

## Urinary iron excretion in thalassaemia after desferrioxamine administration

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**Seshadri, R., Colebatch, J. H., and Fisher, R. (1974).** *Archives of Disease in Childhood*, 49, 195. **Urinary iron excretion in thalassaemia after desferrioxamine administration.** Urinary iron excretion induced with desferrioxamine (DFA) was estimated in 26 children with thalassaemia major. Four separate 24-hour urine collections were made—during a baseline period, after intramuscular injection of DFA 1 g, during blood transfusion with DFA 0.5 g/unit transfused blood, and during the post-transfusion period. Urinary iron, plasma iron, and total iron-binding capacity were estimated by atomic absorption spectrophotometry. Urinary iron excretion in the 24 hours after 1 g DFA ranged from 1.1 to 23 mg/m<sup>2</sup> surface area compared with 0.1 to 1.6 mg/m<sup>2</sup> during the baseline period. A positive correlation was obtained between DFA-induced urinary iron excretion and the amount of blood transfused, plasma iron level, and size of the spleen. Splenectomized patients excreted less iron after DFA than those who were not splenectomized. DFA-induced urinary iron was measured before and after splenectomy in 6 patients. In 5 of the 6 patients a drop in iron excretion was observed and the analysis of the 6 pairs of results indicated that splenectomy produced a decrease in DFA-induced urinary iron excretion.

These findings indicate that the enlarged spleen in thalassaemia is a major source of iron chelated by DFA. It is suggested that treatment with DFA in the presence of a large spleen may be ineffective in removing excess iron from the myocardium and liver.

The survival of children with thalassaemia major depends on repeated blood transfusions. Marked iron deposition develops in various vital organs, and if this is untreated it leads to a severe degree of morbidity and finally death, commonly during the second decade of life. Hence, assessment of the degree of iron overload is important in the prognosis and management of children with thalassaemia major. In recent years desferrioxamine (DFA), a specific and effective chelator of iron, has been used for the assessment of iron stores (Ploem *et al.*, 1966; Hedenberg, 1969). Several tests have been devised based on parenteral injection of a standard dose of DFA followed by measurement of the increased excretion of iron in the urine. We present our experience with the use of DFA for assessment of the degree of iron overload in 26 children with thalassaemia major. The results indicate a positive correlation of urinary iron excretion with the amount of blood transfused, plasma iron level, and spleen

size. They also suggest that splenectomy diminishes urinary iron excretion, which indicates that the spleen plays a major role in iron chelation by DFA in thalassaemia major.

### Patients

In 1972–73, 26 patients with thalassaemia major in the Royal Children's Hospital, Melbourne, were studied. There were 13 boys and 13 girls, their ages at the time of the study ranging from 2 to 21 years with a median of 10 years. The 3 oldest patients, aged 15, 21, and 21 years, will be discussed separately because in addition to splenectomy they had been receiving DFA daily for 5 to 8 years before the present study was carried out. This prolonged chelation therapy may have altered significantly the degree of iron overload compared with the remaining 23 patients. Those 23 patients, except for the ones who had received less than 50 units of blood, had clinical evidence of haemosiderosis such as skin pigmentation, cardiomegaly, and liver enlargement. 6 of them had undergone splenectomy before this investigation. The others had splenomegaly, with the lower edge palpable 4 to 19 cm below the costal margin.

All the patients in this study were transfused at a haemoglobin level of about 6 g/100 ml. They were usually transfused up to an Hb level that was normal for the patient's age. At the time of investigation the total volume of blood received by the patients ranged from 7 to 250 units (1 unit blood equivalent to 250 ml packed red cells).

The 3 patients who had had long-term DFA treatment had received injections of 1 g DFA 6 days a week intramuscularly for 5 to 8 years. In addition, they also received DFA 500 mg/unit blood at the time of each transfusion. All 3 had undergone splenectomy before DFA treatment. They had a moderate degree of liver enlargement, with lower edge 2 to 6 cm below the costal margin. The degree of skin pigmentation in these patients was mild. None had cardiomegaly. Daily DFA injections were discontinued 3 days before the present study.

