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ORIGINAL ARTICLE

Biochemical monitoring after haemopoietic stem cell transplant for Hurler syndrome (MPSIH): implications for functional outcome after transplant in metabolic disease

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Hurler Syndrome is corrected by allogeneic BMT by the action of donor enzyme on recipient tissue. In this paper, we describe monitoring of 39 patients transplanted in two centres to determine donor chimerism, enzyme level and residual substrate – expressed as dermatan sulphate to chondroitin sulphate ratio. We show that in fully engrafted recipients, the enzyme level, expressed as μmol/g total protein/h, post-transplant is 24.2 from an unrelated donor and 10.2 from a heterozygote family donor ($P<0.0001$). There is a tight relationship between mean post-transplant enzyme level and residual substrate – Spearman's rank correlation coefficient (Rho) was −0.76 and −0.80 at 12 and 24 months, respectively ($P<0.0001$). We propose that these differences affect patient outcome. As unrelated donor transplant outcomes improve and especially given the higher levels of donor cell engraftment following cord transplants, our data might influence donor selection where only heterozygote-matched family members are available.

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Introduction

MPSIH (Hurler Syndrome) is an autosomal recessive disorder caused by deficiency of a lysosomal enzyme, α-iduronidase leading to the accumulation of its catabolic substrates, heparan and dermatan sulphate (DS) in tissues. This results in progressive multi-organ dysfunction and death in early childhood.[1]

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Allogeneic haematopoietic stem cell transplantation is an effective therapy for the condition as engrafted donor leucocytes secrete enzyme, which is taken up by deficient host cells. The ability of secreted enzyme to correct such deficient host cells is known as cross correction[2] and forms the basis for the correction of metabolic diseases by both transplantation and exogenous enzyme replacement therapy.[3]

The efficacy of donor cell engraftment has been well documented in Hurler Syndrome, the commonest metabolic indication for transplant.[4] Many factors, including genotype, donor status, age at transplant, peritransplant complications and post-transplant care and schooling have influenced the long term outcome of these patients.

The incidence of mixed chimerism and graft failure in MPSIH transplant is, however, relatively high,[5–7] especially with reduced intensity preparative regimens and T-depleted grafts,[8] necessitating second transplant in a significant proportion. The aim of this paper was to review the graft outcome in terms of circulating enzyme levels and residual substrate in an effort to clarify the optimal choice of donor for this metabolic disorder.

Materials and methods

Patients

A total of 39 patients from two transplant centres were serially monitored in the same laboratory following allogeneic transplant. All were at least 1-year post-transplant. The genotype of each patient was known.

Donors

Donors were classified as related or unrelated and the related further classified as normal (unaffected) or heterozygote (carrier). Unrelated donors were selected on the basis of high resolution typing at HLA class I and II for adult donors and low resolution class I and high resolution for class II for cord donors as is current practice. All unrelated donors were assumed to be unaffected.

Monitoring the graft outcome
The graft is serially measured in all patients in the following way:

Assay for α-L-iduronidase activity
The activity of α-L-iduronidase was measured on leucocytes isolated from peripheral blood at diagnosis and monitored on a regular basis post-transplant. Leucocyte lysates were prepared and assayed for α-L-iduronidase activity using the fluorescent substrate 4-methylumbelliferyl-α-L-iduronide (Glycosynth, Warrington, UK) as described previously.[9] Iduronidase activity was expressed as μmol/g total protein/h (normal reference range 10–50, heterozygote range 5–25).

Measurement of urinary glycosaminoglycans
The total urinary glycosaminoglycans (GAGs) were measured using dimethyl methylene blue as described previously. Extracted GAGs were analysed by two-dimensional electrophoresis based on the method of Whiteman.[10]

The ratio of DS to chondroitin sulphate (CS) was obtained by measuring the size and density of spots seen from two-dimensional electrophoresis of extracted GAGs. The spots were measured under white light using the UVP Gel Photography System and the LabWorks software (Anachem, Luton, UK) and the ratio of DS/chondriotin sulphate was calculated from the data obtained. A representative pattern of GAG from a normal subject, an untreated MPS subject and a patient after transplant is shown in Figure 1.

