Medical Hypotheses 79 (2012) 157–164

Contents lists available at SciVerse ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Epilepsy as a pyridoxine-dependent condition: Quantified urinary biomarkers for status evaluation and monitoring antiepileptic treatment

Svetlana Dolina *, Dov Margalit, Sergey Malitsky, Eugeny Pressman, Aharon Rabinkov

Advanced Neuroprotective Systems, Ltd., Kaplan's Medical Center Pediatric Department, Rehovot, Israel

ARTICLE INFO

Article history:
Received 30 November 2011
Accepted 16 April 2012

ABSTRACT

The study testifies an assumption on epilepsy as an inborn error of pyridoxine metabolism and suggests non-invasive quantitative biomarkers for clarified evaluation of clinical status and monitoring an individual treatment by antiepileptic drugs.

Urinary parameters of pyridoxal-phosphate (PLP)-dependent tryptophan degradation and the level of 4-pyridoxic acid, the end product of pyridoxine metabolism, were measured by HPLC method with simultaneous ultraviolet and fluorimetric detection in children with different forms of epilepsy and matched healthy controls.

The concentrations of compounds formed or metabolized in the course of tryptophan degradation (kynurenines, indoxyl-sulfate) along with correlations between them turned out to be quantitative biomarkers useful for both clarifying patient's clinical state and monitoring antiepileptic treatment. In particular, the value of the ratio of 4-pyridoxic acid to kynurenine appears to be an index of an experienced seizure attack, while the ratio of 3-hydroxyanthranilic acid to 3-hydroxykynurenine reflects activity of kynureninase, the enzyme of critical sensitivity to PLP supply. Growing progressively worse, epilepsy is accompanied by aggravation of PLP-dependent disturbances of tryptophan metabolism and expanding inhibition of kynureninase. The affected pyridoxine metabolism is discussed as an inborn genetic trait in epilepsy in general, rather than a specific sign of pyridoxine-dependent epilepsy solely.

Introduction

Up to recently PDE was the only form of epilepsy for which the principal pathogenetic role of pyridoxine (vitamin B6) was accepted. Described more than 50 years ago [1], PDE has been considered a rare (1:100.000) autosomal recessive genetic disorder, occurring in utero or subsequently in infancy or early childhood. Familial cases are repeatedly described. The resistance to conventional antiepileptic drugs (AED) and response to pyridoxine administration are the main characteristics of PDE. [2–4].

An inborn abnormality of the pyridoxal phosphate (PLP)-dependent synthesis of GABA had been postulated as a cause of the disease and life-long pyridoxine administration had been recommended [5–7]. Later on in the search for the gene responsible for PDE, the primary involvement of glutamate decarboxylase was excluded [8]. At present PDE is considered a result of PLP inactivation caused by accumulation of L-Δ^1-piperidein-6-carboxylate in the pipecolic acid pathway of lysine degradation, when mutations in ALDH7A1/antiquitin gene occur. The raised levels of pipecolic acid and alpha-amino adipic semialdehyde in urine, plasma and CSF are accepted biochemical markers of PDE [9–13]. Recently, seizures dependent on pyridox(am)ine 5-phospate oxidase deficiency and type 2 hyperprolinemia have been also described as inborn errors of vitamin B6 metabolism [14,15].

However, neither recommendations for pyridoxine administration in early started and intractable cases of epilepsy [3,16,17], nor adoption of pyridoxine as the first line drug for infantile spasms in Japan [18–21] changed conventional perception of the strictly limited role of pyridoxine in the pathogenesis of epilepsy in general. Meanwhile disturbances in the metabolism of glutamate, GABA, tryptophan, serotonin, taurine, dopamine and norepinephrine- all of them synthesized and/or metabolized by PLP-dependent enzymes [22,23] – have been repeatedly found in epileptic patients. The increased levels of excitatory amino acids – glutamate, aspartate, and glycine [24–30] and the reduced levels of inhibitory amino acids and amines – GABA, serotonin and taurine [31–36] – were detected in the plasma, CSF and epileptogenic foci of patients with

Abbreviations: PDE, pyridoxine-dependent epilepsy; PLP, pyridoxal-5-phosphate; AED, antiepileptic drug(s); GAD, glutamic acid decarboxylase; TRP, tryptophan; KYN, kynurenine; IDO, indoleamine 2,3-dioxygenase; 3-HOKYN, 3-hydroxykynurenine; 3-HOAA, 3-hydroxyanthranilic acid; KA, kynurenic acid; AA, anthranilic acid; XA, xanthurenic acid; IND, indoxyl sulfate; 4-PA, 4-pyridoxic acid; KAT, kynurenine aminotranspherase; ALP, alkaline phosphatase.

* Corresponding author. Address: 3 Dan street, Ness-Ziona, Israel. Tel.: +972 54 6354688.

E-mail address: dolina.svetlana@gmail.com (S. Dolina).

0306-9877/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.mehy.2012.04.022

different forms of epilepsy. Moreover, a moderate increase in the activity of glutamic acid dehydrogenase, the glutamate synthesizing enzyme specifically inhibited by PLP, has been found in epileptic foci [23]. The similar disorders, i.e., increased levels of plasma glutamate and aspartate and reduced urinary taurine, were also found in asymptomatic first degree relatives of epileptic patients [28–30].

These clinical data along with experimental results obtained in genetically epilepsy-prone seizure-naïve animals [40–43] enable us to hypothesize that an inborn error of pyridoxine metabolism (accentuated by high pyridoxyne requirement during early development) is inherent to epilepsy in a whole. As a starting point for neurotransmitter disorders, such an error may be a key determinant of epileptic diathesis. Evidently, an impairment of GABA (as well as serotonin and taurine) – mediated inhibition and enhancement of glutamate (and aspartate) – mediated excitatory transmission facilitate generation of seizures and spreading of ictal activity throughout the brain.

