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Effects of Food Intake on the Relative Bioavailability of Amifampridine Phosphate Salt in Healthy Adults

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ABSTRACT

Purpose: Amifampridine (3,4-diaminopyridine) has been approved in the European Union for the treatment of Lambert-Eaton myasthenic syndrome. Amifampridine has a narrow therapeutic index, and supratherapeutic exposure has been associated with dose-dependent adverse events, including an increased risk for seizure. This study assessed the effect of food on the relative bioavailability of amifampridine in healthy subjects and informed on conditions that can alter exposure.

Methods: This randomized, open-labeled, 2-treatment, 2-period crossover study enrolled 47 healthy male and female subjects. Subjects were randomly assigned to receive 2 single oral doses of amifampridine phosphate salt (20 mg base equivalents per dose) under fed or fasted conditions separated by a washout period. Blood and urine samples for pharmacokinetic analyses were taken before and after dosing. Plasma concentrations of amifampridine and an inactive 3-N-acetyl metabolite were determined. The relative bioavailability values of amifampridine and metabolite were assessed based on the plasma PK parameters AUC0–1, AUC0–t, and Cmax in the fed and fasted states using noncompartmental pharmacokinetic analysis. Parent drug and metabolite excretion were calculated from urinary concentrations. A food effect on bioavailability would be established if the 90% CI of the ratio of population geometric mean value of AUC0–∞, AUC0–t, or Cmax between fed and fasted administration was not within the bioequivalence range of 80% to 125%. Tolerability was assessed based on adverse-event reporting, clinical laboratory assessments, physical examination including vital sign measurements, 12-lead ECG, and concurrent medication use.

Findings: Food slowed and somewhat decreased the absorption of amifampridine. There was a decrease in exposure (Cmax 44%; AUC 20%) after oral administration of amifampridine phosphate salt in the presence of food, and mean Tmax was 2-fold longer in the fed state. The extent of exposure and plasma elimination half-life of the major metabolite was greater than those of amifampridine in the fed and fasted conditions. Mean AUCs in the fed and fasted states were slightly greater in women than men, with no difference in mean Cmax. Orally administered amifampridine was renally eliminated (> 93%) as the parent compound and metabolite within 24 hours. Single oral doses of 20 mg of amifampridine phosphate salt were considered well tolerated in both the fed and fasted conditions. High intersubject variability (%CVs, > 30%) in amifampridine pharmacokinetic parameter values was observed.

Implications: At the intended dose under fasting conditions, amifampridine exposure may be increased. European Union Drug Regulating Authorities Clinical Trials identifier: 2011-000596-13. (Clin Ther. 2015; 37:1555–1563) © 2015 The Authors. Published by Elsevier HS Journals, Inc.

Key words: amifampridine, food effects, pharmacokinetics, 3,4-diaminopyridine, 3,4-DAP, Lambert-Eaton Myasthenic Syndrome.

INTRODUCTION

3,4-Diaminopyridine (3,4-DAP) has been used for >25 years for treating a variety of neurologic disorders of axonal or synaptic transmission, including Lambert-Eaton myasthenic syndrome (LEMS),1,2 myasthenia gravis, congenital myasthenia, multiple sclerosis, and downbeat nystagmus.3–5 3,4-DAP blocks voltage-dependent potassium ion channels, thereby prolonging the action potential and presynaptic cell
membrane depolarization, enhancing influx or transport of calcium into the nerve ending. The resulting increase in intracellular calcium concentration facilitates exocytosis of acetylcholine-containing vesicles, leading to an increased concentration of acetylcholine at the motor end plate, which in turn results in improved neuromuscular transmission and augmented muscle contraction and strength. Over the past 25 years, a considerable amount of clinical experience with 3,4-DAP has been gained, providing a strong body of evidence supporting its efficacy and tolerability in the treatment of patients with LEMS. 3,4-DAP is a standard of care for LEMS in the United States and has been recommended by the European Academy of Neurology (formerly, European Federation of Neurological Societies) for first-line symptomatic treatment of patients with LEMS. For many years, the absence of a product approved for the treatment of this condition led physicians to prescribe ad hoc preparations of amifampridine base from compounding pharmacies or independent small-scale manufacturers. The process of producing these products was associated with considerable batch variability, lack of reliability in drug quality, safety concerns for the people manipulating the free base, and a risk for overdose due to compounding errors.

