Bone marrow and peripheral blood globin chain synthesis in sickle cell β^0 thalassaemia

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SUMMARY A similar imbalance of globin chain synthesis, with low non- α/α ratios, was shown in peripheral blood and in bone marrow of compound heterozygotes for both the Hb S and β^0 thalassaemia genes (S/ β^0 thalassaemia). Previous purification of whole cell globin obtained from the bone marrow did not change the non- α/α ratio. The mean non- α/α ratios were 0.57 ± 0.13 ($\bar{x} \pm SD$) for the peripheral blood of 12 patients, 0.52 ± 0.10 for five patients using bone marrow globin purified on Sephadex G100, and 0.55 ± 0.16 for the unfiltered bone marrow globin of five patients. The data show that patients with S/ β^0 thalassaemia have a similar β chain deficiency in reticulocytes and in bone marrow cells, provided whole cell globin is used which avoids the removal of the free α chains. The non- α/α ratios in the peripheral blood of an S/ β^0 thalassaemia patient and a β thalassaemia heterozygote from the same family were compared in seven families and no significant difference was found.

Persons heterozygous for both the haemoglobin S and the β thalassaemia genes (S/ β thalassaemia) display a clinical picture that ranges from mild to severe sickle cell syndrome.¹⁻³ Globin chain synthesis in the peripheral blood of these patients is unbalanced irrespective of the type of β thalassaemia gene inherited, that is, β^+ or β^0 .^{2 4-7} In contrast, the results obtained from bone marrow cells are controversial, since both reduced and normal non- α/α ratios have been reported.^{4 6 8-13} The same discrepancy between peripheral blood and bone marrow globin chain synthesis has been observed in heterozygous β thalassaemia.^{10 13-18}

This paper describes the results of bone marrow and peripheral blood globin chain synthesis in a homogeneous group of S/ β^0 thalassaemia patients and a comparison with simple β thalassaemia heterozygotes from the same families.

Patients and methods

The study included 12 patients with S/ β^0 thalassaemia, seven relatives with heterozygous β^0 thalassaemia, and five Hb S homozygotes. Controls consisted of 10 subjects with idiopathic thrombocytopenic purpura, autoimmune haemolytic anaemia, hereditary spherocytic anaemia, or gastrointestinal bleeding. Consent was obtained from all patients (or

from one parent) after full explanation of the procedure. Diagnosis was based on clinical and laboratory data, including haematological findings, electrophoresis on starch gel at pH 8.9 and agar gel at 6.1, quantification of Hb A₂ by elution after electrophoresis on cellulose acetate, measurement of Hb F by alkali denaturation, and solubility of the haemolysate in concentrated phosphate buffer. Diagnosis of S/ β^0 thalassaemia in each case was confirmed by family study, by demonstrating a β thalassaemia gene in one parent or in one sib.³ Haematological data are shown in table 1.

Peripheral blood globin chain synthesis was measured in 34 subjects. After centrifugation of the red cells at 15 000 g for 60 minutes at 4°C, reticulocytes were washed twice in reticulocyte saline (RS) (130 mmol/l NaCl, 5 mmol/l KCl, 7.4 mmol/l MgCl₂·6H₂O), and incubated for one hour at 37°C in the presence of 50 to 100 μ Ci L-[4,5-³H]-leucine.¹⁹ For measuring bone marrow globin synthesis, about 0.5 ml bone marrow obtained by aspiration was diluted with 5 ml RS containing 1000 units heparin and washed twice in RS. After incubation, the cells were washed and lysed by freezing and thawing and the addition of distilled water. Whole cell globin was prepared by acetone-HCl precipitation immediately after the completion of the labelling experiment. Whole cell globin (30 to 60 mg) from bone marrow of five patients was fractionated on a 2.5 cm²×50 cm column of

TABLE 1 Haematological data and globin synthesis ratios in whole cell globin samples from S/β^0 thalassaemia, sickle cell anaemia, and controls.

