preparation containing haem is utilised in therapy.

According to the solution characteristics of iron (fig. 1), the presence of trivalent iron requires solubilization of the element to permit absorption. Most of the metals present in food and drugs are either soluble throughout the pH range of the lumen, (eg. Na, K, Ca or Mg), or are susceptible to precipitation as a consequence of hydrolytic reactions. This is the case for iron (as well as Al, Cu, Mn and Zn (Powell *et al.* 1999). The presence of chelators in the intestine (either endogenous or exogenous, table 1) can, however, allow the iron to exist as soluble complexes in the intestine.

Mechanisms of iron uptake

A large number of studies, some very recent, have been conducted to delineate the biochemical mechanism associated with iron uptake (and absorption) by enterocytes. These have implied the involvement of at least three different component models: a) iron–binding components, b) enzymatic iron reduction, c) specific membrane transport molecules.

Iron binding components.

Proteins characterised by the capacity for binding iron have been found in the brush border membrane of epithelial cells (Wessling-Resnick 1999 and references therein). In particular the paraferritin system formed by β-integrin,

Table 1.

Compounds indicated as chelators for facilitating iron absorption.

Class of chelator	Examples	References
Organic acids	Lactic, citric, succinic, ascorbic, malic, pyruvic	Gillooly *et al.* (1983) Conrad & Schade (1968)
Bile acids and fatty acids	Taurocholate	Powell *et al.* (1990) Jacobs & Miles (1970) Huebers (1983) Simpson *et al.* (1988) Sanyal *et al.* (1990)
Amino acids	Asparagine, glycine, histidine	Christensen *et al.* (1984) Whitehead *et al.* (1996)
Membrane lipids	Fatty acids	Simpson *et al.* (1987)
Proteins	Apotransferrin	Huebers *et al.* (1983) Cazzola *et al.*(1985)
Elutable factors	Mucin	Conrad *et al.* (1991)

mobilferrin and a flavin monooxygenase, has been suggested to take up mucin-bound iron coming from the digestive tract. Mucin is a large glycoprotein endowed with sulphate and carboxy groups that plays a role in metal binding (Hunter *et al.* 1989). It is able to form colloidal polyhydroxy iron cores that hinder further polymerisation and precipitation of large iron hydroxide structures. Fe-mucin is easily dissociable and the released iron is available to β-integrin for membrane uptake. β-integrin is a model for iron absorption that emphasizes the importance of iron release from soluble carriers as an initial step of iron uptake, as insoluble iron is not absorbed. According to this model the iron is taken up in the trivalent state, reduced after internalization and transported in the cytosolic compartment of the cell and finally released to the iron storage proteins (Conrad *et al.* 1999). The precise regulatory function of these proteins in iron trafficking is not completely defined, and only recently (Conrad & Umbreit 2000) has there been an evaluation of the role of the cytosolic iron binding protein, mobilferrin.

Enzymatic iron reduction.

Other recent studies support the view that trivalent iron can be absorbed with the contribution of a reductive step catalysed by the enzyme ferrireductase (Raja *et al.* 1992; Riedel *et al.* 1995). The enzymatic activity, identified in duodenal fragments (Raja *et al.* 1992) and in cultured intestinal cells (Ekemkcioglu *et al.* 1996), acts on different trivalent iron substrates leading to the formation of divalent iron with the V_{max} of the enzyme increased by iron deficiency. Differences in reaction rate have been observed depending on the nature of the substrate with lower reactivity observed for higher molecular weight iron carriers (Pountney *et al.* 1996; Raja *et al.* 2000). The enzyme, termed Dcytb (duodenal Cytochrome b) expressed by human epithelial cells, has now been characterised and cloned (McKie *et al.* 2001). Unlike the ferrireductase previously identified in plants and yeast, the enzyme lacks the cofactors (NADH and NADPH) that usually act as intracellular electron donors. The isolation of Dcytb provides an important mechanism for trivalent iron absorption that could supply ferrous iron to specific transport proteins as described below. Ascorbic acid is well known to promote iron absorption by solubilizing iron in the gastrointestinal lumen (Lynch & Cook 1980), but another, possibly intracellular, role for ascorbic acid in iron transport may also be important (Wienke *et al.* 1997). Dcytb, the candidate iron-regulated duodenal reductase, is highly homologous to cytochrome b561, which utilises cytoplasmic ascorbate as an electron source for transmembrane electron transport (McKie *et al.* 2001). Ascorbate likely also plays a role inside cells as an antioxidant and thus, perhaps indirectly helps maintain Fe as FeII. In man, ascorbate is exclusively derived from the diet leaving the question of a regulatory role for this compound in iron absorption somewhat problematic. More work is required to clarify the involvement of ascorbate in iron absorption.

