ORIGINAL ARTICLE

Taylor & Francis
healthsciences

Ferrous Fumarate Deteriorated Plasma Antioxidant Status in Patients with Crohn Disease

K. Erichsen, T. Hausken, R. J. Ulvik, A. Svardal, A. Berstad & R. K. Berge
Institute of Medicine, Haukeland University Hospital, University of Bergen, and Institute of Marine Research, Section for Marine Chemistry, Bergen, Norway

Erichsen K, Hausken T, Ulvik RJ, Svardal A, Berstad A, Berge RK. Ferrous fumarate deteriorated plasma antioxidant status in patients with Crohn disease. Scand J Gastroenterol 2003;38:543–548.

Background: Iron deficiency anaemia is a frequent complication of Crohn disease. Treatment with ferrous iron (Fe^{2+}) compounds is often unsatisfactory and is associated with gastrointestinal side effects. Theoretically, oral iron supplementation may even be harmful, because iron may reinforce intestinal inflammation by catalysing production of reactive oxygen species. We investigated the effect of ferrous iron on disease activity and plasma antioxidant status in patients with active Crohn disease. **Methods:** Ten patients with Crohn disease and iron deficiency and 10 healthy controls were given ferrous fumarate 120 mg for 7 days. The Crohn Disease Activity Index, gastrointestinal complaints and blood samples for antioxidant status, anaemia, inflammation and iron absorption were investigated on day 1 and day 8. **Results:** During 1 week of ferrous fumarate supplementation, the Crohn Disease Activity Index tended to increase ($P = 0.071$). Patients experienced aggravation of diarrhoea, abdominal pain and nausea. Plasma-reduced cysteine was lower ($P = 0.038$) in patients than it was in controls. One week of ferrous iron supplementation further decreased reduced cysteine ($P < 0.001$) and significantly decreased plasma-reduced glutathione ($P = 0.004$) in the patients. Serum iron increased significantly in patients after an oral iron load test (from 5.8 ± 3.2 μmol/L to 30.9 ± 13.1 μmol/L). **Conclusions:** Treatment of iron deficiency with ferrous fumarate deteriorated plasma antioxidant status and increased specific clinical symptoms in patients with active Crohn disease. Plasma reduced cysteine may be a sensitive indicator for oxidative stress in the intestine.

Key words: Antioxidants; Crohn disease; ferrous compounds; intestinal absorption; iron

Kari Erichsen, M.D., Institute of Medicine, Haukeland University Hospital, University of Bergen, NO-5021 Bergen, Norway (fax. +47 55974973, e-mail. kari.erichsen@helse-bergen.no)

Anaemia is a frequent complication of Crohn disease. It occurs in about one-third of patients (1). Iron deficiency and chronic inflammation are regarded as the most important mechanisms. However, oral iron supplementation as treatment for iron deficiency anaemia in Crohn disease has been unsatisfactory (2). Nausea, bloating, diarrhoea and upper gastrointestinal pain are frequent side effects, leading to poor compliance (3). The cause of these symptoms is not well understood, but may involve intestinal formation of reactive oxygen species (ROS) (4).

Tissue damage due to oxidative stress is considered to play an important role in the pathogenesis of chronic inflammatory bowel disease (IBD). The abundant accumulation of active macrophages and neutrophilic leucocytes in the intestinal mucosa are major sources of ROS (5–8). Iron is a strong catalytic promoter of pro-oxidant reactions. Harmful redox reactions may be initiated and aggravated by free iron supplied by minor bleedings and breakdown of haemoglobin within both the mucosa and the intestinal lumen, and by oral iron.

The antioxidant defence system may become weaker in situations of sustained oxidative stress. Of special relevance to the present study are the aminothiols glutathione and cysteine. The tripeptide glutathione (γ-glutamyl-cysteinyl-glycine) is the most important intracellular antioxidant (9). Cysteine is itself a potent scavenger of ROS, but also the limiting precursor of glutathione synthesis (10–12). The content of glutathione has been reported to be decreased in both inflamed and non-inflamed mucosa in patients with Crohn disease (13). Plasma cysteine was found to be significantly decreased in IBD patients with active intestinal inflammation compared to healthy control subjects (13).

We hypothesized that ferrous iron (Fe^{2+}) may reinforce oxidative stress in the inflamed mucosa of patients with IBD. It was the object of this study to compare plasma antioxidant status in patients with Crohn disease with that of healthy controls, and to see if oral intake of ferrous iron changed plasma antioxidant status. In addition, we evaluated the effect of ferrous iron therapy on disease activity, and also iron absorption, in patients with Crohn disease.

DOI 10.1080/00365520310000771

Materials and Methods

Patients

Ten patients with Crohn disease (7 F and 3 M) with a mean age of 38 (range 23–72 years) were recruited from our outpatient clinic. Clinical disease activity was evaluated according to the Crohn Disease Activity Index (CDAI). Eight patients had active disease with CDAI > 150. All but one patient had iron deficiency anaemia. None of the patients received iron therapy or blood transfusions during the 3 months prior to the study.

Six patients were treated with 5-ASA, four with low-dose steroids and two with low-dose azathioprine. Three patients had small-bowel involvement only; six patients had lesions in the terminal ileum and colon; and one patient had total colitis. Two patients reported arthralgia; three patients had fistulating disease; and one had amyloidosis. None of the patients had indications of haemolysis or deficiency of cobalamin or folic acid.

