Adenine Therapy for Lesch-Nyhan Syndrome

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Extract

A child with the Lesch-Nyhan syndrome was identified at birth by demonstrating a
gross deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase
(HPRT) in cord blood. Therapy with adenine, begun during the 1st month of life,
failed to prevent the development of the severe neurological damage characteristic of
this disease.

Speculation

Since this work was completed, Dr. Grant Bartlett of San Diego has found (unpub-
lished work) a very poor uptake of intravenously administered adenine-^{14}C in the brain
of rabbits. This raises a possible reason for the failure of adenine to prevent the neuro-
logical dysfunction in this disease. A purine compound that is more readily taken up by
brain tissue might, therefore, offer a more reasonable approach to treatment.

Introduction

Children with the severe form of X-linked uric acidu-
ria, the Lesch-Nyhan syndrome, suffer from incapaci-
tating neurological disease and have marked over-
production of uric acid [12]. Treatment of the
hyperuricemia of this condition with uricosuric agents
or allopurinol, however, does not alter the course of the
neurological disease [2, 14]. These patients have a defi-
ciency of the enzyme hypoxanthine-guanine phospho-
ribosyltransferase (EC. 2.4.2.8) (HPRT) [18], which
catalyzes the formation of purine nucleotides from the
free purine bases hypoxanthine and guanine in the
brain and all other tissues. Both the neurological man-
ifestations of this disorder and the aberrations of pu-
rine metabolism are thought to be consequences of this
enzyme deficiency, [9, 27].

The precise mechanism whereby HPRT deficiency
results in neurological dysfunction is not known. Defi-
ciency of this enzyme could lead to insufficient produc-
tion of guanosine 5′-monophosphate (GMP), a neces-
sary precursor of nucleic acid synthesis, or damage

could result from an excessive quantity of some precur-
sor of purine synthesis. A deficiency of HPRT is asso-
ciated with an increased amount of substrate, 5-phos-
phoribosylpyrophosphate (PRPP) in erythrocytes [8]
and skin fibroblasts [16] from HPRT-deficient pa-
tients. Both the neurological dysfunction and the ex-
cessive purine production in these patients could be
related to increased PRPP concentration. Alterna-
tively, a metabolic by-product of the excessive purine
synthesis may have a toxic effect on the developing
brain, or other substrates involved in purine synthesis
de novo may be depleted.

Several lines of evidence have suggested that the
purine base, adenine, may be of benefit in treatment of
patients with Lesch-Nyhan syndrome. Megaloblastic
anemia develops in a small portion of such patients,
and the anemia was corrected promptly in one child
when treated with adenine [19]. Demars et al. [3] have
shown that additional adenine, folic acid, or both are
required for optimal growth of HPRT-deficient fibro-
blasts in tissue culture. In mammalian tissues, adenine

is rapidly converted to its nucleotide by adenine phosphoribosyltransferase, an enzyme present in normal or increased quantities in patients with the Lesch-Nyhan syndrome [8]. 5-Phosphoribosylpyrophosphate is also utilized in this conversion. The nucleotide adenosine 5'-monophosphate (AMP) can then be converted to GMP and other purine nucleotides (Fig. 1).

We report here the results of adenine therapy in two patients with virtually complete HPRT deficiency. In one of these patients, adenine administration began in the 3rd week of life, when he was clinically normal [20].

Case Histories

Case 1

LVT (NIH no. 07-99-83) was the 7-lb 7-oz product of an uncomplicated term pregnancy and delivery in a 24-year-old gravida II, para 1 Caucasian of German descent. He was normally active and fed well without excessive regurgitation in the immediate newborn period. An older brother was normal. Although a maternal first cousin was known to be afflicted with Lesch-Nyhan syndrome, the patient's mother had declined the opportunity for prenatal evaluation of her infant by means of cultured amniotic fluid cells [3, 6, 26].

