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Tyrosine metabolism in health and disease: slow-release amino acids therapy improves tyrosine homeostasis in phenylketonuria

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Abstract

Objectives: Phenylalanine (Phe) hydroxylase (PAH) deficiency leads to hyperphenylalaninemia (HPA) and tyrosine (Tyr) depletion. We investigated Tyr homeostasis in patients with PAH deficiency and the effect of a slow-release amino acids therapy in phenylketonuria (PKU).

Methods: We performed four complementary investigations: (1) Tyr concentrations were monitored in 114 patients (10.6 ± 11.9 years) with PKU on dietary treatment supplemented with traditional amino acid formulations (n=52, 1175 samples) or non-PKU HPA on a free diet (n=62, 430 samples); (2) Tyr metabolism in PKU was quantitatively evaluated in three patients by a simple Tyr oral loading test (100 mg/kg); (3) diurnal and (4) long-term Tyr concentrations were evaluated in 5 and 13 patients with PKU, respectively, who switched from traditional to slow-release amino acids therapy.

Results: 1) Tyr concentrations in the PKU population were subnormal and significantly lower than in non-PKU HPA ($p<0.01$); (2) the response to a Tyr loading test in PKU was normal, with basal Tyr concentrations reached within 12 h; (3) the diurnal metabolic profile in patients on slow-release amino acids therapy revealed higher morning fasting and nocturnal Tyr concentrations with respect to traditional therapy ($p<0.01$); (4) this picture was confirmed at follow-up, with normalization of morning fasting Tyr concentrations in patients on slow-release amino acids therapy ($p<0.01$) and unchanged Phe control ($p=0.19$).

Conclusions: Slow-release amino acids therapy can improve Tyr homeostasis in PKU. If associated to optimized Phe control, such a metabolic goal may allow long-term clinical benefits in patients with PKU.

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Introduction

Phenylketonuria (PKU, OMIM 261600) is an autosomal recessive disorder due to more than 1000 mutations in the gene encoding phenylalanine hydroxylase (PAH), the hepatic enzyme converting phenylalanine (Phe) into tyrosine (Tyr). Newborn screening enables early diagnosis and treatment of PKU, allowing the prevention of irreversible brain damage due to severe persistent hyperphenylalaninemia (HPA). A low-Phe diet able to maintain blood Phe concentrations within safe ranges is the cornerstone of treatment of severe forms of PAH deficiency, including classic and mild PKU, whereas no dietary restriction is necessary in milder variants (non-PKU HPA). In PKU, dietary treatment is recommended for life [1]. Restriction of natural proteins is supplemented with Phe-free medical foods, generally L-amino acid mixtures, to prevent nutritional deficiencies and ensure adequate growth and development [2–4]. As Tyr is promoted to essential or semiessential amino acid in PKU, artificial Phe-free amino acid formulations are generally enriched in Tyr [5]. Few data are available on Tyr homeostasis in PKU. Suboptimal and highly fluctuant Tyr concentrations were reported in small cohorts of PKU patients [6, 7]. This picture can be worsened by commonly observed incomplete compliance to supplementation with artificial amino acids (especially during the adolescent and adult age), mainly related to their unpleasant odor and taste [8–10]. Poor therapeutic compliance, moreover, has been related to the late complications of PKU [11–13]. Recently, new protein substitutes with improved organoleptic characteristics and bioavailability became available for treatment of PKU, including glycomacropeptide, large neutral amino acids mixtures, and slow-release amino acid formulations [14–16]. As Tyr is the key physiological substrate for the synthesis of different metabolites (dopamine, epinephrine, thyroxin, and melatonin), there are good theoretical reasons for considering an improvement of Tyr homeostasis as an

additional metabolic target in PKU, besides the primary therapeutic goal of optimal Phe control.

In this study, we investigated Tyr metabolism in health and in different forms of PAH deficiency. Additionally, we studied the effect of a new slow-release amino acids therapy on diurnal and long-term Tyr homeostasis in PKU.

Methods

Evaluation of Tyr concentration in PKU and non-PKU HPA

Blood Tyr concentration were evaluated in 1605 morning fasting dried blood spots collected in 114 patients (age 10.6 ± 11.9 years; 14.1 ± 24.4 samples per patient) with different forms of PAH deficiency followed at our center. Of them, 52 had classic or mild PKU (1175 samples) and were treated with individualized low-Phe diet supplemented with traditional Phe-free amino acid mixtures according to recommendations [1], and 62 subjects had non-PKU HPA (430 samples) on a free diet.