Variable number tandem repeat analysis
Engraftment status was assessed by polymerase chain reaction analysis. DNA was extracted from peripheral blood and amplified for the following variable number tandem repeat (VNTR) alleles; Apo B, D1S80, COL2A1 and MD as described previously.[11] For each analysis, the patient DNA was analysed both pre- and post-transplant along with DNA from the donor. The resultant amplification products were separated using agarose gel electrophoresis with ethidium bromide and the migration pattern was analysed under ultraviolet light. Quantitative analysis was performed on the amplification products using the UVP Gel Photography System and the LabWorks software

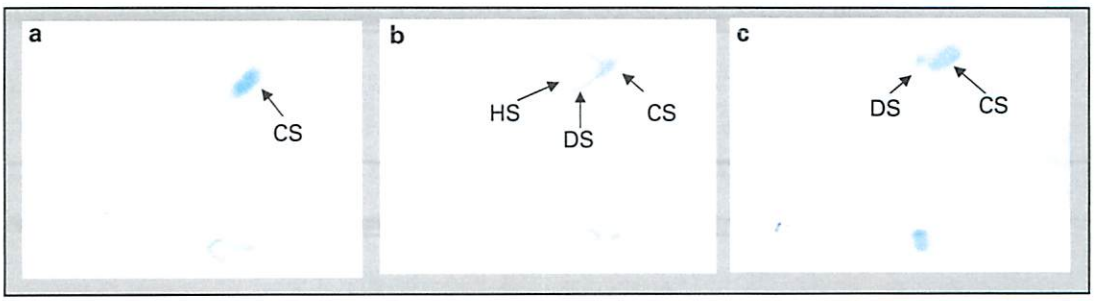

Figure 1 Two-dimensional electrophoresis of extracted GAGs of normal subjects and MPS subjects before and after transplant. Children with MPS I show grossly elevated amounts of urinary DS and trace amounts of heparan sulphate (HS) (**b**) as compared with normal individuals (**a**) who show only CS. After HSCT, the amount of DS detected in the urine decreases with time (**c**); this can be analysed as a semiquantitative technique and expressed as a DS/CS ratio.

Table 1 Biochemical data from the four patient groups after 12 months of stable engraftment

Patient Group	*Iduronidase activity μmol/g/h (range and mean)*	*GAGs DS/CS ratio (range and mean)*
Group 1 $n=19$ MUD = 8, MUC = 6, (Normal sib = 5)	10.7–42.0 mean = 24.8	0.2–0.77 mean = 0.38
Group 2 $n=8$	5.3–20.5 mean = 10.2	0.5–0.87 mean = 0.67
Group 3 $n=5$ (MUD = 4, normal sib = 1)	7.1–38 mean = 17.0	0.31–0.71 mean = 0.49
Group 4 $n=8$	7.4–13.2 mean = 7.1	0.45–1.06 mean = 0.77

Abbreviations: CS = chondroitin sulphate; DS = dermatan sulphate; GAG = glycosaminoglycan; MUC = matched unrelated cord; MUD = matched unrelated donor.
The biochemical data is averaged enzyme level following transplant and residual storage product, expressed as DS/CS ratio.
Group 1 where a normal donor is used – MUD, MUC or normal family donor – and there is full donor chimerism. No difference in engrafted enzyme level was seen between unrelated cord ($n=6$, range 12.5–34.6, mean = 24.3) and unrelated adult donors ($n=13$, range 10.7–42.0, mean = 25.1) with full engraftment. In Group 2, a heterozygous family donor has been used and there is full donor chimerism. In Group 3, a normal donor is used and there is mixed chimerism (range 20–90% donor, median 70%, mean 62%). No mixed chimerism was seen where in recipients of unrelated cord transplants. In group 4, there is mixed chimerism from a heterozygous donor (range 20–70% donor, median 55%, mean 50%).

