Iron and hepcidin: a story of recycling and balance

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Il sottoscritto DOMENICO GIRELLI, in qualità di Relatore dichiara che

nell’esercizio della Sua funzione e per levento in oggetto, DI NON ESSERE in alcun modo portatore di interessi commerciali propri o di terzi; e che gli eventuali rapporti avuti negli ultimi due anni con soggetti portatori di interessi commerciali non sono tali da permettere a tali soggetti di influenzare le mie funzioni al fine di trarne vantaggio.

NOTHING TO DISCLOSE
Overview of iron metabolism and its regulation by the hepatic hormone hepcidin

The 3 main signals regulating hepcidin (iron status, inflammations/infections, and iron requirements from BM erythroid precursors)

The recent discovery of the erythroferrone (ERFE), the long sought “erythroid regulator” of iron metabolism

Possibile usefulness of hepcidin assay (in iron deficiency)

Future Targeted Treatments through pharmacological modulation of hepcidin
Iron: essential but potentially dangerous

easily exchange electrons
\[ \text{Fe}^{3+} \leftrightarrow \text{Fe}^{2+} \]
useful redox properties

key-component of enzymes crucial for \( \text{O}_2 \) transport and energy production (Hb, cytochromes...)

free radicals generation
\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^\bullet \]

strict regulation of body iron content needed

low

anemia

neuromuscular impairment

excess

iron overload

toxic organ damage
IRON “ECOLOGY”

Liver 1000 mg

- hepcidin

Splenic macrophages
≈600 mg

Circulating RBCs
≈2 g

Erythroid precursors in Bone Marrow

Plasma Transferrin

- losses (1-2 mg/day)
- mucosal exfoliation, menses

Duodenal absorption (1-2 mg/day)

- FERROPORTIN

Intestinal lumen

- enterocytes

Duodenal absorption (1-2 mg/day)

- FERROPORTIN
Physiology

Red blood cells
Bone marrow
Reticuloendothelial macrophage

Fe
Fe-Transferrin

Hepcidin

plasma Fe ↑

↑ Inflammation
↓ Hypoxia
↑ Erythropoietic drive
↑ Plasma Fe

Castagna A, J Proteomics 2010 (adapted)
Physiology

Red blood cells

Bone marrow

Reticuloendothelial macrophage

Fe

Fe-Tf

Hepcidin

Final effect: negative feedback

plasma Fe ↑

IL-6

Castagna A, J Proteomics 2010 (adapted)
Iron transferrin from portal vein enters the sinusoids → BMP6 production by SC, KC and HSC.

Both iron transferrin and BMP6 activate a multi-molecular signaling complex, composed of several molecules like BMP receptors, HJV (co-receptor), HFE and TFR2.

hepcidin transcription

Pietrangelo A, Gastroenterology 2015
Pathology

HFE C282Y +/+  

Hepcidin  

Bone marrow  

Red blood cells  

Reticuloendothelial macrophage  

Fe-Tf  

Castagna A, J Proteomics 2010 (adapted)
Pathology

HFE C282Y +/+  

Hepcidin

Intestinal absorption of Fe ↑

Expanded plasma Fe pool → ↑TS% → tissue Fe accumulation (Fe total 3-5 g → >20 g)

Castagna A, J Proteomics 2010 (adapted)
Non-transferrin bound iron (NTBI) in hemochromatosis

Transferrin saturation occurs due to continuously increased iron absorption

Subsequent formation of NTBI in plasma

Uncontrolled iron loading of organs, such as:

100% Normal: No NTBI produced

30% Iron overload
The spectrum of genetic dysregulation of hepcidin

Iron stores

Hepcidin levels

Hereditary hemochromatosis (HH)

Iron Refractory Iron Deficiency Anemia (IRIDA)

- Post-natal microcytic hypochromic anemia with low TS%
- Refractoriness to oral iron
- Slow response to i.v. iron
- Sometimes diagnosed in adulthood
- Normal/high hepcidin levels (diagnosis)

HFE, TFR2, SLC40A1, HAMP, HJV

TMPRSS6
HEP-(atic) CIDIN (antimicrobial)

- small (25 aa) peptide
- defensin-like (innate immunity-related peptides with natural antimicrobial activity)

Ganz T, Physiol Rev 2013
A second level of balance in pathological conditions: the host-pathogen battle for iron

- Essential
- Fe
- Fe\(^{3+}\)
- LPS
- hepcidin
- FERROPORTIN
A second level of balance in pathological conditions: the host-pathogen battle for iron.