Ten healthy age- and sex-matched volunteers served as controls. Informed consent was obtained and the study protocol was approved by the local Ethics Committee.

Procedure

Patients and controls were given ferrous fumarate (Neo-Fer®, Nycomed Pharma, Oslo, Norway) 120 mg/day for 7 days. Iron tablets were taken every morning at the same time, between 0800 and 1000 h. Blood samples to evaluate anaemia and inflammation parameters were collected after an overnight fast on day 1 and on day 8.

To evaluate iron absorption, serum iron was measured before and 2 h after iron administration on day 1 and on day 8. Serum iron measured 2 h after iron administration is considered representative of the peak level (14), when fasting is continued during this period.

In patients, the CDAI was recorded the week prior to and during the week of iron supplementation. Controls registered bowel habit, abdominal pain and general well-being during the same 2 weeks. After iron supplementation, both patients and controls were questioned about adverse effects.

Laboratory investigations

Routine laboratory investigations included haemoglobin concentration, reticulocyte count, leucocyte count, platelet count, serum iron, ferritin, total iron binding capacity (TIBC), transferrin saturation and C-reactive protein (CRP).

Measurement of antioxidant status

Briefly, to determine aminothiols, blood was routinely collected into evacuated tubes containing heparin as an anticoagulant and either monobromobrimane (mBrB) or N-ethylmaleimide (NEM) as thiol derivatizing agents, or no addition was made. The tubes were placed in melting ice and centrifuged within 15 min. Aliquots were drawn from the plasma thus obtained. The total, reduced and oxidized forms of glutathione, cysteine, cysteinyl-glycine and homocysteine were quantified by reversed-phase HPLC, as previously described by Svardal et al. (15). The amounts of reduced and oxidized thiols were assayed in plasma treated with mBrB and NEM, respectively. The total amount of thiol components (reduced + oxidized + protein bound form) was assayed in non-treated plasma.

Plasma vitamin E, beta-carotene (16–18) and vitamin C (19) levels were measured by HPLC as previously described.

Statistics

Data were analysed using the GraphPad Prism (GraphPad Software Inc., USA) statistical software package. Differences between means were evaluated with Student's *t* test for paired and non-paired comparisons. Comparison of proportions was evaluated with Fisher's exact test, and associations between variables were calculated with Pearson's correlation coefficient.

Mean with standard deviation and 95% confidence interval (CI) are given if not otherwise stated. *P* values less then 0.05 were considered statistically significant.

Results

Disease activity

After 1 week of ferrous iron supplementation the CDAI tended to increase (from 218 ± 69 to 261 ± 87; mean of differences 44 with CI −5 to 92; $P = 0.071$).

Symptoms

Eight of 10 patients experienced an increase in the number of liquid and loose stools during the week of ferrous iron therapy (mean of differences 4.8 with CI −0.4 to 10.0; $P = 0.067$). On the contrary, 8 of 10 controls experienced decreased or unchanged number of liquid and loose stools (mean of differences −1.9 with CI −4.5 to 0.7; $P = 0.13$). When comparing the two groups, there was a statistically significant difference in the change of bowel habit (difference between means 6.7 ± 2.6 with CI 1.3 to 12.1; $P = 0.018$). Five of 10 controls, but only 1 of the patients, became constipated while taking ferrous iron tablets ($P = 0.14$).

Seven of 10 patients, but none of the controls, reported exacerbation of abdominal pain during ferrous iron supplementation ($P = 0.003$). Abdominal pain score (subscore of the CDAI) in patients increased from 31 ± 24 to 45 ± 34 (mean of differences 14.5 with CI −5 to 34; $P = 0.13$). Six of 10 patients, but none of the controls, experienced nausea during ferrous iron administration ($P = 0.011$). One patient reported arthralgia after ferrous iron intake. No other alterations in extraintestinal manifestations were noticed.

Laboratory evaluation and iron absorption

Blood haemoglobin did not change in either the patient or the control group after 1 week of ferrous iron therapy. Serum ferritin increased significantly in patients with Crohn disease

Table I. Laboratory evaluation of patients with Crohn disease and healthy controls before and after 1 week of ferrous iron supplementation

Parameter (normal)	Group	Before iron Mean (*s*)	After iron Mean (*s*)
S-CRP (<10 mg/L)	Patients	15.5 (15.8)	15.1 (26.0)
	Controls	3.5 (0.5)	4.4 (2.4)
B-leucocyte count (3.5 to 11.0 10^{-9}/L)	Patients	7.1 (1.9)	6.8 (1.9)
	Controls	5.4 (1.3)	6.5 (2.4)
B-platelet count (140 to 400 10^{-9}/L)	Patients	362 (70)	358 (64)
	Controls	234 (37)	248 (25)
B-haemoglobin (female 11.6 to 16.0 g/dl, male 13.2 to 16.6 g/dL)	Patients	10.6 (1.5)	10.6 (1.2)
	Controls	14.1 (1.0)	14.2 (1.1)
B-reticulocyte count (0.03 to 0.10 10^{-12}/L)	Patients	0.063 (0.026)	0.103 (0.058)
	Controls	0.054 (0.017)	0.061 (0.015)
S-ferritin (female 15 to 150 μg/L, male 28 to 150 μg/L)	Patients	11.0 (12.2)	26.1 (14.7)
	Controls	64.1 (46.3)	62.2 (43.5)
TIBC (45 to 72 μmol/L)	Patients	67.4 (13.4)	64.8 (13.4)
	Controls	56.2 (6.3)	56.7 (8.3)
Transferrin saturation (15 to 45%)	Patients	8.9 (5.0)	11.1 (6.1)
	Controls	33.2 (14.1)	28.4 (10.7)

s = standard deviation.

