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**Research Article**

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## Plasma Deoxyadenosine, Adenosine, and Erythrocyte deoxyATP are Elevated at Birth in an Adenosine Deaminase-deficient Child

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**ABSTRACT** We have determined concentrations of adenosine, deoxyadenosine, and deoxyATP (dATP) in cord blood from an infant prenatally diagnosed as ADA deficient. Plasma deoxyadenosine and adenosine were already elevated in cord blood (0.7 and 0.5  $\mu\text{M}$  vs. normal of  $<0.07 \mu\text{M}$ ). Elevation of plasma deoxyadenosine has not previously been documented in these children. Erythrocyte dATP content was also elevated at birth (215 nmol/ml packed erythrocytes vs. normal of 2.9). These elevated concentrations of adenosine, deoxyadenosine, and dATP are similar to those we observed in another older adenosine deaminase-deficient patient and may explain the impaired immune function and lymphopenia seen at birth.

### INTRODUCTION

Inherited deficiency of adenosine deaminase (ADA)<sup>1</sup> results in the syndrome of severe combined immunodeficiency, fatal in early childhood (1–3). ADA-deficient children accumulate the substrate adenosine in their plasma and erythrocytes, and excrete moderately increased amounts of adenosine and markedly increased

amounts of deoxyadenosine, another substrate of ADA, in their urine (4–11). Additionally, deoxyATP (dATP), a metabolite of deoxyadenosine, is markedly increased in erythrocytes and lymphocytes (7, 10–13).

These children can be returned to completely normal immune function by bone marrow transplantation from a histocompatible sibling donor (14). Concomitant with engraftment, concentrations of metabolites markedly diminish (13, 15). Unfortunately, over half the children do not have a suitable sibling donor. Therefore, based upon the results of *in vitro* experiments (16), children have been treated with multiple partial exchange transfusions with normal frozen, irradiated erythrocytes (4). These erythrocytes contain both the missing enzyme and nucleoside transport sites for the accumulated substrates (7). Such therapy is capable of reducing metabolite concentrations markedly, but not completely to normal (15). Although there is improvement in immune function, and some children are alive after almost 3 yr of therapy, in none of the erythrocyte-transfused patients has there been complete restitution of immune function (18, 19).

Metabolite concentrations can thus be lowered either with erythrocytes or engrafted lymphocytes, providing an enzyme loaded substrate trap for "dialysis." We have now determined plasma deoxyadenosine, adenosine, and erythrocyte dATP in cord blood of a

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<sup>1</sup>Abbreviations used in this paper: ADA, adenosine deaminase; HPLC, high-pressure liquid chromatography.

child diagnosed prenatally as ADA deficient (19). Such a child would be considered to have been "dialyzed" by its normal, albeit ADA heterozygous mother. We were surprised to find marked elevation of erythrocyte dATP and increased plasma adenosine at birth. During these investigations we were also able, for the first time, to demonstrate elevation of plasma deoxyadenosine in this child and other ADA-deficient children. Concentrations of the three metabolites were similar to those observed in untreated, older ADA-deficient children, and considerably higher than those seen after institution of erythrocyte transfusion in this child.

## METHODS

Adenosine, deoxyadenosine, calf thymus DNA, and deoxynucleotide triphosphates were obtained from Sigma Chemical Co., St. Louis, Mo., and/or P-L Biochemicals, Inc., West Palm Beach, Fla. DNA polymerase was from Worthington Biochemical Corp., Freehold, N. J., [<sup>14</sup>C]deoxycytidine triphosphate from Amersham Corp., Arlington Heights, Ill., and methanol (high-pressure liquid chromatography [HPLC] grade) from Burdick & Jackson Laboratories Inc., Muskegon, Mich. dATP in erythrocytes was determined as previously described (11), essentially by the method of Solter and Handschumacher (20).

