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Developmental disorder associated with increased cellular nucleotidase activity

(purine-pyrimidine metabolism/uridine/brain diseases)

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ABSTRACT **Four unrelated patients are described with a syndrome that included developmental delay, seizures, ataxia, recurrent infections, severe language deficit, and an unusual behavioral phenotype characterized by hyperactivity, short attention span, and poor social interaction. These manifestations appeared within the first few years of life. Each patient displayed abnormalities on EEG. No unusual metabolites were found in plasma or urine, and metabolic testing was normal except for persistent hypouricosuria. Investigation of purine and pyrimidine metabolism in cultured fibroblasts derived from these patients showed normal incorporation of purine bases into nucleotides but decreased incorporation of uridine. *De novo* synthesis of purines and cellular phosphoribosyl pyrophosphate content also were moderately decreased. The distribution of incorporated purines and pyrimidines did not reveal a pattern suggestive of a deficient enzyme activity. Assay of individual enzymes in fibroblast lysates showed no deficiencies. However, the activity of cytosolic 5′-nucleotidase was elevated 6- to 10-fold. Based on the possibility that the observed increased catabolic activity and decreased pyrimidine salvage might be causing a deficiency of pyrimidine nucleotides, the patients were treated with oral pyrimidine nucleoside or nucleotide compounds. All patients showed remarkable improvement in speech and behavior as well as decreased seizure activity and frequency of infections. A double-blind placebo trial was undertaken to ascertain the efficacy of this supplementation regimen. Upon replacement of the supplements with placebo, all patients showed rapid regression to their pretreatment states. These observations suggest that increased nucleotide catabolism is related to the symptoms of these patients, and that the effects of this increased catabolism are reversed by administration of uridine.**

Behavioral abnormalities, seizures, developmental delay, and immunodeficiency are known to be associated with several defects of purine and pyrimidine metabolism (1). Seizures and autistic behavior are seen in some patients with dihydropyrimidine dehydrogenase deficiency (2). Individuals with a deficiency of adenylosuccinate lyase activity also display autistic behavior, seizures, and other neurological abnormalities (3). A deficiency of either adenosine deaminase or purine nucleoside phosphorylase causes severe immunodeficiency; neurological symptoms also have been reported with these two enzyme deficiencies (4). Finally, deficiency of hypoxanthine phosphoribosyltransferase causes Lesch–Nyhan syndrome with its characteristic self-injurious behavior, mental retardation, and dystonic posturing (5). Cases of excessive uric acid excretion associated with seizures and autistic behavior are likely to represent defects of purine metabolism, although no specific enzyme abnormality has been identified in these cases (6). In none of these disorders has it been possible to delineate the mechanism through which the enzyme deficiency produces the neurological or behavioral abnormalities. Therapeutic strategies designed to treat the behavioral and neurological abnormalities of these disorders by replacing the supposed deficient metabolites have not been successful in any case.

This report describes four unrelated patients in whom developmental delay, seizures, ataxia, recurrent infections, speech deficit, and an unusual behavioral phenotype were associated with highly elevated activity of cytosolic 5′nucleotidase. Metabolic therapy with pyrimidine compounds appeared to be highly effective in reversing these manifestations.

CLINICAL PRESENTATION

The clinical presentation of four patients is given below and is summarized in Table 1. Details of the clinical course of each patient as well as results of neuropsychological testing and treatment protocols will be published in a separate clinical paper.

The presentation was fairly consistent. All patients were markedly delayed in their developmental milestones, especially language. All had seizures, ataxia, an awkward gait, and mildly impaired fine motor control. All four displayed an unusual behavioral phenotype that was characterized by extreme hyperactivity, distractability, a strange "delirious" quality to their affect, and abnormal social interaction. All four patients experienced frequent ear and sinus infections, but no consistent reason for immunodeficiency (such as reduced antibody titre or abnormal T cell response) could be found. It was noted that during infection, behavioral, language, and neurological abnormalities would worsen. All patients excreted reduced quantities of uric acid when compared with age-matched controls (7). A number of laboratory tests were performed and found to be within normal limits. These included plasma amino acids and organic acids, urinary amino acids and organic acids, plasma and urinary HPLC analysis, biotin, carnitine, folate, and B12. All patients had unaffected parents and siblings; one patient's history was positive for a similarly affected cousin.

