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Non-transferrin-bound iron in plasma following administration of oral iron drugs

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Abstract Non-transferrin-bound iron (NTBI) was detected in serum samples from volunteers with normal iron stores or from patients with iron deficiency anaemia after oral application of pharmaceutical iron preparations. Following a 100 mg ferrous iron dosage, NTBI values up to 9 μM were found within the time period of 1–4 h after administration whereas transferrin saturation was clearly below 100%. Smaller iron dosages (10 and 30 mg) gave lower but still measurable NTBI values. The physiological relevance of this finding for patients under iron medication has to be elucidated.

Keywords Non-transferrin-bound iron—NTBI · Oral iron drugs · Transferrin saturation · Iron medication · Risk factor

Introduction

Plasma iron that is not associated with the iron transport protein transferrin is generally termed as non-transferrin-bound iron (NTBI) (Herschko et al. 1978). NTBI is thought to play an important role in iron induced cell damage by generating highly reactive hydroxyl radicals via the Fenton reaction with resultant peroxidation of cell membrane lipids and other biomolecules (Halliwell and Gutteridge 1986). Such free radical-induced oxidative damage is increasingly implicated also as an important contributor in the pathogenesis of cancer, cardiovascular disease, aging and other degenerative diseases (Halliwell and Gutteridge 1990; Esposito et al. 2003).

NTBI was first recognized in patients with β-thalassaemia major (Herschko et al. 1978) whose transferrin iron-binding capacity has been surpassed, later on in all kinds of iron overload, including haemochromatosis (Gutteridge et al. 1985). Moreover, NTBI was found in blood of patients with leukaemia under conventional chemotherapy and in patients receiving bone marrow transplantation (Bradley et al. 1997; Dürken et al. 1997). Recently plasma NTBI was also measured after parenteral iron application (Breuer et al. 2000) and in haemodialysis patients receiving intravenous (i.v.) iron at the end of haemodialysis session (Scheiber-Mojdehkar et al. 2004). Appearance of NTBI-iron accompanied by an increase of malondialdehyde after i.v.-iron was further described in the same patient group (van-Campenhout et al. 2007). More recently it was also detected in patients with type 2 diabetes (Lee et al. 2006). In this study we describe for the first time the occurrence of NTBI after oral iron medication.

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Probands and methods

Serum samples from healthy adult volunteers or from patients with iron deficiency anaemia were analyzed 0–8 h after administration of an oral test dose of 10–150 mg iron. All probands were tested negative for the C282Y-mutation in the HFE gene, indicating the absence of a haemochromatosis gene, which, if present, could interfere with the individual iron absorption.

NTBI was measured by an ultrafiltration method according to Singh et al. with the modification of using atomic absorption spectroscopy (AAS) for detecting iron instead of HPLC (Singh et al. 1990; Dürken et al. 1997). All plastic and glass ware used was soaked and rinsed with a solution containing Triton X-100 and nitric acid in order to remove traces of iron. An aqueous solution of nitrilotriacetic acid (trisodium salt, Aldrich, Steinheim, Germany), pH 7.0, was added to serum samples to achieve a final concentration of 80 mM. It has been demonstrated that NTA at that concentration is not able to compete with apotransferrin for iron (Gosriwanata et al. 1999).

The mixture was incubated at room temperature for 30 min and transferred to a microfilter system (Ultrafree-MC filter unit, MW cut-off: 10.000 Dalton, Millipore Corporation, Bedford, MA, USA). After centrifugation at room temperature for 120 min at 700 × g, ultrafiltrates were diluted 1:3 with matrix modifier (1% Triton-X-1 00, 20 mM nitric acid). Iron in the ultrafiltrate was measured using AAS with graphite furnace technique (Perkin Elmer, HGA 700).

Serum iron concentrations and total iron binding capacities were determined as described (Gabbe et al. 1982). The U-test according to Wilcoxon, Mann, Whitney was performed for statistical analysis of the results from different iron compounds. The correlation of serum NTBI with transferrin saturation (tfs) was performed by transforming the transferrin saturation into $tfs^{\#} = tfs/(tfs_{max}\text{-}tfs)$ with $tfs_{max} = 115\%$.