Dynamic study of Tyr metabolism and disposal in health and PKU

The outcome of a simple Tyr oral loading test (100 mg/kg) was compared in four healthy subjects and in three patients with classic PKU (genotypes IVS10-11G>A/IVS4+5G>T, IVS10-11G>A/R158Q, and IVS10-11G>A/R158Q). The loading tests were performed after an overnight fast following the procedures already reported for the simple Phe loading test with some modifications [17, 18]. In particular, (a) duration of the tests was 24 h after Tyr administration; (b) a normocaloric nonprotein diet was administered during the test to avoid additional Phe and Tyr intake; (c) blood Tyr concentration was measured at 0, 1, 3, 6, 12, and 24 h after the oral loading.

Diurnal Tyr profile in PKU on traditional or slow-release amino acids therapy

Diurnal Tyr concentrations on dried blood spots in five patients with classic PKU (genotypes R158Q/P211Hfs*130, R158Q/P211Hfs*130, R282W/E280 K, I283F/Y356X, and IVS4+5G>T/IVS10-11G>A) were assessed both before and after a therapeutic switch from traditional amino acid formulations (Tyr content 5.6 ± 1.5 mg/100 g) to a slow-release amino acid formulation (Tyr content 7.8 mg/100 g). In all patients, the switch was due to unsatisfactory palatability of the traditional mixtures. Amino acid formulations were administered 0.9 ± 0.1 g/kg/day subdivided in three administrations at main meals. The employed slow-release product was a sodium alginate amino acid granulate formulation, characterized by an absorption rate similar to natural protein and neutral odor and taste (Afenil Micro 3H, PIAM). Samples were collected before meals (h 8.00, 12.00, and 20.00) and 3 h after dinner (h 24.00).

Longitudinal comparison of Tyr concentrations in PKU patients on traditional or slow-release amino acids therapy

Morning fasting Tyr concentrations were compared in 13 patients with PKU (seven classic PKU, six mild PKU, age 20.6 ± 9.0 years) before and after a switch from traditional to slow-release amino acid formulation. In all patients, the therapeutic switch was due to unsatisfactory palatability of the traditional products. For both therapeutic regimens, a 6-month follow-up period was considered (traditional therapy: 66 samples; slow-release therapy: 63 samples). As the slow-release formulation only contains amino acids, patients on this formulation required additional supplementation with multivitamins and minerals.

Biochemical and statistical analyses

All biochemical measurements were performed on dried blood spots by tandem mass spectrometry.

Statistical analysis was performed with R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna, Austria). The Shapiro–Wilk test was used for testing normality of data distribution. Differences between groups were established using the Student's *t*-test or the Mann–Whitney *U* test. Statistical significance for all calculations was considered achieved when the two-tailed p-value was less than 0.05. The study was conducted according to the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects.

Results

Tyr concentration in PKU patients on traditional amino acids supplementation was subnormal and significantly lower than in subjects with non-PKU HPA on a free diet ($p<0.01$); Tyr concentration in non-PKU HPA was within the normal range (40–150 μmol/L) (Figure 1). Phe concentration in PKU was higher than in non-PKU HPA (263 ± 266 *vs.* 153 ± 90 μmol/L, $p<0.01$).

In PKU patients, the time course of Tyr absorption, distribution, and disposal after an oral Tyr loading test was normal. In particular, basal Tyr concentrations were reached within 12 h after the Tyr loading both in health and in PKU (Figure 2).

A diurnal metabolic profile in PKU patients revealed higher morning fasting and nocturnal Tyr concentrations while on slow-release amino acids therapy with respect to traditional treatment ($p<0.01$) and overlapping Tyr concentrations during the day (h 12.00, $p=0.71$; h 20.00, $p=0.94$) (Figure 3).