Patient 1, a white female, was first studied at 2 years of age because of recurrent sinus infections, seizures, and developmental delay. A preliminary report of her case has been published (8). At 20 months, height and weight were both at the 10th percentile, and head circumference was at the 50% percentile. Seizures began at 19 months of age and consisted predominantly of jerking of the upper extremities, head tilt, and eye deviation. Seizures were not well controlled by carbamazapine or valproate. EEG revealed seizure activity over

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Abbreviations: PRPP, phosphoribosyl pyrophosphate; FBS, fetal bovine serum.

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Table 1. Clinical presentation of four patients

	Symptom	Patient 1	Patient 2	Patient 3	Patient 4
General	Sex	F	F	M	M
	Age first studied, years	3	4	2	8
	Growth retardation	+	−	+	+
Behavior	Hyperactive	+	+	+	+
	Inability to focus	+	+	+	+
	Extreme distractibility	+	+	+	+
	Occasionally aggressive	+	−	+	+
	Impulsive	+	+	+	+
	"Delirious" affect	+	+	+	+
	Compulsiveness	+	+	+	+
	Abnormal social interaction	+	+	+	+
Speech	Speech delay	+	+	+	+
	Slurred speech	+	+	+	+
	Tremulous speech	+	+	+	+
	Short, telegraphic sentences	+	+	+	+
Neurological	Seizures	+	+	+	+
	Abnormal EEG	+	+	+	+
	Ataxia	+	+	+	+
	Impaired fine motor control	+	+	+	+
	Awkward gait	+	+	+	+
Immunological	Frequent infections	+	+	+	+
	Abnormal immunoglobulins	+	+	−	−
	Abnormal T cell response	+	+	−	−
Other	Developmentally delayed	+	+	+	+
	Sparse hair/hair loss	+	+	−	−
	Skin rash	+	+	+	−
	Hypouricosuria	+	+	+	+

the right temporal region. Neurological examination also revealed truncal ataxia, an awkward gait, and mild impairment of fine motor control. Hair loss began at approximately 22 months and continued until age 3.5 years. An intermittant erythema was noted on her forehead and cheeks. Severe recurrent sinusitis required aggressive antibiotic therapy and repeated surgical drainage and lavage.

Speech began at 3 years with monosyllables only and remained profoundly delayed. An unusual behavioral phenotype was noted, which was characterized by extreme hyperactivity, marked impulsivity, perseveration, short attention span, and inability to focus on tasks. She was noted to have a "delirious" quality to her affect. Poor social intereaction was noted with occasional aggression in the form of hitting, poking, pinching, or biting others. Intelligence as measured by the Leiter International Performance Scale gave a value of 84.

Immunological studies were undertaken to find a cause for the apparently increased susceptability to infection. Studies showed an initial IgA of 15 (normal for age: 47 ± 15 mg/dl), after which levels were normal; levels of IgG ranged from 200 to 504 (normal for age: 925 ± 230 mg/dl). Other Ig levels were within normal limits. Intermittant T cell dysfunction was manifested as decreased response to mitogen on several occasions.

Urinary urate was 0.63 mg/mg creatinine (normal for age: 1.19 ± 0.22; ref. 7).

Patient 2, a white female, was first studied at 4 years of age because of recurrent infections, seizures, and delayed speech. Family history was positive for a cousin with a similar presentation. At 4 years of age height and weight were at the 95th and 60th percentile, respectively. At 3 years of age she began having seizures, which were not well controlled by carbamazapine or valproate. EEG showed atypical spike and wave forms with 1- to 2-sec bursts. Neurological examination also revealed truncal ataxia and brisk deep tendon reflexes in the lower extremities. Fine motor control was mildly impaired. Her hair was fair and sparse, and she had a persistent scaly rash on the cheeks, as well as keratosis follicularis on the trunk. The patient suffered from frequent bouts of sinusitis and otitis media and had been treated with antibiotics almost continuously since birth. Speech at 4 years was delayed, with a small vocabulary, slurred and tremulous pronunciation, and improper syntax. Behavior was characterized by hyperactivity, short attention span, and lack of perseverance. Social intereaction with other children was poor, but not notably aggressive. Intelligence as measured by the Leiter International Performance Scale was 85.

Ig studies were notable for slightly decreased IgG_2 at 50, IgM at 96, and IgA at 14 (normal for age: 631 ± 125, 58 ± 19, and 97 ± 15 mg/dl, respectively). After pneumovax she responded only to type IV pneumococcal polysaccharide antigen. However, she had a strong response to *haemophilus influenza* vaccine. Phytohemagglutinin stimulation index was within normal limits, and she had positive skin tests to tetanus, diphtheria, and candida. T cell response to antigens was subnormal on several occasions. Urinary urate was between 0.11 and 0.5 mg/mg creatinine (normal for age: 1.01 ± 0.24; ref. 7).