(Anachem, Luton, UK) and the data obtained was used to calculate the percentage donor pattern observed in patients with a chimeric graft.

Patterns of graft outcome

According to the VNTR – fully engrafted defined as more than 95% donor cells – and the heterozygote state of the donor, the stable graft outcome was defined as follows:

(i) Normal donor, full donor chimerism
(ii) Heterozygote donor, full donor chimerism
(iii) Normal donor, stable but mixed chimerism
(iv) Heterozygote donor, stable but mixed chimerism

Results

The range and mean post-transplant enzyme level, the range and mean DS/CS ratio and the donor source for the 39 patients from the two transplant centres is summarized in Table 1. The patients are grouped as above according to chimerism (full or mixed) and donor (normal or heterozygote).

The relationship between time from transplant, engrafted enzyme level and residual substrate, expressed as DS/CS ratio, in a typical patient is shown in Figure 2.

The relationship between enzyme levels, expressed as μmol/g total protein/h, in the four transplant groups as defined above is shown in Figure 3. The mean for group 1 – fully engrafted normal donor – is 24.2 and the mean for group 2 – fully engrafted heterozygote donor – is 10.2. This difference is highly significant using a *t*-test ($P<0.0001$).

The relationship between leucocyte α-iduronidase and the DS/CS ratio for each patient was studied and is shown in Figure 4. Iduronidase was taken as the mean over the initial 12 months and the subsequent 12–24 months and plotted against the DS/CS ratio at the end of each time period. Spearman's rank correlation coefficient (Rho) was −0.76 at 12 month, $P<0.0001$ and −0.80 at 12–24 months, $P<0.0001$.

Discussion

These data indicate a markedly different transplant outcome, expressed in terms of deficient enzyme level and residual substrate, depending on the donor utilized and the donor chimerism achieved in allografted MPSIH patients. Where a normal donor was used, there is significantly higher enzyme than where a heterozygous donor is used or where there is mixed chimerism after transplant. This higher enzyme level is associated with significantly reduced substrate.

Considerable variability in patient phenotype and outcome has been observed after transplant, despite sustained

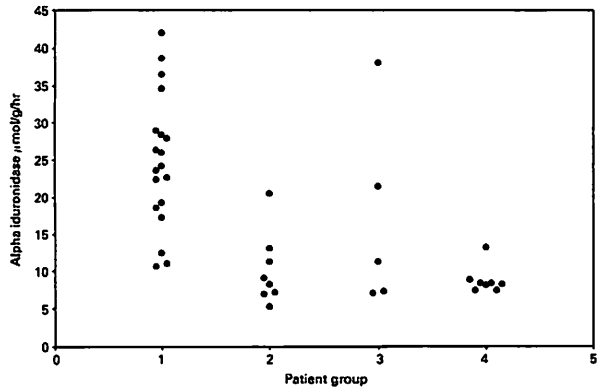

Figure 3 Iduronidase levels with different donors and different donor cell engraftment level. Individual mean α-iduronidase measurements for each patient over the initial 12 months post-HSCT. Group 1 mean = 24.2, Group 2 mean = 10.2, Group 3 mean = 17.0, Group 4 mean = 7.1. Results are expressed as μmol/g total protein/h (normal reference range 10–50, heterozygote range 5–25).

