Acta Paediatrica Hungarica, 26 (4), pp. 327–333 (1985)

Adenine Therapy in Lesch-Nyhan Syndrome

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In a 7-year-old patient with Lesch-Nyhan syndrome (LNS) the ^{15}N excess frequency was determined in the excreted uric acid after oral application of 27 mg ^{15}N glycine/kg body weight, using emission spectrometry. Incorporation of glycine into uric acid was considerably increased in untreated LNS in comparison with the control. This was due to the extremely increased endogenous de novo synthesis of purine. Allopurinol therapy caused only a gradual decrease of uric acid excretion. The pattern of purine excretion changed in favour of the better soluble oxipurines hypoxanthine and xanthine, by competitive inhibition of xanthine oxidase. In LNS, however, allopurinol had no uricostatic effect.

Therapy with adenine is an alternative to influence the de novo synthesis. After adenine application a decrease of the cumulative ^{15}N uric acid excretion occurs and the percentual proportion of ^{15}N uric acid in total ^{15}N excretion decreases. These changes are due to an inhibition of de novo purine biosynthesis. Adenine, however, must be applied in combination with allopurinol in order to avoid the formation of nephrotoxic 2,8-dioxiadenine by xanthine oxidase. Adenine therapy led to an improvement of the clinical course. No side-effects were observed.

Lesch-Nyhan syndrome (LNS) is a disease of purine metabolism with recessive inheritance. The de novo synthesis of purine is increased due to a defect or gross deficiency of the activity of hypoxanthine-guanine phosphoribosyltransferase (HG-PRT), and the daily turnover of uric acid is considerably increased. The excessive juvenile hyperuricaemia and hyperuricuria may lead to the formation of urate nephrolithiasis and nephropathy, gouty arthritis and tophi.

This is regularly associated with progressive neurological signs and symptoms such as aggressive automutilation, choreoathetosis, muscle spasticity and mental retardation. Whereas the complications of hyperuricaemia can be prevented by allopurinol, the drug has no effect on the development of CNS symptoms [18, 22]. Since children with LNS do not show signs of cerebral lesions at birth, there should be some means of prevention but numerous attempts [1, 3, 6, 10, 18] have failed to affect the progression of CNS lesions.

Therapy with adenine is a possibility to increase the nucleotide supply to the CNS. An inhibition of the de novo purine synthesis is anticipated due to the rise of purine nucleotide concentration through a feed-back mechanism. The present paper will describe the effect of adenine therapy

on purine metabolism by means of ^{15}N-tracerkinetic measurements and on the course of the disease of a 7-year-old patient with LNS.

Report of a Case

T. M., a girl, was born on 28. 9. 1976 and admitted at the age of 7 months. At that time the patient showed clear statomotor retardation, no traction response, no rotation from supine into prone position, no grasping, persisting infantile reflexes, jittery arms, athetotic movements.

The blood urea level was 490 μmol/l. Activity of HG-PRT in erythrocytes was reduced to 4% and in skin fibroblasts to 5% of normal (Table I). Thus, the diagnosis of LNS was confirmed. After administration of allopurinol (10 mg/kg body weight) and adjusting the blood urea level to 200—300 μmol/l the child was discharged. She was admitted again at the age of 5 years when she was moderately atrophic (11.2 kg) and had a short stature (3 sigma too small). Her oral mucosa, lips and both forefingers showed scars after bites.

Neurological findings. Vigilant visual contact to persons, speech consists only of some syllables; frequent changes between periods of motor rest and abrupt abnormal patterns of movement, absent traction response, stepping reflex not possible due to crossing of legs, spasm of adductors of fingers and hands, opisthotonus, response to pain and tactile contacts.

Paraclinical findings. Serum urea and creatinine, electrolytes, acid-base status and endogenous creatinine clearance were normal. No evidence of megalocytic anaemia, urea concentration on allopurinol therapy was between 200 and 300 μmol/l. No evidence of calculi in the urinary system on contrast urography and renal sonography. Isotope nephrogram normal on both sides, no evidence of gouty arthritis or tophi. EEG: mild to moderate diffuse disturbances, low voltage, no focal signs or evidence of convulsions.

Combined therapy with adenine and allopurinol has so far been continued for 18 months. During this time, a clear improvement has been observed. The child was more interested and open to contacts from the environment, her understanding of words had improved and the trend for autoaggression has clearly diminished. Only once were mild bites seen on the lips.

Methods

^{15}N tracerkinetic examination. Glycine is an essential element of de novo purine biosynthesis. It was used as ^{15}N glycine (96 at.-% ^{15}N); the oral dose was 27 mg/kg body mass. Then the incorporation rate into uric acid was determined. ^{15}N glycine was administered at 8 a.m. with fluid. Urine was collected over the following 3 days, the daily urine volume was measured, the excretion of uric acid was determined and aliquot samples were deep-frozen (−21°C) for ^{15}N-tracerkinetic examinations. A child with normal metabolism and with the same body mass served as a control.

a) LNS without therapy
b) LNS on adenine therapy
c) LNS on allopurinol therapy
d) LNS on combination therapy with adenine and allopurinol
e) control without therapy
f) control on adenine therapy.