### Methods

Hb was estimated by the cyanmethaemoglobin method using a Coulter haemoglobinometer. At the time of study Hb ranged from 5.5 to 6.5 g/100 ml. Plasma iron, total iron-binding capacity, and percentage saturation of transferrin were estimated by atomic absorption spectrophotometry (Olson and Hamlin, 1969).

The DFA-induced urinary iron excretion test was performed as follows. On the first day after admission a 24-hour specimen of urine was collected for the

estimation of basal urinary iron excretion. The following day 1 g DFA was administered intramuscularly and urine was collected for a further 24 hours. During the third 24-hour period, in which the patient was transfused with 2 to 5 units of blood containing 500 mg DFA/unit, a similar urine collection was made, and again during the next (fourth) 24-hour period after transfusion. Urine samples were collected in acid-washed iron-free polyethylene receptacles and the iron content was estimated using atomic absorption spectrophotometry (Zetner and Mansbach, 1965). Iron excretion was calculated in mg/m<sup>2</sup> body surface area per g DFA administered.

Early observations suggested that DFA-induced urinary iron excretion may be influenced by splenectomy. Therefore the excretion was measured for 24 hours 2 days before and 2 days after the splenectomy in 6 patients. Hb level in these patients was 13 to 14 g/100 ml at the time of study. The dose of DFA in each case was 1 g intramuscularly.

Standard statistical methods were used for calculation of correlation coefficients and partial correlation coefficients. Wilcoxon tests were used for analysing DFA-induced urinary iron excretion in splenectomized patients and in those not splenectomized.

### Results

Table I shows the clinical data, plasma iron levels, and urinary iron excretion in the patients. Plasma

TABLE I  
Details of patients

| Case no. | Age (yr)/sex | Spleen size (cm) | Total blood transfusion (units) | Plasma iron ( $\mu\text{g}/100\text{ ml}$ ) | Plasma iron saturation (%) | Urinary iron excretion* (mg/m <sup>2</sup> per 24 hr) |       |       |       |
|----------|--------------|------------------|---------------------------------|---|----------------------------|---|-------|-------|-------|
|          |              |                  |                                 |   |                            | Day 1   | Day 2 | Day 3 | Day 4 |
| 1        | 2 M          | 13               | 7                               | 135   | 47                         | 0.1   | 2.4   | 8.4   | 4.3   |
| 2        | 2 F          | 6                | 8                               | 190   | 65                         | 0.1   | 4.0   | 1.6   | —     |
| 3        | 3 M          | 10               | 16                              | 73  | 32                         | 0.2   | 1.2   | 1.1   | 0.3   |
| 4        | 3 F          | 4                | 16                              | 219   | 46                         | 0.2   | 1.1   | 3.8   | 0.3   |
| 5        | 3 M          | 15               | 61                              | 205   | 68                         | 0.2   | 4.4   | 2.3   | 0.1   |
| 6        | 3 M          | 4                | 44                              | 195   | 93                         | 0.2   | 2.6   | 3.0   | 0.6   |
| 7        | 5 M          | 10               | 84                              | 118   | 50                         | 0.1   | 4.4   | 3.0   | 0.3   |
| 8        | 5 F          | 9                | 64                              | 128   | 44                         | 0.1   | 3.8   | —     | —     |
| 9        | 6 F          | 12               | 110                             | —   | —                          | 0.1   | 10.0  | 8.5   | 1.5   |
| 10       | 6 F          | 10               | 100                             | 165   | 49                         | 0.1   | 16.0  | 14.6  | 5.0   |
| 11       | 9 M          | 10               | 122                             | 290   | 96                         | 0.9   | 13.8  | 15.3  | 5.0   |
| 12       | 10 M         | 12               | 115                             | 240   | 66                         | 0.2   | 23.0  | 18.7  | —     |
| 13       | 10 F         | 10               | 119                             | 440   | 81                         | 1.5   | 13.2  | 14.0  | 1.0   |
| 14       | 10 F         | Splenectomy      | 136                             | 236   | 88                         | 0.3   | 2.4   | 3.4   | 0.9   |
| 15       | 10 M         | "                | 120                             | 225   | 62                         | 0.4   | 6.6   | 16.4  | 3.3   |
| 16       | 11 F         | "                | 72                              | 280   | 80                         | 0.7   | 7.1   | 5.4   | 5.0   |
| 17       | 11 M         | 19               | 160                             | 225   | 64                         | 1.3   | 19.1  | 17.5  | 12.5  |
| 18       | 12 M         | 8                | 113                             | 286   | 86                         | 0.2   | 11.0  | 9.0   | 2.3   |
| 19       | 12 F         | 9                | 164                             | 217   | 93                         | 0.3   | 5.5   | —     | —     |
| 20       | 12 F         | 10               | 210                             | 185   | 83                         | 0.4   | 4.0   | 4.4   | 2.4   |
| 21       | 12 M         | Splenectomy      | 131                             | 257   | 64                         | 1.1   | 8.0   | 11.7  | 8.8   |
| 22       | 13 F         | "                | 165                             | 350   | 70                         | 1.6   | 8.7   | 3.9   | 1.4   |
| 23       | 14 F         | "                | 160                             | 400   | 97                         | 0.5   | 8.6   | 7.2   | 1.1   |
| 24†      | 15 M         | "                | 140                             | 245   | 59                         | 0.1   | 4.6   | 5.9   | 2.2   |
| 25†      | 21 F         | "                | 240                             | 262   | 88                         | 0.9   | 6.4   | —     | —     |
| 26†      | 21 M         | "                | 250                             | 150   | 47                         | 0.7   | 4.5   | —     | —     |