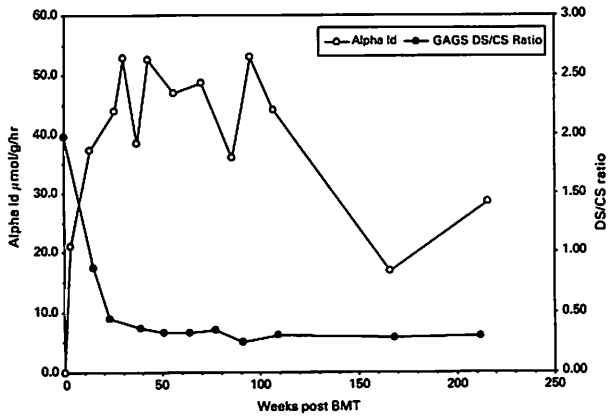

Figure 2 A biochemical profile of an MPS patient who has undergone HSCT. The donor here is a normal related donor, and there is full donor cell engraftment. Leucocyte α-iduronidase activity shows natural fluctuation within the normal range. Residual GAGs have fallen in the initial 6 month period post-transplant, reaching a plateau that shows a DS/CS ratio consistently <0.5. These data are typical of patients showing full engraftment from normal donors.

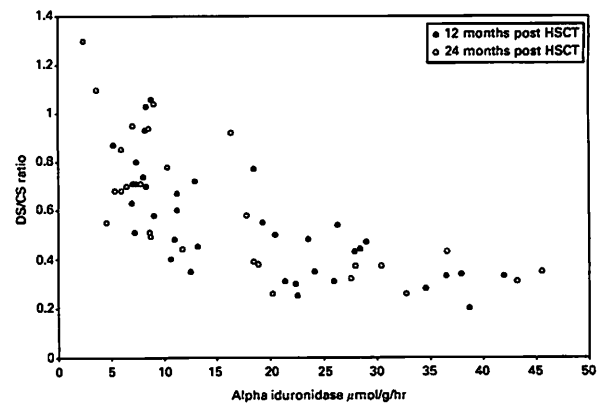

Figure 4 The relationship between leucocyte α-iduronidase activity and the DS/CS ratio. The relationship between leucocyte α-iduronidase activity and the DS/CS ratio for each patient. Iduronidase activity has been taken as the mean over the initial 12 months, and 12–24 months post-HSCT, and plotted against the DS/CS ratio at the end of each time period. Spearman's rank correlation coefficient (Rho) 12 months = −0.759, $P<0.0001$, $n=38$; 12–24 months = −0.792, $P<0.0001$, $n=27$.

engraftment. Although many factors contribute to such variability, these data show that a poorer enzyme level leads to poorer clearance of substrate in the host tissue and probably to a poorer clinical outcome. It is notoriously difficult to determine clinical outcome in these patients after transplant. However, we believe that before our data can be used to influence current transplant practice, including donor selection criteria, clinical outcome scores should be correlated with different variables, including biochemical outcome such as we detail.

These findings will be applicable to other inherited metabolic diseases that respond to allogeneic HSC transplantation and where the metabolic correction is mediated by uptake of secreted enzyme. It is intriguing to speculate that conditions in which transplant has been abandoned for limited clinical efficacy might be more responsive where the transplant is manipulated for optimal outcome with full donor cell engraftment of a normal donor.

One would expect a higher enzyme level where full donor chimerism using an unaffected donor is achieved. There is published data to indicate successful sustained engraftment following cord blood transplant (CBT)[12] and this has been substantiated by the European Group for Blood and Marrow Transplantation (EBMT) group who have noted similar results and reduced mixed chimerism in CBT recipients, relative to other donors (Boelens JJ, Personal Communication, 2006). These observations support the current practice within EBMT of choosing an unrelated cord blood as a first source of stem cells where there is no suitable family donor.[8] The time may have come, however, to consider cord blood as a preferred donor option when only heterozygote matched family donors are available. Clearly more discussion is warranted in this area.

It has been our and others' practice to offer repeat transplant where there is insufficient enzyme (below range for heterozygote) to stabilize GAG reduction.[13] Sustained engraftment has been achieved in this situation with second transplant, using a reduced intensity conditioning procedure and is preferred to donor lymphocyte infusion. Data in this study would appear to support this strategy.

This report emphasizes the relationship between engraftment status and clearance of substrate. Further multicentre, long-term data are urgently required to investigate the long-term benefit of HSCT in MPS1, so that optimal levels of enzyme can be sustained and quality of life improved for this group of patients.

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