0031-3998/90/2802-0127$02.00/0
PEDIATRIC RESEARCH

Vol. 28, No. 2, 1990
Printed in U.S.A.

Comparison of Red Cell Transfusion and Polyethylene Glycol-Modified Adenosine Deaminase Therapy in an Adenosine Deaminase-Deficient Child: Measurement of Erythrocyte Deoxyadenosine Triphosphate as a Useful Tool

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ABSTRACT. The effect of red cell transfusion and polyethylene glycol-modified adenosine deaminase therapy on biochemical abnormalities, clinical status, and immunologic function in an adenosine deaminase-deficient child was investigated. After red cell transfusions, erythrocyte deoxyadenosine triphosphate (dATP) concentrations decreased about 95% and were closely related to adenosine deaminase activities; deoxyadenosine diphosphate concentrations decreased only approximately 30%. The evolution of dATP levels was also closely related to the improvement in clinical status of the patient. However, immune function was not restored. After polyethyelene glycol-modified adenosine deaminase therapy, the concentration of erythrocyte dATP decreased to undetectable levels correlated with an increase of T lymphocyte counts and an increase of lymphocyte responses to mitogens. Immune functions were restored only when dATP levels were below 15 μmol/L. It appears that red cell transfusion therapy is not sufficiently effective to reduce and maintain erythrocyte dATP levels at values compatible with normal immune function. On the contrary, polyethylene glycol-modified adenosine deaminase therapy is a suitable treatment to reduce dATP levels to near undetectable values, allowing the immune function to be restored. dATP measurement is a very useful tool for monitoring and evaluating the degree of efficiency of therapy in adenosine deaminase deficiency. (*Pediatr Res* 28: 127–130, 1990)

Abbreviations

ADA, adenosine deaminase
dADP, deoxyadenosine diphosphate
dATP, deoxyadenosine triphosphate
PEG-ADA, polyethylene glycol-modified adenosine deaminase
SAH, S-adenosyl homocysteine
SCID, severe combined immunodeficiency disease

Received November 20, 1989; accepted March 10, 1990.
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ADA deficiency is usually associated with SCID and results in clinical, immunologic, and biochemical abnormalities. Biochemical abnormalities are characterized by accumulation of purine metabolites, adenosine, deoxyadenosine, dADP, and dATP, in biologic fluids and cells (1–5). Untreated, this disease is usually fatal during the first few months of life (6).

The therapy of choice for ADA deficiency associated with SCID is transplantation with HLA-histocompatible bone marrow (7, 8).

However, in most instances, no suitable donor is available. Thus, other therapeutic approaches have to be considered. For the past 10 years, repeated transfusions with normal, frozen, irradiated red cells have been used as a form of enzyme replacement therapy (9).

Recently, a new enzyme replacement therapy, polyethylene glycol-modified bovine ADA, has been tested by Hershfield *et al.* (10) in six children who have ADA deficiency and SCID.

In our paper, we compare the effect of red cell transfusion and PEG-ADA therapy on biochemical abnormalities, clinical status, and immunologic function of an ADA-deficient child. Furthermore, using a specific HPLC method for the determination of dATP concentrations, a correlation between the evolution of dATP levels and the evolution of immunologic function is shown.

CASE REPORT AND PROTOCOL

The patient is a 31-mo-old girl hospitalized at birth for cyanosis, respiratory distress, and severe lymphopenia (65 to 100 cells/mm^3). On admission to the hospital, she weighed 2900 g and her ht was 47 cm. At 2 mo, she was diagnosed as an ADA-deficient patient with SCID. ADA activity in erythrocytes was not detectable (<2 nmol/h/mg Hb). Immunologic evaluation revealed a reduced number of T and B lymphocytes and reduced lymphocyte response to mitogens. Ig levels were below normal limits. The child presented severe pulmonary candidiasis requiring intensive care (intubation, assisted ventilation) with repeated episodes of apnea and, indeed, an episode of respiratory arrest. Hypotonia persisted with postanoxic renal failure. The child was transferred to a germ-free unit and kept in protective isolation until the age of 28 mo when she could be discharged from the hospital.

