OPEN

Top Clin Nutr
Vol. 29, No. 4, pp. 325–331

PHENYLKETONURIA

Sapropterin Dihydrochloride Mixed With Common Foods and Beverages

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Sapropterin dihydrochloride is used to lower blood phenylalanine levels in tetrahydrobiopterin-responsive phenylketonuria in conjunction with a phenylalanine-restricted diet. This study investigated the solubility and stability of sapropterin tablets and a sapropterin powder formulation when mixed in selected beverages and foods. Solubility was partial for the tablets and complete for the powder. The stability testing showed that 93% or more of active sapropterin dihydrochloride is present at 1 hour after either tablets or powders are mixed with certain foods and beverages. Mixing sapropterin powder with foods and beverages might facilitate its administration in patients who have difficulty swallowing the drug according to prescribing information. **Key words:** *phenylalanine*, *phenylketonuria*, *sapropterin*, *tetrahydrobiopterin*

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This study was supported by BioMarin Pharmaceutical, Inc. BioMarin Pharmaceutical, Inc, provided the materials, conducted the study, and provided editorial and writing support.

J.J.M., E.R.J., J.L.C.P., N.J., D.T., and S.C. are employees and shareholders of BioMarin Pharmaceutical Inc. A.C. is a paid consultant for BioMarin Pharmaceutical, Inc, and participates on BioMarin Pharmaceutical/Merck-Serono advisory boards. BioMarin Pharmaceutical, Inc, manufactures and markets KUVAN (sapropterin dihydrochloride) tablets for oral use and powder for oral solution.

PHENYLKETONURIA (PKU) is caused by a defective phenylalanine hydroxylase enzyme, and unless the dietary phenylalanine (Phe) intake is controlled, it can lead to the toxic accumulation of blood Phe.[1] As recently reported in management guidelines for this disorder, therapy for PKU requires dietary manipulation to maintain blood Phe levels in the

N.J., E.R.J., and J.L.C.P. designed research; E.R.J., J.J.M., D.T., and S.C. conducted the research; D.T., J.J.M., and S.C. analyzed the data; J.J.M., J.L.C.P., D.T., S.C., A.C., E.R.J., and N.J. contributed to applicability of results to clinical use and in reviewing the drafts, J.J.M. and E.R.J. wrote the manuscript; and E.R.J. had primary responsibility for the final content. All authors read and approved the final manuscript.

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DOI: 10.1097/TIN.0000000000000010

recommended range of 120 to 360 μmol/L.[2,3] However, individuals with PKU may have difficulties maintaining dietary therapy, which entails adhering to prescribed Phe-free amino acid–fortified medical foods and/or maintaining a Phe-restricted diet. For patients with PKU, maintaining blood Phe levels within clinically recommended ranges is critical due to the physiologic and neurophysiologic toxicity associated with elevated blood Phe levels.[4]

Sapropterin dihydrochloride (sapropterin, KUVAN®; BioMarin Pharmaceutical, Inc, Novato, California), a synthetically prepared salt of naturally occurring tetrahydrobiopterin (BH_4), is used to treat hyperphenylalaninemia in BH_4-responsive PKU in conjunction with a Phe-restricted diet.[5,6] A 6-week, multicenter, double-blind, placebo-controlled trial of 88 sapropterin-responsive patients with PKU showed that 18 of 41 patients (44%) on sapropterin (10 mg/kg/d) had a 30% or more reduction in blood Phe levels from baseline compared with 4 of 47 controls (9%).[7]

Sapropterin is available as tablets for oral use and in a new powder formulation for oral solution. Sapropterin prescribing information stipulates that tablets and powder be mixed in 120 to 240 mL of water or apple juice and consumed within 15 minutes of preparation for tablets or 30 minutes for powder.[6] Sapropterin is also recommended to be taken with food to allow optimal absorption. The stomach capacity of most 1-month-old infants (~90-150 mL) and many 1-year-old children (~210-360 mL) is smaller than this prescribed fluid volume.[8] Therefore, if small children consume 120 to 240 mL of fluid volume to take their medication, they most likely will not ingest their scheduled feeding, thus possibly affecting their total recommended daily intake of medical food. These restrictions have been addressed somewhat in the new label that allows smaller volumes (minimum 5 mL) of drug-food mixtures for infants weighing less than 10 kg and permitting greater food and beverage choices.[6] Because the 120- to 240-mL fluid volume and the 2 fluid choices for mixing the drug may be impractical for infants and some children, some clinicians have recommended mixing sapropterin with either small fluid volumes or small amounts of soft food.[9,10]