Patient 3, a white male, first came to the attention of the investigators at age 8 years because of seizures, speech delay, and behavioral abnormalities. Seizure activity consisted of status epilepticus and individual tonic-clonic seizures. Seizures were not well controlled by carbamazapine or depakene but fairly well controlled by lorazapam. An EEG at 10 years showed generalized epileptogenic brain dysfunction. Neurological examination revealed truncal ataxia, rotatory nystagmus, and mild to moderate impairment of fine motor control. Hair was normal, but perioral eczema, an eczema-like rash on the trunk, and thickened skin on the soles of the feet were noted. The patient suffered from recurrent sinusitis, and a croup-like condition required intubation. Speech began at 3.5 years and was noted to be delayed for his age, with slurred and tremulous pronunciation. Behavior was hyperactive and impulsive, with extreme distractability and short attention span.

The patient showed little interest in social interaction but was occasionally aggressive toward other children.

Ig levels and T cell function were within normal limits. Urinary urate was between 0.2 and 0.4 mg/mg creatinine (normal for age: 0.80 ± 0.29; ref. 7).

Patient 4, a white male, was first studied at age 2.5 years because of seizures, ataxia, and frequent infections. At 24 months, height and weight were at the 10th and 5th percentile, respectively, and head circumference was at the 50th percentile. Seizures were prolonged and generalized, and they typically began with focal hand jerking and became generalized. An MRI revealed asymmetrical ventricles. Seizures were not well controlled by carbamazapine or valproate, but fairly well controlled by felbamate. Neurological examination revealed truncal ataxia, hypotonia, spontaneous ankle clonus, and a positive Babinski response; toe-walking and mild impairment of fine motor control also were noted. Hair was normal, and no skin rashes were found. The patient suffered from recurrent sinusitis and otitis media, as well as asthma and bronchitis. Speech began at 18 months but remained delayed and difficult to understand; pronunciation was slurred and tremulous, and vocabulary was small. Behavior was hyperactive and impulsive, with marked perseveration and poor social interaction.

T cell function and Ig levels were within normal limits. Urinary uric acid was between 0.45 and 0.6 mg/mg creatinine (normal for age: 1.32 ± 0.24; ref. 7).

MATERIALS AND METHODS

^{14}C-Monolabeled adenine, guanine, hypoxanthine, uridine, sodium formate, orotic acid, adenosine monophosphate, uridine monophosphate, inosine, deoxycytidine, deoxyuridine monophosphate, and deoxycytidine monophosphate of approximately 50 μCi/μmol were obtained from New England Nuclear. All other reagents were obtained from Sigma.

Analysis of purine and pyrimidine compounds and incorporation studies of radiolabeled purine and pyrimidine precursors into the corresponding nucleotides in intact fibroblasts was performed as described (9). *De novo* purine synthesis was measured by quantification of the radiolabeled purine nucleotides produced by incubation of cultured fibroblasts with radiolabeled formate. For these studies, cells were grown in Coon's F12 medium with 10% fetal bovine serum (FBS), harvested in the log phase of growth, and replated in 100-mm plates at a density of 10^6 cells/plate in Earl's minimal essential medium (MEM) with 10% dialyzed FBS. Cells were again harvested in the log phase of growth and replated in 100-mm plates at 10^6 cells/plate with 10 ml MEM, 10% dialyzed FBS, and 10 μCi of sodium [^{14}C]formate. The cells were harvested by trypsinization after 24 hr, extracted with 100 μl of 0.5 M perchloric acid, neutralized with 50 μl of 2 M potassium phosphate, and analyzed by HPLC as described (9).

Individual enzymes also were assayed in fibroblast lysates. For these studies, fibroblasts were grown in Coon's F12 medium with 10% FBS. Cells were harvested in the log phase of growth and lysed at a concentration of 10^7 cells/ml in a buffer that contained 0.1 M sodium phosphate and 0.02 M magnesium chloride at pH 7.2 by three cycles of freeze-thaw. Cell membranes were removed by centrifugation at 50,000 × *g* for 20 min. The lysates were dialyzed for 3 hr against this buffer with two buffer changes at 4°C. Protein concentration was determined by the method of Lowry (10). The conditions for each of the enzyme assays is given in Table 2. The assays were incubated for 30 min at 37°C. Reactions were terminated by addition of 10 μl of 4 M perchloric acid. The precipitated protein was removed by centrifugation, and the neutralized supernatant was analyzed by HPLC. Each assay was done in triplicate.