Diurnal Phe concentrations on traditional and slow-release therapy were not different (h 8.00, 224 ± 127 *vs.* 236 ± 141 μmol/L, $p=0.93$; h 12.00, 167 ± 139 *vs.*

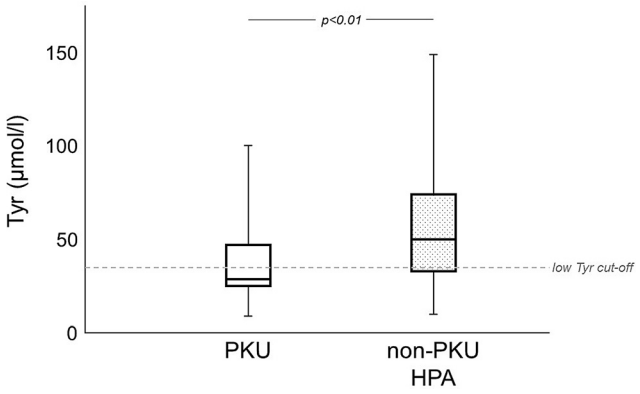

Figure 1: Tyrosine (Tyr) concentration in 1175 samples from 52 patients with phenylketonuria (PKU) on dietary treatment supplemented with traditional amino acids mixtures (withe box) and in 430 samples from 62 patients with non-PKU HPA on a free diet (dotted box). Boxes represent 75% of measurements with medians; error bars are the 1st and 99th percentiles. Dotted line represents the lowest normal Tyr concentration.

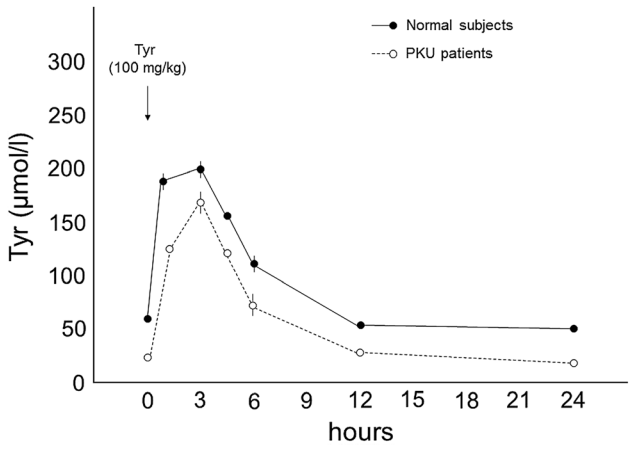

Figure 2: Tyrosine (Tyr) concentrations (medians with ranges) after a simple Tyr oral loading (100 mg/kg) in four healthy subjects (continuous line, black circles) and in three patients with phenylketonuria (PKU, dotted line, white circles).

224 ± 164 µmol/L, p=0.70; h 20.00, 236 ± 166 *vs.* 164 ± 159 µmol/L, p=0.66; h 24.00, 343 ± 33 *vs.* 175 ± 178 µmol/L, p=0.30)

At longitudinal follow-up, slow-release amino acids therapy was associated with significantly higher Tyr concentrations in PKU patients with respect to traditional formulations (p<0.01, Figure 4) and unchanged Phe concentrations (379 ± 260 *vs.* 448 ± 332, p=0.19). In particular, PKU patients on slow-release amino acids therapy showed normal Tyr concentration (Figure 4).

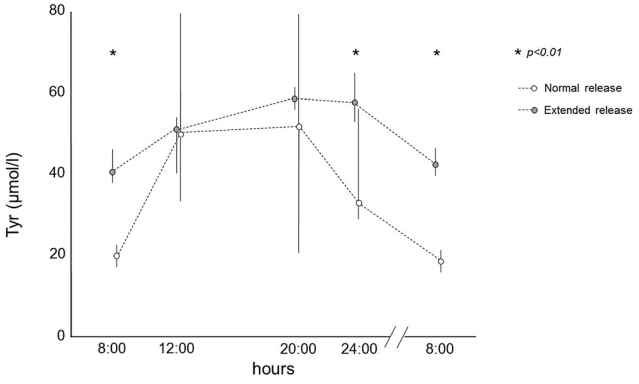

Figure 3: Diurnal tyrosine (Tyr) concentrations (medians with ranges) in five patients with phenylketonuria (PKU) while on traditional amino acids therapy (white circles) or slow-release amino acids therapy (gray circles).

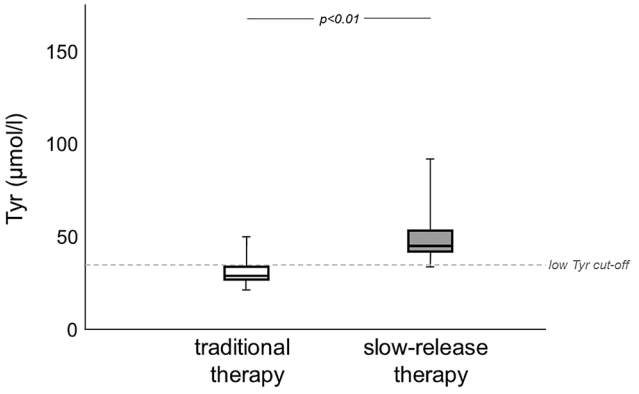

Figure 4: Tyrosine (Tyr) concentrations in 13 patients with phenylketonuria (PKU) while on traditional (withe box) or slow-release amino acids supplementation (grey box). Boxes represent 75% of measurements with medians; error bars are the 1st and 99th percentiles. Dotted line represents the lowest normal Tyr concentration.

