Vitamin B6 in Primary Hyperoxaluria I: First Prospective Trial after 40 Years of Practice

Heike Hoyer-Kuhn, Sina Kohbrok,* Ruth Volland,† Jeremy Franklin,‡ Barbara Hero,† Bodo B. Beck,§ and Bernd Hoppe‖*

Abstract

Background and objectives Primary hyperoxaluria type I (PH I) is caused by deficiency of the liver-specific enzyme alanine-glyoxylate:aminotransferase (AGT). Many mutations are known to perturb AGT protein folding. Vitamin B6 (B6) is the only specific drug available for treatment. Although B6 has been used for >40 years, controlled data on B6 efficacy are lacking. Therefore, this study investigated the absolute and relative change of urinary oxalate (Uox) excretion under increasing dosages of B6, the first prospective trial to do so.

Design, setting, participants, & measurements B6 response was studied in 12 patients (7 male patients) with genetically confirmed PH I (3 Gly170Arg homozygous, 5 compound Gly170Arg and/or Phe152Ile heterozygous, and 4 negative for Gly170Arg and/or Phe152Ile mutations) and noncompromised renal function. Efficacy was defined as a 30% relative reduction in Uox excretion. B6 was administered orally starting at 5 mg/kg body weight per day and given in increments of 5 mg/kg every 6 weeks, up to a final dosage of 20 mg/kg per day at week 24. Uox and serum B6 levels were measured every 6 weeks.

Results Mean relative Uox reduction was 25.5%. Uox declined from 2.09±0.55 (mean±SD) at baseline to 1.52±0.60 mmol/1.73 m^2 per day (P=0.01) at week 24. Serum B6 levels increased from 22.5±8.7 to 1217±776 ng/ml (P<0.001). Six patients showed a ≥30% relative reduction of Uox at week 24.

Conclusion This first prospective trial confirmed B6 efficacy in 50% of patients (three of three homozygous, one of five heterozygous, and two of four patients negative for the Gly170Arg and/or Phe152Ile mutations). Interestingly, no complete biochemical remission was observed, even in the homozygous Gly170Arg study participants. Future trials are necessary to learn more about genotype-related B6 response and B6 metabolism.

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*Division of Pediatric Nephrolgy and †Pediatric Clinical Trial Unit, Department of Pediatrics and Adolescents Medicine, University Hospital Cologne, Cologne, Germany; ‡Institute of Medical Statistics, Informatics and Epidemiology, and §Institute of Human Genetics, University of Cologne, Cologne, Germany; and ‖Department of Pediatrics, Division of Pediatric Nephrology, University Hospital Bonn, Bonn, Germany

Correspondence: Dr. Heike Hoyer-Kuhn, Department of Pediatrics and Adolescents Medicine, University Hospital Cologne, Kerpenerstr. 62, 50924 Cologne, Germany. Email: heike-katharina.hoyer-kuhn@uk-koeln.de

Introduction

Primary hyperoxaluria type I (PH I; Online Mendelian Inheritance in Man #604285) is caused by deficiency of the liver-specific peroxisomal enzyme alanine-glyoxylate-aminotransferase (AGT) that requires pyridoxal-phosphate (PLP, active vitamin B6 derivative) as a cofactor (1–3). Reduced AGT activity results in massive accumulation of glyoxylate, which is rapidly converted to oxalate and subsequently is excreted in the urine. Urinary calcium oxalate supersaturation in turn produces the clinical hallmarks of recurrent urolithiasis and/or progressive nephrocalcinosis (4–6).

PH I is the most disastrous of the three types of hyperoxaluria; the disease accounts for most (70%–80%) PH cases and regularly causes ESRD and death if left untreated (7–11).

More than 150 causative mutations in the AGT gene (*AGXT*) have been reported (12,13). The majority constitute nontruncating, predominantly missense mutations, which negatively affect protein folding and stability or subcellular localization but do not inevitably abolish the active site or affect the production of AGT (13,14).

In vitro studies of the consequences of frequently found mutations (12,15–17) provide sufficient data for the two most common mutations: the missense variants c.508G>A (p.Gly170Arg) and c.731T>C (p.Ile244Thr). Both demonstrate *in vitro* susceptibility to treatment with various pharmacoperones (*e.g.*, vitamin B6, betaine, glycerol) (16,17). Most patients will exhibit at least one missense mutation (18).