(mean of differences 15.1 μg/L with CI 4.7 to 25.5; $P = 0.01$). As for the controls, there was no change. CRP did not alter in either patients or controls after 1 week of ferrous iron therapy (Table I).

In patients with Crohn disease, serum iron increased from 5.8 ± 3.2 μmol/L to 30.9 ± 13.1 μmol/L (mean of differences 25.1 μmol/L with CI 14.3 to 35.9; $P < 0.001$) after the oral iron load test on day 1. In healthy controls, serum iron increased from 18.4 ± 8.0 μmol/L to 34.5 ± 12.5 μmol/L (mean of differences 16.1 μmol/L with CI 8.0 to 24.2; $P = 0.002$). After 1 week of iron supplementation, serum iron increased from 7.2 ± 4.1 μmol/L to 30.9 ± 30.4 μmol/L (mean of differences 23.7 μmol/L with CI 1.1 to 46.2; $P = 0.042$) in patients, and from 15.7 ± 5.4 μmol/L to 27.7 ± 10.3 μmol/L (mean of differences 12.0 μmol/L with CI 6.7 to 17.2; $P < 0.001$) in controls.

Plasma levels of aminothiols, beta-carotene and vitamins prior to ferrous fumarate intake

Plasma reduced cysteine was lower in patients with Crohn disease compared to healthy controls (difference between means -1.1 ± 0.5 μmol/L with CI −2.13 to −0.07; $P = 0.038$). Glutathione, cysteinyl-glycine and homocysteine did not differ between the two groups (Table II). Neither was there any significant correlation between any of the circulating aminothiol species in patients (data not shown).

Plasma levels of beta-carotene were significantly lower (difference between means -0.35 ± 0.01 μmol/L with CI −0.49 to −0.22; $P < 0.001$) in patients with Crohn disease compared to controls, while plasma levels of vitamins C and E were only slightly lower in patients (Table III).

Effect of ferrous fumarate

One week of ferrous iron supplementation significantly decreased plasma levels of reduced cysteine in patients with Crohn disease (mean of differences −1.03 μmol/L with CI −1.47 to −0.60; $P < 0.001$; Fig. 1). The difference between patients and controls in plasma-reduced cysteine increased (difference between means -1.31 ± 0.37 μmol/L with CI −2.10 to −0.53; $P = 0.003$). Furthermore, in patients, the total amount of cysteine decreased (mean of differences −15.62 μmol/L with CI −27.78 to −3.46; $P = 0.02$) after ferrous iron.

Regarding glutathione, similar alterations as for cysteine were observed in patients, with reductions in plasma levels of reduced glutathione (mean of differences −1.45 with CI −2.30 to −0.60; $P = 0.004$). In addition, mean reduced glutathione level became lower in patients compared with controls after ferrous iron supplementation (difference between means -0.83 ± 0.39 μmol/L with CI −1.65 to −0.01; $P = 0.047$). Only minor changes were observed with regard to other thiol species. Moreover, there was no correlation between any of the cysteine and glutathione parameters (data not shown).

Plasma levels of beta-carotene were decreased (mean of differences −0.07 μmol/L with CI −0.11 to −0.02; $P = 0.008$) in controls after ferrous iron administration. In patients with Crohn disease, beta-carotene levels were low at baseline and there was no further decline after ferrous iron. As for vitamins C and E, no alteration was noticed.

Discussion

After 1 week of ferrous fumarate supplementation in iron-deficient patients with active Crohn disease, the CDAI tended to increase. Patients experienced an increase in the number of loose stools, while healthy controls on the contrary experienced harder stools. Patients also reported nausea and aggravated abdominal pain during ferrous iron therapy, whereas none of the controls reported any of these symptoms.

Several mechanisms may be involved in gastrointestinal intolerance to oral iron therapy. Oral iron supplementation renders high faecal iron concentrations. Since only a fraction of supplemented iron will be absorbed, virtually the entire

Table II. Plasma levels of total, reduced, and oxidized forms of aminothiol species before and after one week of ferrous iron therapy in patients with Crohn disease and in healthy controls

