ADA ACTIVITY AND dATP LEVELS IN ERYTHROCYTES AFTER BONE MARROW TRANSPLANTATION

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1. INTRODUCTION

Adenosine deaminase (ADA) is an enzyme of the purine catabolic pathway essential for the degradation of deoxyadenosine (dAdo) derived from the turnover of nucleated cells, principally lymphocytes, in humans ¹. A deficiency of ADA results in life-threatening severe combined immunodeficiency in patients with the severest phenotype ². Consanguineous marriages are also a common finding in this group. Although the majority of patients with ADA deficiency present clinically from birth up to two years, milder forms are now known and the defect has been identified in adults diagnosed as late as the mid-thirties ²,³. Various treatment modalities have been tried, mostly with limited success. The treatment of choice is still by allogenic bone marrow transplantation (BMT) ⁴. Enzyme replacement therapy is an alternative option for those for whom no compatible donor can be found.
(see this symposium). Somatic gene therapy has been attempted by transfecting bone marrow progenitor cells with the ADA gene using a modified retroviral vector but with limited success.

Two biochemical markers are used to monitor the success of either BMT or enzyme replacement: the level of red cell ADA activity, coupled with the concentration of red cell deoxyadenosine triphosphate (dATP). We report studies in several patients before and at regular intervals after successful BMT.

2. METHODS

Blood was collected into EDTA tubes and delivered to the laboratory at room temperature within 24 hours. After centrifuging, the plasma was removed and the red cells washed twice with saline (the buffy coat was removed, but not the top fifth of cells as the sample size was always small). Aliquots of 100µl washed red cells were prepared and stored at –70°C for enzyme assay, or extracted with 200ul of 10% TCA for nucleotide analysis as reported.

The red cell extracts were analysed by ion exchange HPLC. Washed red cells were diluted 1/36 (20µl + 700µl water) freeze-thawed twice, and 25µl incubated 15 min. with adenosine (0.1mM) in 50mM phosphate buffer. The assay products were measured using isocratic ion-pair HPLC.

Patient Treatment: All patients received whole marrow except for patient 1 where the marrow was T-cell depleted. Patient 1 received conditioning with bulsulphan and cyclophosphamide and patient 8 with fludarabine/melphalan/antithymocyte globulin; the others received no conditioning.

3. RESULTS

Two of the patients studied had been treated with PEG-ADA pre BMT, explaining the lower dATP concentrations. In the other patients, the dATP in the red cells fell almost immediately post BMT. The levels then rose to a stable post BMT level after 3-6 months (Fig. 1, Table 1).