Long before the molecular basis of PH I was known, beneficial effects of vitamin B6 (B6) treatment were observed (19–21). Over time, B6 has evolved into a therapeutic standard in addition to hyperhydration and use of crystallization inhibitors, but the level of evidence remained low because it was based on expert opinion (22). The only curative treatment for PH I is (pre-emptive) liver transplantation (23). The outcome of renal replacement therapy is poor compared with that for patients without PH I (24,25). Altogether, substantial risks are associated with the current treatment, and many patients worldwide have no regular access to dialysis and organ transplantation.

There is a dire need to analyze drug interventions more precisely and find innovative therapeutic approaches

(26,27), because conservative therapy has not improved in the past few decades (28). This view is reflected by a surprising absence of controlled clinical trials with potential pharmacoperones (http://clinicaltrials.gov). Therefore, we conducted the first prospective clinical trial to systematically evaluate efficacy, safety, dose-response relationship, and response in relation to *AGXT* genotype of B6 treatment.

Materials and Methods

Study participants were eligible if they fulfilled the following entry criteria: genetically confirmed diagnosis of PH I; no chronic intestinal disease; no liver, kidney, or combined transplantation; chronologic age between 5 and 60 years; renal function defined as an estimated GFR (eGFR)≥60 ml/min per 1.73 m^2 (Schwartz formula). Study participants receiving B6 before the study had to discontinue therapy for a washout period that lasted at least 6 weeks, but always until normalization of serum B6 levels (metabolites measured: pyridoxal and pyridoxal-phosphate; normal range, 7–30 ng/ml [sum of both metabolites]).

The study was designed as a 28-week (drug administration, 24 weeks; follow-up, 4 weeks), single-group, open-label trial with 12 participants. After baseline examinations, participants received intravenous B6 (pyridoxine-hydrochloride) solution orally to administer body weight–correlated accurate dosages. This formulation contained a solution of 100 mg pyridoxine-hydrochloride per 3 ml and was prepared in accordance with good clinical practice criteria. It was given twice a day at 5 mg/kg body weight per day for 6 weeks. Increments of 5 mg/kg every 6 weeks were used, up to a maximum dosage of 20 mg/kg body weight per day.

The primary objective was to investigate the relative reduction of urinary oxalate (Uox) excretion under increasing dosages of B6 at week 24 compared with baseline. A relative reduction of ≥30% (percentage change in Uox expressed as mmol/1.73 m^2 per day) was arbitrarily defined earlier as an adequate response (29), which was recently confirmed in the OXALEurope guidelines (22).

The secondary objective was to analyze the dose-response relationship by repeated measurements of serum B6 levels in correlation to the administered dosage and in correlation to change in Uox.

B6 response was related to the underlying genotype by descriptive statistics. Three genotype groups were defined: c.508G>A (p.Gly170Arg) homozygous (n=3), c.508G>A (p.Gly170Arg) and/or c.454T>A (p.Phe152Ile) compound heterozygous (n=5), and c.508G>A (p.Gly170Arg) negative (n=4).

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the ethics committee of the participating institution (approval number 09–275). Written informed consent was obtained from each participant's parent or legal guardian and children before study-related procedures. The trial was registered on January 20, 2011 (NCT01281878), in the ClinicalTrials.gov Protocol Registration System (http://clinicaltrials.gov/ct2/show/NCT01281878?term=NCT01281878&rank=1).

Enrolled patients were evaluated at baseline and at weeks 6, 12, 18, and 24 for standing height, weight, BP, preexisting conditions/concomitant medications, routine blood chemistry, and hematology. The eGFR was calculated by using the Schwartz formula in children and the Modification for Diet in Renal Disease study equation for adults (30,31).

To reduce variability in oxalate measurement, Uox was analyzed in two consecutive 24-hour urine collections every 6 weeks as previously described, and the mean of these samples was reported as mmol/1.73 m^2 per day (32). Collecting bottles were prefilled with 10 ml thymol 5% in isopropanol per expected liter of urine excretion. Participants were asked to keep the urine samples cooled until they were picked up *via* a courier service within 12 hours. To confirm complete collections, appropriate creatinine excretion was used as control measure, as was done in two recent multicenter PH trials (33,34). Patients with PH I have a lower intestinal oxalate absorption compared with healthy people (35); therefore, the dietary influence on variability of oxalate excretion is minor. However, patients had to avoid diets high in oxalate and vitamin C supplementation.

B6 levels (metabolites measured: pyridoxal and pyridoxal phosphate; normal range, 7–30 ng/ml [sum of both metabolites]) were acquired in parallel every 6 weeks and were analyzed by HPLC.