Phosphoribosyl pyrophosphate (PRPP) was quantified in cultured fibroblasts by measuring the radiolabeled uridine monophosphate produced from radiolabeled orotic acid. Fibroblasts were harvested by trypsinization, washed with PBS, resuspended at a density of 10^7 cells/ml in Earl's minimal essential medium, and incubated at 37°C for 30 min. The suspensions then were immersed in boiling water for 60 sec, cooled on ice, and centrifuged at 2,000 × *g* for 10 min. The supernatant (50 μl) was combined with 50 μl of an assay mixture that contained 50 mM tris (pH 7.4), 20 mM magnesium chloride, 2 mM EDTA, 200 μM [^{14}C]orotic acid, and 0.5 units of orotidine monophosphate pyrophosphorylase/orotidine monophosphate decarboxylase, and incubated for 30 min at 37°C. Uridine monophosphate produced from PRPP then was quantified by HPLC as described (9).

Table 2. Assay of enzymes of purine and pyrimidine metabolism

Enzyme	Cells	Substrates
5′-Nucleotidase	10^5	AMP* 12 μM
5′-Nucleotidase	10^5	UMP* 12 μM
Adenosine kinase	10^5	Adenosine* 12 μM, ATP 1 mM
Adenosine deaminase	10^4	Adenosine* 12 μM
Nucleoside phosphorylase	10^5	Uridine* 12 μM
Nucleoside phosphorylase	10^4	Inosine* 12 μM
Nucleoside phosphorylase	10^5	Thymidine* 12 μM
UMP synthetase	10^5	Orotate* 12 μM, PRPP 1 mM
dCMP deaminase	10^5	dCMP* 5 μM
dCMP deaminase	10^5	dCMP* 5 μM, dCTP 100 μM
Deoxycytidine deaminase	10^5	Deoxycytidine* 12 μM
TMP synthetase	10^6	dUMP* 10 μM, FTHF
Uridine kinase	10^5	Uridine* 12 μM, ATP 1 mM
Deoxyuridine kinase	10^6	Deoxyuridine* 12 μM, ATP 1 mM

All assays (with the exception of TMP synthetase) were performed in a buffer that consisted of 0.1 M sodium phosphate, 0.02 M magnesium chloride at pH 7.2; TMP synthetase was assayed in a buffer consisting of 0.1 M Tris at pH 7.4. For the assay of TMP synthetase, formyl tetrahydrofolate (FTHF) was generated by incubating 2.67 mg of tetrahydrofolate, 9.6 μl of formaldehyde, and 12 mg of DTT in buffer B for 15 min; 10 μl of this mixture was added to the assay.
*Labeled with ^{14}C at approximately 50 μCi/μmol.

For pharmacokinetic studies, patients and one age-matched control were given a single oral dose of 1,000 mg of uridine, and blood was drawn at the designated times. The plasma was separated by centrifugation, deproteinized with perchloric acid, neutralized with sodium hydroxide, and analyzed by HPLC.

The double-blind placebo trial was initiated after patients had been receiving uridine for more than 1 year. Informed consent was obtained from the parents. The trial started with a 1-month period during which each patient received either the uridine (1,000 mg/kg per day) or a placebo mixture that had been prepared to have the same taste. After this 1-month period, patients were returned to uridine for 1 month. After this, patients who had received uridine or placebo during the first month were given placebo or uridine, respectively, for 1 month. The patients received general, neurological, and neuropsychiatric evaluations at end of each of these 1-month nucleoside-placebo periods. At the time of the trial, the nucleoside-placebo schedule was unknown to any of the parents, caregivers, or investigators.

An unblinded trial of ribose was undertaken with two patients. These patients were treated for approximately 10 months with oral ribose at doses ranging from 500 to 2,000 mg/kg per day.