The daily dose of allopurinol was 10 mg/kg body weight, that of adenine, 100 mg/kg body weight. The patient was given a normal diet with restriction of foods rich in purine.

HG-PRT was determined in the haemolysate and in fibroblast homogenates using the radiochemical method of Wehnert et al [20]. Serum and urine uric acid levels were determined enzymatically with uricase (katalase according to Kageyama [8]) (Fermagnost®-uric acid test). The concentrations of hypoxanthine and xanthine were determined in a parallel sample as uric acid equivalent by addition of 0.01 U/ml xanthine oxidase (Boehringer, Mannheim).

Isolation of uric acid. Determination of the ^{15}N labelling of uric acid requires its preparative separation and purification. The separation of uric acid from the collected urine followed a separation scheme designed for ^{15}N isotope analysis in NPN compounds [5]. Uric acid was separated from the other NPN constituents by means of adsorption chromatography to polystyrene sulphonic acid (column: 10×170 mm Dowex 50 WX8; eluent: water). After passage of the column, uric acid was crystallized in a 10 ml fraction and purified by transcrystallization in water.

^{15}N isotope analysis. ^{15}N isotope analysis requires transformation of the nitrogen compound studied into ammonia. Urea N and the N-compounds of urine were transformed into ammonium chloride by the Kjeldahl procedure and alkaline distillation. Determination of the relative ^{15}N frequency was performed by emission spectrometry with the ^{15}N analyser NOI-6 [12]. Isotope analysis required 55 μg ammonium chloride substance. The labelling values determined for uric acid and total N in the urine were mean values of a threefold determination with a relative standard error of 2.5%.

Results and Discussion

In the patient with LNS, HG-PRT activity decreased to 4% in erythrocytes and 5% in skin fibroblasts (Table I). The lack of HG-PRT activity prevented the re-use of the purine bases hypoxanthine and guanine and their synthesis into the respective nucleotides. The decrease of intra-

Table I

HG-PRT activity in erythrocytes and skin fibroblasts

	GMP nmol/l/h and μl erythrocytes	GMP nmol/l/h and g protein
Controls	$\bar{x}=105\pm11$ n=40	$\bar{x}=816\pm107$ n=4
Father	110	absent
Mother	101	273
Patient T. J.	4	40

Table II

Uric acid blood level in a patient with LNS without therapy and on treatment with adenine, allopurinol and their combination

Therapy	Uric acid blood level μmol/l
No therapy	617
Adenine 4×0.5 g daily	1073
Allopurinol 3×50 mg daily	256 ± 71 n=10
Adenine, 4×0.5 g and allopurinol, 3×50 mg, daily	n.s. 277 ± 84 n=6

cellular purine nucleotide concentration reduces the feed-back inhibition of phosphoribosyl-pyrophosphate-aminotransferase (PRPP-AT). Additionally, the HG-PRT defect leads to a reduced reutilization of phosphoribosyl-pyrophosphate (PRPP); thus, more of it is available for de novo purine synthesis. The uncontrolled de novo synthesis of purine nucleotides leads to a massive overproduction of uric acid with a rise of the blood level (Table II) and elimination with urine (Fig. 1). The uric acid elimination (in mmol/l per day) was increased in our patients as compared with the control (Fig. 1). This was even clearer for the proportion of the de novo synthetized uric acid, indicated by the cumulative (^{15}N)uric acid excretion. After the reduction of allopurinol we found a gradual decrease of uric acid excretion in the patient (Fig. 1). The proportion of cumulative (^{15}N)uric acid excretion as compared to the cumulative ^{15}N total excretion was not affected and remained high (Fig. 2).

Allopurinol has no uricostatic effect in LNS, since the increased supply of hypoxanthine with the HG-PRT defect cannot be transformed into IMP, and allopurinol cannot be transformed into allopurinol-MP. Therefore, allopurinol therapy affects only the inhibition of xanthine oxidase. A considerable part of the precursors hypoxanthine and xanthine is excreted instead of uric acid due to the inhibition of xanthine oxidase.

Adenine therapy is an alternative to affect the nucleotide concentration and thus, the de novo synthesis. Exogenous adenine is metabolized into adenosine monophosphate (AMP) by means of adenine-phosphoribosyl-transferase (A-PRT), and part of it is transformed into GMP and IMP through the purine nucleotide cycles.