Parameter		Total before iron	Total after iron		Reduced before iron	Reduced after iron		Oxidized before iron	Oxidized after iron	
GSH	Patients	4.61 (1.30)	4.31 (1.33)	$P = 0.36$	4.48 (1.37)	3.03 (1.05)	$P = 0.004$	1.25 (0.50)	1.06 (0.32)	$P = 0.08$
(μmol/L)	Controls	4.26 (0.90)	4.27 (0.74)	$P = 0.96$	4.69 (2.03)	3.86 (0.61)	$P = 0.30$	1.08 (0.30)	1.15 (0.31)	$P = 0.28$
		$P = 0.49$	$P = 0.95$		$P = 0.83$	$P = 0.047$		$P = 0.35$	$P = 0.53$	
Cysteine	Patients	132.2 (37.4)	116.6 (26.5)	$P = 0.02$	5.85 (0.67)	4.82 (1.00)	$P < 0.001$	57.5 (12.6)	55.5 (13.3)	$P = 0.43$
(μmol/L)	Controls	121.8 (30.8)	133.5 (33.0)	$P = 0.33$	6.95 (1.40)	6.13 (0.62)	$P = 0.09$	63.2 (11.4)	58.2 (10.2)	$P = 0.04$
		$P = 0.51$	$P = 0.22$		$P = 0.038$	$P = 0.003$		$P = 0.30$	$P = 0.63$	
Cys-gly	Patients	24.0 (5.4)	21.2 (5.1)	$P = 0.07$	2.14 (0.73)	1.75 (0.75)	$P = 0.01$	6.51 (2.04)	6.16 (1.72)	$P = 0.40$
(μmol/L)	Controls	21.9 (3.7)	24.7 (5.5)	$P = 0.30$	1.76 (0.40)	1.59 (0.30)	$P = 0.14$	6.61 (1.18)	6.40 (1.34)	$P = 0.51$
		$P = 0.31$	$P = 0.16$		$P = 0.17$	$P = 0.54$		$P = 0.74$	$P = 0.89$	
Homocysteine	Patients	7.59 (2.49)	7.07 (2.84)	$P = 0.32$	0.72 (0.30)	0.67 (0.44)	$P = 0.77$	1.54 (0.36)	1.57 (0.50)	$P = 0.71$
(μmol/L)	Controls	6.43 (2.05)	6.91 (1.72)	$P = 0.55$	0.59 (0.15)	0.60 (0.09)	$P = 0.87$	1.45 (0.35)	1.30 (0.32)	$P = 0.03$
		$P = 0.88$	$P = 0.27$		$P = 0.26$	$P = 0.64$		$P = 0.58$	$P = 0.16$	

Mean and (SD). GSH = glutathione. Cys-gly = cysteinyl-glycine.

Table III. Plasma levels of nutritional antioxidants before and after one week of ferrous iron therapy in patients with Crohn disease and in healthy controls

Parameter		Before iron	After iron	
Betacarotene	Patients	0.08 (0.05)	0.07 (0.06)	$P = 0.58$
(μmol/L)	Controls	0.43 (0.17)	0.37 (0.13)	$P = 0.008$
		$P < 0.001$	$P < 0.001$	
Vitamin C	Patients	37.8 (26.5)	38.7 (26.1)	$P = 0.84$
(μmol/L)	Controls	52.8 (11.3)	56.4 (17.2)	$P = 0.38$
		$P = 0.12$	$P = 0.09$	
Vitamin E	Patients	18.6 (7.6)	17.2 (7.7)	$P = 0.10$
(μmol/L)	Controls	22.2 (5.9)	22.2 (5.4)	$P = 0.98$
		$P = 0.25$	$P = 0.11$	

Mean and (SD).

dose winds up in the distal parts of the bowels (4). In an already inflamed bowel this may reinforce the inflammation by catalysing production of ROS through the Haber-Weiss reaction. Also, iron availability is a key factor in bacterial growth. Administration of ferrous sulphate gave substantial alterations in faecal flora in rats (20). Furthermore, ferrous sulphate increased gut motor activity in dogs (21). Theoretically, increased ROS production, altered bowel flora and increased gut motility together might contribute to oral iron intolerance in Crohn disease.

The lower plasma levels of reduced cysteine in patients with Crohn disease compared to healthy controls suggest that patients were exposed to oxidative stress, most likely due to inflammatory activity in the intestine. The significant fall in plasma levels of reduced cysteine and reduced glutathione in patients after ferrous iron supplementation indicates an iron-induced increase in oxidative stress. Our findings are consistent with those of Sido et al. (13), who found plasma cysteine levels to be decreased in patients with IBD, and normalization of cysteine levels after removal of inflamed bowel. During ongoing inflammation, plasma cysteine levels could be decreased for several reasons. As a potent free radical scavenger, the amino acid cysteine could be consumed

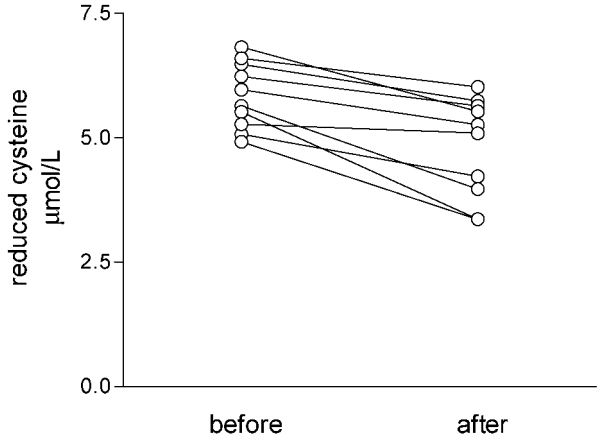

Fig. 1. Effect of ferrous fumarate on plasma-reduced cysteine in individual patients with Crohn disease.

due to oxidative stress in the inflamed gut. Furthermore, being the limiting precursor of glutathione synthesis, plasma cysteine deficiency could be due to consumption to replenish intracellular glutathione stores.