Renal ultrasonography was performed at baseline and week 24 to determine the presence of nephrocalcinosis or urolithiasis. Nephrocalcinosis was described according to Hofmann *et al.* (36). In addition to standard reporting of all adverse events, data were collected by use of biweekly telephone interviews.

To analyze the primary endpoint (level of Uox reduction from baseline to week 24), each patient's change in Uox was analyzed. The mean±SD of these differences was calculated, and a paired t test was performed to test for a mean change from baseline. Means±SD and paired t tests were also used to analyze differences in Uox and B6 levels. The main analysis was performed on the intention-to-treat population, defined as all enrolled patients with at least one measurement of Uox after baseline. Individual series of serum B6 levels over time are presented descriptively. Associations between (*1*) B6 levels and administered dosages and (*2*) time-integrated B6 levels from baseline to week 24 (area under the curve) and Uox level reduction (0–24 weeks) were depicted graphically and quantified using linear correlation coefficients.

For safety aspects, cumulative lists of adverse events and serious adverse events and results of renal ultrasonography were presented descriptively. P values<0.05 were considered to represent statistically significant results. For statistical analyses, R and SPSS were used (R 2.15.0; IBM SPSS statistics version 20).

Results

All study participants completed the 24 weeks of B6 treatment. A synopsis of clinical data on all study participants is given in Table 1.

All 12 enrolled patients were included in the intention-to-treat analysis. Absolute Uox levels declined significantly

Table 1. Baseline characteristics of the study cohort.

Patient/ Family	Sex, Age (yr)	GFR (ml/min/ 1.73 m^2)	*AGXT* Mutation (AGT protein)	Minor/Major Allele (p.P11L status)	Vit B6 before Study	Grade of Nephrocalcinosis	Urolithiasis
Pat 1/F1	m, 16	113	c.508G>A, Missense (p.G170R) c.959_960delCA, Frameshift (p.T320Sfs*11)	Minor allele (p.11L) major allele (p.11P)	Y	1	N
Pat 2/F1	m, 18	129	c.508G>A, Missense (p.G170R) c.959_960delCA, Frameshift (p.T320Sfs*11)	Minor allele (p.11L) major allele (p.11P)	Y	—	Y
Pat 3/F2	f, 12	152	c.508G>A, Missense (p.G170R) c.364C>T, Stop (p.R122*)	Minor allele (p.11L) minor allele (p.11L)	Y	—	Y
Pat 4/F3	f, 11	100	c.33delC, Frameshift (p.K12Rfs*34) c.33delC, Frameshift (p.K12Rfs*34)	Major allele (p.11P) major allele (p.11P)	Y	1	Y
Pat 5/F4	m, 13	127	c.454T>A, Missense (p.F152I) c.1151T>C, Missense (p.L384P)	Minor allele (p.11L) major allele (p.11P)	Y	1	Y
Pat 6/F5	m, 17	217	c.508G>A, Missense (p.G170R) c.508G>A, Missense (p.G170R)	Minor allele (p.11L) minor allele (p.11L)	N	2	Y
Pat 7/F5	m, 15	214	c.508G>A, Missense (p.G170R) c.508G>A, Missense (p.G170R)	Minor allele (p.11L) minor allele (p.11L)	N	—	Y
Pat 8/F6	m, 7	142	c.327delG, Frameshift (p.Q110Sfs*10) c.327delG, Frameshift (p.Q110Sfs*10)	Major allele (p.11P) major allele (p.11P)	N	—	Y
Pat 9/F7	f, 12	91	c.560C>T, Missense (p.S187F) c.836T>C, Missense (p.I279T)	Minor allele (p.11L) minor allele (p.11L)	N	2	Y
Pat 10/F8	m, 10	118	c.482G>A, Missense (p.G161D) c.976delG, Frameshift (p.V326Yfs*15)	Major allele (p.11P) major allele (p.11P)	Y	—	N

Table 1. (Continued)

Patient/ Family	Sex, Age (yr)	GFR (ml/min/ 1.73 m^2)	*AGXT* Mutation (AGT protein)	Minor/Major Allele (p.P11L status)	Vit B6 before Study	Grade of Nephrocalcinosis	Urolithiasis
Pat 11/F9	f, 15	182	c.508G>A, Missense (p.G170R) c.847-3C>G, Splice site loss of splice acceptor exon 9	Minor allele (p.11L) major allele (p.11P)	Y	—	N
Pat 12/F10	f, 11	153	c.508G>A, Missense (p.G170R) c.508G>A, Missense (p.G170R)	Minor allele (p.11L) minor allele (p.11L)	Y	—	N

AGT, alanine-glyoxylate:aminotransferase; Vit B6, vitamin B6; Pat, patient; m, male; Y, yes; N, no; f, female.