A new oral phosphate salt formulation of 3,4-DAP (amifampridine) was approved by the European Medicines Agency in 2009. Comparability of the base versus the phosphate salt formulation of 3,4-DAP was demonstrated in a previously conducted double-blind, single-dose, crossover, bioavailability/bioequivalence study conducted in 27 healthy male subjects. The pharmacokinetic (PK) profiles of both the 3,4-DAP free base and phosphate salt forms in this study were highly variable, with up to 10-fold differences in $C_{\text{max}}$, AUC, and $t_{1/2}$ between subjects. The most probable explanation for this variability appears to be the single metabolic disposition of 3,4-DAP via the activity of N-acetyl transferases (NATs) to form a single major 3-N-acetyl metabolite, which is inactive. NAT enzymes are highly polymorphic in humans and vary considerably with ethnicity. Phenotypically, a patient’s NAT status can be classified, through a range of acetylation activities, from slow to rapid. Variations of polymorphic NAT corresponding with fast and slow acetylator phenotypes have been found to significantly affect the PK and tolerability profiles of amifampridine.

Understanding the PK properties of amifampridine is particularly important because amifampridine has a narrow therapeutic index, and supratherapeutic exposure has been associated with an increase in the risk for seizures. The purpose of this study was to evaluate the effects of food on the PK properties and relative bioavailability of amifampridine phosphate salt.

**SUBJECTS AND METHODS**

**Subjects**

This study enrolled healthy subjects, both men and women, aged 18 to 65 years and with a body mass index between 18.5 and 30 kg/m$^2$, inclusive. Subjects underwent screening to confirm eligibility within 28 days before the administration of the first dose of study drug. Screening included physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and ECG. Women of childbearing potential must have had a negative pregnancy test at screening. Sexually active subjects of childbearing potential must have been willing to use 2 acceptable methods of contraception while participating in the study, with 1 method spermicidal.

Subjects were excluded if they could not tolerate the study-specific diet or were breast-feeding. Subjects were also excluded if they had received any prescribed systemic or topical medication; nonprescribed systemic or topical medication (including herbal remedies, but excluding vitamin/mineral supplements); any medication (including St. John’s wort or other herbal remedy) known to chronically alter drug absorption or elimination processes; medication that prolongs the QT interval or QT interval corrected for heart rate; alcohol, caffeine, poppy seed, or grapefruit containing products; or amifampridine (base or phosphate salt) or fampridine (4-aminopyridine) within defined time periods before planned administration of the first dose of study drug.

Other exclusion criteria included a variety of significant diseases, disorders, or illnesses; high or low blood pressure; ECG abnormalities; a history of cardiac disease; and current or history of alcohol abuse, drug abuse, or smoking within defined time periods before planned administration of the first dose of study drug.

**Study Design**

This was a randomized (1:1), open-labeled, 2-treatment, 2-period crossover study to assess the tolerability and effect of food on the relative bioavailability of amifampridine phosphate salt in healthy subjects after single-dose administration. Written informed consent was obtained from each subject before
any study-related activities were conducted. The study protocol was approved by the institutional review board (Reading Independent Ethics Committee). The study was conducted in accordance with the sections of the US Code of Federal Regulations relating to clinical research studies and/or other national and local regulations, including the Good Clinical Practice guideline and the ethical principles established by the Declaration of Helsinki (2000).