Specific transport proteins.

The third model deals with Fe(II) absorption. By a functional expression cloning approach, a factor mediating Fe(II) uptake has recently been identified (Gunshin *et al.* 1997). The protein able to transport divalent cations through the intestine membrane has been variously termed divalent metal ion transporter (DMT1), Nramp2 or DCT1 (divalent cation transporter). The role of the factor has been supported by a genetic approach, with *mk* mice and Belgrade rats suffering from a microcytic anaemia related to a mutation of the Nramp2 gene that hinders intestinal uptake of dietary iron (Fleming *et al.* 1998).

DMT1 is a proton-dependent protein located in the apical surface of the epithelial cell membrane, able to internalise to the cytosol Fe^{2+} and perhaps other divalent metals, with a stoichiometry of 1 metal ion: 1 proton. This protein is expressed by epithelial cells in normal conditions, but the production and thus the transport of iron increases under anemic conditions (Andrews *et al.* 1999a & b; Canonne-Hergaux *et al.* 1999).

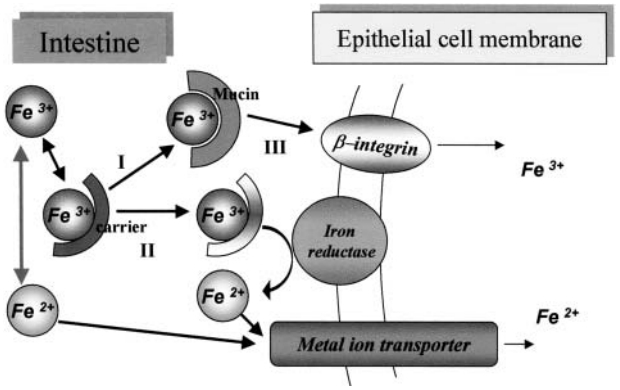

Fig. 6. Model for uptake of iron by the mucosal cell.

This mechanism is able to explain the transport of the iron into the cell and to the storage proteins within the cell but does not explain how the iron from the lumen, especially when is present in the trivalent state, can be taken up by DMT1 or any other transport protein.

Taken together the studies conducted recently suggest a multi-faced aspect of iron absorption and an overall mechanism is depicted in fig. 6.

Ingested iron, either as food or drug, is mobilised in the stomach and released in the less acidic duodenum as soluble divalent Fe ions/complex or trivalent iron carrier forms or as insoluble trivalent polyhydroxy compound.

FeII is taken up by enteric cells through a pathway mediated by the interaction with a specialised divalent metal transporter. Trivalent compounds require a more complex absorption pathway. Insoluble forms do not appear to be absorbed while soluble forms (iron carrier complexes) could enter the cell membrane either by interaction with specialised proteins (e.g. iron-mucin: β-integrin:mobilferrin sequence, Step III) or more likely through a reductive enzymatic reaction (Step II).

Also other mechanisms of iron absorption have been suggested including the one based on a pinocytosis mechanism. Transferrin, either as holoprotein or apoprotein, can be internalised by enterocytes, suggesting a potential route for iron absorption (Alvarez-Hernandez *et al.* 1994; Nunez & Tapia 1999; Oates *et al.* 2000) even though this protein does not seem to promote and regulate iron uptake in the duodenum (Simpson *et al.* 1986). Similarly, the homologue protein lactoferrin, is potentially able to mediate FeIII delivery to enteric cells with a mechanism and a role yet to be elucidated (Lonnerdal & Iyer 1995).

The multiplicity of the mechanisms of iron absorption generally does not affect the efficacy of the treatment of moderate forms of anaemia conducted either with ferrous or ferric drugs but perhaps reflects on the tolerability of these different forms.

These new molecular insights into the mechanism of iron uptake from the intestinal lumen allow us to understand the importance of the various iron species in the intestinal lumen and will enable us to design improved pharmacotherapeutic approaches to iron deficiency.

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