(P=0.01) from 2.09±0.55 at baseline to 1.53±0.60 mmol/1.73 m^2 per day in week 24. Figure 1 presents individual data at baseline and study week 24 based on genotype and Figure 2 on relative change of Uox between baseline and study week 24.

The mean percentage Uox change from baseline to week 24 was −25.5%±26.10% in the whole cohort. At the end of treatment, 6 of 12 patients showed a relative Uox reduction >30% and were considered to be treatment responders (Figure 2). Two of 12 patients were complete nonresponders (patients 04 and 10; Figure 2).

Serum B6 levels at study week 24 versus baseline were significantly increased from 22.47±8.69 to 1217.42±775.95 ng/ml (P<0.001). Individual series of serum B6 levels are depicted in Figure 3. The correlation coefficient relating administered dosages and B6 levels was r=0.66 (95% confidence interval [95% CI], 0.48 to 0.78; P<0.001). Table 2 shows individual response under increasing dosages, with seven of nine patients responding already to the lowest dosage of 5 mg/kg per day. The relationship between time-integrated B6 levels and Uox change (0–24 weeks) is presented in a scatterplot (Figure 4). The correlation coefficient was r=0.27 (95% CI, −0.36 to 0.73; P=0.4).

Uox declined significantly (P<0.05) from 1.80±0.40 at baseline to 0.93±0.06 mmol/1.73 m^2 per day at week 24 in the three c.508G>A homozygous patients. Mean relative Uox change was −47.00%±47.89% (Figure 1). All responded at week 24.

Uox declined from 1.89±0.34 at baseline to 1.48±0.38 mmol/1.73 m^2 per day at week 24 in the five heterozygous patients (P=0.07). Mean relative Uox change was −20.89%±17.78% (Figure 1). One patient responded at week 24. Two patients were siblings (patients 01 and 02): one was a responder and the other a nonresponder.

Uox declined from 2.55±0.64 at baseline to 2.03±0.65 mmol/1.73 m^2 per day at week 24 in the four patients who were c.508G>A negative (P=0.42). Mean relative Uox reduction was −15.1%±37.35% (Figure 1). Two of four responded at week 24.

Oral administration of the liquid B6 formula was well tolerated. No patients discontinued trial medication because of adverse events. One serious adverse event was reported (infectious gastroenteritis). In summary, 70 adverse events (common childhood illnesses) were reported for 83.3% of the study population. Two patients experienced one episode of stone passage, both without complications. Sixty-two of 70 adverse events were declared unlikely to be related to trial medication, and eight were declared to be possibly related: nausea, fatigue, mucositis, gastroenteritis, light sensitivity, pain during voiding, and two episodes of stone passage.

Symptoms indicating severe neurotoxicity were not reported in the short period of 24 weeks, especially not under the highest dosage. Renal imaging at baseline and study week 24 revealed no specific changes regarding the degree of nephrocalcinosis or urolithiasis burden.

Discussion

The overall relative reduction of Uox reached 25.5% in the whole study cohort, a degree of reduction similar to that seen in the initial *Oxalobacter formigenes* trials

(33,34,37), the compound with the largest amount of controlled clinical data on Uox in PH available to date.

Our prospective study partially confirmed previous accounts reporting efficacy of B6 treatment in up to 50% of patients with PH I (18,29,38). The 12 patients studied, although not selected for a specific composition of genotypes, well match the German cohort with an allelic frequency of the c.508G>A mutation of 45.8% (Dr. Bodo B. Beck, personal communication, June 20, 2013) compared with 39% within the whole cohort.

In the past, somewhat conflicting results about B6 efficacy and genotype-phenotype correlations were reported (39,40). Homozygous c.508G>A mutations and a few other specific missense mutations were clearly associated with a more pronounced response to B6 treatment, and consequently it was hypothesized and subsequently shown that homozygosity for c.508G>A resulted in better long-term conservation of renal function (39,41,42). Additionally, carrying the Pro11Leu polymorphism (major allele) in combination with the c.508G>A mutation results in a synergistic mistargeting of AGT and influences the metabolic activity (12). In our 12 patients, significant differences regarding the major/minor allele status were not obvious (Table 1).

All three homozygous c.508G>A patients responded promptly to B6, and, as expected, the decline in Uox from baseline to week 24 was highest in this group (47.0%). Of note, a complete biochemical remission of Uox, as reported earlier in homozygous c.508G>A patients under varying B6 doses, was not observed here (29).