Each subject was randomly assigned to a treatment period (interval) and treatment sequence (AB or BA) and received 2 single doses of amifampridine phosphate salt. Treatment A was a single dose of amifampridine phosphate salt consisting of 2 tablets (10 mg of active pharmaceutical ingredient [API] in each tablet, for a total dose of 20 mg API) administered orally in a fasting state. The fasting state was established using an overnight fast of ≥10 hours. Treatment B was a single dose of amifampridine phosphate salt consisting of 2 tablets (20 mg total API) administered orally in a fed state. Subjects fasted overnight for ≥10 hours and then received a high-fat (~60% of total caloric content), high-calorie (~800–1000 kcal) test meal 30 minutes before dosing. A washout period of 6 to 10 days separated the 2 dose administrations.

**Blood and Urine Sampling**

Blood and urine samples were collected at specific times during the study for the measurement of plasma and urinary concentrations of amifampridine and its major metabolite, 3-N-acetyl amifampridine.

Blood samples for PK analyses were taken at 90 ± 5 minutes before and at 10, 15, 30, 45, 60, 75 minutes and 1.5, 2, 4, 6, 8, 10, 12, 18, and 24 hours after dose administration. Blood samples were collected into lithium heparin tubes (Vacutainer; Becton, Dickinson and Company, Franklin Lakes, New Jersey) and, after mixing, were placed into a cool box containing crushed ice/water. Blood samples were centrifuged within 1 hour of collection, and 1 mL of the separated plasma was transferred into 2 prechilled polypropylene tubes within 2 hours of collection and subsequently stored at ~−70°C until quantification by LC-MS/MS analysis. Sample processing and storage was completed in compliance with Good Laboratory Practice standards.

Urine samples were collected at ≤90 minutes before dose administration (single sample) and at 0 to 4, 4 to 8, and 8 to 24 hours after dose administration to quantify total dose excreted as nonmetabolized amifampridine or 3-N-acetyl amifampridine. During each collection period, the containers were stored in a refrigerator at 2°C to 8°C. The weight of each urine sample was recorded before the removal of 2 subsamples, which were stored, within 4 hours of the end of the collection, at approximately −70°C. The remaining urine was discarded.

**Analytical Methods**

Plasma and urinary concentrations of amifampridine and 3-N-acetyl amifampridine were determined using acetonitrile precipitation extraction followed by HPLC-MS/MS detection (SGS Bioanalytical Services, Cephaç, Saint-Benoit, France).

Briefly, 25 µL of plasma sample was mixed with 300 µL of acetonitrile containing the internal standards and 0.1% formic acid. After vortex-mixing and centrifugation, 300 µL of supernatant was transferred to a clean 96-well plate, and a portion of this amount was directly injected into the LC-MS/MS system. For urine quantification, a 20-µL sample was mixed with 1.2 mL of acetonitrile containing 0.1% formic acid and internal standards. After vortex-mixing and centrifugation, 300 µL of supernatant was transferred to a clean 96-well plate, and a portion of this amount was directly injected into the LC-MS/MS system. The method employed individual stable isotope-labeled internal standards for amifampridine ([2H3]-3,4-DAP) and 3-N-acetyl amifampridine ([2H3]-3-N-acetyl). The HPLC system consisted of two 20-AD pumps (Shimadzu Corporation, Tokyo, Japan) operated in a high-organic (acetonitrile) to high-aqueous (0.1% formic acid in water) gradient flow at 0.8 mL/min with a Waters Atlantis HILIC silica column (3 × 100 mm, 3-µm particle size; Waters Corporation, Milford, Massachusetts) held at 45°C. A portion of the eluent (0.3 mL) was transferred directly to an API 3000 mass spectrometer (AB Sciex, Foster City, California) operated in a positive ESI mode with tandem quadrupole mass filtering. The settings for monitoring each analyte consisted of a characteristic protonated precursor ion (M + H+) to product ion mass transition, as follows: amifampridine, 110 → 93 Da; [2H3-amifampridine, 113 → 96 Da; 3-N-acetyl amifampridine, 152 → 110 Da; and 3-N-[2H3-acetyl amifampridine, 155 → 111 Da. Each precursor–product ion transition was optimized to a particular collision energy (27–30 eV) in the quadrupole collision cell, using nitrogen as the collision gas. In human plasma, the lower limits of quantification (LLOQs) of
amifampridine and the metabolite were 0.5 and 2 ng/mL, respectively. In urine, the LLOQs of both amifampridine and the metabolite were 150 ng/mL.