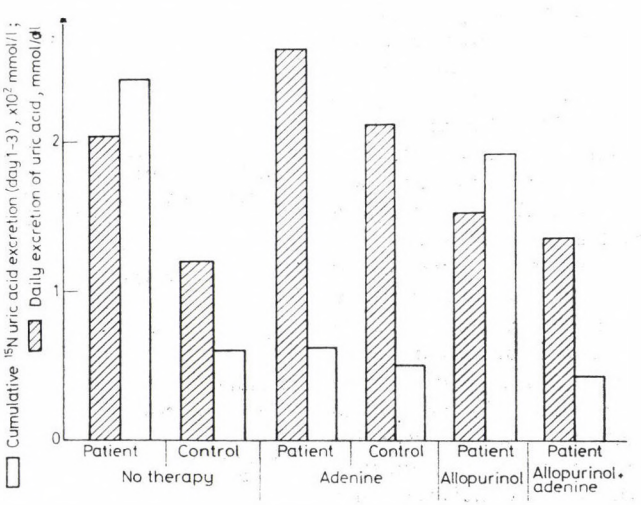

FIG. 1. Daily excretion of uric acid and cumulative ^{15}N uric acid excretion in the urine of a patient with LNS without therapy and on treatment with adenine, allopurinol and a combination of both in comparison with the control

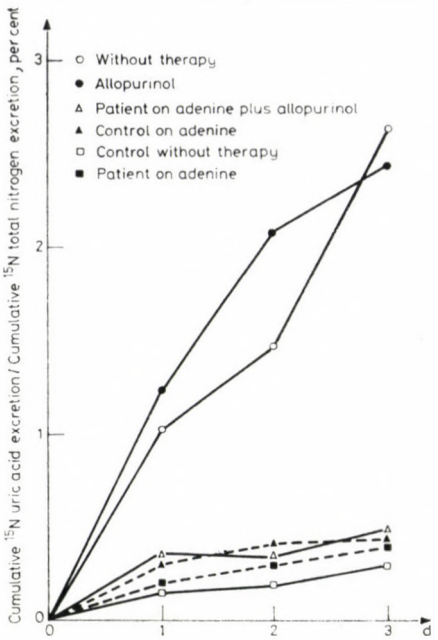

FIG. 2. Quotient of cumulative ^{15}N uric acid excretion to ^{15}N total nitrogen excretion in the urine of a patient with LNS without therapy and on treatment with adenine, allopurinol and a combination of both in comparison with the control

In LNS, the activity of A-PRT is particularly high in the liver, erythrocytes and basal ganglia [16] as well as in HG-PRT deficient fibroblasts [15], due to the stabilizing effect of the increased PRPP concentration on the activity of A-PRT.

After administration of adenine sulphate in a dose of 100 mg/kg body weight daily, we observed an increased elimination of uric acid in the urine of our patient and also in the control subject (Fig. 1). Similarly, the uric acid blood level showed a significant increase (Table II). This rise was induced by that part of adenine which is metabolized directly into uric acid. Since both the cumulative (^{15}N)uric acid elimination (Fig. 1) and the percentual proportion of the total ^{15}N elimination in urine decreased to the values of the control (Fig. 2), an inhibition of adenine of the de novo synthesis of purines may be postulated, and so an inhibition of the de novo synthesis of uric acid in the LNS patient.

Although the actual uricostatic effect originates from adenine, adenine must be applied only in combination with allopurinol. Only on their simultaneous application does the blood uric acid level decrease to normal values (Table II); the quotient oxipurine/uric acid elimination increased from 0.13 to 1.46 in comparison to the administration of adenine alone, and the formation of

nephrotoxic 2,8-dioxiadenine from adenine by xanthine oxidase was prevented [4, 14].

The clinical course was dominated by a progressive neurological picture with dystonic-athetotic infantile cerebral palsy. Whereas the sequelae of juvenile gout could be prevented by allopurinol therapy during the first seven years of life, the cerebral symptoms were not influenced. It is not known by what mechanism the HG-PRT defect leads to such severe neurological lesions [11]. A normal brain function, however, depends on an adequate supply of purine nucleotides [9]. In LNS the synthesis of GMP and IMP through the salvage pathway is reduced; this plays a more important part in comparison with the de novo synthesis in the nerve cell [7, 16]. A decrease of the intracellular concentration of these nucleotides in HG-PRT defects may be prevented by adenine. The activity of A-PRT is increased in compensation [2, 19]. The exogenous intake of adenine leads to an increase of the concentration of AMP and IMP [13], to an inhibition of PRPP-AT and, as we have shown, to a reduction of the de novo synthesis of purine.

Attempts of treatment with adenine in LNS were made in some cases [4, 17, 21] but without success and in a few cases therapy had to be discontinued since kidney lesions developed due to the formation of 2,8-dioxiadenine [14]. Therefore, simultaneous administration of allopurinol is indispensable.

Our patient has been given allopurinol and adenine for 18 months so far; no side-effects have been observed during this time and we could observe a clear improvement of her mental and motor development. Thus, therapy with adenine and allopurinol can be recommended in LNS. Still, certain irreversible cerebral lesions could not be changed, and our studies did not answer the question, whether neurological complications may be prevented by an onset of therapy in early infancy.

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Received 19 December 1984

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