Decreased levels of reduced glutathione were demonstrated in both inflamed and non-inflamed ileal mucosa in patients with Crohn disease (13). Impaired glutathione synthesis in gut mucosa and loss of glutathione due to oxidative stress contribute to intestinal glutathione deficiency (13). In healthy subjects, cysteine is the most abundant aminothiol in plasma, with a total concentration about 40 times that of glutathione (22). By comparison, most circulating glutathione is inside red blood cells (23). Evidently, our findings suggest that, in plasma, cysteine may be a more sensitive marker than glutathione for oxidative stress in the gut.

Excess iron may be toxic. In the presence of oxidative stress associated with inflammation or ischaemic injury, the toxicity is amplified because the presence of substantial amounts of the free radical superoxide allows iron-catalysed production of the hydroxyl radical. This in turn may initiate lipid peroxidation (24). Oral iron supplementation resulted in aggravated colonic injury caused by experimental colitis in rats, as shown by histological scores, quantitative evaluation of rectal bleeding and colon length (25, 26). There was also an increase in lipid peroxidation and a decrease in antioxidant vitamins (25).

Our observation of a pro-oxidant shift in plasma redox balance of cysteine and glutathione after ferrous iron supplementation in patients with Crohn disease, but not in healthy controls, supports the view that exogenously administered iron may exert increased toxicity in Crohn disease.

In our study, all 10 patients were treated with drugs that modulate the inflammatory process. This may have decreased the toxic effect of iron. Reifen et al. (27) showed that treatment with 5-ASA and or lycopene decreased the inflammatory reaction induced by iron supplementation in rats with experimental colitis.

The plant pigment beta-carotene exerts antioxidant events in vitro, but its in vivo relevance is unknown (28). Poor dietary intake may explain some of the lowered plasma levels of the plant pigment beta-carotene in patients, as there was also a tendency to low plasma levels of the dietary vitamins C and E. These findings are consistent with those of Geerling et al. (29), who found deficiencies for vitamin C, vitamin E, beta-carotene, selenium and zinc in patients with Crohn disease in remission. The failure of iron to further decrease beta-carotene levels in patients, in contrast to controls, is probably due to their initially very low levels. Beta-carotene was shown to increase solubility of ferrous fumarate, suggesting that beta-carotene may form a complex with iron (30). In control subjects, we found plasma levels of beta-carotene, but not aminothiols, to be decreased after ferrous iron intake. Binding of beta-carotene to luminal iron may have prevented absorption of, and thus reduced plasma levels of beta-carotene in the controls.

Available compounds of oral iron supplementation are generally ferrous salts, and there is no significant difference in their side effects (31) or efficacy (32). Modern compounds of stable complexes with ferric iron (Fe^{3+}) are potentially less toxic (33). One such chelate, ferric trimaltol, showed limited pro-oxidant properties in vitro (34) and corrected iron deficiency with a low incidence of side effects in patients with inflammatory bowel disease in remission (33).

Patients with iron deficiency have increased iron absorption compared to healthy subjects with normal iron status (14). We found higher absorption of a ferrous salt in patients with Crohn disease and iron deficiency anaemia of mild to moderate degree compared to healthy controls with normal iron stores. These findings are consistent with those of Bartels et al. (35), who found a preserved ability to absorb iron even in cases of severe chronic inflammatory bowel disease. After 1 week of administration of relatively high doses of ferrous iron we found great variation in iron absorption, especially in the patients. The variation may be due to individual increases in iron stores, as reflected by an increase in serum ferritin and no sign of acute phase reaction, limiting uptake in some patients. Furthermore, a mucosal block of iron absorption due to saturation of the enterocytes has been described (36, 37).

In conclusion, ferrous fumarate supplementation increased specific clinical symptoms in patients with active Crohn disease. Plasma cysteine may be an interesting biomarker of oxidative stress in the intestine. The significant fall in plasma levels of reduced cysteine and reduced glutathione after ferrous iron intake supports the view that oral intake of iron may increase the oxidative stress in patients with Crohn disease.

References

1. Gasché C, Dejaco C, Waldhoer T, Tillinger W, Reinisch W, Fueger GF, Gangl A, et al. Intravenous iron and erythropoietin for anemia associated with Crohn's disease. Ann Intern Med 1997;126:182–7.
2. Gasché C, Reinisch W, Lochs H, Parsaei B, Bakos S, Wyatt J, et al. Anemia in Crohn's disease. Importance of inadequate erythropoietin production and iron deficiency. Dig Dis Sci 1994;39:1930–4.
3. Gasché C. Anemia in IBD: The overlooked villain. Inflamm Bowel Dis 2000;6:142–50.
4. Babbs CF. Oxygen radicals in ulcerative colitis. Free Radic Biol Med 1992;13:169–81.
5. Mahida YR, Wu KU, Jewell DP. Respiratory burst activity of intestinal macrophages in normal and inflammatory bowel disease. Gut 1989;30:1362–70.
6. Williams JG. Phagocytes, toxic oxygen metabolites and inflammatory disease: implications for treatment. Ann R Coll Surg Engl 1990;72:253–62.
7. Grisham MB, Granger DN. Neutrophil-mediated mucosal injury. Role of reactive oxygen metabolites. Dig Dis Sci 1988; 33:6S–15S.
8. Weiss SJ. Tissue destruction by neutrophils. N Engl J Med 1989; 320:365–76.
9. Meister A, Anderson ME. Glutathione. Ann Rev Biochem 1983; 52:711–60.
10. Meister A. Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. Pharmacol Ther 1991;51:155.