\*Day 1, baseline period; day 2, after 1 g DFA i.m.; day 3, during blood transfusion; day 4, post-transfusion period.

†Received long-term DFA treatment for 5–8 years.

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iron levels ranged from 73 to 440  $\mu\text{g}/100\text{ ml}$  and the percentage saturation of transferrin ranged from 32 to 97%. These levels increased with age and amount of blood received in all patients except the 3 who had received long-term DFA treatment.

The amounts of urinary iron excreted during the baseline period (day 1) ranged from 0.1 to 1.6  $\text{mg}/\text{m}^2$ . After 1 g DFA intramuscularly (day 2) the amounts of urinary iron excreted ranged from 1.1 to 23.0  $\text{mg}/\text{m}^2$ . These were 6- to 160-fold greater than the excretion during the baseline period. During blood transfusion with administration of DFA (day 3) the amounts of urinary iron excreted were greater than after 1 g DFA alone in 10 patients and were less in 12. In the 24 hours after transfusion (day 4) the amounts of iron excreted ranged from 0.1 to 12.5  $\text{mg}/\text{m}^2$ . These amounts were slightly greater in 17 patients and less in 3 patients than excretion during the baseline period.

Table II shows the results of a statistical analysis that correlated DFA-induced urinary iron excretion after 1 g intramuscular DFA (day 2) with the amount of blood transfused, plasma iron level, plasma iron saturation index, and spleen size based on 17 patients who were not splenectomized. All

factors except the percentage saturation of transferrin were significantly correlated with DFA-induced urinary iron excretion ( $P < 0.05$ ). Calculation of partial correlation coefficients of each factor conditional on the other 3, followed by elimination of nonsignificant factors, showed the spleen size and plasma iron levels to be the main factors influencing the amount of DFA-induced urinary iron excretion.

Table III shows the DFA-induced urinary iron excretion in splenectomized patients and in those who were not splenectomized, together with data comparing the 2 groups with respect to age and amount of blood transfused. The splenectomy patients excreted significantly less iron than the patients with a large spleen (Wilcoxon one-sided test for two samples,  $P < 0.05$ ).

Table IV shows the DFA-induced urinary iron excretion before and after splenectomy. 5 of the 6 patients excreted less iron after splenectomy and the analysis of the 6 pairs of results indicated that splenectomy produced a decrease in DFA-induced urinary iron excretion. (Wilcoxon one-sided test for pair differences,  $P < 0.05$ ).

