

INTRAVENOUS CHELATION THERAPY DURING TRANSPLANTATION FOR THALASSEMIA

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ABSTRACT

Background. Thalassemia patients with heavy iron overload risk further increase of body iron stores after bone marrow transplantation (BMT) due to intensive red-cell transfusions in the post BMT course and to massive mobilization of iron deposits from marrow cells following the conditioning regimen. Nevertheless, iron chelation has not yet been used during the transplant period, mainly for concerns related to the toxicity and antiproliferative properties of the drug.

Methods. Fifteen thalassemic patients received desferrioxamine (DFO) before and during BMT according to two different schedules (first: from day -9 to day +60, and second: from day -9 to day -2, then from day +28 to day +60) at a dose of 40 mg/kg/day as a 24-hour intravenous infusion.

Results. The median time to neutrophil, platelet and erythrocyte recovery showed no difference between DFO-treated patients and the control group (18 days vs. 15, 16 vs. 18 and 22 vs. 23, respectively; *p*: N.S.). The incidence of acute GVHD was 23% in the DFO group and 13% in controls (*p*: N.S.). The median serum ferritin (SF) at 6 months after BMT was significantly lower in the DFO-treated patients (2081 versus 4187; *p*: 0.007) than in the control group. This difference continued to be evident, though not statistically significant, during longer follow-up.

Conclusions. Intravenous DFO therapy during BMT does not seem to have affected the engraftment parameters or the incidence of infections or GVHD. No adverse effects were observed during the therapy. Therefore thalassemic patients with heavy iron overload can be candidates for a course of i.v. chelation during the transplant period. This therapy could also be followed by post-BMT iron removal (i.e. phlebotomies or desferrioxamine) to accelerate the clearance of body iron deposits.

Key words: desferrioxamine, thalassemia, BMT

Iron overload predictably complicates long-term red-cell transfusions in homozygous β -thalassemia. Excess deposition of iron in various organs such as the liver, heart, pancreas, joints, endocrine glands results in cellular injury and functional insufficiency.¹ Desferrioxamine (DFO) therapy has been used extensively for over the last two decades in patients with iron overload and has resulted in prolonged survival.²

Allogeneic BMT has been increasingly adopted, starting in the 1980s, as a radical cure for thalassemia.^{3,4} However, the use of DFO imme-

diately preceding and during the transplant period has not yet been explored, mainly for concerns related to the toxicity and antiproliferative activity of the drug. Nevertheless, intensive transfusional support during the period of marrow aplasia, and the acute mobilization of iron deposits from marrow cells as a result of the conditioning regimen lead to a possible further increase of body iron stores.

Studies of long-term follow-up demonstrate that iron deposits are slowly cleared after transplant; however, serum ferritin takes almost two years to reach pre-transplant levels.⁵ Moreover,

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the subsequent decrease in serum ferritin is slow due to the very limited capability of iron excretion of the organism.⁵ On the basis of these observations and an increasing interest in the outcome of iron deposits after BMT, we began a pilot trial to explore the possibility of intravenous DFO therapy before and early post-BMT in patients with homozygous β -thalassemia.

Patients and Methods

Patients

Fifteen patients with homozygous β -thalassemia (8 males and 7 females, age 9 to 15 years, mean 12) who underwent allogeneic BMT from HLA-identical siblings in 1988 and 1989 were selected to receive concomitant intravenous DFO therapy. Seven of them had been splenectomized before transplant. Three were retrospectively assigned to Class 1, 8 to Class 2 and 4 to Class 3 according to previously described criteria.³

The control group included 15 consecutive patients transplanted from HLA identical siblings during the same period: seven were males and 8 females, aged from 10 to 15 years (mean 12). Four of these patients had been splenectomized before transplant. One was in Class 1, 9 were in Class 2 and 5 patients were in Class 3.

All patients were conditioned with busulfan 14 mg/kg for 4 consecutive days and cyclophosphamide 200 mg/kg over the next 4 consecutive days. Cyclosporin A was administered from day -2 till day +365 as GVHD prophylaxis. One patient who received DFO developed cardiac tamponade (on day -3) during the conditioning regimen and was not included in this study.⁶

Desferrioxamine protocols

A Broviac catheter was inserted in an external jugular or subclavian vein in all patients before the start of therapy and maintained for the entire treatment period. All patients received DFO at a dose of 40 mg/kg/day (24-hour intravenous infusion) according to two different chelation protocols: 1) eight patients received DFO continuously from day -9 to day +60; 2) seven received DFO from day -9 to day -2, then

from day +28 to day +60, avoiding the period of marrow infusion, marrow aplasia and early reconstitution.