Concentration results were calculated using a linear calibration curve of drug-to-internal standard or metabolite-to-internal standard peak area ratios and calibration curves were generated using 1/X²-weighted linear least squares (LS) regression. The assay performance was monitored daily with quality-control (QC) samples using blank plasma or urine samples spiked with reference standards as follows for amifampridine in samples using blank plasma or urine samples spiked with reference standards as follows for amifampridine in plasma: low QC, 1.0 ng/mL (mean accuracy, 97.2% [N = 34]; range, 89.5%–109% nominal); mid QC, 75 ng/mL (mean accuracy, 103% [N = 32]; range, 94.8%–109% nominal); and high QC, 350 ng/mL (mean accuracy, 98.9% [N = 34]; range, 87.4%–108% nominal); for amifampridine in urine: low QC, 300 ng/mL (mean accuracy, 109% [N = 10]; range, 101%–119% nominal); mid QC, 6000 ng/mL (mean accuracy, 105% [N = 10]; range, 105%–112% nominal); and high QC, 12,000 ng/mL (mean accuracy, 103% [N = 10]; range, 89.2%–111%), and for 3-N-acetyl amifampridine metabolite in plasma: low QC, 6 ng/mL (mean accuracy, 98.7% [N = 32]; range, 87.6%–105% nominal); mid QC, 100 ng/mL (mean accuracy, 96.8% [N = 31]; range, 90.4%–100% nominal); and high QC, 1500 ng/mL (mean accuracy, 96.0% [N = 33]; range, 92.6%–97.3% nominal), for 3-N-acetyl amifampridine metabolite in urine: low QC, 300 ng/mL (mean accuracy, 109% [N = 12]; range, 98.3%–121% nominal); mid QC, 6000 ng/mL (mean accuracy, 103% [N = 12]; range, 90.5%–110% nominal); and high QC, 12,000 ng/mL (mean accuracy, 103% [N = 11]; range, 90.0%–109% nominal).

Tolerability Assessment

Tolerability was assessed throughout the study and determined from the evaluation of adverse events (AEs), clinical laboratory assessments (hematology, chemistry, and urinalysis), physical examination including vital sign assessments, 12-lead ECG, and concurrent medication use. All AEs were assessed and coded by the investigator using the Medical Dictionary for Regulatory Activities version 13.1 (Northrop Grumman Corporation, Falls Church, Virginia).

Pharmacokinetic Analysis

The PK population consisted of all subjects who received at least 1 dose of study drug and had evaluable PK data. The PK analysis was conducted using WinNonlin Enterprise version 5.2 (Pharsight Corporation, Mountain View, California). PK parameter values were determined using noncompartmental analysis. The total amount of amifampridine or 3-N-acetyl DAP metabolite drug equivalent excretion into 24-hour urine collections was calculated. The total amount of parent drug or 3-N-acetyl amifampridine metabolite excreted into the urine was calculated by multiplying total urine volume gathered during a collection interval by the concentration of amifampridine quantified during that collection interval (0–4, 4–8, or 8–24 hours). To determine the total amount of drug or metabolite excreted into urine in the full 24-hour collection period, the 3 collection intervals from 0 to 24 hours were separately summed together for each component. For the conversion of metabolite to amifampridine equivalents (due to a mass increase of 42 Da via acetylation), the total mass of the 3-N-acetyl metabolite excreted in a given interval was multiplied by a conversion factor of 0.722 (109.1/151.1).

