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Effect of D-ribose on purine synthesis and neurological symptoms in a patient with adenylosuccinase deficiency

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Abstract

Oral supplementation of 10 mmol/kg/day of D-ribose to a patient with an inherited deficit of adenylosuccinase, severe psychomotor retardation, and epilepsy caused a marked increase in plasma concentration and urinary excretion of urate, while minor changes in succinylpurine levels were observed. D-Ribose administration was accompanied by a slight improvement of behaviour and a progressive reduction of seizure frequency, which increased dramatically upon two attempts to withdraw the drug. Substitution of D-ribose with an equivalent amount of D-glucose did not result in an increase of seizure frequency.

Keywords: Adenylosuccinate lyase; Psychomotor retardation; Epilepsy; Purine metabolism; D-Ribose

1. Introduction

Adenylosuccinase (adenylosuccinate lyase; EC 4.3.2.2) catalyses two steps in the synthesis of purine nucleotides involving the nonhydrolytic cleavage of succinyl groups to yield fumarate: the conversion of succinylaminoimidazole carboxamide (SAICA) ribotide into aminoimidazole carboxamide (AICA) ribotide along the de novo pathway, and the formation of AMP from adenylosuccinate in the conversion of IMP into adenine nucleotides. Partial adenylosuccinase deficiency in humans [1–4] leads to the accumulation in body fluids of two normally undetectable compounds, SAICA riboside and succinyladenosine (S-Ado), that are the dephosphorylation products of the two substrates of the enzyme. The defect is transmitted as an autosomal recessive trait [5–7] and associated with psychomotor retardation, epilepsy and, in some cases, autistic features. In addition, some of the subjects display cerebral and cerebellar hypotrophy, growth failure, and muscular wasting. The prognosis for survival of profoundly retarded patients is poor. At present, the oldest of these patients has reached about 20 years of age, but two others died at 8 and 13 years, respectively [4]. The pathogenesis of the symptoms is still debated, since accumulation of intermediates proximally from the enzyme defect, as well as deficiency of metabolites which are normally formed distally thereof, might have deleterious effects. With the aim of replenishing the adenine nucleotide pool without increasing the level of hypothetically toxic metabolites above the

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enzyme defect, some patients were treated [2] for several months with oral supplements of purine bases, e.g. adenine combined with allopurinol, which avoids the oxidation of adenine into its poorly soluble nephrotoxic product, 2,8-dihydroxyadenine [8]. No clinical improvement or reduction of succinylpurine synthesis was recorded, with the exception of some acceleration of body growth.

In this work, we attempted to stimulate purine de novo synthesis in a subject with inherited deficiency of adenylosuccinase by giving D-ribose orally. The rationale is that D-ribose is immediately phosphorylated [9–12] to ribose 5-phosphate and then extensively converted to glucose and 5-phosphoribosyl 1-pyrophosphate, a key regulatory substrate in purine metabolism (Fig. 1) [13].

2. Materials and methods

The patient, a 13-year-old female (weight: 31 kg), was described previously elsewhere [3,7,14]. She presented severe psychomotor retardation and epilepsy (about two seizures per month). Febrile infections increased seizure frequency. At the time of study, she was treated with 200 mg/day carbamazepine (Tegretol, Ciba-Geigy Co.). Seizure frequency had not changed for at least 6 months before she began taking D-ribose, and no changes had been made in her medication during this interval. Normal adult controls, free of any neurological or muscular disorders, volunteered for the study. Experimental protocols were approved by the Institution's Committee on human experiments. Informed consent was obtained in all cases.