Graft documentation

Globin-chain synthesis of erythroid marrow and peripheral blood reticulocytes was examined by measuring the incorporation of [H^3] leucine, followed by column or high-performance liquid chromatography. Cytogenetic analysis was performed on unstimulated marrow and phytohemagglutinin-stimulated peripheral blood when donor and recipient were not of the same sex.⁷

Serum iron, unbound iron binding capacity and ferritin assay

Serum iron (SI) and unbound iron binding capacity (UIBC) were determined before BMT, at 6 months after BMT and then yearly by means of routine laboratory tests. Serum ferritin (SF) was evaluated by enzyme-linked immunosorbent assay (Eurogenetics, Turin, Italy).

Liver histology

Percutaneous liver needle biopsies were performed in all patients before BMT and then yearly after BMT. Liver hemosiderosis after Pearls' staining of the specimen was graded as absent, mild, moderate or severe using previously published criteria.⁸

Statistical analysis

Differences between the DFO and control groups were analyzed by the Wilcoxon test for independent data.

Results

The median time to neutrophil recovery $>0.5 \times 10^9/L$ in the DFO group and the control patients was not statistically different (18 versus 15 days, respectively; *p*: N.S.). The median time to neutrophil recovery for the 8 patients who received DFO continuously (from day -9 to +60) was 17 days versus 15 days for the 7 patients who received DFO discontinuously (from day -9 to -2, then from +28 to +60); this difference was not statistically significant either.

Furthermore, the time to platelet and erythrocyte recovery did not differ between the DFO and the control groups (16 versus 18 days and 22 versus 23, respectively).

The median time to graft documentation was the same in the DFO-treated and the control group. There were no significant differences regarding red-cell and platelet support (median: 5 and 7 units versus 5 and 6 units, respectively).

Four patients in the DFO group (26%) developed grade ≥ 2 acute GVHD, and 3 out of 13 evaluable patients (22%) developed mild chronic GVHD, which has now resolved. In the control group 2 patients (13%) developed grade 2 acute GVHD, while 2 out of 13 evaluable patients had mild and 1 patient had moderate chronic GVHD (22%), which has also resolved. Two patients in the DFO group (13%) rejected their grafts during the first 60 days post-BMT and one of them died. Another patient in this group died from grade IV acute GVHD. In the control group one patient (6%) rejected his graft and one patient died of CMV interstitial pneumonia and encephalitis.

The infective complications (bacteremia or fungemia) in both the DFO group and controls were similar: 1 *Staphylococcus anaemolyticus*, 1 *Staphylococcus epidermidis*, 2 *Pseudomonas aeruginosa* and 1 *Candida albicans* (total 5 episodes) in the DFO Group and 1 *Staphylococcus epidermidis*, 1 *Pseudomonas aeruginosa* and 3 *Candida* (1 *albicans* and 2 *guilliermondi*) (total 5) in the control group.

During DFO therapy one patient developed transitory disturbances of vision that resolved spontaneously when the drug infusion was stopped; this patient was also under cyclosporine therapy for GVHD prophylaxis. No other adverse effects or toxicities were noted in the group of treated patients.

Interestingly, data from long-term post-transplant follow-up show that the mean value of SF at 6 months after transplant in patients who received DFO was significantly lower than that of the control group (2081 versus 4187; $p: 0.007$) (Figure 1). The median value of SF at 1, 2, 3 and 4 yrs after transplant of patients who received DFO was still lower than that of the control group but this difference was not statistically significant (Figure 1).

Iron grading of liver specimens in both the DFO group and control patients revealed iron overload before BMT (Table 1). Four years after BMT there was no change regarding liver hemosiderosis in the DFO group, while the percentage of patients with severe liver hemosiderosis had increased in the control group.

Regarding liver inflammatory activity, 6 patients (40%) in the DFO group suffered from chronic persistent hepatitis and 4 patients (27%) had chronic active hepatitis before BMT, while 5 patients (33%) showed no inflammatory activity on liver biopsy. Four years after BMT 4 out of 11 evaluable patients (36%) exhibited chronic persistent hepatitis and 5 (46%) chronic active hepatitis. Three patients (27%) developed

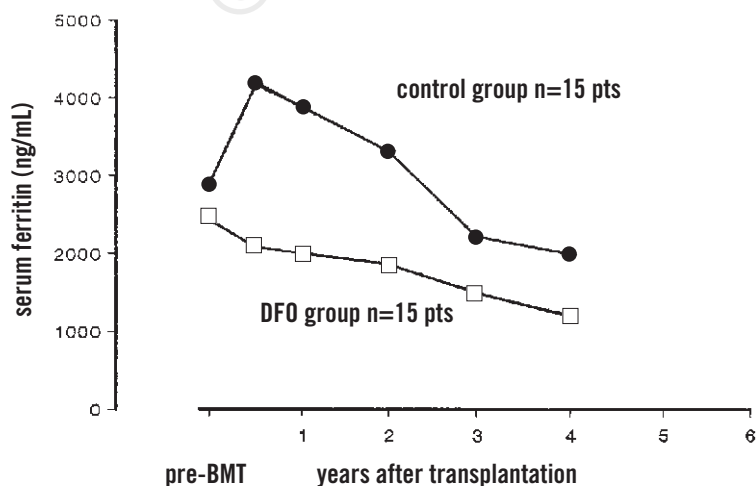


Figure 1. Median serum ferritin in DFO-treated and control patients.

