Changes in transferrin saturation after treatment with the oral iron chelator deferiprone in patients with iron overload

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Abstract

Aims—To evaluate the changes in transferrin saturation in patients with iron overload following the oral administration of the iron chelator deferiprone; to assess the correlation between the degree of transferrin desaturation, the deferiprone dose, and urinary iron excretion.

Methods—Serum samples were obtained from 16 patients with iron overload at different time intervals following the oral administration of deferiprone (50 mg/kg). These samples were analysed using 6M urea/polyacrylamide gel electrophoresis (UPAGE). This method is able to resolve serum transferrin into four different forms (free iron, two forms of monoferric, and diferric). The deferiprone concentration in these samples was estimated using high pressure liquid chromatography (HPLC). Zero time samples (t_0) from 10 patients were incubated with 150 μ M deferiprone or normal saline either at room temperature or at 37°C for 30 minutes and 24 hours, and also at -20°C for six weeks. Samples were then analysed using UPAGE.

Results—A maximum decrease in transferrin saturation from (mean (SD)) 93.0 (10.6)% to 54.5 (17.2)% was observed 72.5 (50.0) minutes after deferiprone administration and in most of the patients coincided with peak deferiprone concentration. This was associated with a maximum rise in the percentage of iron free transferrin (apotransferrin) from 2.9 (7.0)% to 27.3 (17.8)%. The total amount of iron estimated to be removed from transferrin constituted 21.3 (20.2)% of the 24 hour urinary iron excretion measured during the study. When deferiprone (150 μ mol/l) was incubated in vitro with t_0 samples from 10 patients for 30 minutes and 24 hours at room temperature, 37°C, and at -20°C for six weeks, deferiprone was more efficient at removing iron from transferrin at 37°C, with maximum transferrin desaturation accomplished within 30 minutes compared with 24 hours at room temperature.

Conclusions—The results confirm that deferiprone can remove iron from transferrin when administered orally to patients with iron overload and that transferrin

bound iron may, therefore, be a significant source of the iron chelated by deferiprone in vivo.

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The oral iron chelator deferiprone (L_1 , 1,2-dimethyl-3-hydroxypyrid-4-one, CP20) has now been given to a large number of patients with iron overload in many centres worldwide and shown to have an efficacy comparable to that of desferrioxamine (DFX).¹ The use of both chelators is associated with a wide variation between patients, and even from day to day in individual patients, in the amount of iron chelated and excreted in urine. The exact body pools of iron available for chelation by deferiprone or DFX have not been established. However, transferrin has been suggested as one source of iron available for chelation by deferiprone but not by DFX.^{2,3}

Transferrin has a molecular weight of 80 000 and two binding sites for iron, one at each of its two terminals. In normal individuals transferrin is only one third saturated, whereas in patients with iron overload it is usually completely saturated. Transferrin saturation is tested routinely by estimating the difference between the total iron content of serum before and after the addition of a saturating concentration of iron. Although this method is adequate for most purposes, it can be inaccurate. Other forms of iron such as non-transferrin bound or ferritin iron can cause over estimation of transferrin saturation.^{4,5} Therefore, if minor changes in transferrin saturation are to be estimated, such as those caused by an iron chelator, a more sensitive method is required. Furthermore, the presence of an excess of the chelator in a serum sample might interfere with the routine measurement of transferrin saturation.

Mackey and Seal⁶ showed that partially saturated human transferrin separates into four bands on urea/polyacrylamide gel electrophoresis (UPAGE). The slowest and fastest represent the iron free transferrin (apotransferrin) and diferric transferrin (transferrin-Fe₂), respectively, whereas the two intermediate bands represent the C and N terminal monoferric forms.⁷

In contrast to DFX, deferiprone at concentrations similar to those observed in plasma of patients receiving deferiprone treatment can

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Patient characteristics.

