Review

The influence of lactoferrin, orally administered, on systemic iron homeostasis in pregnant women suffering of iron deficiency and iron deficiency anaemia

Rosalba Paesano a, Miriam Pietropaoli b, Sandra Gessani c, Piera Valenti d,*

a Department of Obstetrician and Gynecology, Sapienza, University of Rome, Rome, Italy
b Microbo srl Biotechnology Company, Rome, Italy
c Istituto Superiore di Sanità, Department of Cell Biology and Neurosciences, Rome, Italy
d Department of Public Health Sciences, Sapienza, University of Rome, Rome, Italy

Received 14 March 2008; accepted 6 June 2008
Available online 14 June 2008

Abstract

Iron is a fundamental element for humans as it represents an essential component of many proteins and enzymes. However, this element can also be toxic when present in excess because of its ability to generate reactive oxygen species. This dual nature imposes a tight regulation of iron concentration in the body. In humans, systemic iron homeostasis is mainly regulated at the level of intestinal absorption and, until now, no regulated pathways for the excretion of iron have been found.

The regulation and maintenance of systemic iron homeostasis is critical to human health. Excessive iron absorption leads to iron-overload in parenchyma, while low iron absorption leads to plasma iron deficiency, which manifests as hypoferremia (iron deficiency, ID) and ID anaemia (IDA). ID and IDA are still a major health problem in pregnant women. To cure ID and IDA, iron supplements are routinely prescribed. The preferred treatment of ID/IDA, consisting in oral administration of iron as ferrous sulphate, often fails to exert significant effects on hypoferremia and may also cause adverse effects.

Lactoferrin (Lf), an iron-binding glycoprotein abundantly found in exocrine secretions of mammals, is emerging as an important regulator of systemic iron homeostasis. Recent data suggest that this natural compound, capable of interacting with the most important components of iron homeostasis, may represent a valuable alternative to iron supplements in the prevention and cure of pregnancy-associated ID and IDA.

In this review, recent advances in the molecular circuits involved in the complex cellular and systemic iron homeostasis will be summarised. The role of Lf in curing ID and IDA in pregnancy and in the maintenance of iron homeostasis will also be discussed. Understanding these mechanisms will provide the rationale for the development of novel therapeutic alternatives to ferrous sulphate oral administration in the prevention and cure of ID and IDA.

© 2008 Elsevier Masson SAS. All rights reserved.

Keywords: Lactoferrin; Iron; Hypoferremia; Anaemia; Pregnancy

1. Iron homeostasis and disorders

Iron is an essential element for living cells, owing to its ability to gain and lose electrons. However, iron can also be toxic when present in excess because of its capacity to donate electrons to oxygen, thus causing the generation of reactive oxygen species (ROS), such as superoxide anions and hydroxyl radicals. Hydroxyl radicals are known to cause tissue injury and organ failure by damaging a number of cellular...
components, including DNA, proteins and membrane lipids. Accordingly, all organisms have developed strategies that allow acquiring, binding and storing elemental iron in a nontoxic, readily available form. In this respect, lactoferrin (Lf) in the secretions and transferrin (Tf) in the serum, ensure that the proper $10^{-18}$ M free iron concentration in human fluids is maintained, thus avoiding iron precipitation, ROS induction and microbial colonisation [1,2].

The total body iron, about 3 g in women and 4 g in men, is mainly incorporated as haemic-iron in the haemoglobin, myoglobin and cytochromes (2–2.7 g), and as non-haemic form in various enzymes. Every day 20 mg of iron, derived primarily from lyses of senescent erythrocytes, are utilised for the de novo synthesis of haem.

The systemic iron homeostasis is tightly regulated through iron absorption and storage. Absorption of nearly all dietary iron (1–2 mg daily) takes place in the proximal duodenum and includes the following steps: (i) reduction of iron from the ferric state (III) to the ferrous state (II); (ii) apical uptake by enterocytes followed by transcellular trafficking; and (iii) basolateral efflux by the ferrous iron transporter ferroportin (FPN). The fate of iron absorbed by enterocytes can be either storage as ferritin followed by excretion in the faeces when the senescent erythrocytes, are utilised for the de novo synthesis of haem.

The systemic iron homeostasis is tightly regulated through iron absorption and storage. Absorption of nearly all dietary iron (1–2 mg daily) takes place in the proximal duodenum and includes the following steps: (i) reduction of iron from the ferric state (III) to the ferrous state (II); (ii) apical uptake by enterocytes followed by transcellular trafficking; and (iii) basolateral efflux by the ferrous iron transporter ferroportin (FPN). The fate of iron absorbed by enterocytes can be either storage as ferritin followed by excretion in the faeces when the senescent erythrocytes, are utilised for the de novo synthesis of haem.

