Hematologic response in type I Gaucher's disease after enzyme replacement therapy

Sir,

Gaucher's disease is a sphingolipidosis characterized by recessive autosomal transmission. It is often accompanied by normochromic normocytic anemia which almost never requires transfusion therapy. Continued therapy using enzyme replacement is currently a treatment choice for this rare and as yet little studied disease. The neurologic and liver damage linked to metabolic abnormalities are particularly poor understood.[1-4] We monitored hemoglobin and reticulocyte behavior after 6 months of enzyme replacement therapy in 13 patients, age between 6 and 46 years, with type I non-neuropathic Gaucher's disease followed in our Institute. The patients were divided into two groups by age; one group of 7 patients aged between 6 and 18 years, the other group of 6 patients aged between 19 and 46 years (no longer pediatric, but continuing to reveice treatment at our Gaucher's Disease Center). Reticulocyte RNA was stained and examined under Argon light using a Dasit® 3000 analyzer. The reading is based on the principle of cytometric flow and provides, both quantitative (absolute and percentage values) and qualitative information on reticulocytes, breaking them down into three subgroups according to intensity of fluorescence and, therefore, state of maturity (high fluorescence-HFR, middle fluorescence-MFR, low fluorescence-LFR). Blood samples were stored with EDTA 2K and analyzed within two hours of being taken. The Wilcoxon test for paired data, before and after therapy, was used for statistical analysis.

After 6 months of enzyme therapy the hematologic findings of the first group (Table 1) had improved, with increased hemoglobin and MCHC and a reduction in both total reticulocytes and HFR; conversely, the number of LFR increased. In the second group, however, (Table 2), hemoglobin did not change significantly following therapy and the patients seemed

Table 1. Variations of hematologic parameters before (1) and after 6 months (2) of enzyme replacement therapy in 7 young patients affected by Gaucher's disease type I with normal levels of serum ferritin.

	Reticulocytes								RBC data						Ferritin
Pts.	Total 1 $10^6/\mu L$	Total 2	LFR 1 %	LFR 2	MFR 1 %	MFR 2	HFR 1 %	HFR 2	Hb 1 g/dl	Hb 2	MCV 1 fL	MCV 2	MCHC 1 g/dL	MCHC 2	2 ng/mL
1	0.0702	0.0755	92.2	96.2	7.4	3.8	0.4	0	11.1	11.8	85.9	81.9	31.6	35.4	344.1
2	0.0533	0.0517	73.9	98	21	2	5.1	0	13.1	14.4	93.6	97.2	30.4	31.9	278.5
3	0.0727	0.0477	88.3	95.6	9.9	4.4	1.8	0	12.9	13.4	79.4	84.4	33.6	34	283.1
4	0.0408	0.0393	77.9	92.1	19.4	7.2	2.7	0.7	12.1	14.3	86.3	93.1	31.2	32.2	52.2
5	0.0709	0.066	80.7	90.5	15.7	9	3.6	0.5	11.7	13	77.4	76.6	31.4	35.1	18.6
6	0.076	0.05	81.7	95.3	16.2	4.7	2.1	0	13.2	15.1	80.2	86.9	34.6	35.1	243.5
7	0.0717	0.0325	83.9	96.9	13.9	3.1	2.2	0	10.9	12.5	77	83.5	31.6	32	144.5
mean	0.065	0.052	82.7	94.9	14.8	4.9	2.6	0.2	12.1	13.5	82.8	86.2	32.1	33.7	194.9
S.D.	0.013	0.015	6.2		4.9		1.5		1.0		6.0		1.5		124.8
p 1 vs 2	0.054		<0.05		<0.05		<0.05		<0.05		NS		<0.05		

NS: not significant.

Table 2. Variations of hematologic parameters before (1) and after 6 months (2) of enzyme replacement therapy in 6 older patients affected by Gaucher's disease type I with high levels of serum ferritin.