Plasma adenosine and deoxyadenosine were determined by HPLC using a Laboratory Data Control dual pump system (Milton Roy Co., Riviera Beach, Fla.) as previously described (11), monitoring the effluent at 260 nm. Plasma proteins were precipitated and nucleosides were extracted by addition of an equal volume of 100% methanol, followed by vortexing vigorously and storage 1–2 h at 4°C. The supernate derived from 0.5 ml of plasma was diluted to 10 ml with H<sub>2</sub>O, brought to pH 10, and applied to Bio-Rad AG 1-X2 columns (Bio-Rad Laboratories, Richmond, Calif.), essentially by the method of Kuttesch et al. (6). The column fractions containing adenosine and deoxyadenosine were lyophilized and reconstituted with H<sub>2</sub>O, usually to 0.4 ml. A 100- $\mu$ l sample was then analyzed by HPLC with and without prior incubation with calf intestinal adenosine deaminase (Sigma Chemical Co.). Adenosine and deoxyadenosine were identified by elution volume, by disappearance after treatment with ADA and subsequent appearance of peaks with the elution volume of the expected products, inosine and/or deoxyinosine. All samples analyzed were treated in this fashion. In preliminary experiments, <sup>14</sup>C-labeled and/or cold adenosine and deoxyadenosine were added to normal plasma at final concentrations in the micromolar range. We determined the counts per minute recovered as well as adenosine and deoxyadenosine as measured by HPLC. Recovery (taking into account measured volume losses) was ~90% (81–112, *n* = 3). Under ideal conditions, as little as 5 pmol/100  $\mu$ l (0.05 nmol/ml) could be detected. However, the presence of an unidentified compound in plasma, eluting between adenosine and deoxyadenosine, can raise the lower limits of detection to as high as 0.5 nmol/ml).

*Case histories.* Patient 1 was the younger male sibling of an ADA-deficient child with severe combined immunodeficiency. The ADA deficiency of the patient was diagnosed prenatally and confirmed at birth. Further clinical details have been previously reported (19). The child was lymphopenic at birth (740 lymphocytes/mm<sup>3</sup>), had diminished T cells (10%), and diminished response to phytohemagglutinin (20% of normal).

TABLE I

	Plasma		Erythrocytes
	Adenosine	Deoxyadenosine	dATP
	$\mu$ M	$\mu$ M	nmol/ml packed erythrocytes
Normal individuals ( <i>n</i> )	<0.07 (3)	<0.07 (3)	2.9±0.96 (7)
Normal cord blood ( <i>n</i> )	<0.07 (3)	<0.07 (3)	3.8; 3.5 (2)
Patient 1 (ADA <sup>-</sup> )			
Pretransfusion			
Cord blood	0.68	0.47	215.0
3 d old	0.07*	0.15*	391.0
10 d old	—	—	374.0
Posttransfusion			
3 mo old	—	—	31.0
15 mo old	2.07	<0.07	100.0
Patient 2 (ADA <sup>-</sup> )			
Pretransfusion			
6 mo old	0.31	1.80	608.0
6.5 mo old	1.29	1.65	588.0

\* Perchloric acid extract.

Patient 2 was a 4-mo-old female child with pulmonary infections, diarrhea, and neurologic abnormalities. The child was found to be profoundly lymphopenic, immunodeficient, and ADA deficient. Further details will be reported elsewhere.<sup>2</sup>

## RESULTS

*Plasma adenosine and deoxyadenosine.* Cord blood plasma from three normal neonates contained no detectable adenosine or deoxyadenosine. Cord blood plasma from the ADA-deficient child contained 1.08  $\mu$ M adenosine and 0.75  $\mu$ M deoxyadenosine (Table I). Elevated adenosine and deoxyadenosine were also detected at 3 d of age. Both deoxyadenosine and adenosine in plasma of a 6-mo-old, severely ill, ADA-deficient patient were elevated and ranged between 0.49 and 2.05  $\mu$ M adenosine, and 2.64 and 2.88  $\mu$ M deoxyadenosine. We were unable to detect adenosine or deoxyadenosine in plasma from non-ADA-deficient individuals, although normal plasma adenosine has been reported to be 0.07  $\mu$ M (6). After institution of erythrocyte transfusion therapy at 6 wk of age, deoxyadenosine could no longer be detected in ~25 samples of plasma from this child or other similarly treated ADA-deficient patients, even at times when adenosine was still elevated and increased dATP was present in erythrocytes.