Patient No.	Age(years)/ Sex	Diagnosis	Serum ferritin (µg/l)	TIBC (µmol/l)	Transferrin saturation(%)*	UIE (mg/24 hours)
1	21/F	BTM	8130	33	78.8	15.8
2	23/F	BTM	7400	NA	NA	NA
3	13/M	CSA	3131	39	100	30
4	60/F	SCD	4006	36	100	8.7
5	81/M	MDS	5650	36	83	12.5
6	15/M	SCD	5950	66	36	8.1
7	46/M	CSA	2055	38	81.6	17.3
8	26/M	BTM	9060	48	79	11
9	17/M	PKD	3050	36	94.4	32.7
10	22/M	BTM	3350	NA	NA	NA
11	23/M	BTM	3520	30	100	16.5
12	27/F	BTM	3980	33	100	11.5
13	30/F	BTM	3850	54	100	5.2
14	43/F	ASA	1285	36	83.3	9.8
15	15/M	BTM	1320	27	100	3.1
16	31/F	BTM	4000	47	100	NA

BTM = β -thalassaemia major; CSA = congenital sideroblastic anaemia; SCD = sickle cell disease; MDS = myelodysplastic syndrome; PKD = pyruvate kinase deficiency; ASA = acquired sideroblastic anaemia; UIE = urinary iron excretion.
* Measured by routine laboratory technique.

remove an appreciable amount of iron from transferrin in vitro.² Evans *et al*³ recently observed a progressive fall in transferrin saturation after the administration of deferiprone to a patient with iron overload. In this study we have explored the interaction between deferiprone and transferrin both in vivo in a larger group of patients and in vitro. The results help to establish the degree of transferrin desaturation in vivo following administration of deferiprone and its relation to the amount of iron excreted in urine.

Methods

Deferiprone was synthesised at the Royal Free

Hospital as described previously.⁸ Acrylamide/bisacrylamide (19:1), as a ready-made solution of 40% (w/v), and rivanol were obtained from Sigma (Poole, Dorset, UK). This study had the approval of the Ethical Committee of the Royal Free Hospital. Blood samples were obtained from 16 patients with iron overload (table) at different time intervals (zero, 10, 20, 30, 45, 60, 75, 90, 120, 180, 240, and 300 minutes) following the oral administration of deferiprone (50 mg/kg), separated within 30 minutes of collection and stored at -20°C until analysis. Serum samples were thawed within four weeks of collection and immediately analysed using UPAGE, as described by Williams *et al*.⁹ Serum samples were also obtained from normal volunteers and used as controls with each run of UPAGE.

For analysis, samples were treated with rivanol as described previously¹⁰ and applied to the gel. After staining and destaining, gels were scanned using a laser densitometer (Molecular Dynamics, Sunnyvale, California, USA). The deferiprone concentration in these samples was estimated using high pressure liquid chromatography (HPLC), as described before.¹¹
A further experiment was performed: zero time samples (*t*₀) from 10 patients were incubated with 150 µM deferiprone or normal saline, either at room temperature or at 37°C for 30 minutes and 24 hours, and also at -20°C for six weeks. This concentration of deferiprone was chosen to be comparable to the mean of peak deferiprone concentration observed in the

Figure 1 Changes in serum transferrin saturation (□) and in the percentages of apotransferrin (◆), transferrin-Fe(C) (◇), and transferrin-Fe₂ (■) in four cases (cases 6, 10, 14, and 15) after the oral administration of deferiprone (50 mg/kg).

patients' sera. Samples were then treated with rivanol and analysed using UPAGE as above.

Serum iron and the total iron binding capacity (TIBC) were measured by routine laboratory techniques.^{12,13} Urinary iron was measured using atomic absorption spectrophotometry. Serum ferritin was estimated by an enzyme linked immunosorbent assay (ELISA) technique.¹⁴ Statistical significance was assessed using Student's *t* test.

Results

Using the UPAGE method, three bands were observed in patients' sera following deferiprone administration. These represent iron free transferrin (apotransferrin), C terminal monoferric transferrin (transferrin-Fe(C)), and diferric transferrin (transferrin-Fe₂) in order of increasing mobility.⁷ Adding an excess of iron to the sera caused complete disappearance of the first two bands and an increase in the density of the third band.