Accordingly, all organisms have developed strategies that allow acquiring, binding and storing elemental iron in a nontoxic, readily available form. In this respect, lactoferrin (Lf) in the secretions and transferrin (Tf) in the serum, ensure that the proper $10^{-18}$ M free iron concentration in human fluids is maintained, thus avoiding iron precipitation, ROS induction and microbial colonisation [1,2].

The total body iron, about 3 g in women and 4 g in men, is mainly incorporated as haemic-iron in the haemoglobin, myoglobin and cytochromes (2–2.7 g), and as non-haemic form in various enzymes. Every day 20 mg of iron, derived primarily from lyses of senescent erythrocytes, are utilised for the de novo synthesis of haem.

The systemic iron homeostasis is tightly regulated through iron absorption and storage. Absorption of nearly all dietary iron (1–2 mg daily) takes place in the proximal duodenum and includes the following steps: (i) reduction of iron from the ferric state (III) to the ferrous state (II); (ii) apical uptake by enterocytes followed by transcellular trafficking; and (iii) basolateral efflux by the ferrous iron transporter ferroportin (FPN). The fate of iron absorbed by enterocytes can be either storage as ferritin followed by excretion in the faeces when the senescent erythrocytes, are utilised for the de novo synthesis of haem.

Accordingly, all organisms have developed strategies that allow acquiring, binding and storing elemental iron in a nontoxic, readily available form. In this respect, lactoferrin (Lf) in the secretions and transferrin (Tf) in the serum, ensure that the proper $10^{-18}$ M free iron concentration in human fluids is maintained, thus avoiding iron precipitation, ROS induction and microbial colonisation [1,2].

The total body iron, about 3 g in women and 4 g in men, is mainly incorporated as haemic-iron in the haemoglobin, myoglobin and cytochromes (2–2.7 g), and as non-haemic form in various enzymes. Every day 20 mg of iron, derived primarily from lyses of senescent erythrocytes, are utilised for the de novo synthesis of haem.

The systemic iron homeostasis is tightly regulated through iron absorption and storage. Absorption of nearly all dietary iron (1–2 mg daily) takes place in the proximal duodenum and includes the following steps: (i) reduction of iron from the ferric state (III) to the ferrous state (II); (ii) apical uptake by enterocytes followed by transcellular trafficking; and (iii) basolateral efflux by the ferrous iron transporter ferroportin (FPN). The fate of iron absorbed by enterocytes can be either storage as ferritin followed by excretion in the faeces when the senescent erythrocytes, are utilised for the de novo synthesis of haem.
types that are involved in iron export, including the duodenal cells, hepatocytes, macrophages, and placental cells [14]. Tissue-specific deletion of the FPN gene causes iron accumulation in enterocytes, hepatocytes and above all in macrophages, which require FPN to efficiently recycle iron from lysed erythrocytes [15]. FPN has a molecular weight of 67 kDa and 12 putative transmembrane domains and seems to function as a dimer [16]. The synthesis of FPN is modulated by transcriptional and post-transcriptional mechanisms. IRE/IRP regulate FPN mRNA. FPN transports Fe(II), which is converted to Fe(III) by multicopper oxidases, including ceruloplasmin and hephaestin, using oxygen as an electron acceptor [17,18]. In the absence of a ferroxidase, iron does not get exported through FPN. As a matter of fact, subjects carrying mutations in ceruloplasmin gene show iron-overload in parenchymal tissues and, in the absence of hephaestin, iron remains within the mucosa [19]. The iron transport across the enterocytes and its export into the blood by FPN are shown in Fig. 1.