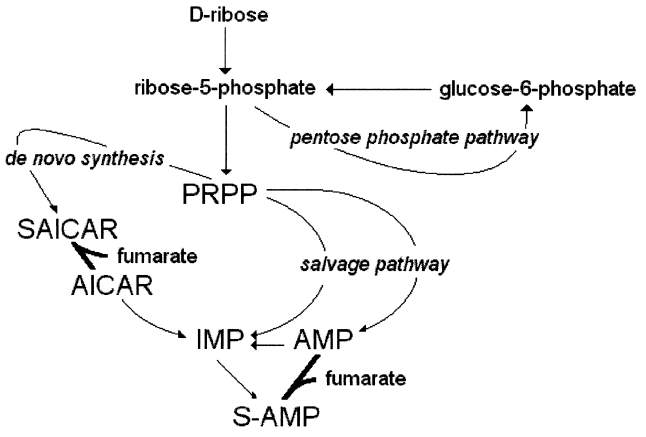

Fig. 1. General scheme showing the role of adenylosuccinase in purine metabolism and the stimulation of 5-phosphoribosyl 1-pyrophosphate (PRPP) synthesis via the pentose phosphate pathway. SAICAR, succinylaminoimidazole carboxamide ribotide; AICAR, aminoimidazole carboxamide ribotide; S-AMP, adenylosuccinate.

Erythrocyte subpopulations of different ages were separated by centrifugation on a discontinuous NaCl/Percoll (Pharmacia A.G.) density gradient [15]. Mononuclear preparations containing 75–80% lymphocytes and apparently free of contaminating erythrocytes were obtained by a one-step procedure involving centrifugation of heparinised blood layered on Histopaque (Sigma Chemical Co.) [3]. Adenylosuccinase activity was determined by following the conversion of adenylosuccinate into AMP after separation of the reaction product from the substrate by high-performance zone electrophoresis [16]. SAICA riboside and S-Ado were measured in plasma and urine by high-performance liquid chromatography [1]. The concentration of D-ribose was determined by reacting its furfural derivative with *p*-bromoaniline [11,17]. Oxypurine concentration ([xanthine]+[hypoxanthine]) was measured by the enzymatic method of Tattersall et al. [18] with minor modifications. Carbamazepine, glucose, urate, and creatinine in plasma and/or urine were measured by standard laboratory methods.

Standard electroencephalograms (EEG) were performed with a computerised Brain Quick Micromed – 24 channels; the patient kept her eyes open during the analysis. Patient's skill to perform simple exercises (to go up and down the stairs, to wash her hands, to take off her jacket, to blow bubbles) was documented every 15 days by means of 30-min shots.

3. Results

D-Ribose was administered orally (four times daily) to healthy individuals and to the patient with adenylosuccinase deficiency, who had been restricted to an isocaloric purine-free diet.

In good agreement with previously reported data [10–12], the mean plasma [D-ribose] in normal adult volunteers was 0.2–0.5 mM, if the subjects were treated with 13 mmol/kg per day of D-ribose, whereas double amounts resulted in a concentration of 1–3

Table 1
D-Ribose administration to the adenylosuccinase-deficient patient, restricted to an isocaloric purine-free diet

		D-Ribose supplement[a]	
		none	10 mmol/kg per day
Plasma			
	urate, mM	0.12 ± 0.1	0.28 ± 0.1
	xanthine+hypoxanthine, μM	1.8 ± 0.4	1.9 ± 0.5
	SAICA riboside, μM	2.5 ± 0.1	2.5 ± 0.1
	S-Ado, μM	3.0 ± 0.1	3.0 ± 0.1
	glucose, mM	5.4 ± 0.2	4.8 ± 0.2
	ribose, mM	0.0	0.3 ± 0.05
	carbamazepine, μg/ml	6.7	6.2
Urine, mol/mol			
	urate/creatinine	0.26 ± 0.05	0.93 ± 0.04
	SAICA riboside/creatinine	0.062 ± 0.007	0.052 ± 0.007
	S-Ado/creatinine	0.088 ± 0.006	0.094 ± 0.006

[a]Quadruplicate measurements are reported as mean ± S.D.

mM. Depending on the dosage, between 5 and 15% of D-ribose was eliminated in the urine. The administration of 13 mmol/kg per day of D-ribose caused a 10–20% decrease in serum glucose, while both the uricaemia and the urate-to-creatinine ratio of morning urine samples rose 2–3 times above the starting levels (data not shown). Aside from occasional cases of diarrhoea under higher dosages, the oral administration of D-ribose at concentrations up to 30 mmol/kg per day was tolerated without complaints.