Because of the maternal family history, blood was obtained from the umbilical cord at birth and showed no detectable HPRT activity either by a screening assay [5] or in the conventional quantitative assay [18]. In addition, a urine sample collected when the patient was 14 days old showed a markedly elevated uric acid-to-creatinine ratio [11] of 6.0.

At 13 days of age the patient was admitted to the National Institutes of Health. His head circumference was 34.5 cm, and general physical and neurological examinations were
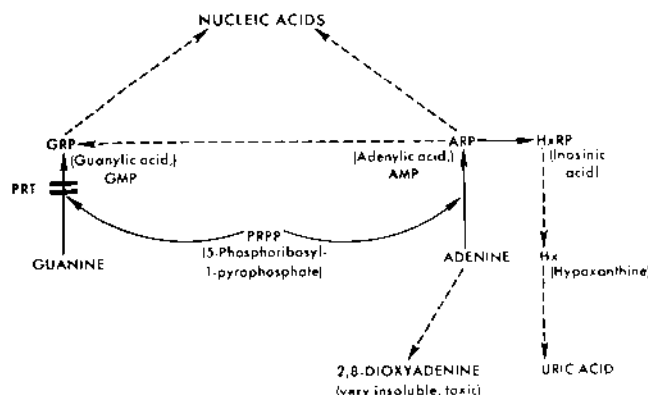

Fig. 1. Pathways available for adenine metabolism in mammalian tissues. Broken lines indicate multiple reaction sequences, and all intermediates are not included. The site of the metabolic block in patients with the Lesch-Nyhan syndrome and deficiency of the enzyme HPRT is indicated by the heavy double line.

Table I. Initial laboratory values for patient LVT

Blood:	
Hemoglobin	15.2 g/100 ml
Red blood cells	4.4 × 10⁶/mm³
Hematocrit	43%
Platelets	372,000/mm³
Reticulocytes	0.5%
White blood cells	8,500/mm³
differential (%):	
Neutrophiles	40
Bands	6
Lymphocytes	44
Monocytes	9
Basophiles	1
Mean corpuscular volume	98
Mean corpuscular hemoglobin	34.6
Mean corpuscular hemoglobin concentration	35.3
Urine:	
Specific gravity	1.004
pH	5.0
Protein	negative
Sugar	negative
Acetone	negative
White blood cells/high power field	1-5
Slight amorphous sediment including urate crystals	
Cerebrospinal fluid:	
8 white blood cells/mm³ (7 lymphocytes)	
6 red blood cells/mm³	
Glucose	56 mg/100 ml
Protein	62 mg/100 ml
Chemical values in blood:	
1. Blood urea nitrogen	9 mg/100 ml
2. Glucose	88 mg/100 ml
3. Phosphate	7.8 mg/100 ml
4. Uric acid	8.7 mg/100 ml
5. Creatinine	0.7 mg/100 ml
6. CO₂	19 mEq/liter
7. Na	140 mEq/liter
8. K	3.7 mEq/liter
9. Cl	106 mEq/liter
10. Calcium	5.15 mEq/liter

entirely normal. Initial laboratory data, also normal except for the high levels of urate in serum, are shown in Table I. Electrocardiogram, electroencephalograms, and skull roentgenograms were unremarkable. No evidence of megaloblastic changes was found in a bone marrow aspirate.

When the patient was 23 days old he received oral adenine therapy in divided doses every 4 hr. Toxicity was monitored by determining blood counts, blood urea nitrogen, creatinine and uric acid levels in serum, and urinalysis three times weekly. Urinary sediment was examined daily for the presence of the toxic metabolite of adenine, 2,8-dioxyadenine, as described below. The dosage was gradually increased from initial doses of 1.6 mg/kg/24 hr until evidence of toxicity was detected. Despite a high fluid intake,

azotemia, increasing urate levels in serum, and increasing presence of 2,8-dioxyadenine in the urine occurred at an adenine dose of 75 mg/kg/24 hr. At this point, allopurinol, 10 mg/kg/24 hr, administration was begun and adenine was discontinued for 3 days. The azotemia disappeared within 1 day after adenine discontinuation and did not recur when adenine was resumed at a dose of 25 mg/kg/24 hr and gradually increased to 60 mg/kg/24 hr. The patient was discharged from the hospital at 4 months of age and continued taking allopurinol 10 mg/kg/24 hr and adenine 60 mg/kg/24 hr at home.