The mean DFA-induced urinary iron excretion in the 3 oldest patients who had received long-term treatment was 5.2  $\text{mg}/\text{m}^2$  per 24 hours (4.6, 6.4, 4.5). This was 1.7 mg less than the mean urinary iron excretion (mean 6.9, range 2.4-8.7) in 6 younger patients who had undergone splenectomy but had not received any DFA treatment.

TABLE II

*Correlation coefficients based on 17 patients who had no splenectomy*

|                          | Blood transfused     | Plasma iron          | Plasma iron saturation (%) | Spleen size          |
|--------------------------|----------------------|----------------------|----------------------------|----------------------|
| DFA-induced urinary iron | +0.518<br>$P < 0.05$ | +0.495<br>$P < 0.05$ | +0.234<br>$P > 0.05$       | +0.479<br>$P < 0.05$ |

### Discussion

Desferrioxamine is trihydroxamic acid, a white, water-soluble solid. It combines with trivalent

TABLE III

*DFA-induced\* urinary iron excretion in splenectomized and nonsplenectomized patients*

| Nonsplenectomized group |          |                  |                                  |  | Splenectomized group |          |                         |                                  |  |
|-------------------------|----------|------------------|----------------------------------|--|----------------------|----------|-------------------------|----------------------------------|--|
| Case no.                | Age (yr) | Spleen size (cm) | Total blood transfusion† (units) | Iron excretion ( $\text{mg}/\text{m}^2$ per 24 hr) | Case no.             | Age (yr) | Age at splenectomy (yr) | Total blood transfusion† (units) | Iron excretion ( $\text{mg}/\text{m}^2$ per 24 hr) |
| 9                       | 6        | 12               | 110 (125)                        | 10.0   | 14                   | 10       | 9                       | 136 (151)                        | 2.4  |
| 10                      | 6        | 10               | 100 (105)                        | 16.0   | 15                   | 10       | 7                       | 120 (133)                        | 6.6  |
| 11                      | 9        | 10               | 122 (122)                        | 13.8   | 16                   | 11       | 2                       | 72 (68)                          | 7.1  |
| 12                      | 10       | 12               | 115 (115)                        | 23.0   | 21                   | 12       | 7                       | 131 (112)                        | 8.0  |
| 13                      | 10       | 10               | 119 (126)                        | 13.2   | 22                   | 13       | 5                       | 165 (150)                        | 8.7  |
| 17                      | 11       | 19               | 160 (133)                        | 19.1   | 23                   | 14       | 5                       | 160 (120)                        | 8.6  |
| 18                      | 12       | 8                | 113 (94)                         | 11.0   |                      |          |                         |                                  |  |
| 19                      | 12       | 10               | 164 (164)                        | 5.5  |                      |          |                         |                                  |  |
| 20                      | 12       | 10               | 210 (182)                        | 4.0  |                      |          |                         |                                  |  |
| Mean                    | 9.8      | 11               | 135 (129)                        | 12.8   |                      | 11.6     | 5.8                     | 131 (122)                        | 6.9  |

\*DFA dose 1 g i.m.

†Figures in parentheses indicate blood transfusion in units/ $\text{m}^2$  body surface area.

TABLE IV  
DFA-induced\* urinary iron excretion before and after splenectomy

| Case no. | Age (yr) | Total blood transfusion (units) | Spleen size (cm) | Iron excretion (mg/m <sup>2</sup> per 24 hr) |                   |
|----------|----------|---------------------------------|------------------|--|-------------------|
|          |          |                                 |                  | Before splenectomy                           | After splenectomy |
| 7        | 6        | 104                             | 10               | 5.0  | 1.3               |
| 9        | 7        | 134                             | 13               | 6.0  | 1.7               |
| 11       | 10       | 156                             | 14               | 7.2  | 2.0               |
| 13       | 10       | 144                             | 10               | 5.0  | 5.4               |
| 17       | 12       | 170                             | 19               | 8.8  | 1.5               |
| 20       | 13       | 230                             | 12               | 5.0  | 2.0               |

\*DFA dose 1 g i.m.

iron to form the red pigment ferrioxamine which is excreted in the urine and bile. While the exact site of action of DFA remains uncertain, the results of recent investigations suggest that the storage compounds ferritin and haemosiderin represent the major source of the iron it chelates (Ploem *et al.*, 1966; Wohler, 1964; Fielding, 1965; Hallberg, Hedenberg, and Weinfeld, 1966; Balcerzak *et al.*, 1968; Cumming *et al.*, 1969; Lipschitz *et al.*, 1971). It has also been shown that haem iron is readily chelatable by DFA during catabolism of Hb (Fielding, 1965; Karabus and Fielding, 1967; Hedenberg, 1969).