Statistical Analysis

AUC₀₋₄₈, AUC₀₋₄₈, and Cmax were subject to statistical analysis by fed/fasted status. These PK parameter values were logarithm-transformed (base e) before analysis and were analyzed using a mixed model. The model included sequence, period, and treatment as fixed effects and subject as a random effect. For these parameters, LS means and mean differences were calculated for the fed and fasted states. The residual variance from the mixed model was used for calculating the 90% CI of the difference between the fed and fasted states. These values were back-transformed to give geometric LS means, a point estimate, and 90% CI of the ratio of the fed relative to the fasted treatment. A food effect on bioavailability was established if the 90% CI of the difference between the fed and fasted states. These values were back-transformed to give geometric LS means, a point estimate, and 90% CI of the ratio of the fed relative to the fasted state. A food effect on bioavailability was established if the 90% CI of the ratio of population geometric mean AUC₀₋₄₈, AUC₀₋₄₈, or Cmax value between the fed and fasted states was not contained within the bioequivalence range of 80% to 125%.

RESULTS

Subjects

A total of 47 subjects took part in this study. One subject was withdrawn from the study due to a positive cotinine test result at check-in for the fed period of the study and was not included in the analysis of the fed state. Two subjects were not included in the fasted...
group; 1 subject was withdrawn from the study due to a severe AE of gastroenteritis during the fed portion of the study (described subsequently), and the other subject was withdrawn from the study due to a positive cotinine test result at check-in for the fasted period of the study. The mean age of the subjects was 37 years; mean body mass index, 24.6 kg/m². The study population was predominately male, white, and non-Hispanic. The overall summary statistics for the baseline demographic characteristics are presented in Table I.

Pharmacokinetic Properties of Amifampridine and the Inactive 3-N-Acetyl Amifampridine Metabolite under Fasting and Fed Conditions

Single oral dose administration of amifampridine phosphate salt tablets (2 tablets, 20 mg total API) with a high-fat meal was associated with a decrease in systemic exposure (Cmax, AUC) compared with administration in the fasted state (Table II and Figure). The mean Tmax was longer under fed conditions, indicating that food decreases the rate of amifampridine absorption. The mean terminal t½ of amifampridine was not substantially altered by food.

The extent of exposure based on geometric mean ratios (fed-to-fasted) suggests that the administration of amifampridine under fed conditions resulted in a decrease in absorption, with an ~18% to 20% decrease in overall exposure (AUC) and a 44% decrease in Cmax in the presence of food compared with those in fasted conditions (Table II). Geometric LS mean ratios (point estimates) with 90% CIs for bioequivalence bounds of 80% to 125% suggested a significant food effect on Cmax because the CI (47.0%–67.5%) was not contained within the bioequivalence limits. However, for AUC0–t and AUC0–∞, the CIs overlapped the bioequivalence range; the upper limits of the CIs were contained within, whereas the lower limits of the CIs were outside the bioequivalence range (73.1%–87.6% and 76.0%–89.2%, respectively). By this strict criterion of CI limits, the AUC exposures were not significantly different between the fed and fasted states. The results from the linear mixed-effects model suggest a lack of sequence and period effects (P > 0.1) of the crossover treatment design. A carryover effect was also absent in the study due to amifampridine levels below the LLOQ before dosing in treatment period 1 (drug naïve) and treatment period 2 (with a washout interval of 6–8 days between treatment periods 1 and 2).

Intersubject variability in amifampridine PK exposure (Cmax and AUCs) was high. PK parameter values ranged broadly across subjects, from 8- to 45-fold for AUC0–t under fed (8.3–282 ng · h/mL) and fasted (20.6–267 ng · h/mL) conditions, AUC0–∞ under fed (9.66–292 ng · h/mL) and fasted (22.1–271 ng · h/mL) conditions, and Cmax under fed (2.81–132 ng/mL) and fasted (16.0–137 ng/mL) conditions. For the terminal t½, the ranges across subjects varied from 3.5- to 4.5-fold under fed (0.82–3.78 hours) and fasted (1.23–4.31 hours) conditions.