Discussion

A substrate reduction therapy through a low-Phe diet is the mainstay of treatment of PKU, allowing the prevention of neurotoxicity of severe persistent HPA and the achievement of normal cognitive development. Dietary restriction of natural protein in PKU should be integrated with artificial Phe-free amino acids mixtures to prevent nutritional deficiencies and ensure adequate Tyr supply [1].

We showed that low blood Tyr concentration is common in PKU patients on treatment with traditional Phe-free mixtures and that subjects with non-PKU HPA on a free diet have normal Tyr availability. Our findings in a large PKU population are consistent with previous observations in

smaller cohorts [6, 7], straightening the opportunity of longitudinal Tyr monitoring and the need of new therapeutic approaches to improve Tyr homeostasis in PKU [19]. Although peripheral Tyr deficiency has nonobvious clinical effects in PKU, different pathophysiological considerations address the opportunity of its correction. As Phe has the highest affinity for the blood–brain barrier countertransporter for large neutral amino acids (LAT1), peripheral HPA leads not only to increased brain Phe levels (which directly inhibit both Tyr and tryptophan hydroxylases) but also to reduced brain influx of Tyr and tryptophan, the key substrates for the synthesis of L-Dopa and serotonin, respectively [20, 21]. In this context, suboptimal peripheral Tyr concentration can worsen Tyr deficiency at the central level, with multiple potential metabolic derangements [22]. Different experimental observations are in agreement with this pathophysiological hypothesis. First, brain uptake of F-dihydroxyphenylalanine, a substance carried by the LAT1, is reduced in PKU [23]. Second, low neurotransmitter levels were reported in PKU patients either on good or poor Phe control and regarded as a determinant of brain damage [21, 24–26]. Third, specific Tyr deficiency into the brain of PKU patients was demonstrated both *in vivo* and post mortem [27, 28] and related to impaired cerebral protein synthesis [29].

Therapeutic supplementations with high-dose Tyr (100 mg/kg/day) were attempted in PKU patients with inconsistent results, being not currently recommended in the clinical practice [30]. In this study, we showed that Tyr deficiency in PKU patients is not due to accelerated disposal, suggesting that high-dose Tyr supplementation is unnecessary in PKU. This finding is in agreement with the observed normal Tyr availability in non-PKU HPA and with previous observations on Tyr metabolism after oral Phe loadings with or without tetrahydrobiopterin and in PKU heterozygotes [17, 31]. Moreover, we showed that a continuous rather than pulsatile amino acids absorption through a slow-release technology can substantially improve peripheral Tyr availability in PKU, with potential long-term benefits in affected patients. Actually, functional deficits in PKU appear more strictly related to the combination of both HPA and low Tyr concentration with respect to HPA alone [19, 32]. However, the simple use of the Phe/Tyr ratio for the biochemical monitoring of PKU patients can be misleading, due to the critical importance of HPA with respect to Tyr deficiency [33]. By this approach, indeed, either safe or unsafe HPA could be theoretically associated to the same ratio depending on Tyr concentration, with potential perpetuation of inadequate Phe control. To avoid this risk, we suggest that blood Phe monitoring should remain the primary indicator of metabolic control in PKU, whereas Tyr monitoring (facilitated by dried blood spots analysis by tandem mass spectrometry) should be considered an ancillary test, normalization of which should be endeavored as a complementation of adequate Phe control. The use of slow-release amino acids therapy to supplement Phe dietary restriction tailored to individual Phe tolerance can be functional to this purpose. In particular, the described Phe and Tyr concentration ranges in non-PKU HPA, virtually not associated to clinical complications [34], could likely represent a combined therapeutic target for PKU patients.

In conclusion, slow-release amino acids therapy can improve Tyr homeostasis in patients with PKU, with potential long-term clinical benefits if associated to optimized Phe control.

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Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Ethical statement: The study was conducted according to the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects.

Competing interests: Authors state no conflict of interest.

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