The assessment of iron stores in thalassaemia by DFA-induced urinary iron excretion is more complex than in other conditions of iron overload. In thalassaemia the iron store is constantly increasing, due largely to repeated blood transfusions. Increased haem catabolism due to intramedullary haemolysis and hypersplenism may also influence the amount of iron chelated by DFA. Moreover, the age of the patient and the degree of anaemia also affect the urinary iron excretion (Fielding, 1970). For these reasons wide variations may occur in the amount of iron excreted after DFA in thalassaemia. Thus, previous reports have mentioned DFA-induced urinary iron excretion in thalassaemia major ranging from 0.84 to 24.1 mg in 24 hours per g/DFA (Smith, 1962; Hwang and Brown, 1964; McDonald, 1966; Diwany *et al.*, 1968; Markum *et al.*, 1969).

In the present study, by measuring the DFA-induced urinary iron excretion in each patient when Hb was around 6 g/100 ml, the influence of anaemia was kept constant or standardized. The amounts of DFA-induced urinary iron excretion ranged from 1.1 to 23 mg/m<sup>2</sup> per 24 hours after 1 g DFA (Table I). The degree of variation correlated well with the amount of blood received and the plasma iron level in patients who had not been splenectomized (Table II). Apart from these factors, the size of the spleen

also influenced DFA-induced urinary iron excretion. This was especially noticeable above 6 years of age when the clinical signs of sequestration of red cells due to hypersplenism were present. When DFA-induced urinary iron excretion in patients above 6 years of age who had not been splenectomized was compared with that in splenectomized patients, the latter excreted significantly less iron (Table III).

Evidence that splenectomy influences DFA-induced urinary iron excretion was also provided by the measurement of iron excretion during the pre- and postoperative periods (Table IV). Thus, 5 of 6 patients showed 60 to 83% decrease in DFA-induced urinary iron excretion after splenectomy. Markum *et al.* (1969), in a study on 8 patients, suggested that splenectomy did not influence the chelation of iron by DFA. Their study, however, in contrast to the present study, compared children below the age of 5½ years who had not been splenectomized with splenectomized children who were above that age.

This observation may be important in clinical practice when iron chelation therapy is used. In thalassaemia the spleen is one of the major sites of destruction of erythrocytes and contains a large reservoir of iron released from haem catabolism. Since the iron-chelating action by DFA is short-lived (about 30 minutes) and quantitatively limited (90 mg iron/g DFA), all the DFA available may readily chelate splenic iron in metabolically active form, leaving no free DFA to combine with the iron present in heart and liver. Hence, significant benefit in cardiac and liver function from DFA treatment may not be demonstrable in the presence of a large spleen, especially when there is evidence of hypersplenism.

It has been routine in our clinic since 1965 to add DFA to each unit of donor blood at the time of transfusion. So far we have not had any side-effects from this procedure. During blood transfusion the amount of iron excreted per g DFA was not

significantly different from that excreted after intramuscular DFA alone (Table I). During the 24-hour period after transfusion, iron excretion decreased conspicuously but not to the baseline levels, indicating that some iron chelated on the previous day of transfusion continued to be excreted during the next day. Wohler (1964), using radioactive  $^{59}\text{Fe}$ , observed that 90% of the chelated iron was excreted within 48 hours.

Plasma iron and percentage saturation of transferrin tended to increase with the age of the patients (Table I). This study showed a significant correlation between the plasma iron level and the amounts of DFA-induced urinary iron excreted in patients who were not splenectomized (Table II). However, the degree of transferrin iron saturation did not seem to influence the amounts of DFA-induced urinary iron excreted. A similar observation was made by Karabus and Fielding (1967).