Sapropterin is hygroscopic, highly soluble in water (>1 g/mL), and has optimal stability in either dry conditions or acidic solutions.[11] Sapropterin is prone to auto-oxidation under neutral or alkaline conditions[12,13]; therefore, sapropterin product labeling states that the drug should be stored in a dry environment and then ingested 15 to 30 minutes (depending on the drug formulation) after dissolution in water or apple juice.[6]

To date, one study has investigated the stability of crushed sapropterin tablets with 2 foods and 3 brands of Phe-free infant medical food formulas.[14] It is not known whether other beverages or foods, some of which might be neutral or alkaline, may accelerate sapropterin oxidation and degrade the active ingredient (sapropterin dihydrochloride) of the drug. The goal of this study was to investigate the solubility and stability of sapropterin powder and tablets when mixed with 15 common, low-protein beverages and soft foods. This study did not evaluate the bioavailability and bioequivalence of sapropterin powder or tablets.

MATERIALS AND METHODS

Solubility testing

Sapropterin crushed tablets (using a tablet crusher combination pill container) and sapropterin powder were mixed separately with 5 mL of room temperature water and assessed visually for solubility. This 5-mL volume is equivalent to a 20-mg/mL drug concentration.

Assay method

An ion-exchange chromatography method for the sapropterin dihydrochloride assay was developed for batch-release and stability testing of sapropterin tablets and powder. A

test sample was prepared by initially dissolving the 10 dosage units in 1.0-L sample diluent (prepared by dissolving 400 mg of thioglycerol and 2.00 g of L-cysteine-HCl in 2.0 L of water). A 1:10 dilution of the initial solution was prepared in additional sample diluent to bring the drug substance concentration to 100 μg/mL. From this concentration, 50-μL samples for high-performance liquid chromatography (HPLC) injections were prepared. Assay performance was determined by comparison of the sapropterin dihydrochloride peak area in the test sample with a standard curve generated using main peak areas from solutions of the reference material at known concentrations extending from 50 to 150 μg/mL.

Stability testing

For stability testing, all food and beverage mixtures were prepared in duplicate. The 4 powdered medical foods were first prepared using volumes of water specified by each of the respective manufacturers: 8 g of Abbott Phenex-1 Phe-free infant medical food formula (Abbott Laboratories, Columbus, Ohio) was mixed with 60 mL of water, 25 g of Vitaflo PKU Express Phe-free medical food (Vitaflo International Ltd, Liverpool, England) was mixed with 80 mL of water, 14 g of Nutricia LoPhlex Phe-free medical food (Nutricia North America, Rockville, Maryland) was mixed with 70 mL of water, and 49 g of Bettermilk (unflavored) medical food (Cambrooke Foods, Inc, Ayer, Massachusetts) was mixed with 120 mL of water. Foods and beverages that did not require special preparation were as follows: 20 mL of room temperature water, 20 mL of hot water, 20 mL of lemon-lime soda (Sprite; The Coca-Cola Company, Atlanta, Georgia), 20 mL of 1814-g carton rice milk (Rice Dream Rice Drink; The Hain Celestial Group, Inc, Melville, New York), 20 mL of apple juice (Safeway, Inc, Pleasanton, California), 20 g of lemon pudding (Hunt's Snack Pack pudding; ConAgra Foods, Inc, Omaha, Nebraska), 20 g of coconut milk yogurt (So Delicious Coconut Milk Yogurt, vanilla flavor, 113-g container; Turtle Mountain, LLC, Eugene, Oregon). A 127.6 g quantity of mashed banana was prepared. Oatmeal (Quaker Cinnamon & Spice Instant Oatmeal, 43-g packet; Quaker Oats Company, Chicago, Illinois) and rice cereal (Cream of Rice Hot Cereal, 45-g packet; B&G Foods, Inc, Parsippany, New Jersey) were each prepared using 250 mL of hot water (73°C—from a hot water dispenser) according to instructions provided by the manufacturer.

Sapropterin tablets or powder were then added to each of the foods and beverages. For the sapropterin tablets, 10 tablets (1000 mg of sapropterin dihydrochloride) were ground with a mortar and pestle into a powder and mixed for 2 to 5 minutes in a 1-L beaker containing one each of the foods and beverages. For sapropterin powder, 10 packets of powder were opened and the packet contents combined to produce 1000 mg of sapropterin dihydrochloride and then mixed for 2 to 5 minutes, using a spatula, in a 1-L beaker containing one each of the foods and beverages. The first set of duplicates (T = 0 samples) were extracted, filtered, and immediately diluted to 100 μg/mL of sapropterin dihydrochloride in a solution of thioglycerol, L-cysteine-HCl hydrate, and HPLC-grade water. The filtrates were analyzed in duplicate with a Waters Alliance 2695 HPLC (Waters Corp, Milford, Massachusetts) using a Whatman Partisil 10 SCX 4.6 × 250-mm column (Hichrom Ltd, Reading, Berkshire, England). The second set of duplicates were incubated for 1 hour at room temperature (T = 1-h samples) and then extracted, filtered, and diluted in a solution of thioglycerol, L-cysteine-HCl hydrate, and HPLC-grade water. A larger-volume extraction, and sonication (20 minutes), was required for complete separation of the oatmeal samples due to their fibrous nature; the larger dilution was taken into account during analysis. Stability of all foods and beverages was determined by comparing T = 1-h analysis values to the amount of sapropterin in the pure tablet batch and calculating the percentage difference.