Case	Hb (g/dl)	MCH (pg)	Hb F (%)	Hb A ₂ (%)	Non-α/α ratio (total counts)		
					Peripheral blood	Bone marrow	
						Original	After Sephadex
<i>S/β° thalassaemia</i>							
1	9.0	23.7	13.5	3.9	0.41	0.45	0.39
2	7.2	24.0	6.3	4.1	0.64	0.43	0.55
3	8.6	21.0	7.8	6.0	0.76	0.53	0.50
4	8.8	25.1	6.2	4.8	0.52	0.82	
5	8.5	21.8	9.9	6.4	0.61	0.51	
6	8.4	22.7	2.9	5.4	0.41		0.68
7	8.6	20.1	7.9	5.6	0.42		0.50
8	4.6	24.2	6.9	4.2	0.67		
9	8.5	26.6	21.4	3.9	0.52		
10	9.5	22.6	1.0	5.8	0.77		
11	5.5	22.5	9.5	3.5	0.65		
12	8.7	20.2	5.7	5.6	0.52		
Mean±SD	8.0±1.5	22.9±1.9	8.2±5.2	4.9±1.0	0.57±0.13	0.55±0.16	0.52±0.10
<i>SS homozygotes (n=5)</i>							
Mean±SD	8.5±0.9	33.4±3.1	4.7±3.4	2.8±0.3	1.00±0.08	0.98±0.12*	
<i>Controls (n=10)</i>							
Mean±SD	10.0±2.8	30.4±3.4	0.7±0.3	2.7±0.4	1.02±0.08	1.10±0.12†	

*n=4, †n=8

TABLE 2 Comparison of peripheral blood non- α/α ratios for an S/β^0 thalassaemia patient and a β thalassaemia heterozygote from the same family.

Family	Non- α/α ratio (total counts)	
	Patient	Relative
01	0.41	0.45
02	0.64	0.47
05	0.61	0.51
06	0.41	0.38
08	0.67	0.80
10	0.77	0.51
11	0.65	0.43
Mean $\pm$ SD	0.59 $\pm$ 0.13	0.51 $\pm$ 0.14

Sephadex G100²⁰ in 1% v/v formic acid at a flow rate of 0.15 ml/minute at room temperature. Blue Dextran was added as a marker and a considerable proportion of the radioactivity eluted with the unretarded blue Dextran peak. All material that eluted after this initial fraction was freeze dried and subsequently analysed on CM-cellulose. Globin separation was carried out by CM-cellulose chromatography.¹⁹ The non- α/α ratios were calculated on the basis of the total count in each entire chromatographic peak corresponding to the γ , β , β^S , and α chains.

Results

The results of the globin synthesis ratios are summarised in table 1. The non- α/α ratios in peripheral blood were 0.57 $\pm$ 0.13 (mean $\pm$ SD) for 12 patients with S/β^0 thalassaemia, 1.00 $\pm$ 0.08 for five Hb S homozygotes, and 1.02 $\pm$ 0.08 for 10 controls.

The morphological observation of bone marrow smears from S/β^0 thalassaemia patients showed a significant erythroid hyperplasia and the specific activity of the globin obtained from bone marrow was five to 100 times higher than that of peripheral blood of the same patient. Bone marrow globin synthesis ratios were measured in whole cell globin from seven patients with S/β^0 thalassaemia, and there was no significant difference between the mean non- α/α ratios in globin after purification on Sephadex G100 and those in unfiltered globin (0.52 $\pm$ 0.10 and 0.55 $\pm$ 0.16 respectively). These values are similar to those observed in peripheral blood of the same patients ($p>0.05$, rank sum test on paired data).

Discussion

Although the β thalassaemias are highly heterogeneous at the molecular level, these Brazilian patients with S/β^0 thalassaemia represent a

homogeneous group, at least from the ethnic standpoint, since in all cases the β^0 thalassaemia gene was of Italian origin. Furthermore, the globin synthesis ratios analysed in the present study were not strictly dependent on the molecular defect of the β^0 thalassaemia gene since no synthesis of β^A chains was observed. The occurrence of α thalassaemia has not been excluded, but this does not invalidate our results because α thalassaemia would be expected to increase the non- α/α ratios.