Mean (SD) AUC0–t values were numerically higher in women than in men under fed (120 [98.3] vs 94.6 [58.8] ng · h/mL) and fasted (129 [93.4] vs 104 [62.7] ng · h/mL) conditions. However, the variability in these values was sufficiently high (%CVs, 63%–88%) that the differences were most likely

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Table I. Baseline demographic and clinical characteristics of the subjects in this study of the effects of food intake on the relative bioavailability of amifampridine 20 mg (N = 47).*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tr>
<td>Age, y</td>
<td>Mean (SD) 37 (13.4)</td>
</tr>
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<td></td>
<td>Range 19–62</td>
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<tr>
<td>Sex, no. (%)</td>
<td>Male 31 (66.0)</td>
</tr>
<tr>
<td></td>
<td>Female 16 (34.0)</td>
</tr>
<tr>
<td>Race, no. (%)</td>
<td>White 41 (87)</td>
</tr>
<tr>
<td></td>
<td>Black 3 (6)</td>
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<tr>
<td></td>
<td>Other 3 (6)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>Mean (SD) 72.5 (11.78)</td>
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<tr>
<td></td>
<td>Range 49.5–98.5</td>
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<tr>
<td>Height, cm</td>
<td>Mean (SD) 171 (9.9)</td>
</tr>
<tr>
<td></td>
<td>Range 140–189</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>Mean (SD) 24.6 (2.60)</td>
</tr>
<tr>
<td></td>
<td>Range 19.4–29.5</td>
</tr>
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</table>

*All patients were of non-Hispanic ethnicity.
not significant. Differences in mean C\text{max} values between women and men were small in both the fed (41.5 [36.6] vs 40.1 [28.7] ng/mL, respectively) and fasted (59.0 [37.3] vs 59.1 [33.4] ng/mL) conditions.

Amifampridine is metabolized by NAT to form a single major 3-N-acetyl metabolite, which is inactive.12 As with amifampridine PK under fed conditions, a decrease in systemic exposure (AUC, C\text{max}) to the 3-N-acetyl metabolite was observed, with a corresponding longer mean T\text{max} under fed conditions, indicating that the food effect decreased the rate of amifampridine absorption and consequently the rate of 3-N-acetyl metabolite formation (Table II and Figure). The 3-N-acetyl plasma amifampridine metabolite concentrations were greater than amifampridine concentrations in all subjects at all time points (Table II). The resulting plasma PK exposure parameter measurements were greater than amifampridine AUC\text{0–∞} and C\text{max} under fed (11.2- and 4.66-fold) and fasting (12.3- and 4.53-fold) conditions. The mean terminal t\text{½} of the 3-N-acetyl metabolite was also longer than the t\text{½} for amifampridine under fed (4.03 vs 2.28 hours) and fasted (4.10 vs 2.50 hours) conditions. The mean metabolite exposure indexes (based on ratios of metabolite to parent drug) were greater for the PK exposure parameters C\text{max} and AUC in both the fasted and fed conditions (AUC\text{0–t}: fed, 11.6; fasted, 12.3; AUC\text{0–1}: fed, 11.2; fasted, 12.3; and C\text{max}: fed, 4.66; fasted, 4.53).

PK exposure ranges of the 3-N-acetyl amifampridine metabolite across subjects varied widely and were less under fed conditions (AUC\text{0–t}: fed, 703–2364 ng · h/mL; fasted, 816–2534 ng · h/mL; AUC\text{0–1}: fed, 715–2408 ng · h/mL; fasted, 83–2595 ng · h/mL; and C\text{max}: fed, 90.1–406 ng/mL; fasted, 119–609 ng/mL).