Three of five study participants who were compound heterozygous for c.508G>A/c.454T>A (patients 01, 03, and 11) showed at least an intermittent response. Of two brothers, one showed persistent B6 response and the other was almost a complete nonresponder. Because the nonresponder had higher B6 levels compared with the responding sibling, we excluded compliance problems as the cause of the different response. Urine was collected during the same time periods. Hence, results appear plausible according to reports of discordant clinical course in PH I families despite the same genotype (7,10).

In patient 05 (c.454T>A heterozygous), some B6 response was expected. But only an intermittent response was detected under the lowest dosage and the lowest serum B6 levels, which cannot be explained yet. This underlines the need for detailed elucidation of B6 mechanism and pharmacological action. In the c.508G>A negative group, two of four patients responded intermittently and the other were clear nonresponders.

How were the absolute Uox levels influenced by the underlying genotypes? The homozygous c.508G>A patients started from the lowest mean absolute Uox but reached the highest mean reduction. The heterozygous c.508G>A and/or c.454T>A patients started from an intermediate level and the c.508G>A negative group with the highest mean level of absolute Uox versus the homozygous group (95% CI, −1.78 to 0.27; P=0.115). In particular, the higher Uox levels at baseline in the c.508G>A negative patients may express differences in severity of disease and consequences for long-term outcome.

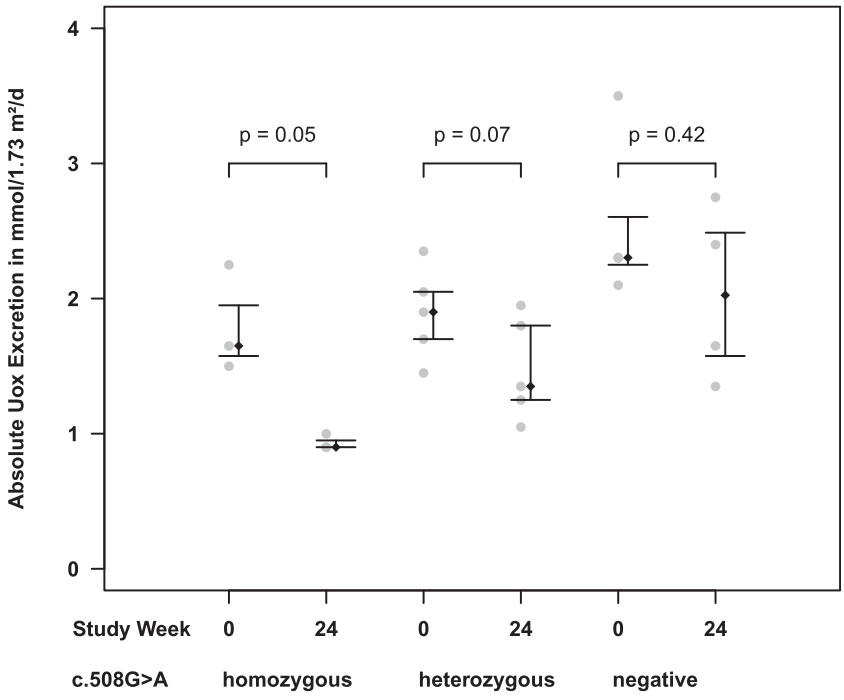

Figure 1. | Absolute urinary oxalate (Uox) excretion at study week 0 versus study week 24 among the three different genotype groups (c.508G>A homozygous *n*=3; c.508G>A and/or c.454T>A compound heterozygous *n*=5; c.508G>A negative *n*=4). Shown are medians and 25th and 75th percentiles. Gray circles represent individual study participants.