By 8 months of age, despite this therapeutic regimen, the patient did not appear to be developing normally. He was unable to roll from back to front and could sustain his head in the upright position for only brief intervals. He did not laugh and did not transfer objects from hand to hand. His head circumference was 41 cm (less than 3rd percentile). He was unable to support his head when he was raised to a sitting position and was able to maintain the sitting position only with difficulty even when supported. Hyperextension and pronation of the arms were present during feeding. The direct tendon reflexes in the legs were hyperactive, and unsustained clonus was present at both ankles. His tongue had a fresh raw notch 0.5 cm wide and approximately as deep at the tip; this had apparently been chiselled out by the action of his only teeth, the two lower central incisors.

At 10 months of age the patient had progressed little from his developmental level at 8 months. Clear-cut evidence of spasticity was present. No additional self-mutilation was noted.

Case 2

SM (NIH no. 07-60-55). This 13-year-old, 26-kg Negro male had all the clinical and biochemical manifestations of the Lesch-Nyhan syndrome, including undetectable HPRT activity in his erythrocytes and a urinary uric acid-to-creatinine ratio of 4.4. He had scars and deformities of the fingers from biting, but he was unusual in that his lips and tongue showed no lesions. No change in the patient's clinical state was noted during his therapy with progressively increasing doses of adenine over a 20-day period; he continued to show spasticity, athetoid movements, and self-mutilating behavior. At an adenine dose of 35 mg/kg/24 hr the patient developed a decline in urine output, marked crystalluria, and dysuria. He also showed evidence of increased excretion of 2,8-dioxyadenine in his urine. Administration of adenine was decreased and allopurinol begun; there followed a rapid reversal of these evidences of toxicity (Figure 2).

Special Studies

Animal Studies of Adenine Nephrotoxicity

Adenine is oxidized by the enzyme xanthine oxidase to 2,8-dioxyadenine, an exceedingly insoluble purine
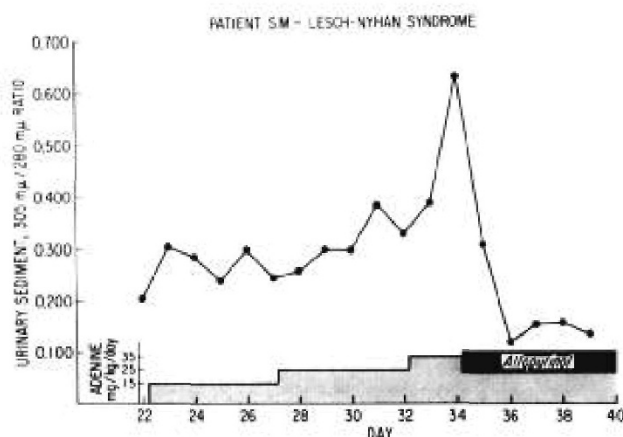

Fig. 2. Urinary dioxyadenine content, measured by ratio of absorbances of solution of urinary sediment at 305 mμ/280 mμ, plotted against dose of adenine in *patient SM*. See text for details of measurements of ratio. The sharp rise in the ratio at the highest dose of adenine was taken as evidence of toxicity.

Table II. Effect in rats of allopurinol on renal toxicity of adenine

Group[1]	Adenine dosage, mg/kg	No. of rats	Mean kidney weight, g/rat	Crystals, 1-4+
A_1	70	3	1.78	0, 3+, 1+
A_2	250	3	1.81	3+, 4+, 3+
B_1	70	3	1.44	0, 0, 0
B_2	250	3	1.90	3+, 2+, 2+
C	0	3	1.47	0, 0, 0

[1] *Groups A_1 and A_2 received adenine only. Groups B_1 and B_2 were pretreated with allopurinol and then received adenine. Group C was used as a control. See text for details of study.*

compound [1]. Renal failure accompanying the precipitation of dioxyadenine crystals in renal tubules is the principal toxic effect observed in animals after adenine administration [15]. Since allopurinol, a potent inhibitor of xanthine oxidase, would be expected to prevent the oxidation of adenine to its toxic metabolite, we undertook a limited study to test whether allopurinol would protect against adenine toxicity.

