

alanine, tyrosine, and tryptophan) blood levels, and routine laboratory tests (chemistry, hematology, and urinalysis) before inclusion in the study.

The drug tested was (6R)-5,6,7,8-tetrahydrobiopterin, also known as 2-amino-6-(1,2-dihydroxypropyl)-5,6,7,8-tetrahydro-3H-pteridin-4-one tetrahydrobiopterin, or sapropterin (BH4 or 6R-BH4). The drug was obtained in 10 mg or 50 mg oral tablets from Schircks Laboratories, Switzerland (product no. 11.212 (6R)-5,6,7,8-Tetrahydro-L-biopterin dihydrochloride). The half-life of the Schircks 6R-BH4 dihydrochloride salt is approximately 3.5 hours.

Drugs known to inhibit folate synthesis such as bactrim, methotrexate, or 5-FU were not permitted to be administered during the study. Before initiation of 6R-BH4 dosing, a 7 day washout period was required for any drugs known to inhibit folate synthesis. No investigational drugs were permitted to be taken during study participation or within 30 days prior to study enrollment.

Within a maximum of 4 weeks following the completion of baseline assessments, eligible subjects began the first stage of the study. Single ascending doses of 10 mg/kg, 20 mg/kg and 40 mg/kg of 6R-BH4 were administered orally, with a washout period of at least 7 days between each dose, and subjects were monitored 24 hours after each dose. Subjects underwent a safety assessment and blood amino acid (i.e. phenylalanine, tyrosine, and tryptophan) level measurements before and 24 hours after each 6R-BH4 dose. Blood pressure was measured 30 minutes and 1 hour after each dose. Safety assessments included physical examinations, vital signs, serial assessment of PKU or HPA related signs and symptoms, recording of adverse events, and monitoring of changes in laboratory parameters (chemistry, hematology, and urinalysis). Subjects were instructed to continue their usual diet without any modification, and to record daily intake of food and beverages throughout the study.

After the first stage of the study was completed, subjects entered the second stage of the study, during which they received 10 mg/kg of 6R-BH4 daily in an oral dosage form, for a total of 7 days. After a washout period of at least 7 days, each subject received 20 mg/kg of 6R-BH4 daily for a total of 7 days. During the second stage of the study, subjects were monitored before dosing, at 24 and 72 hours after first dose, and on the 7th day of dosing at each of the two dose levels. Monitoring included a safety assessment as described above, measurement of serum blood amino acid (i.e. phenylalanine, tyrosine, and tryptophan) levels and evaluation of phenylalanine and tyrosine oral intake. Subjects were instructed to continue their usual diet without any modification, and to record daily intake of food and beverages throughout the study.

After a single dose of 6R-BH4 (10 mg/kg), blood Phe declined $10\% \pm 0.26\%$ from baseline. Single doses of 6R-BH4 at 20 mg/kg and 40 mg/kg showed mean declines of $17\% \pm 0.28\%$ and $27\% \pm 0.25\%$ respectively. The reduction in blood Phe levels appeared to be dose dependent.

FIG. 16 shows mean blood phenylalanine level after 10 and 20 mg/kg 6R-BH4 daily for 7 days, in the 14 of 20 patients who responded to treatment. For the purposes of this study, a decline in blood Phe levels of 30% was considered

to be "responsive", although patients who exhibit less of a decline would still benefit from BH4 treatment. The seven-day trial showed a sustained decrease in blood Phe concentration in 70% of the patients (14/20) taking 20 mg/kg. Of those 14 patients, 10 (71%) responded favorably to 10 mg/kg/day. Blood tyrosine was observed to increase in some but not all patients; some patients had increases of >80% from baseline tyrosine levels. The individual blood Phe responses to multiple doses of 10 mg/kg BH4 are shown in 11 adults (FIG. 17) and 9 children (FIG. 19). The individual blood Phe responses to multiple doses of 20 mg/kg BH4 are shown in 11 adults (FIG. 18) and in 9 children (FIG. 20).

Thus, a single-dose loading test was inadequate to identify patients who responded to BH4 treatment with a reduction in blood Phe level of 30% or more. A 7-day loading test successfully identified a high percentage of responsive patients. The 20 mg/kg, 7-day loading test with 6R-BH4 identified 70% of the PKU patients that responded to 20 mg/kg of BH4. Of the 14 responders, 71% also showed a 30% or greater reduction in blood Phe level with the lower dose of 10 mg/kg 6R-BH4.

The references cited herein throughout, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are all specifically incorporated herein by reference.

What is claimed is:

1. A method for treating a subject suffering from hyperphenylalaninemia (HPA) due to BH4-responsive phenylketonuria (PKU) comprising administering to said subject tetrahydrobiopterin (BH4) or pharmaceutically acceptable salt thereof at a daily dose of 10 mg/kg to 20 mg/kg, wherein the administering is multiday, oral, and only once per day.
2. The method of claim 1, wherein said subject is administered BH4 for at least 7 days.
3. The method of claim 1, wherein the subject is administered BH4 for at least 2 weeks.
4. The method of claim 1, wherein said subject is administered BH4 for at least 6 weeks.
5. The method of claim 1, wherein said BH4 is administered as a crystallized form stable for at least 3 months at 40° C. and 75% relative humidity.
6. The method of claim 5, wherein said crystallized form of BH4 comprises at least 99.5% pure (6R)-5,6,7,8-tetrahydrobiopterin.
7. The method of claim 1, further comprising administering to said subject a protein-restricted diet.
8. The method of claim 1, wherein said subject is pregnant, is an infant of 0 to 3 years, or has a plasma phenylalanine concentration of greater than 600 μM prior to treatment with BH4.
9. The method of claim 1, wherein said BH4 is in the form of a tablet.
10. The method of claim 1, wherein said BH4 is dissolved in a liquid.
11. The method of claim 1, wherein the BH4 is administered at a dose of about 10 mg/kg.
12. The method of claim 1, wherein the BH4 is administered at a dose of about 20 mg/kg.

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