- Essential iron sequestration into macrophages.
- Hepcidin inhibition of ferroportin.
- LPS stimulation of iron uptake by pathogens.

Special Conference SIMTI – Rome, October 15, 2015 – D.G.
ANEMIA OF CHRONIC DISEASE OR “ANEMIA OF INFLAMMATION”

- Impaired iron metabolism (hepcidin-induced “macrophage block and hypoferremia reducing iron availability to invading pathogens)
- Cytokine-induced impaired proliferation of erythroid progenitors
- Blunted EPO response

Pathophysiological meaning:
1 (+) iron sequestering (during infections)
2 (+) classic homeostatic loop
3 (-) matching iron absorption with erythropoiesis requirements
Regulators of Iron Balance in Humans

By Clement Finch

THE STORE REGULATOR

The normal US adult male with no unphysiologic blood loss has iron stores of 1,000 ± 300 mg as derived from plasma ferritin and phlebotomy studies.\textsuperscript{14} Whereas it is not known whether excretion exerts any regulatory effect in the normal individual, it has been repeatedly shown by radioiron measurements, using radioiron salts or food labeled biosynthetically with radioiron, that non-heme iron absorption is inversely related to iron stores.\textsuperscript{15,16} Absorption from a test meal is high if iron stores are depleted and is suppressed if iron stores are enlarged.\textsuperscript{17} This regulation is so predictable in normal subjects that plasma ferritin measurements of iron stores have been used to predict absorption from a meal of known availability.\textsuperscript{18} The highly available heme iron is much less affected by the status of iron stores,\textsuperscript{19,20} but has seemed of secondary importance in considerations of iron deficiency because of its limited intake by most of the world’s needy population.

...
Ferrokinetic studies in humans:

✓ Normal iron absorption = 1-2 mg/day

✓ Pts. with Iron deficiency anemia receiving therapeutic doses of iron can absorb > 20 mg/day

✓ Similar amount can be absorbed by subjects with normal iron stores when erythropoiesis is stimulated (i.e. after blood loss or by EPO administration)

Finch C, Blood 1994
BLOOD LOSS SUPPRESSES HEPCIDIN TO INCREASE IRON AVAILABILITY FOR “STRESS” ERYTHROPOIESIS

Fe ↑

- ↑ Inflammation
- ↓ Erythropoietic drive
- ↓ O₂
- ↑ Fe

Fe-Transferrin

Reticuloendothelial macrophage

Bone Marrow normal erythropoiesis

RBCs precursors

RBCs

Western diet: ~15 mg Fe/die
only ~ 10% absorbed!

Adapted from Castagna A, J Proteomics 2010
BLOOD LOSS SUPPRESSES HEPCIDIN TO INCREASE IRON AVAILABILITY FOR “STRESS” ERYTHROPOIESIS

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Adapted from Castagna A, J Proteomics 2010

Special Conference SIMTI – Rome, October 15, 2015 – D.G.
Erythroferrone (ERFE) the newly identified erythroid regulator

Proposed mechanism of action

- ERFE clearly involved in hepcidin suppression in response to **acute** “stress erythropoiesis” (after hemorrhage/EPO administration)

- Further work needed to confirm whether it is the long-sought erythroid regulator responsible for **chronic** hepcidin suppression in ILAs.

Kautz L, Nat Genet 2014

Kautz L, E Blood 2014

Erythroblasts (bone marrow, spleen)

\[ \text{Hemorrhage} \rightarrow \text{HIF} \rightarrow \text{EPO} \rightarrow \text{JAK2/STAT5} \rightarrow \text{ERFE} \rightarrow \text{haptocytes} \]

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### Genetic disorders leading to systemic Iron overload