Male rats of the Sprague-Dawley strain, 200–300 g, kept in the fed state, received adenine [21] in aqueous suspension by gastric intubation in a single dose (*groups A-1 and A-2*). Other animals (*groups B-1 and B-2*) were pretreated with three doses of allopurinol (10 mg/kg/dose) at 12-hr intervals, and the adenine was administered 2 hr after the last dose of allopurinol. An untreated group (*group C*) was used as a control. All animals were killed 24 hr after adenine administration. The kidneys were removed and weighed, and unstained coded sections were examined under the polarizing microscope for the birefringent crystals
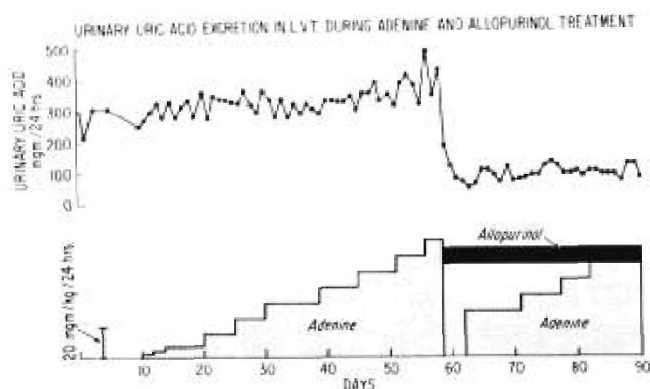
Fig. 3. Urinary uric acid excretion in *patient LVT* during treatment with adenine and allopurinol. Allopurinol, 10 mg/kg/24 hr, was started when signs of adenine nephrotoxicity appeared.
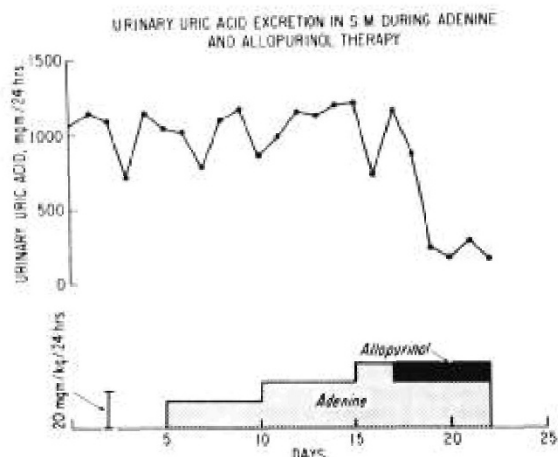

Fig. 4. Urinary uric acid excretion in *patient SM* during treatment with adenine and allopurinol. Allopurinol, 16 mg kg/24 hr, was started when signs of adenine nephrotoxicity appeared.

typical of dioxyadenine. The observer was unaware of the code used, and slides were graded in random order. Results are summarized in Table II. The data suggest that at the lowest dose of adenine, 70 mg/kg (*group B-1*), allopurinol had a significant protective effect against adenine nephrotoxicity. The kidneys of this group of rats did not exhibit the pale, swollen appearance of "adenine kidneys" [15] and had no detectable crystal deposition. At extremely high adenine doses, however, allopurinol in the amount used did not protect against adenine toxicity.

Urinary Dioxyadenine in Patients during Adenine Therapy

Dioxyadenine has a strong ultraviolet absorption at 305 mμ, whereas uric acid and other naturally occur-

ring urinary purines absorb maximally at wave lengths less than 290 mμ in acidic solutions. The high ratio of absorbance at 305 mμ to absorbance at 280 mμ has been used as an indication of the content of dioxyadenine in body fluids [15].