RESULTS

The incorporation of purine and pyrimidine precursors into nucleotides is shown in Table 3. The quantities of labeled purine nucleotides produced from labeled purine bases were comparable to normal controls. The distribution of the various

Table 3. Incorporation of precursors into nucleotides

Precursor	Pt 1	Pt 2	Pt 3	Pt 4	Controls (*n*)
Adenine	9472	9792	8993	9025	9170 ± 982 (6)
Hypoxanthine	3246	3686	3071	3387	3043 ± 618 (6)
Guanine	3429	2970	2893	2913	3242 ± 561 (6)
Formate	5046*	6681†	6269*	5890*	8748 ± 1026 (4)
Uridine	3982*	5840†	4876*	6137†	8294 ± 1142 (4)

Incorporation of formate into nucleotides is in units of pmol/100 nmol UV per 24 hr. All others are in units of pmol/100 nmol UV per 2 hr (14). Statistical significance, †, $P < 0.05$; *, $P < 0.01$.

labeled nucleotides was also comparable to normal controls, and no unusual radiolabeled compounds that would indicate an abnormality of nucleotide interconversion were seen in any of the patients (data not shown). Incorporation of uridine into pyrimidine nucleotides was slightly to moderately decreased in all patients (Table 3), but the distribution of the various pyrimidine nucleotides was comparable to normal controls, and no unusual radiolabeled peak were noted in any of the patients (data not shown). *De novo* purine synthesis, as measured by incorporation of radiolabeled sodium formate into purine nucleotides, was also decreased in all patients. Again, the distribution of labeled nucleotides was comparable to normal controls, and no unusual peaks (such as succinylaminoimidazolecarboxamide ribonucleotide) were noted, which would indicate a deficiency in any of the enzymes of *de novo* purine synthesis.

To further investigate the enzymes of purine and pyrimidine metabolism in these patients, the activities of individual enzymes were measured in dialyzed fibroblast lysates (Table 4). The results of these assays indicate that the activities of the enzymes of purine and pyrimidine salvage, interconversion, and catabolism were not significantly different ($P > 0.1$) in these patients, with the exception of cytosolic 5′-nucleotidase. This activity was always elevated 6- to 10-fold in all of the patients, and the elevation was always significant ($P < 0.001$). This elevation was similar whether a purine (AMP) or a pyrimidine (UMP) was used as a substrate. Although incorporation of uridine into pyrimidine nucleotides was found to be decreased, the activity of uridine kinase was comparable to normal controls.

To determine whether the increased nucleotidase activity altered the intracellular concentrations of the various purine and pyrimidine nucleotides, these concentrations were measured in the cultured fibroblasts of patients and in normal controls (Table 5). No significant differences were seen.

Intracellular PRPP was measured to determine whether the reduced *de novo* purine synthesis and the observed hypouricosuria could be explained by reduced availability of PRPP. The concentration of PRPP in the cultured fibroblasts of all patients was found to be approximately 50–70% of normal controls (Table 6).

Table 5. Nucleotide concentrations in cultured fibroblasts

Nucleotide	Pt 1	Pt 2	Pt 3	Pt 4	Normals (5)
AMP	0.5	0.6	0.2	0.8	0.9 ± 0.4
ADP	8.2	8.9	7.4	9.8	9.6 ± 3.6
ATP	11.1	12.2	10.6	14.6	13.7 ± 4.2
GDP	0.5	0.3	0.4	0.8	0.7 ± 0.3
GTP	3.8	4.7	3.8	5.2	4.9 ± 1.7
UTP	3.2	3.7	3.2	4.2	4.2 ± 1.1
CTP	0.8	0.8	0.6	1.1	1.2 ± 0.6

Nucleotide concentrations are expressed in units of nmol/10^6 cells.

On the basis of these studies of purine and pyrimidine metabolism in the cultured skin fibroblasts of the patients, a trial with oral nucleotide supplementation was begun with patient 1. She received 50 mg/kg per day each of UMP and CMP, and this dose was gradually increased to 150 mg/kg per day. At the higher dose, improvement in speech and behavior as well as greatly decreased seizure activity were noted. She began to pay more attention to her environment, focus better on tasks, and was not hyperactive. Interaction with others became more normal and appropriate for her age. Aggressive behavior ceased. Speech improved from short telegraphic sentences to longer, more complex, and age-appropriate expressions. Seizure activity decreased to the point that valproic acid (625 mg/day) was discontinued entirely, and the carbamazapine dose was decreased from 500 to 50 mg/day. The nucleotide mixture was noted to cause intermittent diarrhea and poor weight gain. A 1-week interruption in the supply of nucleotides caused rapid regression to her pretreatment condition. Speech and behavior deteriorated, and seizures increased to >10 episodes per day.

Treatment was continued with uridine 200 mg/kg per day, and her condition improved. The dose was gradually increased to 1,000 mg/kg per day. Patient 2 was started on the same treatment regimen and showed similar improvement, with fewer seizures, decreased ataxia, improved speech and behavior, and improved performance on standardized tests of cognitive function.