Another pivotal component of systemic iron metabolism is hepcidin, a circulating peptide hormone synthesised by hepatocytes and secreted in plasma and urine. It has been reported that hepcidin mRNA is also present in the heart, pancreas and haematopoietic cells, although the biological relevance of this expression is not understood. The active form of hepcidin, derived from an 84-amino-acid precursor, is a 25-amino-acid peptide containing eight cystein residues. The bioactive human hepcidin is a 25-amino-acid peptide first identified in human urine [20] and plasma [21]. The urine also contains minor 20- and 22-amino-acid forms truncated at the N-terminus [22]. Hepcidin regulates the entry of iron into plasma through FPN [23,24]. Hepcidin, by binding to FPN, causes FPN phosphorylation, internalisation and degradation in lysosomes [25,26], thus preventing iron export and enhancement of cytosolic iron stored in ferritin. The influence of hepcidin concentration on iron export from cells into the blood is shown in Fig. 2. Therefore, increased expression of hepcidin leads to IDA [27,28]. The expression of hepcidin can be regulated in several ways [29]. Hypoxic conditions decrease hepcidin expression, resulting in increased iron export into the plasma [27]. Moreover, hepcidin production is also suppressed when iron stores are low, and consequently, FPN is displayed on basolateral membranes of enterocytes allowing iron transport to plasma. On the contrary, when iron stores are adequate or high or when oral or parenteral iron is loaded, the liver produces hepcidin, which circulates to the small intestine thus hindering iron export to circulation [27]. Similarly, in macrophages recycling senescent red blood cells, hepcidin-induced degradation of FPN results in iron trapping by macrophages. Moreover, hepcidin synthesis can be markedly induced by inflammatory status. In particular, interleukin-6 (IL-6) and probably other cytokines, induce transcription of the hepcidin gene in hepatocytes [30]. Transcriptional activation occurs in response to the binding of signal transducer and activator of transcription-3 (STAT3) to the hepcidin gene promoter. Increased hepcidin gene expression and consequent decrease in FPN levels result in decreased plasma iron concentration. Hypoferremia in this instance is characterised by iron retention in the intestinal mucosa and in macrophages. The inability to export iron leads to a decreased pool of serum transferrin-Fe(III) and iron-limited erythropoiesis. Indeed, iron and inflammation have both been reported to suppress FPN mRNA expression independently of hepcidin [31–33].

Therefore, the dysregulation of hepcidin, FPN, ceruloplasmin or hephaestin results in a spectrum of iron disorders [13]. In inflammatory disorders and infections, cytokine-induced hepcidin up-modulation contributes to the development of anaemia of inflammation, characterised by hypoferremia and anaemia despite adequate iron stores [34].

3. Hypoferremia and iron deficiency anaemia in pregnancy

Pregnancy is characterised by an increased iron requirement, due to enhanced blood volume and development of foetal—placenta unit [35]. Hypoferremia and anaemia are still prevalent among pregnant women in both developing and industrialised countries, and represent an important risk factor for maternal and infant health. However, the degree of foetal ID is not always as severe as that in mother [36].
Iron transfer from the mother to the foetus is supported by a substantial increase in maternal iron absorption during pregnancy and is regulated by the placenta [37,38]. Most iron transfer to the foetus occurs after the 30th week of gestation and likely involves placental expression of those proteins known to mediate systemic iron homeostasis. The placental syncytiotrophoblast acquires ferric iron bound to transferrin at the apical membrane through TfR-1 [11]. The synthesis of TfR-1 increases in pregnant women suffering of ID and IDA [38]. FPN is found on the placental basal foetal-facing membrane, consistent with unidirectional mother–foetus iron transport [39]. Ferritin is strongly expressed in the stroma and contributes to iron accumulation in foetal tissues [39]. In contrast, maternal serum ferritin usually markedly decreases between 12th and 25th week of gestation, probably as a result of iron utilisation for expansion of the maternal red blood cell mass.

Concomitantly with the enhanced placental–foetal iron transport, controlled by foetal hepcidin, an increased expression of placental FPN is also observed. Therefore, similarly to the regulation of systemic iron homeostasis, high levels of hepcidin could induce internalisation and degradation of placental FPN, thus decreasing iron transport to foetus. Interestingly, it has been reported that hepcidin synthesis increases during inflammation, mostly due to the stimulatory effect of IL-6 on hepcidin promoter. The enhanced hepcidin production leads to a decrease in plasma iron level, thus contributing to hypoferraemia and anaemia induction. In this respect, the level of pro-inflammatory cytokines, including IL-6, synthesised by peripheral blood cells in uncomplicated pregnancy and in unexplained recurrent spontaneous abortion has been compared [40]. In this study, significantly increased levels of pro-inflammatory cytokines were found in women undergoing unexplained recurrent spontaneous abortion as compared to normal pregnant women at similar stages of pregnancy. Furthermore, it has been demonstrated that significantly higher levels of IL-6 (range 0–77 pg/ml) are present in the

Fig. 2. Hepcidin regulates iron export from cells into the blood. When hepcidin concentration is low, iron is exported from cells into the blood by ferroportin. When hepcidin concentration is high, hepcidin binds to ferroportin and induces its internalisation and degradation thus inhibiting iron export from cells to the blood.
plasma of patients with preeclampsia as compared to healthy pregnant women (0–19 pg/ml) [41].