Ten-milliliter aliquots of freshly voided morning urine samples from each patient were centrifuged, the supernatant was decanted, and the sediment was dissolved in 5 ml of 1 N HCl. Residual insoluble material was removed by centrifugation, and the ratio of absorption at 305 mμ to that at 280 mμ was determined on a suitable dilution of the supernate with a spectrophotometer [22]. As shown in Figure 2, the 305 mμ-to-280 mμ ratio increased with increasing doses of adenine. In both patients, a sharp increase in this ratio was noted when adenine toxicity occurred.

Urinary Uric Acid Excretion

Twenty-four-hour urine was collected on ice using 3 ml of toluene as preservative. The urine was alkalinized with lithium carbonate and heated to redissolve any precipitated uric acid, and uric acid was then analyzed by a specific enzymatic method [13]. Patients received purine-free diets during all study periods.

Adenine administration resulted in no significant change in total uric acid excretion in the urine in either patient (Figs. 3 and 4). A small increase in uric acid excretion in *LVT* at the highest adenine dose may have reflected conversion of adenine to uric acid; in previous studies, it was found that 15–25% of a single oral dose of 1.0 g adenine-^{13}C given to normal adults was converted to uric acid [17]. Similar information during chronic administration of adenine is not available. Since specialized studies were not performed to ascertain the fraction of the administered adenine that was converted to urate in the patients in this study, the effect of adenine on the patients' endogenous production of uric acid could not be directly measured.

Erythrocyte PRPP Measurements

Patients with HPRT deficiency have a markedly increased concentration of PRPP in erythrocytes [8], presumably reflecting decreased utilization of this substrate in the reaction catalyzed by the missing HPRT enzyme (Fig. 1). A different enzyme which also utilizes PRPP as a substrate, adenine phosphoribosyltransferase, is present in normal or increased amounts in these erythrocytes [7].

Heparinized venous blood was chilled and centrifuged at 800 × g for 10 min, plasma and buffy coat

were removed, and the red cells were washed twice with Krebs-Ringer buffer, pH 7.4, at 4°. A 2% suspension of the washed erythrocytes was then assayed for PRPP content by a modification [8] of the method of Henderson and Khoo [10]. Prior to adenine treatment, erythrocyte PRPP concentrations were elevated far above normal in both patients (Figs. 5 and 6). Oral adenine administration resulted in striking reductions of the erythrocyte PRPP in these subjects.

Discussion

The presence of a lag in development, and such findings as hyperreflexia, posturing of the hands, and biting of the tongue strongly suggest that therapy with high doses of adenine failed to prevent the development of neurological dysfuction in patient LVT, even though treatment was started in the 1st month of life. Similarly, the drug had no effect on the well established neurological deficits in patient SM. The decrease in erythrocyte PRPP levels with adenine administration demonstrates that adenine was absorbed from the gastrointestinal tract in our patients. There is, however, considerable possibility that only a small amount of the administered adenine entered the central nervous system of these patients. In experimental animals, adenine-^{14}C enters the brain to a very limited degree [4]. Furthermore, the uptake of adenine by the brain does not increase linearly with adenine dose. Such factors could explain the failure of adenine to prevent development of neurological dysfunction.

Administration of adenine-^{13}C resulted in a substantial inhibition of purine synthesis de novo in both normal and gouty subjects, including a patient with an incomplete deficiency of HPRT, without diminishing total uric acid excretion in the urine [17]. Likewise, uric acid excretion in urine did not change despite increasing doses of adenine in our two patients; a similar observation has been made by Berman et al. [2]. If it is assumed that a significant proportion of the administered adenine was converted to uric acid and excreted, it may be inferred that adenine administration also caused a reduction in endogenous uric acid production in these patients. The magnitude of this effect, however, is evidently small enough that adenine cannot be recommended as useful for reducing the hyperuricemia or hyperuricosuria seen in patients with Lesch-Nyhan syndrome.