Our results for seven patients with S/ β^0 thalassaemia show that the imbalance of globin synthesis in bone marrow is equivalent to that found in peripheral blood reticulocytes, provided whole cell globin is used, which avoids the removal of free α chains. Balanced globin synthesis in bone marrow has been reported for most of the cases of S/ β thalassaemia studied so far; the majority of the patients had S/ β^+ thalassaemia.^{4 6 8-12} The question of globin synthesis in bone marrow of carriers of a single β thalassaemia gene (simple heterozygotes and S/ β thalassaemia compound heterozygotes) has been complicated by conflicting results since the report of balanced synthesis by Schwartz.¹⁶ The majority of subsequent studies confirmed the finding of balanced synthesis¹⁷ and several hypotheses have been presented to explain the discrepancy between the results obtained in peripheral blood and in bone marrow. The apparently balanced synthesis in bone marrow could be the consequence of unstable β thalassaemic mRNA,¹⁰ a compensatory increase of β chain synthesis by the normal β gene in the erythroid precursors,¹⁰ or increased proteolytic activity removing the excess of α chains from these cells.^{13 18} It could also be artefactual or the result of methodological problems such as the presence of non-globin proteins which co-chromatograph with the β chains^{11 19-21} or the preferential loss of α chains during storage, precipitation of globin, or handling of the material. The contamination of the β peak by non-globin proteins, probably of non-erythroid origin, has been fully documented.²⁰ However, this should not greatly affect the non- α/α ratios of patients with S/ β^0 thalassaemia, since they have a significant erythroid hyperplasia of bone marrow. Moreover, β^S chains elute later than β^A chains during chain separation and Chalevelakis *et al*²⁰ have shown that the bulk of non-globin contaminating proteins run in the early part of the chromatogram. In agreement with this, the filtration of whole cell globin on Sephadex G100 did not significantly change the mean non- α/α ratios of our patients. However, a higher ratio was obtained for one patient in bone marrow (0.82) than in peripheral blood (0.52) with unfiltered globin (case 4, table 1), and Ladas *et al*¹¹ observed that

filtration of globin reduced the bone marrow non- α/α ratio in one of two cases.

The majority of previous studies of globin synthesis in bone marrow of patients with S/ β thalassaemia and of β thalassaemia heterozygotes have been carried out with stroma free globin.^{4 6 8 12 16} So far, unbalanced globin synthesis in bone marrow of patients with β thalassaemia in association with β globin structural variants has been reported for five S/ β^+ thalassaemia patients out of eight investigated by Bank *et al*⁴ and Ladas *et al*,¹¹ as well as for one case each of Hb S/ β^0 thalassaemia,²² Hb D/ β^0 thalassaemia,²³ and Hb E/ β^0 thalassaemia.²⁴ In six of these cases the studies were carried out with whole cell globin. Thus, contamination of the β chain peak and proteolysis of the α chains may contribute in part to changing the ratio towards balance, but it seems that the main reason for obtaining a balanced non- α/α ratio in bone marrow from S/ β thalassaemia patients is the use of stroma free globin with removal of the free α chains with the stroma.

Our results, showing an unbalanced globin synthesis in the bone marrow of S/ β^0 thalassaemia patients, are in agreement with those reported by Chalevelakis *et al*²¹ for simple β thalassaemia heterozygotes. This finding is not unexpected since peripheral blood β^S/α and β^A/α ratios, respectively, are similar for the two diseases.

Finally, the comparison of globin chain synthesis in peripheral blood of patients with S/ β^0 thalassaemia and that of their relatives with heterozygous β^0 thalassaemia has shown that the imbalance of chain synthesis was similar for the two groups (table 2). This finding represents indirect evidence that the genes in trans position with the thalassaemia gene (β^S or β^A , respectively) have similar synthetic activities.

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