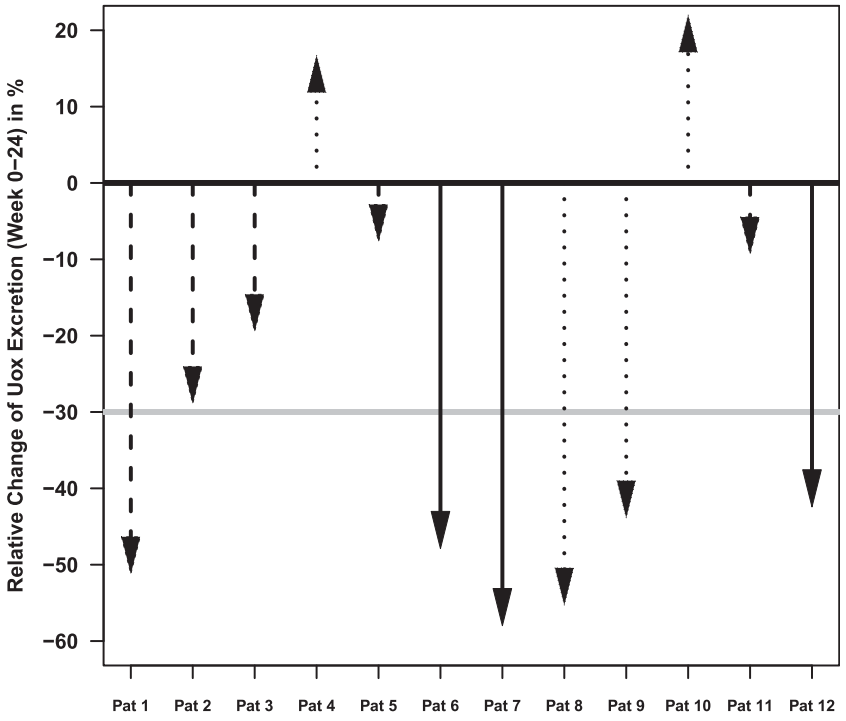

Figure 2. | **Individual relative change in Uox excretion from study week 0 to study week 24 for individual study participants.** Response, defined as relative change of ≥30%, is marked as the gray line. Continuous line represents c.508G>A homozygous study participants, dashed line represents c.508G>A and/or c.454T>A compound heterozygous, spotted line represents c.508G>A negative study participants.

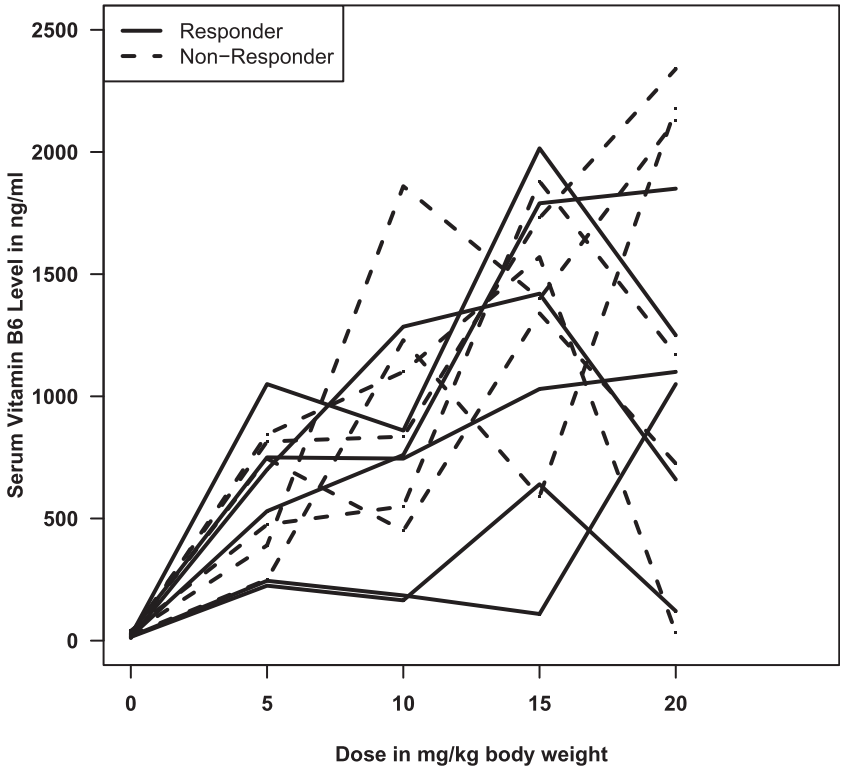

Figure 3. | **Individual series of serum vitamin B6 levels study weeks 0–24 depending on administered dosage of vitamin B6.** r=0.66 (95% confidence interval, 0.48 to 0.78); P<0.001). Responders are depicted as solid lines, and nonresponders are depicted as dashed lines.