After diagnosis, the child, for whom no histocompatible bone marrow donor was found, received six fetal thymus/liver transplants between the ages of 4 and 10 mo. At 10 mo, the child still suffered from lack of appetite, vomiting, and diarrhea. Her

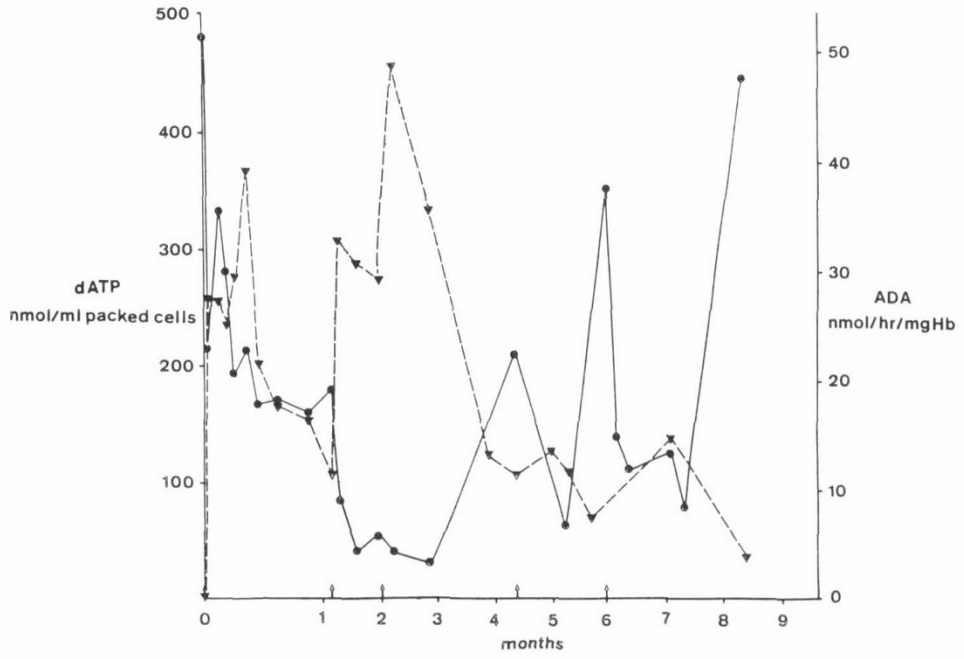

Fig. 1. Relation between erythrocyte dATP concentration (●——●) and erythrocyte ADA activity (▼— — —▼) after red cell transfusions. *Arrows* indicate transfusions.

growth rate was slow (wt, 3400 g; ht, 55 cm). Neurologic examination revealed hypotonia and psychomotor development retardation. Immunologic and biochemical parameters remained abnormal.

Transfusions with irradiated red cells (20 mL/kg body wt) were started at 12 mo and performed monthly for 3 mo and then bimonthly for 5 mo.

Because red cell transfusion therapy failed to restore the immune function significantly, a clinical trial with PEG-ADA was started in our patient. PEG-ADA is a polymer-enzyme conjugate of polyethylene glycol to bovine ADA used for enzyme replacement therapy and supplied by Enzon, Inc. (South Plainfield, IL). The dosing schedule was established according to the biochemical and immunologic responses of the patient. On the basis of these responses, the patient received a single intramuscular injection of PEG-ADA every week. The initial dose was 10 U/kg the 1st wk, followed by 20 U/kg the 2nd wk, 25 U/kg the 3rd wk, and 30 U/kg from wk 4 on.

For biochemical monitoring during the 1st and 2nd wk of treatment, blood samples were drawn before each injection and on d 1, 2, 3, and 5 after injection. Later, blood samples were drawn twice a week, just before and 2 d after each injection. After 6 mo of treatment, samples were obtained once a month. Specific tests of immunity were performed before treatment and repeated approximately every 4–6 wk for the 1st 6 mo, then approximately every 2 mo.

The protocol of this study was approved by the FDA and the Ethics Committee of University Claude Bernard Lyon I and Hospices Civils de Lyon. This clinical trial is included in a multicenter open-label study in children with SCID resulting from ADA deficiency.

METHODS

HPLC analysis of purine metabolites. Blood was collected in a heparinized tube and immediately centrifuged to prevent metabolic changes that occur when plasma is left in contact with erythrocytes (11).