Administration of 10 mmol/kg per day of D-ribose to the patient with adenylosuccinase deficiency did not produce appreciable toxic effects. Urinary losses were around 5% of the assumed dose. As shown in Table 1, plasma [D-ribose] was about 0.3 mM, while glycaemia decreased from 5.4 mM to 4.8 mM upon drug loading. No significant changes in plasma carbamazepine concentration (from 6.7 to 6.2 μg/ml) were noticed. Compared to the starting values, the patient showed a markedly higher urate-to-creatinine ratio in urine (from 0.26 to 0.94), indicating a rise in the urate excretion approximately from 0.78 to 2.8 mmol/day. Uricaemia increased from 0.12 to 0.28 mM. Hyperuricosuria and hyperuricaemia were constantly present during D-ribose supplementation to the purine-free diet over the time assayed (10 days). Urinary succinylnucleoside levels varied scarcely ([SAICA riboside]/[creatinine] changed from 0.062 to 0.052; [S-Ado]/[creatinine] changed from 0.088 to 0.094). No appreciable variations of succinylnucleoside concentrations (2.5 μM SAICA riboside; 3.0 μM S-Ado) were observed in plasma. Plasma oxypurine concentration ([xanthine]+[hypoxanthine]) did not change significantly (from 1.8 μM to 1.9 μM). Higher doses of D-ribose (up to 20 mmol/kg per day) caused mild diarrhoea promptly reversed by reducing drug administration.

Supplements of 10 mmol/kg per day of D-ribose to unrestricted diet were well tolerated by the patient for several months. Adenylosuccinase activity in erythrocytes and mixed peripheral blood lymphocytes remained practically constant under all conditions tested over the time assayed (about 30–40% of that in control subjects [3,16]). Enzyme activities did not vary in erythrocyte subpopulations of different ages. Routine blood analysis did not show appreciable changes in liver enzymes and in haematological parameters (data not shown).

D-Ribose administration was accompanied by a progressive reduction in seizure frequency, which increased dramatically upon two attempts to withdraw the drug (Fig. 2). Substitution of D-ribose with an equivalent amount of D-glucose did not result in an increase of the tendency to have convulsions. Suppression of D-ribose did not coincide with febrile infections.

Before the administration of sugars, the EEG pattern consisted of spikes, sharp waves, and slow waves in different combinations with paroxysmal appearance (pseudoperiodic pattern) Background rhythm

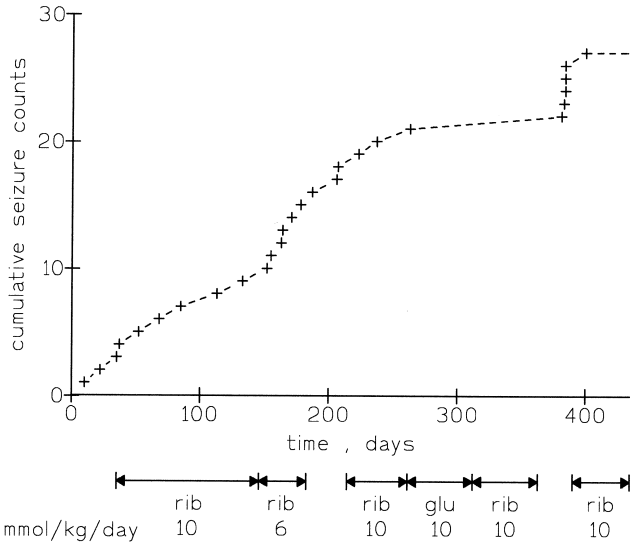

Fig. 2. Effect on seizure frequency of long-term treatments with sugars. D-Ribose (rib) and D-glucose (glu) were administered orally, four times daily, at the doses reported. The numbers of generalised seizures are added to the cumulative total progressing with time.

was also somewhat slow (delta activity). After loading D-ribose or D-glucose, the paroxysmal epileptiform activity was still evident, with a better background activity (theta activity).

According to her parents, the patient showed, during the treatment with D-ribose, greater motor nimbleness and a wish of playing and expressing herself, and a lower frequency of stereotypic body movements. Analysis of videotape records confirmed the slight improvement of patient's motor co-ordination, which was less evident when D-ribose was replaced with D-glucose.