Overall, these data strongly suggest that an increased expression of pro-inflammatory cytokines, including IL-6, occurring during pregnancy could play a role in the establishment of systemic iron homeostasis disorders, such as ID and IDA. In light of these observations, the treatment of ID and IDA during pregnancy and postpartum should be entirely reconsidered taking into account not only the enhanced blood volume and development of foetal-placenta unit, but also the pivotal role that factors regulating iron transport from tissues to blood, i.e. FPN and hepcidin, might play in these events.

4. Oral administration of lactoferrin in pregnant women suffering of ID and IDA

The most used treatment for ID and IDA currently consists in oral administration of iron as ferrous sulphate. However, ferrous sulphate administration often fails to exert any significant effects on these pregnancy-associated pathologies, and frequently causes several adverse effects (gastrointestinal discomfort, nausea, vomiting, diarrhoea, constipation). This is likely due to the poor bio-availability of inorganic iron requiring the administration of large quantity of ferrous sulphate [42,43].

Lf, a prominent protein in milk, many other secretory fluids and white blood cells, is a monomeric 80-kDa glycoprotein, synthesised by exocrine glands and by neutrophils at infection and inflammation sites. The molecule is folded into homologous N- and C-terminal lobes, each comprising two domains that enclose a conserved iron-binding site [44]. Lf by virtue of its iron-binding ability, ensures that free iron concentration in human secretions does not exceed 10⁻¹⁸ M, thus preventing ROS formation and inhibiting microbial growth. Although Lf iron-binding at high affinity (KD ~10/20 M) is, without any doubt, a key property of this protein, and accounts for some of its many biological roles in host defence [2], it is now clear that Lf exhibits other functions besides iron sequestration, such as a strong capacity to modulate the inflammatory response. Lf, by virtue of its capacity to reduce pro-inflammatory cytokine expression in vivo, including IL-6 [45], might represent a novel and promising natural compound to be used in the prevention and cure of pregnancy-associated ID and IDA. In this respect, we have recently reported that oral administration of bovine Lf could represent an effective therapy in preventing and curing ID and IDA in uncomplicated pregnancy [46]. In this study, representing the first clinical trial on the therapeutic effect of bovine Lf on systemic iron homeostasis, we have clearly demonstrated the efficacy of this natural compound in rescuing iron homeostasis in pregnant women suffering of ID and IDA. Independently of pregnancy trimester, oral administration of 100 mg of Lf (about 30% iron-saturated) twice a day before the meal, increased the total serum iron and haemoglobin concentrations at a greater extent than that observed after oral administration of ferrous sulphate. In contrast to the administration of ferrous sulphate, Lf oral administration did not result in any side effect [46]. The potent effect exhibited by Lf as compared with ferrous sulphate was neither due to the amount of iron supplied by Lf (8.8 mg/day), lower than that provided by ferrous sulphate (156 mg/day) [46], nor to enhanced iron absorption, equally well absorbed from Lf and ferrous sulphate [47].

As shown in Table 1, in a further clinical trial involving 143 pregnant women suffering of ID and IDA, we found that oral administration of Lf also increases the number of red blood cells and serum ferritin concentrations at a higher extent with respect to orally administered ferrous sulphate. In order to gain further information on the therapeutic potentiality of orally administered Lf in the control of systemic iron homeostasis, we performed a study on five pregnant women suffering of ID and IDA. The enrolled subjects were treated for 30 days with Lf followed by further 30 days of ferrous sulphate treatment. The number of red blood cells, as well as haemoglobin, total iron, ferritin and IL-6 concentrations in serum were assayed. As shown in Table 2, all the blood parameters tested were found to be increased after Lf therapy, while these values decreased already after 30 days of ferrous sulphate treatment. Concerning IL-6, its concentration significantly decreased after 30 days of Lf treatment, while newly increased after 30 days of ferrous sulphate treatment. In keeping with these results, an inflammatory response to oral ferrous sulphate administration has been also observed in healthy volunteers receiving 120 mg of iron per day [42]. High levels of IL-6 well correlated with low values of total serum iron and serum ferritin. Conversely, low levels of IL-6 detected after Lf treatment well related with increased values of total serum iron and serum ferritin.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Haematological values of 143 pregnant women suffering of ID and IDA untreated or treated with lactoferrin or ferrous sulphate orally administered for 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnant women (33) Refusing therapy P</td>
</tr>
<tr>
<td></td>
<td>Time 0 After 30 days</td>
</tr>
<tr>
<td>Red blood cells (mmc)</td>
<td>3.869.000 3.688.000 0.0940</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.6 10.9 0.0042</td>
</tr>
<tr>
<td>Total serum iron (µg/dl)</td>
<td>52 32 0.0017</td>
</tr>
<tr>
<td>Serum ferritin (ng/ml)</td>
<td>11 6 0.0066</td>
</tr>
</tbody>
</table>