Plasma and erythrocytes were then rapidly deproteinized with 35% perchloric acid. The supernantants were adjusted to pH 6–7 with 5 mol/L sodium hydroxide. The extracts were stored at −20°C while awaiting analysis.

Twenty-four-h urine samples were analyzed without further treatment.

Adenine, adenosine, and deoxyadenosine were measured using a sensitive and specific reversed-phase HPLC method, which we have described previously (12). This method also allows the determination of SAH, a compound that accumulates after SAH hydrolase inhibition by adenosine or deoxyadenosine.

Ribonucleotides and deoxyribonucleotides of adenine were separated and quantified using an anion-exchange HPLC method as previously described (13).

ADA assay. ADA activity in erythrocytes, lymphocytes, and plasma was determined by a spectrophotometric method (14).

Immunologic methods. The evaluation of immunologic function was performed in the Immunology Laboratory of the Neurologic Hospital in Lyon. Lymphocyte subsets were analyzed by a flow cytometric method using monoclonal antibodies (15). Lymphocyte response to nonspecific mitogens, Concanavalin A, phytohemagglutinin, and pokeweed mitogen, was measured by ^{3}H-thymidine incorporation.

RESULTS

Pretreatment findings. ADA activity in erythrocytes and lymphocytes was not detectable before red cell transfusions. In erythrocytes, dATP level was 479 nmol/mL packed cells and dADP was 100 nmol/mL packed cells, whereas these compounds were not detectable in erythrocytes from healthy subjects. (2, 4). The determination of purine nucleotides in lymphocytes was not possible as the peripheral lymphocyte count was too low.

In plasma samples, adenosine was present at a concentration of 1.9 μmol/L (normal values 0.8 ± 0.4 μmol/L, mean ± SD) (12), whereas deoxyadenosine was not detectable. Moreover, SAH was not detected in plasma samples from our ADA-deficient patient.

In urine, high levels of deoxyadenosine were found; the mean value was 515 mmol/mg of uric acid, whereas deoxyadenosine was not detectable in healthy subjects.

To summarize, four abnormal purine metabolites, adenosine, deoxyadenosine, dADP, and dATP, were found in biologic fluids and cells of our ADA-deficient patient before red cell transfusions.

Effect of red cell transfusion therapy. Figure 1 shows the effect of red cell transfusions on dATP concentration and erythrocyte