Results and discussion

This study for the first time demonstrates the occurrence of NTBI iron in human plasma after administration of therapeutic doses of iron. A test dosage of 100 mg ferrous iron in form of both (i) aqueous iron ascorbate solution and (ii) four different pharmaceutical iron preparations resulted for all ferrous compounds in an increase in plasma iron as weIl as in non-transferrin-bound iron (Table 1). Although the transferrin saturation was clearly below 100% in all probands, NTBI values in the range of 6–12 µmol/l were found in plasma 1–8 h after oral administration of iron (Fig. 1). This finding supports the concept that full saturation of transferrin is not

Table 1 Maximal non-transferrin-bound plasma iron after oral iron medication in subjects with normal iron stores, as well as patients with iron deficiency anemia

Test compound and dosage	*n*	NTBI-max (µmol/l)	*P* (U-test)	Max. serum-Fe (µmol/l)	*P* (U-test)	Transferring saturation at NTBImax (%)	*P* (U-test)
(*a*) *Normal iron stores*							
Iron ascorbate solution: 100 mg	8	6.7 (3.0/9.2)	–	50 (41/65)	–	69 (61/76)	–
prep A: 100 mg	6	5.9 (4.0/9.6)	ns	42 (35/51)	ns	67 (58/76)	ns
prep B: 100 mg	7	4.5 (3.9/9.6)	ns	45 (42/47)	ns	76 (63/87)	ns
Iron ascorbate solution: 10 mg	6	2.4 (1,6/2,7)		27.1 (24/34)		48 (46/51)	
prep B: 50 mg	4	1.7 (1.4/2.3)		23.5 (19/25)		33 (25/39)	
prep C: 150 mg	5	0.7 (0.6/0.9)		24.5 (21/25)		33 (32/35)	
prep D: 80.5 mg	5	2.9 (2.1/3.1)		34.9 (30/38)		57 (55/66)	
(*b*) *Iron deficiency anaemia*[a]							
Iron ascorbate solution: 100 mg	2	3.0, 2.6		47, 51		57, 76	

Prep A, Eryfer™ (iron(II)sulfate); prep B, ferro sanol duodenal™ (iron(II) glycine sulfate); prep C, Niferex™ (iron(III)polysaccharide-iron complex); prep D, Lösferron™ (iron(II)gluconate). Median values; in brackets, 25 %-, 75 %-percentiles

[a] Two patients with gastrointestinal blood loss, haemoglobin, 7–8 g/dl; serum ferritin < 10 µg/l

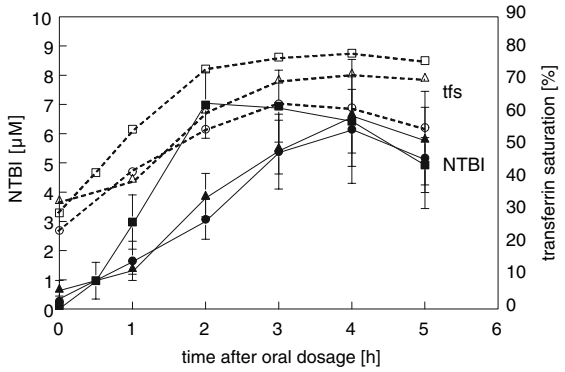

Fig. 1 Increase of non-transferrin-bound plasma iron (NTBI, closed symbols) and transferrin saturation (Tfs, open symbols) after oral dosage of 100 mg Fe in form of aqueous ferrous ascorbate solution (Asc, quadrats), prep A (Eryfer^{TM}, triangles), or prep B (ferro sanol duodenal^{TM}, circles), respectively. Values, mean ± SEM from 6–8 adult volunteers

necessary for the presence of NTBI (Breuer et al. 2000). Maximum values of NTBI and transferrin saturation correlate with each other (Fig. 2).