Because the patients appeared to derive significant benefit from oral uridine, a pharmacokinetic study was undertaken to determine whether absorption and catabolism of this nucleoside were normal. Fig. 1 shows the results of an 8-hour study. Absorption and catabolism in the patients appears to be normal; the different timing of the peak was due to the fact that the patients ingested the dose of uridine at various rates, ranging from all at once (control) to over a period of 1 hr (patient 2). The rate of disappearance of the drug from the plasma is close to the value of 118 min reported for infused uridine (11).

Table 4. Enzyme activities in dialyzed fibroblast lysates

Enzyme (substrate)	Pt 1	Pt 2	Pt 3	Pt 4	Normals (*n*)
5′-Nucleotidase (AMP)	9.54	9.21	7.61	8.67	1.14 ± 0.78 (9)
5′-Nucleotidase (UMP)	7.81	8.38	8.87	9.41	1.54 ± 0.86 (5)
Adenosine kinase (adenosine)	2.91	3.17	ND	ND	3.77 ± 0.79 (5)
Adenosine deaminase (adenosine)	4.43	3.87	ND	ND	4.85 ± 1.17 (5)
Nucleoside phosphorylase (inosine)	4.50	5.52	ND	ND	4.58 ± 0.89 (5)
Nucleoside phosphorylase (uridine)	0.59	0.39	0.65	0.44	0.48 ± 0.13 (5)
UMP synthetase (orotic acid)	2.55	1.56	1.95	2.27	1.99 ± 0.47 (5)
Uridine kinase (uridine)	3.41	3.09	4.06	3.53	3.44 ± 0.66 (4)
dCMP deaminase (dCMP)	0.0016	0.0021	ND	ND	0.0021 (2)
dCMP deaminase (dCMP, + dCTP)	0.039	0.036	ND	ND	0.048 (2)
Deoxycytidine deaminase (deoxycytidine)	0.19	0.099	ND	ND	0.33 (3)
TMP synthetase (dUMP)	0.0013	0.0017	ND	ND	0.0033 (3)
Deoxyuridine kinase (deoxyuridine)	0.039	0.037	ND	ND	0.034 (2)

All activities are in units of nmol/min per mg of protein. ND, not determined.

Table 6. PRPP content of cultured fibroblasts

Patient 1	Patient 2	Patient 3	Patient 4	Controls (5)
112	127	115	149	220 ± 48

Concentration is expressed in units of pmol/10^6 cells.

After approximately 1½ years of oral uridine therapy, a double-blind placebo trial was undertaken to test the efficacy of pyrimidine supplementation. The switch from pyrimidine to placebo was immediately apparent to the patients' parents, teachers, speech therapist, and other clinicians involved in their care. Seizures increased, ataxia returned, and speech and behavior deteriorated to the pretreatment state. Upon return to nucleoside therapy, the patients quickly improved and soon regained their previous condition.

Patients 3 and 4 were treated with oral ribose in an unblinded trial. At a dose of 600 mg/kg per day, decreased seizure activity and ataxia as well as improved speech and behavior were noted. However, ribose was considered to be inferior to uridine for the treatment of this disorder, and the treatment with ribose was discontinued in favor of uridine.

DISCUSSION

These patients are considered to represent a previously unrecognized disorder in which greatly increased nucleotide catabolism is associated with neurological and behavioral abnormalities. The phenotype of the four patients consisted of developmental delay, convulsive disorder, impaired development of speech, unusual behavior that was characterized as impulsive, aggressive, hyperactive, highly distractable, and lacking in normal social connectedness, and recurrent infections, especially acute and chronic sinusitis and otitis media. This phenotype is consistent with other, though varied, abnormalities of the nervous system observed in other disorders of purine and pyrimidine metabolism (1–6). Although these patients fulfill the diagnostic criteria for pervasive developmental disorder, they cannot be considered classically autistic because they do not show the same determined avoidance of social contact.

Studies of purine and pyrimidine metabolism initially were undertaken in patient 1 because of persistent macrocytosis and megaloblastic changes in the marrow and reduced urate excretion. Megaloblastic anemia is a prominent feature of orotic aciduria, the model for pyrimidine nucleotide depletion and effective therapy with nucleosides. On the other hand, macrocytosis was not seen in any of the other patients. These symptoms eventually disappeared in patient 1, suggesting that they might have been an effect of anticonvulsant therapy.