Table 1. Liver biopsy iron grading in DFO-treated and control patients.

	Absent	Mild	Moderate	Severe
DFO group:				
pre BMT	0	4(27)	7(46)	4(27)
4 yrs	0	4(36)	4(36)	3(28)
Control group:				
pre BMT	0	4(47)	10(67)	1(6)
4 yrs	0	1(8)	6(50)	5(42)

Values in parentheses are percentages.

liver cirrhosis: 2 of them showed severe and 1 moderate liver hemosiderosis before transplant. Two out of these 3 patients were positive for hepatitis C virus antibodies (ELISA 2nd generation test).

In the control group 5 patients (33%) presented chronic persistent hepatitis and 7 (47%) had chronic active hepatitis before BMT, while 3 patients (20%) displayed no inflammatory activity on liver biopsy. Four years after BMT 5 out of 12 evaluable patients (42%) demonstrated chronic persistent and 5 (42%) chronic active hepatitis. Of this group, 3 patients (25%) also developed cirrhosis, all of whom had shown moderate liver hemosiderosis before the transplant. Two out of these 3 patients were positive for hepatitis C virus antibodies.

During the long-term post-transplant follow-up, one patient in the DFO group and 6 patients in the control group were forced to recommence iron removal therapy (phlebotomy or desferrioxamine) because of persistent heavy iron overload and deterioration of liver histology.

Discussion

Daily intravenous therapy with high doses of DFO has been reported to remove excessive iron effectively in patients with transfusional iron overload.⁹ DFO has also been observed to have inhibitory properties on cellular proliferation.¹⁰ The immunomodulating properties of DFO have been exploited *in vivo* to inhibit graft rejection and autoreactive T-cells.¹¹ Using a

model of murine pancreatic allografts, DFO administration appeared to reduce chronic islet rejection while not interfering with the acute rejection response.¹² Free radicals may also interfere with successful engraftment;¹³ an intravenous preparation of DFO binds iron to prevent conversion of [O⁻] and [H₂O₂] to free hydroxyl radicals, thus inhibiting direct tissue injury via OH-by activation of complement and neutrophils.¹⁴ Administration of DFO has also been used to treat graft-versus-host disease in human bone marrow transplant allograft recipients.¹⁵

Our data do not indicate any influence of DFO administration on the incidence of acute or chronic GVHD. The incidence of infective complications was likewise similar in the treated and control groups.

We observed 2 marrow rejections in the DFO group and 1 in the control group.

Regarding the time to marrow recovery, the median time for neutrophil, platelet and erythrocyte recovery was not significantly different in DFO-treated patients compared with the control group; patients who received DFO continuously through the entire period of marrow infusion, marrow aplasia and early engraftment showed a delay in neutrophil recovery compared to those who received DFO discontinuously (17 days vs. 15). Although this difference did not reach statistical significance, the effect is most probably due to the antiproliferative properties of the drug and suggests that DFO treatment should be avoided during the immediate peri-transplant period.

The outcome of SF during the post-transplant follow-up in this group of patients is also interesting; we observed that patients who received DFO had a significantly lower median SF value at 6 months after transplant than those patients who did not receive DFO. The behavior of SF in the control group during the post-transplant follow-up is also similar to that seen in a more extensive series of transplanted patients reported in the literature.⁵

Based on these data, we suggest that intravenous DFO therapy during BMT may prevent the increase in iron overload related to the intensive blood support during this period. The

drug probably also acts by binding iron that is acutely mobilized by cytoablative agents.

The long-term outcome of iron overload after BMT for thalassemia has been studied with increasing interest⁸ in recent years; iron removal therapy has been proposed for those patients who maintain heavy liver iron deposits for years after BMT and these new therapeutic approaches are currently under study.¹⁶⁻¹⁸ On the other hand, intensive intravenous chelation therapy may also be considered for thalassemic patients with heavy iron overload in the pre-transplant period. In fact, the lack of toxicity and teratogenicity of DFO has recently been noted.¹⁹

Although this preliminary experience with DFO therapy on the peri-transplant period is very limited, it may contribute to our understanding of the possible beneficial effects as well as the possible clinical indications and toxicity of this therapy for patients with iron overload submitted to bone marrow transplant. The optimal timing and duration of iron removal therapy in these patients remains, for many aspects, an open discussion.

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