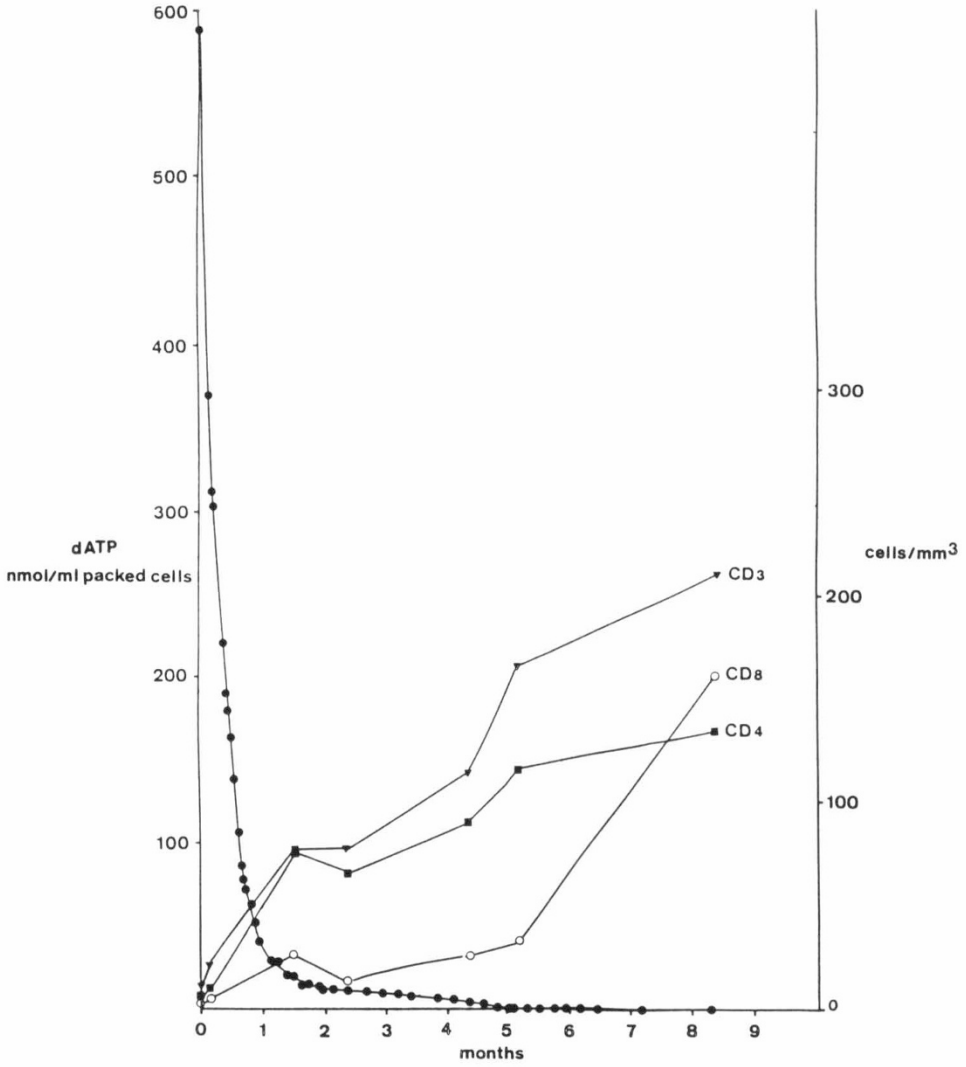

Fig. 2. Effect of PEG-ADA treatment on erythrocyte dATP concentration (●) and circulating counts of T lymphocyte subsets. Normal values ranged from 450 to 2500 cells/mm^3 for CD3, 550 to 1200 cells/mm^3 for CD4, and 350 to 850 cells/mm^3 for CD8.

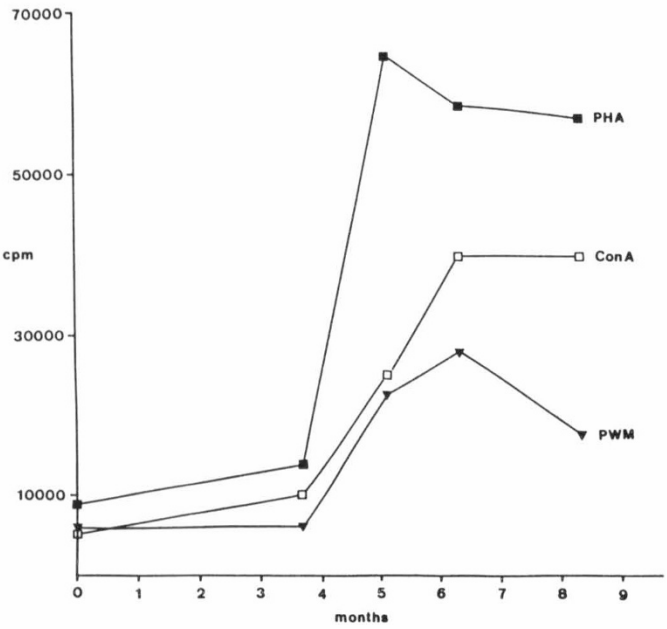

Fig. 3. Lymphocyte response to nonspecific mitogens after PEG-ADA treatment. Incorporation of ^{3}H-thymidine (cpm) is shown.

ADA activity. The concentration of dATP in erythrocytes decreased significantly during the 1st 10 d after red cell transfusion. After the 3rd monthly transfusion, erythrocyte dATP level was approximately 30 nmol/mL packed cells, 95% lower than before therapy. However, as shown in Figure 1, when red cell transfusions were not regularly performed, dATP levels increased again rapidly to high values.

At the same time as dATP levels decreased, ADA activity increased significantly. ADA activity rose from undetectable values before therapy to 49.5 nmol/h/mg Hb (normal values, 61 ± 29 nmol/h/mg Hb, mean ± SD) after the 3rd transfusion. The corresponding dATP was 39 nmol/mL packed cells. Thus, low erythrocyte dATP levels are correlated with high ADA activities, and vice versa.

