THE EFFECT OF DEOXYCYTIDINE AND TETRAHYDROURIDINE IN PURINE NUCLEOSIDE PHOSPHORYLASE DEFICIENCY

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INTRODUCTION

Adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) deficiency have been recognized as the primary cause of an associated immune deficiency syndrome. A number of mechanisms have been proposed to explain the predominant effect of these enzyme deficiencies on the development and function of the lymphoid system. One of the mechanisms concerns the phosphorylation of accumulated metabolic compounds i.e. deoxyadenosine (dAdo) in case of ADA-deficiency and deoxyguanosine (dGuo) in case of PNP deficiency in the lymphoid cells and particularly in thymocytes (1). Indeed increased deoxyATP and deoxyGTP levels have been found in the lymphocytes of ADA- and PNP-deficient patients respectively (2,3). These triphosphates may inhibit the enzyme ribonucleotide reductase which leads to a depletion of deoxyCTP and interference with lymphocytic DNA-synthesis (1).

In ADA-deficiency transplantation of bone marrow derived lymphoid stem cells is the method of choice. However, in most instances no suitable bone marrow donor is available. Enzyme replacement therapy with irradiated normal erythrocytes to lower accumulated dAdo and dGuo has resulted in some ADA- or PNP-deficient patients in improvement of immune function (4,5,6,7). Iron overload and
viral infection (e.g. cytomegalovirus, hepatitis B virus) are some of the complications of this treatment. Administration of deoxycytidine (dCyd), to bypass the ribonucleotide reductase has been suggested as a possibility to overcome the impairment of DNA-synthesis (1). However, a high activity of (deoxy)cytidine-deaminase is found in the mucosa of the gut as well as in the liver and as a consequence orally and intravenously administered dCyd, is rapidly converted to uridine and uracil before reaching the cells where it is needed.

We treated a PNP-deficient patient, born in January 1975, (8), with erythrocyte transfusions for more than 5½ years. This regimen has resulted in a partial restoration of in vitro T cell function. Orally, and lateron intravenously administered dCyd had no additional beneficial effect at all(6). The still existing T cell deficiency is reflected in the clinical condition of the patient: she still shows a vulnerability to infection(9). We therefore have treated the patient again with dCyd, this time in combination with tetrahydro-uridine (THU), a potent non-toxic inhibitor of (deoxy)cytidine deaminase. We present here the metabolic and immunological results during this treatment.

CLINICAL PROTOCOL AND METHODS

Clinical protocol:THU, kindly provided by Dr.Ch.W.Young (Memorial Sloan-Kettering Cancer Center, New York) was administered subcutaneously 50 mg/kg bodyweight/day in 3 doses. dCyd was given from the 3rd day on, 50 mg/kg bodyweight/day in a continuous intravenous infusion, for 3 weeks; during the 4th week the same amount of dCyd was given in a continuous subcutaneous infusion during 10 hours overnight. This enzyme replacement therapy was continued during the administration of THU and dCyd.

At regular intervals the immune status of the patient was assessed as well as the serum content and the urinary excretion of purine- and pyrimidine metabolites according to methods described in detail previously (6,8,10). The (deoxy)ribonucleotide content of the erythrocytes was analyzed by HPLC (11). Perchloric acid extracts of freshly withdrawn blood were made according to Cohen et al. (2) with minor modifications. In order to analyze deoxyribonucleotides the neutralized perchloric acid extracts were treated with sodium periodate according to Garret and Santi (12). 2,3 Di-phosphoglycerate (2,3-DPG) in the erythrocytes was determined as previously described (13). Ecto-5'-nucleotidase on intact lymphocytes was determined as described(14). Adenosine deaminase activity of the lymphocytes was determined essentially according to van Laarhoven et al. (15).