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Gene and Inheritance</th>
<th>Age at Presentation</th>
<th>Neurologic Symptoms</th>
<th>Anemia</th>
<th>Transferrin Saturation</th>
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<tbody>
<tr>
<td>Impaired hepcidin–ferroportin axis</td>
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<tr>
<td>HH type I</td>
<td>HFE, AR</td>
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<td>No</td>
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<td>HH type IIA</td>
<td>HFE2, AR</td>
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<tr>
<td>HH type IVB</td>
<td>FP (GOF), AD</td>
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<td>No</td>
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<td>Impaired iron transport</td>
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<tr>
<td>Inadequate release to erythron: aceruloplasminemia</td>
<td>CP, AR</td>
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<tr>
<td>Inadequate uptake by erythron</td>
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<td>DMT1 mutations</td>
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<td>Hypotransferrinemia</td>
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<td>Ineffective erythropoiesis</td>
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<td>Thalassemia</td>
<td>Globin, AR</td>
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<td>Congenital sideroblastic anemia</td>
<td>ALAS2, XL; SLC25A38, AR; GLRX5, AR; ABCB7, XL</td>
<td>Variable</td>
<td>ALAS2 and SLC25A38: no; GLRX5 and ABCB7: yes</td>
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<td>Congenital dyserythropoietic anemia</td>
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<tr>
<td>Type I</td>
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<td>Type II</td>
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<td>Type III</td>
<td>Unknown, AD</td>
<td>Child</td>
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</table>

**“IRON LOADING ANEMIAS” (ILAs)**
Pathophysiology of iron overload in ILAs

Bone Marrow

- normal erythropoiesis

RBCs precursors

Ineffective/expanded erythropoiesis

- anemia

Portal vein

↑ Iron absorption

hepcidin

↑“erythroid regulator” (ERFE?) overrides signal from iron stores
Pathophysiology of iron overload in ILAs

Bone Marrow

normal erythropoiesis

RBCs precursors

Ineffective/expanded erythropoiesis

anemia

↑ iron absorption

hepcidin

Portal vein

↑ “erythroid regulator” (ERFE?) overrides signal from iron stores
Hepcidin is suppressed in iron deficiency

Drakesmith H, Prentice AM, Science 2012
Hepcidin assays (ELISA and MS-based)

pro-hepcidin and N-terminus truncated isoforms in urine and serum

Kulaksiz H, Gut 2004

Castagna A, J Proteom 2010

Bozzini C, BCMD 2008
Promise of hepcidin assay in the clinic: predict nonresponsiveness to oral iron in IDA

- Substudy of a phase III clinical trial
- NR = Δ Hb < 1 g/dl after 14 days
- hepcidin >20 ng/ml = 81.4% Positive Predictive Value of NR
- NR majority subsequently responded to i.v. iron

Bregman DB, Am J Hematol 2013
Iron deficiency anemia in elderly: revisited in the hepcidin era

Busti F, Front Pharmacol 2014
The **MOST** promising application of hepcidin assay
(from a global health perspective)

- I.D. major health problem in children from low-income countries.
- The “Pemba” trial: “routine” iron supplementation is not the solution, but rather can ▲ mortality due to infections.

- Hepcidin is the major predictor of RBC iron incorporation in anemic African (Gambia) children, indicating iron utilization for children’s growth rather than for the growth of infectious agents.

Hepcidin as a point-of-care index guiding “safe” and effective iron therapy
Pharmacology of hepcidin

The pathophysiology and pharmacology of hepcidin

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<th>Therapeutic approach</th>
<th>Targeted disease</th>
<th>Mode of action</th>
<th>Agents</th>
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<tr>
<td>Hepcidin agonists</td>
<td>Iron overload (hereditary hemochromatosis and iron-loading anemias)</td>
<td>Hepcidin mimics</td>
<td>Minihepcidins [47]</td>
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<td>Stimulators of hepcidin production</td>
<td>Gene silencing of TMPRSS6 [50,51]</td>
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<td>BMP pathway agonists</td>
<td>BMP pathway inhibitors [54,56,74]</td>
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<td>Gene silencing of hepcidin and its regulators [66]</td>
<td>Erythropoiesis-stimulating agents [65]</td>
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<td>Hepcidin peptide neutralizing binders</td>
<td>Anti-hepcidin antibodies [67]</td>
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<td>Agents interfering with hepcidin–ferroportin interaction</td>
<td>Anticalins [68]</td>
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<td>Spiegelmers [69]</td>
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<td>Anti-ferroportin antibodies [71]</td>
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<td>Thiol modifiers [72]</td>
</tr>
</tbody>
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*Ruchala P & Nemeth E, Trends Pharmacol Sci 2014*
Il “Gruppo Interdisciplinare per le Malattie del Ferro” (AOUI Verona)

U.O.C. partecipanti:
1. Medicina Generale a indirizzo Immuno-Ematologico ed Emocoagulativo
2. Laboratorio Analisi
3. Servizio Trasfusionale
4. Radiologia
5. Anatomia Patologica
6. Fisica per Tecnologie Biomediche

http://www.gimferverona.org