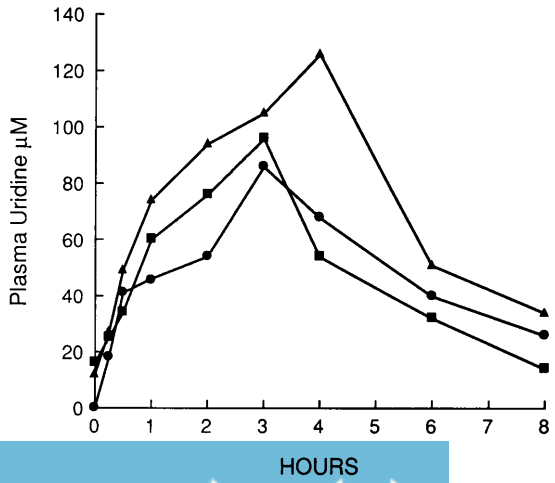

FIG. 1. Pharmacokinetics of uridine after a single oral dose of 1,000 mg/kg. ●, Patient 1. ▲, Patient 2. ■, Control.

Patient 2 initially displayed an elevated reticulocyte count; this eventually resolved and also might have been an effect of anticonvulsant therapy.

Extensive studies of purine and pyrimidine metabolism failed to reveal any deficient enzyme activity. The incorporation of normal amounts of adenine, hypoxanthine, or guanine into both adenine and guanine nucleotides indicated no gross deficiency of any of the enzymes of purine salvage, nucleotide interconversion, or phosphorylation, i.e. adenine phosphoribosyltransferase, hypoxanthine-guanine phosphoribosyltransferase, IMP dehydrogenase, GMP synthetase, GMP reductase, GMP kinase, adenylosuccinate synthetase, adenylosuccinate lyase, AMP deaminase, AMP kinase, or nucleotide diphosphate kinase. The absence of any unusual radioactive peaks from radiolabeled formate and the lack of any unusual ultraviolet-absorbing peaks (such as succinylaminoimidazole carboxamide ribonucleoside) in the urine on HPLC analysis both suggest a lack of deficiency of any of the enzymes of *de novo* purine synthesis. Similarly, a normal distribution of pyrimidine nucleotide products suggests that the absence of gross deficiency in any of the enzymes of pyrimidine salvage or of nucleotide phosphorylation or interconversion, including uridine kinase, UMP kinase, and CTP synthetase. Elevated cytosolic 5′-nucleotidase activity was the only consistently abnormal metabolic finding in these patients. This could represent the primary defect or an adaptational response to an abnormal accumulation of some substrate for this enzyme. Whether the increased activity is due to a greater number of normal enzyme molecules or a genetically altered enzyme has yet to be determined.

Three human 5′-nucleotidases have been described. In addition to the well characterized membrane-bound 5′-nucleotidase (12), which is involved mainly in extracellular signaling, two cytosolic 5′-nucleotidases have been purified from human sources, and these are believed to be responsible for intracellular nucleotide catabolism. One of these cleaves various purine and pyrimidine monophosphates, with K_m values in the low micromolar range (13). This "low K_m" 5′nucleotidase shows conventional hyperbolic kinetics and is competitively inhibited by ATP and ADP. The other cytosolic enzyme, the so-called "high K_m" 5′nucleotidase also cleaves purine and pyrimidine nucleotide monophosphates, but K_m values are higher (14). This enzyme is strongly stimulated by ATP and diphosphoglycerate; magnesium and inorganic phosphate also regulate activity. Because elevated nucleotidase activity was found in dialyzed, membrane-free lysates, one of these two cytosolic nucleotidases is likely to be responsible for the increased activity. A nonspecific, nucleotide-hydrolyzing phosphatase is a lesser possibility.

Clinical syndromes arising from superactivity of nonallosteric enzymes are rare. One case of anemia arising from superactive adenosine deaminase has been reported (15). The patient had approximately 40–70 times the normal activity. A mutation that confers excessive activity on superoxide dismutase and is believed to be responsible for a type of familial amyotrophic lateral sclerosis (16) is also known.

Superactivity of allosteric enzymes is more readily understood. In cases such as PRPP synthetase superactivity (17), mutations cause failure of the regulatory apparatus such that the enzyme is permanently in its high activity state. Considering the complex allosteric regulation of the cytosolic high-K_m 5′-nucleotidase, this would appear to be a distinct possibility.