Table 2. Individual absolute urinary oxalate levels and response depending on administered dosage during study weeks 0–24

Patient	Uox Level per Vitamin B6 Dosage (mmol/1.73 m^2 per day)								Genotype
	Baseline (Vitamin B6, 0 mg/kg per day)	Study Week 6 (Vitamin B6, 5 mg/kg per day)		Study Week 12 (Vitamin B6, 10 mg/kg per day)		Study Week 18 (Vitamin B6, 15 mg/kg per day)		Study Week 24 (Vitamin B6, 20 mg/kg per day)	
		Uox	Response	Uox	Response	Uox	Response	Uox	Response	
01	2.05	1.20	+	1.25	+	0.95	+	1.05	+	c.508G>A; c.959_960delCA
02	1.70	1.30	–	2.0	–	2.25	–	1.25	–	c.508G>A; c.959_960delCA
03	2.35	1.25	+	1.45	+	1.20	+	1.95	–	c.508G>A; c.364C>T
04	2.10	2.60	–	2.15	–	2.85	–	2.4	–	c.33delC; c.33delC
05	1.90	1.20	+	1.35	–	1.50	–	1.8	–	c.454T>A; c.1151T>C
06	1.65	1.05	+	0.68	+	1.00	+	0.9	+	c.508G>A; c.508G>A
07	2.25	0.75	+	1.00	+	1.20	+	1.00	+	c.508G>A; c.508G>A
08	3.50	2.80	–	2.75	–	2.25	+	1.65	+	c.327delG; c.327delG
09	2.31	1.20	+	1.30	+	1.60	+	1.35	+	c.560C>T; c.836T>C
10	2.30	2.80	–	2.60	–	2.85	–	2.75	–	c.482G>A; c.976 del G
11	1.45	1.05	–	1.00	+	0.85	+	1.35	–	c.508G>A; c.847-3C>G
12	1.50	1.02	+	0.60	+	1.05	–	0.90	+	c.508G>A; c.508G>A

Uox, urinary oxalate.

Systematic data on serum B6 levels under treatment in different dosages were not yet available. Analysis of B6 levels in each patient demonstrated that increase of administered dosage tended to result in higher serum levels, but the correlation between serum B6 and dosage was only moderate (r=0.66; Figure 3). Additionally, Uox was not relevantly correlated to cumulative serum B6 levels (r=0.26; Figure 4).

These findings are in accordance with retrospective data demonstrating response under lower dosages than used in this trial (21). Recently, the effect of pyridoxine and pyridoxal-phosphate was explained by a prosthetic group and a minor chaperone effect. Increasing the dosage of vitamin B6 did not lead to an enhanced chaperone effect in this *in vitro* study with transformed AGT cells (16).

For further clarification, all active B6 metabolites should be measured to evaluate their specific influence on oxalate metabolism. It also might help to find explanations for response/nonresponse phenomena in patients with high B6 levels and nonresponse. In addition, measurement of serum B6 and its vitamers is helpful to identify nonadherent patients.

The intravenous formulation is not routinely used, but exact dosing was necessary for this study. The formulation used contained the same active component, pyridoxine-hydrochloride, as the oral pill formulation. Resorption in the upper gastrointestinal tract is the same; thus, there should be no differences regarding serum levels and absorption.

There is international consensus to increase B6 dosage to 20 mg/kg body weight in order to determine the optimal dosage with the highest reduction of Uox (22). Therefore, the lowest dosage might be sufficient in some patients, but there clearly will be others who need the highest dosage to significantly reduce Uox. The optimal dose needs to be assessed individually over a period of 3–6 months.

Milliner *et al.* had reported a gradual reduction of Uox over a longer period of B6 administration (28). This may not be in disagreement with our findings, but would have to be verified in a long-term follow-up study of our patients. In addition, we rarely experienced complete normalization of Uox in c.508G>A homozygous patients; such normalization was, however, previously shown in 100% of patients homozygous for that mutation (29).

Our trial emphasizes that oral administration of an intravenous B6 solution is well tolerated, even at high dosages. The observed adverse effects were those cited in the investigators' brochure. No impairment in renal function or renal imaging was detected. The 6-week period during which study participants received the highest dose, 20 mg/kg, is too short to claim that the solution is safe over the long term because peripheral neurotoxicity may require time to develop.

The study did have some limitations. Uox is frequently used as a surrogate marker to assess efficacy of drug treatment in PH. This is allowable and justified because the endogenous excess of oxalate generation is the pathophysiologic basis of PH I (43). However, this measure is limited. First, it would be more desirable to examine the B6 effect on preservation of renal function (eGFR) directly (*e.g.*, its implication on the chronic inflammatory process leading to impairment of kidney