RESULTS

Analytical method

The HPLC method was found to have the following performance: inaccuracy: ±0.5%; repeatability: relative standard deviation (RSD) 0.1% or less; intermediate precision: RSD = 0.5%; and linearity: R^2 = 1.00 (at 70%-130% of nominal sample concentration). The food and beverage textures and ingredients did not affect the method. Specificity of the batch-release and stability-indicating test method was determined by verification of the absence of coelution of the sapropterin peak with related substance impurities or with sample matrix components. Specificity of the method was determined by preparation and injection of matrix "blank" samples (preparations containing the food or beverage but no sapropterin) to verify absence of coelution with sample matrix components.

Solubility

For solubility testing, sapropterin crushed tablets mixed in room temperature water yielded a slightly milky, particulate solution indicating that full dissolution did not occur—an expected result given the insolubility of some excipients (Table 1). White particles were likely due to crospovidone, sodium stearyl fumarate, and dibasic calcium phosphate that are either insoluble or partially soluble. Sapropterin powder dissolved rapidly (within 15 seconds) and completely in room temperature water, producing a clear, yellow-hued solution. There was no evidence of insolubility when sapropterin powder was mixed with other beverages and foods.

Stability

Table 2 shows sapropterin percent recovery when mixed in foods and beverages at 1 hour relative to values obtained from the manufacturing batch. The percent recovery for all samples ranged from 93% to 105.8%. A 0.9% overall average decrease in the amount of sapropterin powder was present in the tested samples at 1-hour incubation—with water at 22°C. Lemon pudding, mashed banana, and Abbott Phenex-1 all exhibited the least amount of degradation. A 4.1% average decrease in sapropterin recovery was observed for crushed tablets mixed in the same 15 foods and beverages.

DISCUSSION

Sapropterin is indicated to reduce blood Phe levels in patients with hyperphenylalaninemia due to BH_4-responsive PKU when used in conjunction with a Phe-restricted diet.[6] Sapropterin prescribing information stipulates a range of dosages from 5 to 20 mg/kg/d and that sapropterin tablets and

Table 1. Sapropterin Tablet and Powder Ingredients and Functions

Sapropterin Powder	Sapropterin Tablets	Functions
Sapropterin dihydrochloride	Sapropterin dihydrochloride	Active ingredient[a]
Mannitol	Mannitol	Bulking agent/diluent
Ascorbic acid	Ascorbic acid	Flavoring agent/antioxidant
Sucralose		Sweetener
Potassium citrate		Buffering agent
	Crospovidone	Disintegrant[b]
	Dibasic calcium phosphate	Binder[c]
	Riboflavin	Coloring agent
	Sodium stearyl fumarate	Lubricant[b]

[a]A cofactor of phenylalanine hydroxylase.[6]
[b]Insoluble or partially soluble in water.
[c]Partially soluble in water.

Table 2. Foods and Beverages, Mass Quantities Used for Stability Tests, and 1-Hour Stability (Recovery) for Sapropterin Tablets or Sapropterin Powder Are Mixed in Selected Foods and Beverages

Food or Beverage	Mass Used for Stability Testing	1-h Stability of Sapropterin Powder[a]			1-h Stability of Sapropterin Tablets[ab]		
		T = 0	T = 1-h	Δ	T = 0	T = 1-h	Δ
Water (22°C)	20 mL	100	99.7	−0.3			
Water (73°C)	20 mL	100	99	−1			
Apple juice	20 mL	100.9	98.4	−2.5			
Applesauce	20 g	98.3	97.1	−1.2			
Coconut milk yogurt	20 g	99	97.2	−1.8	101.2	96.2	−5
Lemon pudding	20 g	97.5	97.8	0.3			
Lemon-lime soda (Sprite)	20 mL	99.8	98.6	−1.2	103.6	98.0	−5.6
Mashed banana	20 g	98.3	97.5	−0.8	101.7	95.8	−5.9
Rice milk	20 mL	101.6	100.3	−1.3	104.1	98.8	−5.3
Oatmeal, Cinnamon-& Spice Instant	43 g	96.8	95.3	−1.5	98.6	94.1	−4.5
Rice cereal	45 g	101.3	102.6	1.3	104.4	102.7	−1.7
Abbott Phenex-1 Phe-free infant medical food formula	8 g	101.2	100.9	−0.3			
Vitaflo PKU Express Phe-free medical food	25 g	101.5	105.8	4.3	98.1	95.4	−2.7
Nutricia LoPhlex Phe-free medical food	14 g	96.9	93.1	−3.8			
Bettermilk (unflavored) Medical food milk substitute	49 g	96.5	93.0	−3.5	95.3	93.3	−2
Overall average recovery				−0.9			−4.1