Deferiprone was capable of removing iron from transferrin (fig 1). The mean transferrin saturation at *t*₀ in patients' sera ranged between 57.8 and 100% (mean (SD) 93.0 (10.6)), compared with between 12.7 and 20.5% (16.4 (3.0)) for normal volunteers (*n* = 10). The correlation between the transferrin saturation measured by UPAGE and that obtained by using the routine laboratory method (88.3 (17.6)) was significant (*r* = 0.83, *p* = 0.0003). Following deferiprone administration, there was a progressive fall in the degree of transferrin saturation and the percentage of transferrin-Fe₂ and the appearance or rise in the percentage of apotransferrin and transferrin-Fe(C) (fig 2). The lowest transferrin saturation observed following deferiprone administration was 54.5 (17.2)% (range 16.0–74.7%), occurring 72.5 (50.0) minutes (10–180 minutes) after deferiprone administration. Transferrin desaturation (difference between *t*₀ and the lowest values) was 39.5 (17.4)% (14.8–84.0%). In six patients transferrin saturation returned to the *t*₀ value after 1.6–6 hours (4.5 (2.0) hours)) of deferiprone administration whereas in the rest it returned to 86.0–98.0% of the *t*₀ value after six hours of follow up. In case 6 transferrin saturation rose to 170% of the *t*₀ value at six hours (fig 1A) and in case 15 transferrin

Figure 2 The percentages of the various forms of transferrin at zero time (*t*₀) (■) and at the time of maximum change (□) in 16 patients with iron overload following the oral administration of deferiprone (50 mg/kg). Data expressed as mean (SD).

Figure 3 The correlation between the maximum transferrin (Tf) desaturation (the difference between *t*₀ and the lowest values) and the associated deferiprone (L1) concentration in 16 patients following the oral administration of deferiprone (50 mg/kg).

Figure 4 Transferrin desaturation in 10 patients with iron overload following the incubation of their zero time (*t*₀) samples with deferiprone (150 μmol/l) for 30 minutes and 24 hours at room temperature and 37°C and for six weeks at -20°C compared with the maximum *in vivo* desaturation of transferrin (In vivo) observed in their serum samples following the oral administration of deferiprone (50 mg/kg). Data expressed mean (SD).

desaturation was biphasic (fig 1D). Deferiprone concentrations observed at the time of the lowest transferrin saturation ranged between 57 and 310 μmol/l (128.4 (69.6)). In 11 patients the lowest transferrin saturation coincided with the peak deferiprone concentration. There was a significant correlation between maximum transferrin desaturation and the simultaneous deferiprone concentration (*r* = 0.56, *p* = 0.02; fig 3). No significant correlation was found between the degree of transferrin desaturation and the serum ferritin concentration (*p* = 0.25) or 24 hour urinary iron excretion (*p* = 0.64). The amount of iron removed from transferrin

in vivo was 13.8 (5.1) $\mu\text{mol/l}$ (6.7–22.1 $\mu\text{mol/l}$), estimated by multiplying the TIBC value by maximum transferrin desaturation. The total amount of iron chelated from transferrin by deferiprone was approximately estimated by multiplying the amount of iron removed from transferrin by each patient's predicted plasma volume ($\text{weight}(\text{kg}) \times 70(\text{ml/kg}) \times (1-\text{PCV})$ (packed cell volume)) and was 35.0 (15.3) μmol (13.9–67.1 μmol). Urinary iron excretion measured during the study was 250.3 (156.4) μmol (55.4–583.9 μmol). No significant correlation was found between urinary iron excretion and the total amount of iron removed from transferrin ($p=0.9$).

Incubation of t_0 serum samples ($n=10$) with 150 μM transferrin for 30 minutes at room temperature caused a small fall in transferrin saturation (4.8 (4.4)%); when incubation was continued for 24 hours this fall was more pronounced (21.7 (8.2)%, $p=0.004$). By contrast, at 37°C a significant drop in transferrin saturation was observed at 30 minutes (28.6 (11.7)%, $p=0.04$), comparable to that achieved in 24 hours at room temperature ($p=0.31$), but this fall did not significantly increase after 24 hours of incubation (25.6 (10.5)%, $p=0.68$). Only a small fall in transferrin saturation was observed when the samples were incubated at -20°C for six weeks (7.8 (1.7)%) (fig 4). In this experiment a fourth band was observed in the UPAGE gels, corresponding to transferrin-Fe(N), after incubating the samples with transferrin at room temperature and at 37°C for 30 minutes and 24 hours. No similar band was found after six weeks of incubation at -20°C . The percentages of this form of transferrin were 10.8 (4.9), 29.3 (7.4), 25.1 (1.6), and 26.7 (1.7), respectively. These changes were associated with a rise in the transferrin-Fe(C) concentration of 10.8 (4.9), 4.9 (3.0), 8.2 (3.3), and 8.2 (5.3)%, respectively.