The disturbances of PLP dependent TRP metabolism, particularly, over-excess of neurotoxic 3-HOKYN, have been repeatedly shown in epilepsy starting from 50-s [44–47]. Summarizing the data obtained later [48,49] we suggested that correlations between compounds formed in the course of PLP-dependent TRP degradation (Fig. 1), might be indicative of the clinical status in epileptic patients. Some of these correlations are accepted in literature as reflecting activity of enzymes of TRP degradation [50]. Specifically, the ratio of KYN to TRP serves an index of activity of indoleamine 2,3-dioxygenase (IDO),[1] the rate-limiting enzyme of TRP degradation, initiating this pathway (Fig. 1). The ratio between levels of 3-HOAA and 3-HOKYN is considered an index of activity of kynureninase, the enzyme critically sensitive to PLP supply [51–53]. In our study the activities of both of these constitutive enzymes of TRP degradation pathway (IDO and kynureninase), were also evaluated by the values of these ratios. We also accepted the correlation between 4-PA, the end product of pyridoxine metabolism, and KYN as an indicator of recently experienced seizure attack.

The paper discusses application of quantitative urinary biomarkers for further clarification of patient's status at different stages of epilepsy and for tailoring of individualized AED treatment.

Materials and methods

Subjects

Urine samples were studied in children of 4–17 years of age with different clinical forms and stages of epilepsy, but healthy in all other respects. Patients with absence and atonic seizures were not included in the study. Altogether, 109 subjects divided into the following groups were comparatively studied:

1. Newly diagnosed epileptic patients who had experienced their first epileptic attack on the previous day and had not yet been treated with AED ($n = 11$);
2. Epileptic patients successfully treated with AED and seizure-free for at least three months, regardless of the type of epilepsy ($n = 19$);
3. Epileptic patients partially responsive to AED treatment, i.e., those having repeated seizures in spite of antiepileptic treatment ($n = 19$);
4. Control group of healthy children matched by sex and age ($n = 37$).

[1] Being heme-containing enzyme, IDO is, apparently, PLP–dependent, inasmuch as heme synthesis is PLP dependent.

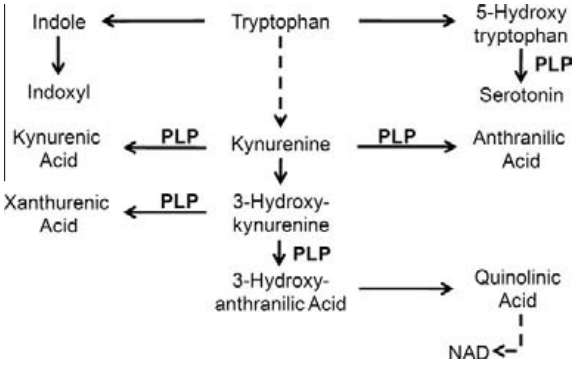

Fig. 1. Outline of the kynurenine pathway of tryptophan degradation. PLP – dependent links are marked.

About 200 urine samples were tested. Patients' samples were provided by the Pediatric Department of Kaplan's Hospital (Rehovot). Control samples were collected from healthy children in local kindergartens and elementary schools.

Determination of tryptophan and its metabolites in urine by HPLC with simultaneous ultraviolet and fluorimetric detection

Urinary TRP and its metabolites were determined by modified Herve et al [54] method. In addition to KYN, 3-HOKYN and 3-HOAA detected by these authors, some other TRP metabolites, i.e., xanthurenic acid (XA), anthranilic acid (AA), kynurenic acid (KA), Indoxyl sulfate (IND), and 4-PA (the end product of pyridoxine metabolism) were measured. All standards were purchased from Sigma. All solvents were HPLC graded.

Sample preparation

Mixed standard solutions (1 mM of each compound) were stored at −80 °C for up to 3 months. Urine samples were collected into 20 mL glass scintillation vials, stored in aliquots at −80 °C. Samples were acidified by addition of 100 μL of 2.4 M perchloric acid to 900 μL of urine. After centrifugation (5000 g, 15 min, 4 °C), supernatants were filtered (0.22 μm Millipore filter) into HPLC vials and analyzed at the same day.

Chromatography

Reverse phase HPLC analysis was performed with Inertsil (C-18, 5 μm) column (250 mm × 4.6 mm) and Merck Hitachi system equipped with Quaternary Pump L-7100 and interface D-7000. Peaks detection and quantification were carried out with a scanning fluorescence detector L-7485 connected to the programmable photodiode array detector L-7450A. Samples were analyzed using the following gradient: 28 min isocratic elution of 100% solvent A, 6 min linear gradient from 100% to 75% of solvent A, 61 min isocratic elution 75% of solvent A, 2 min linear gradient from 75% to 100% of solvent A, and 8 min isocratic elution of 100% solvent A. Solvent A was 1 M ammonium acetate buffer, pH 5.2; solvent B was 6% acetonitrile in 1 M ammonium acetate buffer, pH 5.2. The mobile phase was prepared on the day of analysis. Acquisition and processing of chromatograms were performed using HSM software (Merck-Hitachi). Standard compounds showed a linearity range from 0.03 μM to 10 μM. Concentrations were calculated basing on the peak areas of external standards. TRP, its metabolites, and 4-PA were determined with UV and fluorescence detection at two different excitation and emission wavelengths: 3-HOKYN, KYN and XA were detected by UV absorption at 365 nm and eluted