Lower, but still measurable amounts of NTBI iron were found after oral dosage of 30 and 10 mg of iron (Table 1). No increase of plasma iron as well as NTBI was observed after administration of 150 mg of ferric iron in form of a pharmaceutical iron preparation (polysaccharide-iron complex). Thus, the amount of NTBI in plasma seems to go parallel with the bioavailability of the applied iron compound, being higher with ferrous ascorbate solution than

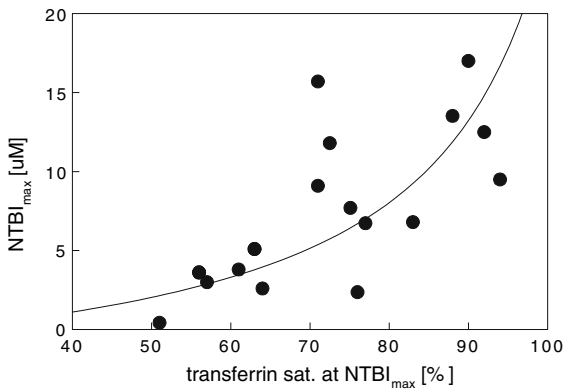

Fig. 2 Transferrin saturation (tfs) at NTBI maximum after oral dosage of 100 mg ferrous iron (ferrous ascorbate solution, or pharmaceutical iron preparations) in healthy volunteers. Significant linear correlation after transforming tfs into tfs/(tfs$_{max}$–tfs) with NTBI = 3.9 ± 0.4 µM at tfs$_{max}$ = 58% (R = 0.76, $P < 0.001$)

pharmaceutical iron preparations, and very low with ferric iron preparations (Nielsen et al. 1994).

Following the 100 mg ferrous iron dosage, which is frequently used in most of the pharmaceutical preparations for treatment of iron deficiency, values for NTBI in plasma are found which are similar to those found in iron overload diseases.

In the two patients with chronic iron deficiency anemia (Table 1) the absorption of iron from the therapeutic dose was in the range of 13–18% from 100 mg iron which is higher than in probands with normal iron stores (5–10%). However, NTBI seemed to be lower in iron deficiency than with normal iron stores. This can be explained by the much higher amount of circulating apotransferrin in iron deficiency. From first results in patients under oral iron therapy (data not shown), it can be estimated that continuously increased NTBI are to be measured throughout the time of iron medication, which can last several weeks up to months in patients with severe anaemia. However, it remains unclear if this finding has any toxicological relevance for iron deficient patients. It can be argued that, in contrast to the situation in iron overload diseases, the NTBI fraction may not be harmful in iron deficiency because this iron enters cells rapidly and is used for the erythropoesis or added to the exhausted iron stores.

On the other hand, NTBI is known as the most toxic iron fraction. It could catalyse the breakdown of most biomolecules such as lipids, sugars, DNA and amino acids (Halliwell and Gutteridge 1986). Very recently there are first studies indicating an elevation of risk from oral iron. Schümann et al. (2005) found elevated urinary levels of thiobarbituric acid reactive substances (TBARs) and 8-hydroxy-2-desoxyguanosine, indicators of oxidative damage, in response to oral ferrous sulphate in healthy human volunteers. Lachili et al. (2001) studied the effect of oral iron (100 mg/d as ferrous fumarate) in combination with vitamin C on lipid peroxidation. They found an increased utilization of Vit E and significantly enhanced TBARs in plasma suggesting that pharmacological doses of iron, at least when associated with high vitamin C intake, can result in uncontrolled lipid peroxidation.

In patients with bowel disease and iron deficiency oral ferrous sulfate increased plasma malondialdehyde, a marker of lipid peroxidation (Erichsen et al.

2005). Low dose (36 mg daily) of oral ferrous iron increased oxidized glutathione in healthy pregnant women with borderline anaemia (Rehema et al. 2004).

Prospective studies are required to clarify the clinical relevance of increased NTBI levels from oral iron medication. A simultaneous intake of the antioxidative vitamin E with/before the oral iron drug could be a therapeutic approach recommended to protect cells and molecules from NTBI induced oxidative damage. That a single oral dose of vitamin E attenuates lipid peroxidation in patients on haemodialysis receiving intravenous iron was demonstrated recently (Roob et al. 2000). The protecting role of vitamin E in iron induced lipid peroxidation in-vivo has also clearly been demonstrated (Dresow et al. 1995).

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