Discussion

The results obtained following deferiprone administration showed that deferiprone is effective in removing an appreciable amount of iron from transferrin in most of the patients studied. These data are comparable to previously reported results.³ The degree of transferrin desaturation was dependent on the deferiprone concentration as there was a significant correlation between maximum transferrin desaturation and the associated deferiprone concentration.

The significant correlation between transferrin desaturation and the deferiprone concentration may suggest that these changes could have occurred in vitro after obtaining the serum samples rather than in vivo. We therefore carried out experiments to establish the speed of iron removal from transferrin by deferiprone at different temperatures. The results indicate that this process is much faster at 37°C than at room temperature or -20°C , being accomplished within 30 minutes (fig 4). There was no significant difference between the degree of transferrin desaturation achieved in vitro after 30 minutes or 24 hours at 37°C or

24 hours at room temperature and maximum transferrin desaturation observed in vivo (27.7 (10.8); fig 4). These results suggest that the observed changes in transferrin saturation are more likely to have occurred in vivo than to be purely caused by the interaction between deferiprone and transferrin in the stored serum sample. Furthermore, changes in transferrin saturation observed in cases 6 and 15 (figs 1A and D) suggest that transferrin saturation is not entirely dependent on deferiprone concentration and other in vivo factors may influence the interaction between transferrin and deferiprone. The absence of transferrin-Fe(N) from the serum samples is consistent with previous reports.^{15,16} Williams and Moreton¹⁵ found that storage of serum samples at -15°C for several days causes redistribution of iron between transferrin-Fe(C) and transferrin-Fe(N), resulting in the disappearance of the latter from serum but with no effect on overall transferrin saturation. Therefore, it was not possible to examine, in vivo, the distribution of iron between the four different forms of transferrin following administration of deferiprone. However, a fourth band representing transferrin-Fe(N) was observed in vitro after incubating serum samples taken from 10 patients with deferiprone. The changes in the concentration of this form of transferrin during incubation with deferiprone were more marked than the concurrent changes in the concentration of transferrin-Fe(C). In keeping with previous reports²³ this suggests that deferiprone preferentially binds the iron atom present in the C terminal site of diferric transferrin. Following deferiprone administration, transferrin saturation usually returned to the t_0 values within six hours whereas in case 6 transferrin saturation continued to increase, reaching 170% of its t_0 value at six hours (fig 1A). As this patient's t_0 transferrin saturation was relatively low (54%), it is possible that deferiprone at an initial high concentration caused a fall in transferrin saturation whereas at a later stage, when the deferiprone concentration fell, it participated in the transport of iron to transferrin. Previous studies have shown that deferiprone given to a normal volunteer can cause a progressive increase in transferrin saturation.³ The cause of the biphasic transferrin desaturation observed in case 15 (fig 1D) is unclear.

The degree of the iron load had no bearing on the degree of transferrin desaturation, as is evident from the lack of a significant correlation between the latter and the serum ferritin concentration. Assuming that all the iron removed from transferrin by deferiprone was excreted in urine in 24 hours, it was found that this comprised 21.3 (20.2)% (5.8–67.1%) of total urinary iron excretion. This calculation does not allow for possible flux of iron from tissue to transferrin when free deferiprone is present in plasma.

These data suggest, therefore, that transferrin is a source of iron available for chelation by deferiprone. Its rapid resaturation within six hours of deferiprone administration indicates that it could act efficiently in recruiting more iron for chelation should further doses of deferi-

prone be given. However, it is not the sole source of chelatable iron. Non-transferrin bound iron may be another form readily available for chelation. Its contribution to the total urinary iron excretion has yet to be evaluated. Other sources of iron such as tissue iron stores presumably also contribute either directly or indirectly through an intermediate vehicle such as transferrin to the iron chelated by deferiprone.

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