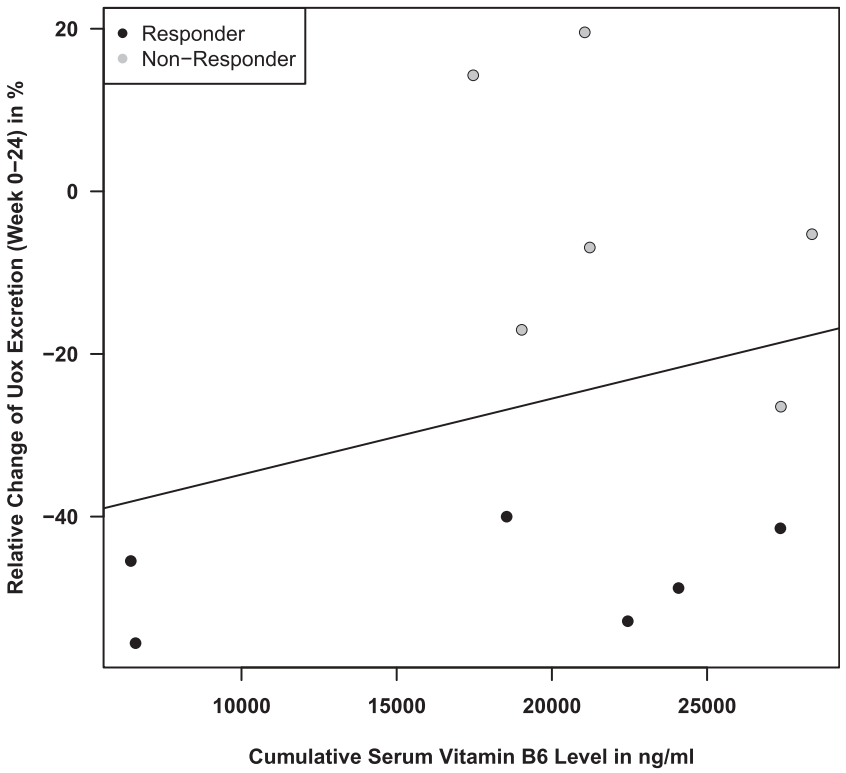

Figure 4. | **Relationship between relative change of Uox excretion and cumulative serum vitamin B6 levels during study weeks 0–24.** Relative change of Uox excretion reduction level in dependence of time-integrated serum vitamin B6 levels (area under the curve) shown for all individual study participants (r=0.26; 95% confidence interval, −0.37 to 0.72); P<0.4). Responders are depicted as solid circles, and nonresponders are depicted as gray circles.

function). However, that would require long-term observational studies.

Second, the natural and handling-associated variability of Uox must be considered in all PH trials (even those using 24-hour timed collections) (44). To obtain the most accurate 24-hour sampling, patients had to repeat urine collections if they were found to be inadequate on the basis of 24-hour creatinine excretion. However, most patients had adequate collections; only 5 of 120 urine collections had to be repeated.

Third, B6 efficacy has only been arbitrarily defined by a ≥30% reduction in Uox (19,20,22,29). How the B6 response correlates with long-term outcome will require larger multinational prospective long-term studies.

Finally, Uox is a measure that accurately reflects endogenous oxalate metabolism only in study participants with renal function that is not severely compromised (45).

Although the sample size consisted of only 12 study participants, this number represented about 50% of children and adolescents known in Germany with stable (normal or near-normal) renal function who had not undergone liver and kidney transplantation. The small sample size available for clinical trials with this rare disease limits the conclusions.

The trial was designed without a control group, but withholding the only available specific medication for PH I would not have been ethically acceptable. Nonadherence in adolescents and study participants with chronic diseases is an ongoing problem. However, measurement of B6 levels detected nonadherence in only one patient.

B6 levels were assessed between 4 and 7 hours after the last morning dosage. Measurement includes the active form pyridoxal-phosphate and pyridoxal as the membrane-passing preactive form. Because there are no data available on metabolization of pyridoxine-hydrochloride to its active form pyridoxal-phosphate in the liver of individuals with PH I, we should be aware of differences in the measured levels based on time of blood collection and metabolizing differences.

In summary, this first prospective clinical trial after 40 years of clinical use demonstrates that B6 is efficacious as a pharmacoperone in PH I, that administered dosage and serum B6 level are moderately correlated, and that serum B6 level and reduction in urine oxalate are not correlated. Analysis of Uox in the three groups indicates that B6 treatment reduces the Uox excretion in patients carrying a mistargeting mutation. The sample size, however, is too low to allow us to assess significant differences. Analysis of Uox in the three groups indicates that the c.508G>A homozygous group started with the lowest mean level of absolute Uox. This may favorably affect the long-term outcome (39,41).

Currently, B6 is the only pharmacoperone available for patients with PH I. Larger trials are needed to clarify the mechanism of B6 action and to assess the effect on rare PH 1 genotypes.

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Disclosures

None.

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B.B.B. and B.H. contributed equally to this work.

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