11. Deneke SM, Fanburg BL. Regulation of cellular glutathione. Am J Physiol 1989;257:L163.
12. Meister A. Glutathione metabolism and its selective modification. J Biol Chem 1988;263:17205–8.
13. Sido B, Hach V, Hochlehnert A, Lipps H, Herfarth C, Droge W. Impairment of intestinal glutathione synthesis in patients with inflammatory bowel disease. Gut 1998;42:485–92.
14. Goch J, Birgegaard G, Danielson BG, Wikström B. Iron absorption in patients with chronic uremia on maintenance hemodialysis and in healthy volunteers measured with a simple oral iron load test. Nephron 1996;73:403–6.
15. Svardal AM, Mansoor MA, Ueland PM. Determination of reduced, oxidized and proteine-bound glutathione in human plasma with precolumn derivatization with monobromobrimane and liquid chromatography. Anal Biochem 1990;184:338–46.
16. Seta K, Nakamura H, Okuyama T. Determination of α-tocopherol, free cholesterol, esterified cholesterol and triacylglycerols in human lipoproteins by high-performance liquid chromatography. J Chromatogr 1990;515:585–95.
17. Arnaud J, Fortis I, Blachier S, Kia D, Favier A. Simultaneous determination of retinol, α-tocopherol and β-carotene in serum by isocratic high-performance liquid chromatography. J Chromatogr 1991;572:103–16.
18. Sierra C, Pastor MC, de Ramon M. Liquid chromatography determination of α-tocopherol in erythrocytes. Clin Chim Acta 1992;208:119–26.
19. Vaagenes H, Muna ZA, Madsen L, Berge RK. Low doses of eicosapentaenoic acid, docosahexaenoic acid, and hypolipidemic eicosapentaenoic acid derivatives have no effect on lipid peroxidation in plasma. Lipids 1998;33:1131–7.
20. Benoni G, Cuzzolin L, Zambreri D, Donini M, Del Soldato P, Caramazza I. Gastrointestinal effects of single and repeated doses of ferrous sulphate in rats. Pharmacol Res 1993;27:73–80.
21. De Ponti F, D'Angelo L, Forster R, Einaudi A, Crema A. Effect of iron succinyl-protein complexes on gastrointestinal motility in the fasting dog. Digestion 1991;50:72–81.
22. Ueland PM. Homocysteine species as components of plasma redox thiol status [editorial]. Clin-Chem 1985;41:340–2.
23. Hoffenberg EJ, Deutsch J, Smith S, Sokol RJ. Circulating antioxidant concentrations in children with inflammatory bowel disease. Am J Clin Nutr 1997;65:1482–8.
24. McCord JM. Iron, free radicals, and oxidative injury. Semin Hematol 1998;35:5–12.
25. Carrier J, Aghdassi E, Cullen J, Allard JP. Effect of oral iron supplementation on oxidative stress and intestinal inflammation in rats with induced colitis. Aliment Pharmacol Ther 2001;15:1989–99.
26. Reifen R, Matas Z, Zeidel L, Berkovitch Z, Bujanover Y. Iron supplementation may aggravate inflammatory status of colitis in a rat model. Dig Dis Sci 2000;45:394–7.
27. Reifen R, Nissenkorn A, Matas Z, Zeidel L, Berkovitz Z, Levine A, Bujanover Y. 5-ASA and or lycopene decrease the oxidative stress and inflammatory reaction induced with iron supplementation. Gastroenterology 1999;116:A804.
28. Britton G. Structure and properties of carotenoids in relation to function. FASEB J 1995;9:1551–9.
29. Geerling BJ, Badart-Smook A, Stockbrügger RW, Brummer R-JM. Comprehensive nutritional status in patients with long-standing Crohn's disease in remission. Am J Clin Nutr 1998;67:919–26.
30. Garcia-Casal MN, Layrisse M, Solano L, Baron MA, Arguello F, Llovera D, et al. Vitamin A and beta-carotene can improve nonheme iron absorption from rice, wheat and corn by humans. J Nutr 1998;128:646–50.
31. Hallberg L, Ryttinger L, Sölvell L. Side-effects of oral iron therapy. A double-blind study of different iron compounds in tablet form. Acta Med Scand 1966;Suppl 459:3–10.
32. Macdougall IC. Strategies for iron supplementation: oral versus intravenous. Kidn Int Suppl 1999;69:S61–6.
33. Harvey RSJ, Reffitt DM, Doig LA, Meenan J, Ellis RD, Thompson RPH, et al. Ferric trimaltol corrects iron deficiency anaemia in patients intolerant of iron. Aliment Pharmacol Ther 1998;12:845–8.
34. Singh S, Hider RC. Calorimetric detection of the hydroxyl radical: comparison of the hydroxyl-radical-generating ability of various iron complexes. Anal Biochem 1988;171:47–54.
35. Bartels U, Pedersen NS, Jarnum S. Iron absorption and serum ferritin in chronic inflammatory bowel disease. Scand J Gastroenterol 1978;13:649–56.
36. Umbreit JN, Conrad ME, Moore EG, Latour LF. Iron absorption and cellular transport: the mobilferrin/paraferritin paradigm. Semin Hematol 1998;35:13–26.
37. Benito P, House W, Miller D. Comparison of oral and intraperitoneal iron supplementation in anemic rats: a re-evaluation of the mucosal block theory of iron absorption. Br J Nutr 1998;79:533–40.