4. Discussion

The effect of D-ribose supplementation in humans has been deeply analysed in the literature. Infusion of both labelled and unlabelled D-ribose showed that this pentose was rapidly and extensively metabolised [9–12], the principal fate being the conversion to glucose via the pentose phosphate pathway. D-Ribose led to an increased provision of 5-phosphoribosyl 1-pyrophosphate and stimulation of de novo purine synthesis in vitro [19] and in vivo [20–23]. D-Ribose was administered to patients with a deficiency of myoadenylate deaminase [24–26] or muscular phosphorylase [27] in order to increase nucleotide synthesis. For the same reason, D-ribose has already been applied successfully in patients with coronary heart disease [28]. Further possible sites of application for D-ribose include transplantation [29] and myocardial scintigraphy [30]. Though converted to glucose, D-ribose caused a sustained lowering of glycaemia, presumably because of the inhibition of phosphoglucomutase [9,12]. The lack of symptomatic response to D-ribose-induced hypoglycaemia suggests that neural tissue could be able to utilise this sugar. The hypothesis is in line with the observation that D-ribose was metabolised to hexose phosphate by brain homogenates [31]. On the other hand, chronic hypoglycaemia was found to increase brain glucose transport [32]. It is also conceivable that D-ribose could elevate the 5-phosphoribosyl 1-pyrophosphate level in brain cells with low or no de novo synthesis and allow these to better salvage purine bases, a very important process in human brain [13].

Our experiments show that D-ribose caused a sustained increase in plasma concentration and urinary excretion of urate both in a patient with adenylosuccinase deficiency and in normal control subjects. Patient's urinary excretion of urate approached, in the presence of pentose, the upper reference limit for her age class [33,34]. The long-lasting hyperuricaemia and hyperuricosuria, which we found in subjects restricted to a purine-free diet, strongly suggest that D-ribose could enhance purine de novo synthesis, although we cannot exclude that an increased hepatic purine catabolism could also contribute, at the beginning of D-ribose supplementation, to increased purine excretion. The hypothesis is in line with the in vitro experiments on partially defective human fibroblasts [35], showing normal fluxes through the conversion of SAICA ribotide into AICA ribotide, and suggests that inherited reduction of adenylosuccinase activity did not make the enzyme limiting, provided that we are considering the overall metabolism in the patient. However, because adenylosuccinase deficiency is not generalised in all the cells [1,2], it is conceivable that the efficiency of purine synthesis, and the capacity of D-ribose to promote it, might be different in the various tissues. Indeed, we have found very small changes of SAICA riboside and S-Ado in plasma and urine, which did not account for

the marked increase in purine metabolic flux. This could be easily explained by assuming that purine de novo synthesis was less operative in tissues severely lacking in adenylosuccinase activity. This hypothesis is consistent with the observation [36] that succinylnucleotides did not accumulate in tissues (liver, kidney), where adenylosuccinase activity was markedly decreased.

The decrease in seizure frequency upon administration of sugars (D-ribose, D-glucose), as well as the exacerbation of symptoms upon stopping the treatment, indicates that the metabolism of carbohydrates might be involved in the inherited damage. This finding is in line with previous observations on patients with adenylosuccinase deficiency. Indeed, positron emission tomography already demonstrated a limited glucose uptake in patient's brain [37], suggesting that the metabolic flux through the glycolytic path was reduced. On the other hand, ^{31}P-nuclear magnetic resonance spectra of patient's muscle showed a reduction of the energy reserve [16]. We suggested that the impairment of energy metabolism was due to defective supply of oxidisable substrates either directly to the citric acid cycle (as fumarate) or from the glycolytic pathway, owing to the lack of adenylosuccinase in the purine nucleotide cycle. This hypothesis is consistent with the in vitro experiments showing that the purine nucleotide cycle controls phosphofructokinase and glycolytic oscillations at least in muscle extracts [38].

This work shows that administration of sugars can improve some of the symptoms in the patient with adenylosuccinase deficiency and gives new insight for managing the inherited disease. Further studies are required to exclude any toxic side-effect, not yet observed, in order to utilise sugar supplementation in clinical practice.

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