*Erythrocyte dATP.* dATP content of normal cord blood erythrocytes was 3.6 nmol/ml packed erythrocytes,

<sup>2</sup> Papageorgiou, P., and R. Hirschhorn. Manuscript in preparation.

similar to findings in normal adults ( $2.9 \pm 1.0$ ). In contrast, dATP content of the ADA-deficient cord blood erythrocytes was 215 nmol. dATP content at 3 and 10 d of age was 391 and 374 nmol. In comparison, dATP content of erythrocytes from a 4-mo-old, severely ill, ADA-deficient patient was 608 and 588 nmol on two separate occasions. After institution of erythrocyte transfusions, dATP fell to 31 nmol at 3 mo of age. With continued, approximately biweekly, erythrocyte transfusions, dATP ranged between 30 and 99 nmol during the first 6 mo of life.

## DISCUSSION

During these studies we were able to show that plasma deoxyadenosine, a substrate of ADA, is elevated in the plasma of ADA-deficient children. Our ability to detect this metabolite, not reported in prior studies, may be the result of the use of methanol rather than perchloric acid extracts, and the known lability of deoxyadenosine in acid.

We have found that plasma adenosine, deoxyadenosine, and erythrocyte dATP are already elevated at birth in an ADA-deficient child. The concentrations of adenosine and deoxyadenosine were not very different from those we had observed in plasma of a 6-mo-old, severely ill, ADA-deficient child. The dATP content of erythrocytes was also markedly elevated at birth but was lower than that seen in the older ADA-deficient child. The significance of these differences in magnitude is difficult to evaluate because the extracts of erythrocytes were prepared by different individuals; thus, technical factors such as length of exposure to acid could account for the differences observed. The concentration of dATP present in cord blood was considerably higher than concentrations observed in the same child after institution of erythrocyte transfusions, but lower than that seen at 3 and 10 d of age.

The presence of increased, potentially toxic metabolites in cord blood could reflect overloading of the maternal clearance system at term by the dying placenta. More likely, the increased metabolites in cord blood reflect increases present in utero, and may explain the lymphopenia seen at birth in some of these children. It is likely that stem cells as well as mature peripheral blood lymphocytes have suffered in utero. These observations raise the question as to whether intrauterine erythrocyte transfusion should be considered when, as in this family, the mother desires to continue the pregnancy despite the diagnosis and no normal sibling donor is available for postnatal bone marrow transplantation. In the future, it will be of interest to determine concentrations of metabolites in amniotic fluid at the time of prenatal diagnosis.

These observations also raise the question as to why the mother's total body ADA could not completely detoxify the infant in utero. Two possibilities can be considered. First, the rate of adenosine and deoxyadenosine production by the fetus may be too great to be completely cleared by the mother's ADA. This seems unlikely because a much smaller erythrocyte mass, transfused postnatally, considerably lowered these metabolites. Alternatively, the limiting factors could be the rate of transplacental transport of adenosine and deoxyadenosine from the fetal circulation and amniotic fluid to the maternal circulation. Similar accumulation of low molecular weight metabolites in amniotic fluid has been reported in other inherited disorders (21, 22). The amniotic fluid in the last two trimesters of pregnancy, when the fetal kidney is functional, may serve as a reservoir for accumulation of any toxic metabolites produced by the fetus and not completely cleared by the mother. This reservoir, derived from urinary deoxyadenosine, could then serve to challenge the fetus (but not the infant) with an added ingested load of deoxyadenosine additional to that produced daily as a result of *de novo* purine biosynthesis and the inherited metabolic block.

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