	Reticulocytes								RBC data						Ferritin
Pts.	Total 1 $10^6/\mu L$	Total 2	LFR 1 %	LFR 2	MFR 1 %	MFR 2	HFR 1 %	HFR 2	Hb 1 g/dL	Hb 2	MCV 1 fL	MCV 2	MCHC 1 g/dL	MCHC 2	2 ng/mL
1	0.0720	0.0335	80.3	86.8	18.2	11.5	1.5	1.7	11.7	11.9	98.3	100.5	32.9	32.7	756.5
2	0.0841	0.0632	74.8	91.4	21.6	8.6	3.6	0	14.3	14	94.2	94.9	33.3	31.9	901.1
3	0.1342	0.0840	60.8	76.5	25.6	22.4	13.6	1.1	12.9	12.4	100.4	97.7	30.6	31.3	2415.2
4	0.0101	0.0776	82.3	95.4	16.1	4.6	1.6	0.0	12.9	12.5	97.2	93.5	30.8	31.6	1431
5	0.0580	0.0435	69.4	91.3	23.3	8.1	7.3	0.6	14.5	12.2	99.6	92.9	33.9	27.4	622.1
6	0.1021	0.0600	84.9	87.4	13.9	11.4	1.2	1.2	13.7	13.9	92.6	92.8	33.6	33.7	420.8
mean	0.0768	0.0603	75.4	88.1	19.8	11.1	4.8	0.8	13.3	12.8	97.1	95.4	32.5	31.4	1091.1
S.D.	0.0420		9.1		4.5		4.9		1.0		3.1		1.4		733.0
p	NS		<0.05		<0.05		NS		NS		NS		NS		

NS: not significant.

to store iron, although hemoglobin values were on the borderline of normal. The number of HFR decreased, but not significantly. The mean values of serum ferritin in the first group of patients was 194.9±124.8 ng/mL, while it was 1091.1±733 ng/mL in the second group.

We believe that the improvement shown in hematologic and reticulocyte parameters in the group of younger patients with normal serum ferritin supports the hypothesis of effectiveness of enzyme replacement therapy.[5,6] The reason for the high levels of serum ferritin in the second group of patients remains to be clarified, but evidence that these levels occur in patients who can no longer be considered *pediatric*, and who have slight hepatosplenomegaly, suggests that these may be due to spleen damage from myeloatrophic-like lipid infiltration. In this second group, it is probable that the dosage used in therapy (30 u/kg/day) was not sufficient for adult subjects, whose storage was greater. Accordingly, for some of these patients, we suggest an increase in the dosage of enzyme replacement therapy and, if necessary, iron chelation therapy with desferrioxamine.

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p53 overexpression in refractory anemia. An immunohistochemical analysis of bone marrow biopsies

Sir,

Genetic abnormalities are common in myelodysplasia (MDS) but molecular mechanisms underlying the latter's genesis and evolution are still largely unknown.[1] The characterization of p53 tumor suppressor gene in MDS patients revealed alterations in 5% to 10% of cases[2-6] which were almost exclusively seen in more advanced phenotypes.

Bone marrow biopsies obtained at diagnosis from 19 patients with MDS in refractory anemia (RA) phase were immunohistochemically studied. The patients' age at diagnosis was in the range from 20 to 82 years. Nine of the patients were men (47.4%). Using a technique for antigen retrieval based on microwave oven heating and an alkaline phosphatase anti-alkaline phosphatase complex, p53 overexpression was detected in two cases (10.5%). P53 positive cells constituted 36% and 39% of total bone marrow cells (Figure 1). Five patients (26%) evolved to a more advanced subtype or overt acute leukemia , including the two cases with abnormal p53 expression (Table 1). Time to progression was 3 and 4 months for the p53-positive patients and 48, 11 and 30 months for the p53-negative patients. RA cases that progressed to a more aggressive subtype later exhibited a significantly higher frequency of p53 overexpression (2/5-40%) than cases that did not transform (0/14-0%) ($p<0.001$).

Immunohistochemical analysis may often tell us more about the functional status of the p53 control pathway than DNA sequencing does.[7] Although mutations of p53 gene can be inferred from immunohistochemical detection of accumulated mutant protein, there is not an absolute correlation. In general, detection by molecular methods yields lower frequencies of abnormality than immunostaining.[8] Possible reasons include: a) a minor subclone, below the detection level of single strand conformation polymorphism analysis, but detected by immunologic techniques, had acquired a mutation; b) non-muta-

Table 1. Summary of patients with disease progression and correlation to p53 status at initial diagnosis.

Case	*Sex/Age*	*Disease progression*	*p53 status**
2	M/22	RAEB	+
5	F/20	RAEB-t	–
7	M/35	RAEB	–
9	M/63	RAEB	–
11	M/68	AML	+

**+ ≥ 5% of positive cells; – = < 5% of positive cells*