That 5′-nucleotidase activity is increased in response to an abnormal accumulation of some nucleotide substrate or that an altered concentration of allosteric affector is the cause of increased activity appear less likely. Activity was increased even in dialyzed extracts. No abnormal nucleotide peaks were found in fibroblasts lysates, all nucleotide concentrations in fibroblasts appeared to to comparable to normal controls, and no unusual radiolabeled nucleotides were produced from any of the purine or pyrimidine precursors in the pulsing studies. A search for an enzyme of

nucleotide metabolism whose deficiency might result in accumulation of a substrate for cytosolic 5′nucleotidase did not reveal any abnormally high or low activities. The activities of all phosphoribosyltransferases and nucleoside kinases were normal. Increased nucleotide concentrations due to increased *de novo* synthesis also seems unlikely. Increased *de novo* purine synthesis would be expected to cause hyperuricosuria, as in PRPP synthetase superactivity (6, 22); increased *de novo* pyrimidine synthesis would be expected to cause orotic aciduria, as in ornithine transcarbamylase deficiency (18). Urate excretion in these patients was, in fact, decreased, and urinary concentrations of orotate, as well as those of cytosine and uracil, appeared to be within normal limits.

The mechanism through which increased 5′-nucleotidase activity produces neurological abnormalities and increased susceptibility to infections is not yet understood. In the other defects of purine metabolism that cause immunodeficiency a demonstrable lack of functional T or B cell, or lack of a thymus (4) is found. In the patients reported here, other than intermittantly low levels of IgG and IgA, and occasionally abnormal T cell responses no such indications of gross immunodeficiency were seen. Nor was there any accumulation of an unusual metabolite that might account for seizures, as in dihydropyrimidine dehydrogenase deficiency or adenylosuccinate lyase deficiency (2, 3).

The metabolic basis of the effectiveness of oral uridine is also unclear. Metabolic therapy with pyrimidine nucleotides was initiated in patient 1 due to megaloblastic anemia and reduced incorporation of uridine into pyrimidine nucleotides. However, further biochemical studies provided no evidence of pyrimidine nucleotide deficiency, and experience with additional patients has shown that this disorder bears little resemblance to orotic aciduria (19). Reduced uridine incorporation in cultured cells is a result of the relative rates of uridine phosphorylation and UMP catabolism and does not necessarily indicate a net deficiency of uridine nucleotides. Furthermore, the optimum dose of uridine for the treatment of these patients is far greater that amount required for pyrimidine replacement in orotic aciduria patients (19), and the pretreatment urinary concentrations of uridine and thymine in these patients were normal. Thus it is considered unlikely that uridine acts to alleviate a deficiency of pyrimidine nucleotides.

Of the reported inborn errors of metabolism, these patients most closely resemble the patient reported by Wada *et al.* (20), who was found to have a deficiency of PRPP synthetase. This patient was developmentally delayed and hypouricosuric, suffered from constant tonic-clonic seizures, and was said to be indifferent to the presence of others. PRPP synthetase activity was approximately 15% of normal, but erythrocyte PRPP concentrations were only moderately reduced. Treatment with corticosteroids increased his erythrocyte PRPP and lessened his symptoms (21). The patients reported here are also hypouricosuric and have reduced cellular PRPP. This PRPP deficiency may arise from increased utilization of PRPP by phosphoribosyltransferases in response to the increased nucleotide catabolism. If clinical symptoms in both disorders are considered to be a result of PRPP deficiency, then uridine may act by increasing PRPP. Production of uridine nucleotides from uridine would be expected to inhibit *de novo* pyrimidine synthesis, thus conserving PRPP, as well as make ribose available for PRPP synthesis through phosphorolysis of uridine. Both extracellular uridine (22) and ribose (23) have been shown to increase intracellular PRPP in a number of model systems. The finding that oral ribose also can cause some degree of neurological improvement in these patients would appear to lend support to this PRPP-increasing mechanism of action.

In addition to its metabolic effects, uridine also has direct effects on the central nervous system. Uridine has been shown to competitively inhibit γ-aminobutyric acid binding to both high- and low-affinity sites in the frontal cortex, hippocampus, and thalamus (24). Indeed, pretreatment of rats with uridine can protect against γ-aminobutyric acid type A antagonist-induced seizures (24). Uridine has dopaminergic effects as well. Pretreatment of rats with uridine reduced amphetamine-induced dopamine release (25). These direct effects also might play a role in reducing the neurological and behavioral symptoms in these patients.

The finding of four unrelated patients in local hospitals within a short period suggests that this syndrome is not rare. The phenotype is fairly distinctive, but at present enzyme analysis is required for a positive diagnosis.

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