Adenine therapy did succeed in correcting one abnormal biochemical variable, for the elevated levels of PRPP in erythrocytes fell toward the normal range. Since PRPP concentrations in brain, liver, and other
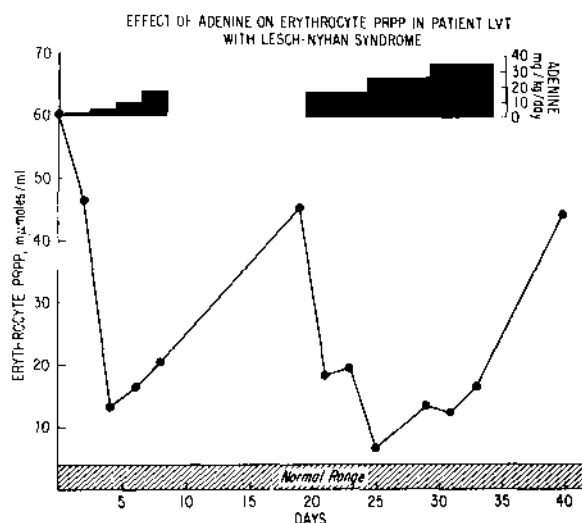

Fig. 5. Effect of adenine on erythrocyte 5-phosphoribosyl-1-pyrophosphate in patient LVT. Normal range was determined from values from 10 normal adults.
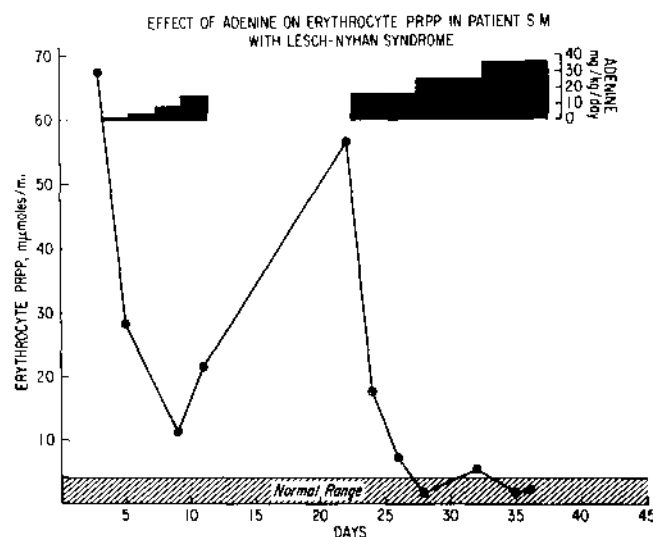

Fig. 6. Effect of adenine on erythrocyte 5-phosphoribosyl-1-pyrophosphate in patient SM. Normal range was determined from values for 10 normal adults.

metabolically active tissues possessing intact purine biosynthetic pathways de novo could not be directly measured, the importance of this compound in explaining the accelerated purine synthesis in HPRT-deficient patients could not be determined. The possibilities therefore remain that in these metabolically active tissues PRPP has a very high synthesis and turnover rate, that HPRT deficiency markedly reduces PRPP degradation and results in its accumulation, and that the amount of adenine administered was insufficient to correct the PRPP abnormalities in these target organs.

Summary

Oral therapy with adenine, begun in the 1st month of life, did not prevent the development of neurological dysfunction by 8 months of age in a child with deficiency of HPRT (Lesch-Nyhan syndrome), and similar treatment had no effect on an older child with the same disorder. Uric acid excretion in the urine did not decrease during adenine therapy; however, there was a dramatic decline in elevated erythrocyte PRPP concentrations during treatment. The absorbance ratio at 305/280 mμ of dissolved urinary sediment was found useful for monitoring the nephrotoxicity which appeared after administration of high doses of adenine. This toxicity was minimized by simultaneous therapy with allopurinol, which inhibits the conversion of adenine to the less soluble derivative, 2,8-dioxyadenine, the substance responsible for nephrotoxicity.

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20. Informed consent was obtained in accordance with the provisions set forth in the Declaration of Helsinki.
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