[a]Stability data shown as percent average recovery relative to the manufacturing batch concentration certificate of analysis (N = 2).
[b]Tablets were crushed and ground before mixing with foods.

powder be mixed in 120 to 240 mL of water or apple juice and administered with food to provide optimal absorption.[6] However, the prescribed mixing volume may be difficult to administer to very young pediatric patients due to their small stomach capacity. As humans grow from infant to adulthood, stomach capacity increases from 10 to 20 mL in newborns, to 210 to 360 mL in 1-year-old infants, and to 2000 to 3000 mL in adults along with ability of the stomach to tolerate larger fluid volumes.[8] Our solubility methods used a 5-mL volume, at a 20 mg/mL concentration, to obtain a total volume that approximates a newborn's stomach capacity. Burton et al[9] suggest that mixing sapropterin tablets with small fluid volumes is a viable drug administration strategy for infants, and this approach has also been addressed in the new label allowing smaller volumes (minimum 5 mL) for infants weighing less than 10 kg.[6] It is important to note that the amount of food or beverage used to mix and administer the sapropterin in this study is small and not meant to constitute or replace a meal. Because prescribing information recommends that sapropterin be taken with food to increase its absorption and tolerability, a meal might need to be consumed

whenever a small amount of dosed food or beverage is ingested.

As expected, the crushed tablets only dissolved partially in water. Table 1 shows that 2 of the sapropterin excipients (ie, crospovidone and sodium stearyl fumarate) are insoluble or partially soluble and were observed as white particles when mixed in water. Dibasic calcium phosphate is only partially soluble and also contributes particulate mass. The powder formulation, which does not contain these additives, dissolved completely.

This study used 15 low-protein foods and beverages that are consistent with a Phe-restricted diet, although some of these test foods and beverages contained Phe that should be taken into account when prescribing daily dietary Phe intake. It has been postulated that a better tasting drug-food or drug-beverage mixture might improve compliance in taking the medication.[15]

The stability of sapropterin is established with the 15 liquids and soft foods listed using standard preparation methods (including the use of hot water). The data show that 93% or more of sapropterin dihydrochloride is present at 1 hour after crushed sapropterin tablets or powder are mixed with low-protein foods and beverages. Both sapropterin powder (mean = −0.9%) and sapropterin tablets (mean = −4.1%) showed slight degradation at 1 hour. The decrease in sapropterin is likely due to auto-oxidation caused by either near-neutral pH or alkaline conditions. Small increases in drug amounts at 1 hour in certain foods and beverages might have been caused by measurement variation or relative differences in the amount of drug compared with the manufacturing batch value. The data showed that even mixing sapropterin with hot foods and/or hot water does not adversely affect stability of the drug.

A major limitation of this study is that the stability testing was done in vitro. Although this study simulated food preparation methods of a home setting, the experiment was carried out in a manner that could not fully represent all situations that patients or their caregivers may encounter. Taste was not deemed appropriate for this article due to its high subjectivity and the presence of sucralose in the Kuvan powder (and not in the tablets), which would be expected to confound the taste results. In addition, this study did not test the safety and efficacy of these preparations in lowering blood Phe levels for treatment of hyperphenylalaninemia.

CONCLUSION

This study found partial solubility for sapropterin tablets and complete solubility for the sapropterin powder formulation at a 20 mg/mL concentration. This study also showed that 93% or more of the active sapropterin dihydrochloride is present after both crushed tablets and powder formulation are mixed with certain commercially available, low-protein foods and beverages and medical foods and formulas at 1 hour, thus indicating that mixing sapropterin with foods and beverages does not significantly degrade stability of the drug. Because stability data at more than 1 hour have not been assessed, the use of sapropterin mixed in food and beverages more than 1 hour after preparation is not recommended. The ability to mix sapropterin in soft foods and beverages may improve the practicality of sapropterin use in infants and small children, as well as benefit other patient populations who have difficulty tolerating administration of sapropterin according to current prescribing information.

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