All the values are expressed as mean values. Statistical analysis was performed using ANOVA test.
5. Conclusions

The result of the clinical trials carried out in pregnant woman suffering of ID and IDA unravelled the strong therapeutic potential of bovine Lf, indicating that this natural compound represents an efficient alternative to ferrous sulphate therapy. As a matter of fact, iron supplementation with ferrous sulphate only restores haemoglobin concentration [6], while does not significantly increase the number of red blood cells, total serum iron and serum ferritin (Table 1). The failure of ferrous sulphate therapy in increasing total serum iron concentration indicates that this therapeutic regimen fails in restoring iron transport from tissues to blood, differently from that observed after Lf therapy. These intriguing results support the idea that Lf can modulate systemic iron homeostasis independently of the concentration of Lf-bound iron, which is considerably lower than that supplied by ferrous sulphate [46]. It is now well established that systemic iron homeostasis is mainly regulated by iron absorption and inflammation. Concerning iron absorption, Lf and ferrous sulphate exhibit a similar capacity in iron absorption [47]. However, a relevant difference resides in the fact that while Lf significantly reduces IL-6 concentration in serum of pregnant woman suffering of ID and IDA, ferrous sulphate rather exhibits an opposite effect (Table 2). The recent advances on the role of inflammation in iron homeostasis, as well as of FPN in iron transport from tissues to plasma, may provide an explanation for the failure of the classical therapy with ferrous sulphate in restoring physiological concentrations of total serum iron and serum ferritin. Under inflammatory conditions, FNP expression at the cell surface might be negatively regulated by its interaction with hepcidin [31–33] as well as by other factors, thus impairing iron release into plasma in patients treated with ferrous sulphate. Since hepcidin synthesis is under the control of various factors, including IL-6, and IL-6 is increased in pregnancy, the hepcin-IL-6 axis could represent another key molecular link between inflammation and hypoferraemia/anaemia. Although a correlation between hepcidin serum levels and haematological parameters characterising ID and IDA has not been found yet [48,49], the capacity of

Table 2

<table>
<thead>
<tr>
<th>PW</th>
<th>Red blood cells $\times 10^3$/mmc</th>
<th>Haemoglobin (g/dl)</th>
<th>Total serum iron (µg/dl)</th>
<th>Serum ferritin (ng/ml)</th>
<th>IL-6 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T30</td>
<td>T60</td>
<td>T0</td>
<td>T30</td>
</tr>
<tr>
<td>1</td>
<td>3.000</td>
<td>3.500</td>
<td>3.600</td>
<td>9.5</td>
<td>10.5</td>
</tr>
<tr>
<td>2</td>
<td>3.450</td>
<td>4.200</td>
<td>4.100</td>
<td>10.8</td>
<td>11.4</td>
</tr>
<tr>
<td>3</td>
<td>3.250</td>
<td>3.900</td>
<td>3.800</td>
<td>10.0</td>
<td>10.5</td>
</tr>
<tr>
<td>4</td>
<td>2.900</td>
<td>3.500</td>
<td>3.600</td>
<td>10.8</td>
<td>11.5</td>
</tr>
<tr>
<td>5</td>
<td>3.340</td>
<td>4.400</td>
<td>3.300</td>
<td>11.0</td>
<td>11.9</td>
</tr>
</tbody>
</table>

PW = pregnant women. T0 corresponds to the beginning of the treatment, T30 corresponds to 30 days of lactoferrin treatment and T60 corresponds to the following 30 days of ferrous sulphate treatment.

Fig. 3. A putative interplay of lactoferrin with key proteins of systemic iron homeostasis. Lactoferrin, orally administered, in pregnant women suffering of iron deficiency anaemia (hypoferraemia) decreases. IL-6 thus restoring iron export from cells into the blood by ferroportin.
Lf to rescue systemic iron homeostasis through factors as hepcidin, other than IL-6 and FPN, cannot be excluded. A putative interplay of lactoferrin with key proteins of systemic iron homeostasis is illustrated in Fig. 3.

In conclusion, these clinical trials demonstrate that oral administration of Lf exerts a potent effect to counteract ID and IDA in pregnant women. We speculate that this effect could be due to the capacity of Lf to decrease pro-inflammatory cytokines leading to the rescue of haematological parameters and reduction of adverse pregnancy outcomes in women suffering of ID and IDA.

References