Besides, compared with the variations of erythrocyte dATP levels, an average decrease of only 30% of dADP values could be observed during the days after red cell transfusions. In urine, deoxyadenosine became undetectable.

Clinically, after red cell transfusions, the child gained weight (approximately 1800 g) and showed an improvement of neurologic and psychomotor development. The child could hold her head up and was able to sit for a few minutes. This clinical improvement was particularly observed after the 3rd transfusion, when erythrocyte dATP levels were lower.

Effect of PEG-ADA therapy. After repeated weekly injections of 30 U/kg PEG-ADA, the average plasma ADA activity was between 30 and 40 μmol/h/mL (therapeutic range: 20–30 μmol/

h/mL). The effect of PEG-ADA therapy on erythrocyte dATP concentration is shown in Figure 2. dATP concentrations decreased rapidly during the 1st wk and then decreased steadily to undetectable levels after 4 mo of treatment. The levels of dADP became undetectable after 1 mo of treatment. Similarly, in urine, deoxyadenosine became undetectable after 1 mo.

The tests of cellular immune function showed a significant improvement throughout treatment. Total lymphocyte count increased to 2546 cells/mm^3 after 1 mo of PEG-ADA therapy and then became stable at a value of approximately 1300 cells/mm^3. Circulating T lymphocytes increased regularly as shown in Figure 2. Figure 3 shows that lymphocyte blastogenic response to nonspecific mitogens increased significantly after 3 mo of treatment.

On the clinical level, the child regained her appetite with a consequent weight increase. However, the weight-height development remained slow (wt 6800 g, ht 69 cm at 30 mo). On the neurologic and psychomotor level, a marked improvement was observed. The child looked alert, could distinguish between unknown and familiar faces, and was able to grasp. There was an improvement in muscular tonus (the child could crawl), but progress was slow.

After 7 mo of PEG-ADA treatment, the child was able to go home at the age of 28 mo with the treatment being maintained at 30 U/kg/wk.

DISCUSSION

The values obtained for erythrocyte dATP levels before red cell transfusions were in the range of those found in untreated ADA-deficient SCID patients reported in literature (2, 4). In plasma, the absence of deoxyadenosine is not surprising. Recently, in their investigations, Morgan *et al.* (4) have noted considerable heterogeneity of clinical, immunologic, and biochemical parameters in ADA-deficient SCID patients. The only consistent abnormalities were the high levels of dATP in erythrocytes and deoxyadenosine in urine.

The decrease of erythrocyte dATP levels after red cell transfusions has been noted by other authors (16, 17). However, our results also show that the levels of erythrocyte dADP are not markedly modified after transfusions. In addition, we show that there is a close correlation between ADA activity and erythrocyte dATP levels. Thus, as previously suggested (18), dATP appears to be the major purine metabolite involved in the pathophysiologic mechanisms of ADA deficiency. Despite a clinical improvement associated with a decrease of erythrocyte dATP concentrations, the immune function was not significantly restored in our patient under transfusion therapy.

The results obtained with PEG-ADA therapy show that the start-up of immune functions, albeit partial, is correlated with dATP erythrocyte concentrations. In a previous study (18) in an ADA-deficient bone marrow transplanted patient, we found that normal immune functions are compatible with very low erythrocyte dATP levels (14 nmol/mL packed cells).

Thus, our comparative study between red cell transfusion and PEG-ADA therapy shows that red cell transfusion therapy is not sufficiently effective to reduce and maintain erythrocyte dATP levels at values compatible with normal immune functions. In addition, repeated red cell transfusion carries the risk of iron overload and viral infection. On the contrary, PEG-ADA therapy is a suitable treatment to reduce dATP levels to near undetectable levels compatible with the immune functions being restored. Furthermore, PEG-ADA had a markedly reduced immunogenicity compared with native ADA permitting long-term administration by weekly intramuscular injection (10).

In conclusion, the results obtained in our study show that the measurement of dATP levels is very useful in monitoring and evaluating the degree of efficiency of enzyme replacement therapy in the treatment of ADA deficiency.

Acknowledgments. The authors thank Enzon Inc. (South Plainfield, NJ) for providing PEG-ADA for the treatment of our patient with ADA deficiency.

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