Received 8 August 2002
Accepted 9 January 2003

EDITORIAL

Taylor & Francis
healthsciences

Believe in Iron

Iron deficiency is the most common disorder in the world. The numbers are staggering. As many as 4–5 billion people may be iron-deficient and 2 billion—over 30% of the world's population—have iron-deficiency anaemia (WHO report 2002). Iron deficiency is a disease not just in developing countries, it affects the whole Western world. Food fortification and iron supplementation are therefore top goals for battling iron-deficiency anaemia.

Iron is a common constituent of our environment. It is the essential participant in many redox processes in eukaryotes and most prokaryotes. Because there is no excretory route, iron homeostasis in organisms is always regulated at the level of iron uptake. This system is conserved through evolution to prevent toxic iron overload. Injuries and diseases, however, may cause excessive loss of iron, mainly due to blood loss. The tight regulation of iron absorption does not allow immediate counteraction, and iron deficiency may develop.

Iron deficiency anaemia is a gastroenterological disease: blood loss that occurs in the stomach or intestine cannot be matched by duodenal iron absorption. Such a negative iron balance often exists in inflammatory bowel diseases (IBD). Though specific investigations are lacking, the intestinal ulcerations are considered to be the site of blood loss in IBD. As a result, iron deficiency and anaemia are common, and it is worthwhile reflecting upon optimization of iron supplementation in this disease (1)—as did Kari Erichsen and co-workers in this issue of the journal (see page 543). Specifically, they looked into the tolerability of oral ferrous fumarate in relation to oxidative stress, and traced some unexpected findings: First, oral ferrous fumarate is better absorbed in iron-deficient Crohn patients than in non-deficient controls; second, it increases specific symptoms of Crohn disease, such as diarrhoea or abdominal pain, and third, it lowers the amount of antioxidant scavenger molecules possibly due to its pro-oxidant capacity at the site of inflammation.

The adaptive increase of iron absorption capacity in iron-deficiency anaemia is relatively small. Most parts of the ingested iron are still not taken up, but are passed on to the ileum and colon, the sites of inflammation in Crohn disease and ulcerative colitis, before appearing in the stool. When this iron comes into contact with the ulcerated intestinal wall, it may enhance the local production of reactive oxygen species and increase inflammation. This has already been demonstrated in animal models of IBD (2).

The undesired effects of oxidative stress in IBD have been known for years (3). In vitro, iron chelation significantly reduced mucosal reactive oxygen species production (4). There are few who still disregard the importance of free oxygen radicals as a cause of tissue injury and of DNA damage (potentially leading to the development of colorectal cancer) in IBD. There are also few who disbelieve in the intolerance of oral ferrous iron in this specific population. As Erichsen and co-workers have created a link between intolerability and pro-oxidant activity of oral ferrous fumarate in Crohn disease, they provide important information for clinical practice, i.e. not to use ferrous fumarate for iron supplementation in IBD. It would be surprising if ferrous sulphate and ferrous gluconate do not cause similar effects.

The time required for uncomplexed ferrous iron, Fe(II), to undergo oxidation to the ferric state, Fe(III), is dependent on many factors, the dominant being pH, temperature and oxygen concentration. Ferric iron has less pro-oxidant potential, but is sparingly insoluble and generally bio-unavailable. Surprisingly, in an open study the oral administration of ferric polymaltose complexes has been found safe and effective (5). Most safety and efficacy data on iron supplementation in IBD exist on iron sucrose (6–8). It is currently unknown whether the good tolerability of iron sucrose is because of the Fe(III) status. Iron sucrose is administered intravenously and thus does not pass the inflamed bowel segments locally, as oral products do. Further studies such as Erichsen's need to directly compare the pro-oxidant effects of ferrous and ferric compounds as well as of the oral and intravenous administration route.

C. Gasche, M.D.
Medical University and Dept. of Medicine IV
General Hospital Vienna
Austria
Fax. +43 1 40400 4735
E-mail. christoph.gasche@akh-wien.ac.at

References

1. Oldenburg B, Koningsberger JC, Berge Henegouwen GP, Van Asbeck BS, Marx JJ. Iron and inflammatory bowel disease. Aliment Pharmacol Ther 2001;15:429–38.
2. Oldenburg B, Berge Henegouwen GP, Rennick D, Van Asbeck BS, Koningsberger JC. Iron supplementation affects the production of pro-inflammatory cytokines in IL-10 deficient mice. Eur J Clin Invest 2000;30:505–10.
3. Lih-Brody L, Powell SR, Collier K P, Reddy GM, Cerchia R, Kahn E, et al. Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. Dig Dis Sci 1996;41:2078–86.
4. Millar AD, Rampton DS, Blake DR. Effects of iron and iron chelation in vitro on mucosal oxidant activity in ulcerative colitis. Aliment Pharmacol Ther 2000;14:1163–8.
5. Harvey RS, Reffitt DM, Doig LA, Meenan J, Ellis RD, Thompson