The actual dose of DFA given in this study was chosen as 1 g, irrespective of the age of the patients, and represented from 568 to 2000 mg/m<sup>2</sup> body surface area. No attempt was made to assess the optimal dose of DFA. McDonald (1966) found no advantage in increasing the dose above 1 g as the greater initial increase in iron excretion that resulted was not maintained.

The 3 patients who had received long-term DFA treatment excreted much less iron after DFA than other patients, who were younger. There are at least three possible reasons for their lower levels of urinary iron excretion. (1) These may be a result of decreased total body iron stores produced by long-term DFA administration. (2) They may be due to the DFA becoming less effective as suggested by McDonald (1966) in his study. (3) The decreased iron excretion after DFA is probably due in part to the size of the test dose of DFA, which in these 3 patients amounted to only 568 to 660 mg/m<sup>2</sup>. The follow-up study of these patients indicated a significant improvement in the degree of haem siderosis, which will be the subject of a separate report.

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REFERENCES

- Balcerzak, S. P., Westerman, M. P., Heinle, E. W., and Taylor, F. H. (1968). Measurement of iron stores using deferoxamine. *Annals of Internal Medicine*, **68**, 518.
- Cumming, R. L. C., Millar, J. A., Smith, J. A., and Goldberg, A. (1969). Clinical and laboratory studies on the action of desferrioxamine. *British Journal of Haematology*, **17**, 257.
- Diwany, M., Gabr, M., El Hefni, A., and Mokhtar, N. (1968). Desferrioxamine in thalassaemia. *Archives of Disease in Childhood*, **43**, 340.
- Fielding, J. (1965). Differential ferrioxamine test for measuring chelatable body iron. *Journal of Clinical Pathology*, **18**, 88.
- Fielding, J. (1970). Storage iron and desferrioxamine. *Proceedings of the Royal Society of Medicine*, **63**, 1218.
- Hallberg, L., Hedenberg, L., and Weinfeld, A. (1966). Liver iron and desferrioxamine-induced urinary iron excretion. *Scandinavian Journal of Haematology*, **3**, 85.
- Hedenberg, L. (1969). Studies on iron metabolism with desferrioxamine in man. *Scandinavian Journal of Haematology*, **Suppl. 6**.
- Hwang, Y. F., and Brown, E. B. (1964). Evaluation of deferoxamine in iron overload. *Archives of Internal Medicine*, **114**, 741.
- Karabus, C. D., and Fielding, J. (1967). Desferrioxamine chelatable iron in haemolytic, megaloblastic and sideroblastic anaemias. *British Journal of Haematology*, **13**, 924.
- Lipschitz, D. A., Dugard, J., Simon, M. O., Bothwell, T. H., and Charlton, R. W. (1971). The site of action of desferrioxamine. *British Journal of Haematology*, **20**, 395.
- McDonald, R. (1966). Deferoxamine and diethylenetriaminepentaacetic acid (DTPA) in thalassaemia. *Journal of Pediatrics*, **69**, 563.
- Markum, A. H., Iskandar, W., Keng, K. L., and Odang, O. (1969). Iron excretion in thalassaemia after the administration of chelating agents. *Paediatrica Indonesiana*, **9**, 89.
- Olson, A. D., and Hamlin, W. B. (1969). A new method for serum iron and total iron-binding capacity by atomic absorption spectrophotometry. *Clinical Chemistry*, **15**, 438.
- Ploem, J. E., de Wael, J., Verloop, M. C., and Punt, K. (1966). Sideruria following a single dose of desferrioxamine-B as a diagnostic test in iron overload. *British Journal of Haematology*, **12**, 396.
- Smith, R. S. (1962). Iron excretion in thalassaemia major after administration of chelating agents. *British Medical Journal*, **2**, 1577.
- Wohler, F. (1964). Diagnosis of iron storage diseases with desferrioxamine. (Desferal test.) *Acta Haematologica*, **31**, 321.
- Zettner, A., and Mansbach, L. (1965). Application of atomic absorption spectrophotometry in the determination of iron in urine. *American Journal of Clinical Pathology*, **44**, 517.

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