LIPID PEROXIDATION AND SPECTRIN OF RBC MEMBRANE IN HYDROXYUREA TREATED $E\beta$ THALASSAEMIA.

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ABSTRACT

The aim of the present work is to understand the effect of low dose of hydroxyurea (HU) therapy on oxidative damage of RBC membrane in non-transfused $E\beta$ thalassaemia. HU was administered at a dose of 30 mg/kg/day for 90 consecutive days. The percentage of spectrin and the level of malondialdehyde (MDA), fetal hemoglobin (HbF), hemoglobin (Hb) and packed cell volume (PCV) were measured. The HbF level was significantly increased after 90 days of HU therapy. MDA level of RBC membrane was decreased. There was no change in PCV, Hb and spectrin content of RBC membrane after HU therapy for 90 days in $E\beta$ thalassaemia. Increment of HbF in HU treated $E\beta$ patient may have some role on the correction of oxidative damage of RBC membrane by inhibiting further degradation of spectrin and by decreasing lipid peroxidation of red cell membrane.

KEY WORDS: Hydroxyurea; Spectrin; Lipid Peroxidation; $E\beta$ thalassaemia.

INTRODUCTION

Stimulation of fetal hemoglobin (HbF) synthesis by hydroxyurea (HU) in experimental animals and in pilot studies of patients with sickle cell disease (1) prompted a series of clinical trials of this agent in sickle cell disease and β thalassaemia (2). HU therapy is associated with significant reduction in painful crises, occurrence of acute chest syndrome and the frequency of transfusion (3). Olivieri (2) stated that chronic use of HU may improve the clinical phenotype of sickle disease. Rodger (4) stated that HU augments HbF levels and improves ineffective erythropoiesis in β thalassaemia/ HbF patients who are transfused before or during the course of HU therapy. The transfused patients may take a longer period of time to evidence their response than non transfused patients.

Spectrin, mainly the erythrocyte membrane skeleton protein, has an ability to maintain the normal shape (5) and is intimately related to the maintenance of phospholipid asymmetry across the erythrocyte membrane bilayer (6). Erythrocytes exposed to higher oxidative stress reflect increased production of 'activated oxygen' or free radicals (7). This activated oxygen increases malondialdehyde (MDA), a secondary breakdown product of lipid peroxidation (8).

The objective of the pilot study is to determine whether low dose of HU therapy in $E\beta$ thalassaemia is associated with the significant reduction of lipid peroxidation of RBC membrane and is also associated with inhibition of further damage of spectrin of RBC membrane cytoskeleton with the increment of HbF.

MATERIALS AND METHODS

The study was with 20 patients of non-transfused

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E β thalassaemia patients. The patients were diagnosed after clinical and hematological investigation which included hemoglobin (Hb) concentration (g/dl), red cell morphology, osmotic fragility test, alkali-resistant Hb estimation and agarose gel electrophoresis of Hb with suitable modification. HU was administered at a dose of 30 mg/kg/day for 90 days consecutively in E β thalassaemics. The consent of all patients and our institute ethical committee clearance were obtained.

Phenylmethyl sulfonyl fluoride (PMSF), DFP, leupeptin, pepstatin, spectrin, sodium dodecyl sulphate (SDS), 2-mercaptoethanol, Tris, EDTA, bromophenol blue, acrylamide, coomassie blue R-250, coomassie blue G-250, bovine serum albumin (BSA) were procured from Sigma Chemical Co, St Louis, Missouri U.S.A. All other reagents used were of analytical grade and were purchased from E. Merck, Germany. Neodrea used as hydroxyurea therapy in the patients was procured from Neon Antibiotics Private Ltd, Calcutta.

Venous blood was collected in EDTA for Hb, HbF and packed cell volume (PCV) estimation and in acid-citrate-dextrose solution for the assay of MDA and spectrin. This sample was collected before HU therapy and after 90 days of treatment. The packed red cells were isolated by centrifugation at 3,000 rpm at 4°C. To red blood cells proteolysis inhibitors (final 2 mmol/L DFP, 10 μ g/ml of leupeptin and pepstatin), were added with gentle shaking and incubated at 37°C for 30 mins (9). Washed red cells were hemolyzed in 5mM sodium phosphate buffer (pH 8.0) containing 100 mg/L of PMSF and 0.1 mmol/L EDTA and centrifuged at 15,000 rpm repeatedly until colourless membranes appeared (10). The RBC membrane ghosts were finally suspended in the same buffer at a concentration of about 3-4 mg of protein/ml and stored at -20°C.

Polyacrylamide gel electrophoresis (PAGE) in the

presence of SDS and 2-mercaptoethanol was performed as described by Laemmli (11) using 50 μ g of protein in each lane. The gel was stained with coomassie blue R-250 and scanned by Beckman Appraise Densitometer (USA) at 600 nm.

MDA by thiobarbituric acid method (12) and protein by dye - binding method (13) of RBC membranes were analysed. HbF is usually assessed in haemolysates by its resistance to alkali denaturation (14). PCV was analysed by cell counter (Sysmex, K. 1000, Japan).

RESULTS AND DISCUSSION

Clinical response associated with administration of low dose of HU in E β thalassaemia has been recorded in 12 patients without blood transfusion. In these non-transfusion dependent E β thalassaemia patients treated with HU for 90 days consecutively, substantial increase in HbF synthesis was observed without an increase in total Hb concentration and PCV (Table 1). The explanation for a lack of increase in total Hb in the face of substantial increase in HbF during HU therapy is not obvious. Possibly, treatment with HU may have a broader effect on globin gene expression (2).

MDA level was decreased after 90 days of HU therapy in E β thalassaemia (Table 1). The α and β bands of spectrin did not show further degradation after 90 days of HU treatment (Table 1, Fig. 1). In studies of quantitative changes in lipid peroxidation and spectrin of RBC membrane after 90 days of HU treatment in E β thalassaemia, HU was shown to be associated with a significant decrease of lipid peroxidation and protect further degradation of spectrin of RBC membrane cytoskeleton. This pilot study indicates that HU therapy may influence fetal hemoglobin production in erythroid cells which may have some role on the correction of oxidative damage of RBC membrane.

Table 1. Hb, HbF, PCV, MDA, α and β spectrin of RBC membranes 90 days after HU treatment in E β thalassaemia (n=20).

Treatment (days)	Hb (g/dl)	HbF (%)	PCV (%)	MDA (nmoles/mg protein)	Spectrin(%)	
					α	β
0 (n=20)	5.8 $\pm$ 0.2**	16.7 $\pm$ 2.6	20.1 $\pm$ 1.1	0.74 $\pm$ 0.01	6.3 $\pm$ 0.2	7.3 $\pm$ 0.2
90 (n=20)	5.6 $\pm$ 0.4 (NS)	27.8 $\pm$ 4.4 P<0.05	20.8 $\pm$ 1.1 (NS)	0.53 $\pm$ 0.02 P<0.005	7.0 $\pm$ 0.2 (NS)	8.0 $\pm$ 0.2* (NS)

*Dose 30 mg/kg/day, **Mean $\pm$ SE, NS -Non significant.

Therefore, in the management of E β thalassaemia, HU therapy may reduce dependence on blood transfusion and may improve overall survival.

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Fig. 1. SDS-PAGE analysis of red cell membrane of E β thalassaemia after 90 days consecutive treatment with hydroxyurea.

- A α band of spectrin (2,40,000 dalton M.W.)
 B β band of spectrin (2,20,000 dalton M.W.)
 2. 0 days of HU treatment of E β thalassaemia.
 3. 90 days after HU treatment of E β thalassaemia Control.

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