DOI 10.1080/00365520310000780

RP, et al. Ferric trimaltol corrects iron deficiency anaemia in patients intolerant of iron. Aliment Pharmacol Ther 1998; 12: 845–8.
6. Gasche C, Dejaco C, Waldhoer T, Tillinger W, Reinisch W, Fueger GF, et al. Intravenous iron and erythropoietin for anemia associated with Crohn disease. A randomized, controlled trial. Ann Intern Med 1997;126:782–7.
7. Gasche C, Dejaco C, Rinisch W, Tillinger W, Waldhoer T, Fueger GF, et al. Sequential treatment of anemia in ulcerative colitis with intravenous iron and erythropoietin. Digestion 1999; 60:262–7.
8. Gasche C, Waldhoer T, Feichtenschlager T, Male C, Mayer A, Mittermaier C, et al. Prediction of response to iron sucrose in inflammatory bowel disease: associated anemia. Am J Gastroenterol 2001;96:2382–7.

Scand J Gastroenterol 2004;39:801–804.

CORRESPONDENCE

Taylor & Francis
healthsciences

A Comment

TO THE EDITOR: In the recent study by Erichsen et al ('Ferrous fumarate deteriorated plasma antioxidant status status in patients with Crohn disease', Scand J Gastroenterol 2003;38: 543–8) and in C. Gasche's editorial entitled 'Believe in iron', in the same issue), evidence was found that a 1-week treatment with ferrous fumarate decreased plasma antioxidant status in patients with active Crohn disease but not in normal controls. The data presented are very interesting and need further study to evaluate the clinical importance of these findings. It is speculated by the authors of this study, and in the accompanying editorial, that the use of ferric compound may have advantages compared with ferrous iron, because ferric iron has no pro-oxidant activities. A modern compound "ferric trimaltol" is mentioned which had shown in one study to be effective in patients with inflammatory bowel disease.

Readers should be informed, however, that 'ferric trimaltol' is not a licensed pharmaceutical drug and could cause serious side effects because of its iron chelator behavior and content of 'maltoi'. It should also be mentioned that all ferric iron drugs on the market, including the soluble iron (III) hydroxyl-polymaltose complex, have to be confronted with well-accepted findings of a very low bioavailability (Nielsen et al., Bioavailability of iron from oral ferric polymaltose in humans. Arzneim Forsch 1994;44:743–8).

At the moment, we are witnessing a renaissance of ferric compounds in oral iron treatment in different countries. In some countries, these non-effective iron drugs are even market leaders. However, in days of limited resources in health care systems these drugs have to be regarded with skepticism. Treatment of iron deficiency in Crohn disease could be one of the rare indications for these compounds, but this has to be clarified in further studies. Low amounts (not 120 mg Fe/d as in the Erichsen study) of a ferrous iron compound or a parental iron administration could also be a better and much cheaper alternative.

P. Nielsen
Zentrum für Experimentelle Medizin
Institut für Biochemie und Molekulerbiologie
Hamburg
Germany
E-mail. Nielsen@uke.uni.hamburg.de

A Further Comment

TO THE EDITOR: We appreciate Dr. Nielsen's interest in our work (Erichsen et al. 'Ferrous fumarate deteriorated plasma antioxidant status in patients with Crohn disease'. Scand J Gastroenterol 2003:38;543–8), and take this opportunity to comment on his statements.

Iron (III)-hydroxide polymaltose complex (IPC) consists of nuclei of ferric iron surrounded by non-covalent molecules of polymaltose. The structure is similar to that of physiologically occurring ferritin. The advantage of this type of formulation is the absence of iron ions. IPC is available for oral iron substitution in several European countries.

In the publication, Dr. Nielsen is referring to area under the curve being used for assessment of the bioavailability of IPC (1). It is not possible to use this method to demonstrate the bioequivalence of IPC and iron salts, because iron administered as IPC shows slow absorption and serum uptake and fast elimination from the serum into the storage compartments (2). Therefore, unlike iron salts, IPC shows no serum iron increase after oral administration.

Two recently published studies (3, 4) concluded that IPC was as effective as ferrous sulphate in the correction of haemoglobin levels in iron deficiency anaemia. However, IPC was associated with significantly fewer gastrointestinal side effects than ferrous sulphate.

Inflammatory bowel disease may indeed be an indication of ferric iron compounds for oral administration. With these compounds, aggravation of pro-oxidant reactions is less likely to occur as a result of the absence of ionic iron. Further study with ferric iron compounds is warranted.

K. Erichsen
Medical Dept.
Division of Gastroenterology
Haukeland University Hospital
Bergen
Norway
E-mail. kari.erichsen@helse-bergen.no

References

1. Nielsen P, Gabbe EE, Fisher R, Heinrich HC. Bioavailibility of iron from oral ferric polymaltose in humans. Arzneim Forsch 1994;44:743–8.
2. Geisser P, Müller A. Pharmacokinetics of iron salts and ferric hydroxide carbohydate complexes. Drug Research 1987;37:100–4.
3. Jacobs P, Wood L, Bird AR. Better tolerance of iron polymaltose complex compared with ferrous sulfate in the treatment of anaemia. Hematology 2000;5:77–83.
4. Borolla JR, Cicero RE, Dibildox M, Sotres D, Gutierrez R. Iron hydroxide polymaltose complex vs iron sulphate in the treatment of iron deficiency anaemia in infants. Revista Mexicana de pediatria 2000;57:63–7.

DOI 10.1080/00365520410008060