ROLE OF NON-TRANSFERRIN-BOUND IRON IN THE PATHOGENESIS OF IRON OVERLOAD AND TOXICITY

Pierre Brissot, Olivier Loréal

Mammalian cells accumulate iron from two main circulating sources. The first one, which is the classical source, consists of iron bound to transferrin. The second one, identified by Hershko and colleagues, is called Non-Transferrin-Bound Iron (NTBI). The latter source is increasingly acknowledged as being of primary importance in iron overload situations, due to its high uptake by parenchymal cells and its potential toxicity.

1. DEFINITION, NATURE AND DOSAGE OF NTBI

1.1. DEFINITION

Four main forms of circulating iron can be individualized:

1.1.1. Transferrin iron. Transferrin is a glyco-protein consisting of a single polypeptide chain with two binding sites (each capable of binding one atom of ferric iron), and of two branched carbohydrate chains (glycans). Plasma transferrin can exist under four molecular forms (apotransferrin, monoferric A, monoferric B, diferric transferrin) but can be considered, physiologically, as a single homogeneous pool. The normal plasma concentrations are 20 \( \mu \text{mol/L} \) for iron and 30 \( \mu \text{mol/L} \) for transferrin (which corresponds to a transferrin saturation rate of approximately 30%).

1.1.2. Haem iron forms. Haem iron is carried as haemoglobin bound to haptoglobin and as haem bound to haemopexin.

1.1.3. Ferritin-iron is likely to represent only a minute amount since the iron content of circulating ferritin is usually low. Serum ferritin levels are normally less than 200 ng/ml in men and 100 in women.

1.1.4. Non-Transferrin-Bound Iron (NTBI).

i) Physiologically: This iron species is either undetectable or at very low concentration (<1\( \mu \text{mol/L} \)) in the normal plasma. It represents, however, a significant iron form in the normal cerebrospinal fluid (CSF). Indeed, likely due to an efficient blood-brain barrier against plasma iron crossing, iron concentration in normal CSF is low close to 0.8
Transferrin levels are estimated\(^2\) between 0.1 and 0.4 μmol/L, and it is established that, in most normal CSFs, transferrin is fully saturated, and therefore that other forms of iron must be present. This is supported by Gutteridge’s data\(^6\) showing normal mean levels of non-transferrin bound iron of 0.55 ± 0.27 μmol/L in CSF.

ii) In case of iron overload, either acute or chronic, plasma NTBI becomes a significant fraction of circulating iron, its concentration reaching several micromoles/L. It should be noted that despite its imperfection the term NTBI is confined to this special iron entity and does not apply to ferritin-iron, haptoglobin-iron or hemopexin-iron, which, strictly thinking, correspond also to iron species which are transferrin-bound.

iii) In various non iron-overloaded diseases, the presence of circulating NTBI has been reported. These conditions include fulminant hepatic failure\(^7\), hematological diseases\(^8\) especially under chemotherapy\(^9\)-\(^12\) and bone marrow transplantation\(^13\)-\(^14\), adult respiratory distress syndrome\(^15\), and cardiopulmonary bypass surgery\(^16\).

1.2. NATURE
The chemical form of NTBI remains poorly defined but is likely to be heterogeneous, involving both non-protein and protein-bound forms. The non-protein ligands appear to correspond to low molecular weight organic compounds such as ascorbate, phosphate, carbonate, organic acids and aminoacids. In the absence of iron overload a low-molecular-weight polypeptide has been isolated and purified from normal human cord and adult sera by gel filtration and HPLC. Its molecular weight has been estimated to be 2.5 KDa\(^17\)-\(^18\). In genetic hemochromatosis, Grootveld et al\(^19\) characterized NTBI by high performance liquid chromatography and nuclear resonance spectroscopy and proposed that NTBI in the plasma of iron-overloaded patients exists largely as complexes with citrate and possibly also as ternary iron-citrate-acetate complexes. Besides these non protein-bound forms, NTBI could be complexed to proteins such as albumin which offers nucleation sites for iron aggregation\(^20\). The investigation of hypotransferrinemic mouse serum\(^21\) revealed a mixture of iron species as demonstrated by differing reactivity to acid extraction, differing elution from sephadex G200 and differing reactivity to NTBI assays. These species could not be identified with existing iron complexes or proteins.

1.3. METHOD OF QUANTIFICATION
A number of assays have been proposed, since the original description by Hershko and colleagues\(^1\). They, schematically, correspond to three main types of methodological approaches.

1.3.1. The chelation-ultrafiltration-detection approach. It is based on the prior mobilization of serum NTBI by agents such as EDTA\(^1\), oxalate\(^22\) and, mostly, nitrilotriacetate (NTA)\(^20\). After ultrafiltration, which separates the chelated NTBI from transferrin-iron, NTBI detection is performed according to colorimetric methods\(^23\), HPLC\(^20\)-\(^24\)-\(^26\) techniques, or graphite furnace atomic absorption spectrometry\(^13\)-\(^27\). When transferrin is incompletely saturated, a significant improvement of the method consists to add a cobalt salt which blocks the free iron binding sites in order to avoid in vitro donation of iron from NTA onto the vacant sites of transferrin and therefore an underestimation of NTBI values\(^28\)-\(^29\).

1.3.2. The bleomycin approach. It is based upon the ability of the antitumor antibiotic bleomycin to degrade DNA via a free radical reaction in the presence of ferrous ions.