Nearly all (>93%) of the administered dose of amifampridine phosphate salt was renally eliminated as either unchanged amifampridine or the 3-N-acetyl metabolite within 24 hours (Table III). The fraction of unchanged amifampridine eliminated in the 0 to 24 hour urine collections averaged 18.8% (fasted) to 19.2% (fed) of the administered dose, whereas the 3-N-acetyl metabolite represented 74.0% (fed) to 81.7% (fasted) of the administered dose.

**Tolerability**

The prevalences of AEs (ie, numbers of subjects with ≥1 AE) were similar between the fed (23/46 [50%]) and fasted (24/45 [53.3%]) conditions (Table III).
and fasted (24/45 [53%]) states, although the frequency of AEs (ie, total number of AEs) was less in the fed state (40 AEs) compared with that in the fasted state (61 AEs). The most frequent AEs (~50% of all AEs) were paresthesias (paresthesia and oral paresthesia), which are well-known adverse effects of amifampridine treatment. In both the fed and fasted states, the majority of AEs reported were mild and resolved without treatment. One severe AE of gastroenteritis, which was not considered by the investigator to be related to the study drug, was associated with subject withdrawal from the study. There were no deaths, serious AEs, or clinically meaningful findings from clinical laboratory evaluations, 12-lead ECGs, or physical examinations including vital sign assessments.

**DISCUSSION**

Overall in this study, there was a decrease in amifampridine exposure (44% in C<sub>max</sub> and ~20% in AUCs) after oral administration of amifampridine phosphate salt in the presence of food. Mean T<sub>max</sub> was longer in the fed state (1.31 hours) than in the fasted state (0.637 hour). The delay in T<sub>max</sub> may have been attributable to differences in gastric emptying time in the presence of food that delayed the introduction of drug to the duodenum for absorption. Together, these

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Figure. Effects of food intake on mean (SD) plasma concentrations of amifampridine (A), inset from 0–6 hours emphasizes the shift in time to maximal concentration and differences in the rate of absorption and 3-N-acetyl amifampridine metabolite (B) over time after the administration of amifampridine 20 mg.
findings suggest that food slows and somewhat decreases the absorption of amifampridine.

This was the first in-human study to identify and quantify the major metabolite 3-N-acetyl amifampridine. In comparison with the parent compound, metabolite levels were greater in all subjects and at all time points, whether fed or fasted. In both the fed and fasted states, C\text{max} and AUC values of the 3-N-acetyl amifampridine metabolite were ~4.5-fold and 12-fold greater than those of amifampridine. The mean plasma elimination t\text{1/2} of the 3-N-acetyl metabolite was longer than that of amifampridine, in both the fed (4.03 vs 2.28 hours) and fasted (4.10 vs 2.50 hours) states. Thus, the duration and extent of exposure to the inactive metabolite were greater than those of the parent compound.

The mean AUCs in the fed and fasted states were slightly greater in women than in men, with no difference in mean C\text{max} values. Due to the high level of variability observed in these PK values, it is not clear whether the sex differences are significant.

Orally administered amifampridine phosphate salt appears to undergo rapid renal elimination, with >93% excretion from the body within 24 hours as either the parent drug or the metabolite.

A high intersubject variability in amifampridine PK parameter values was observed in this study. The most probable explanation for the high variability is the metabolic disposition of amifampridine through a single pathway via NAT enzymes 1 and 2. NAT-1 and NAT-2 are known to be highly polymorphic in humans, with recognized slow, intermediate, and fast acetylator phenotypes. Slow and fast acetylator NAT phenotypes affect the overall PK and disposition of amifampridine, with slow acetylator phenotypes producing less 3-N-acetylamifampridine metabolite and being associated with >80% more AEs than fast acetylator phenotypes.12 In the present study involving a mixture of undifferentiated NAT phenotypes, the %CVs of C\text{max} and AUC were ~60% to 70%, and the PK parameter values in subjects varied over a range of >10-fold.

Single oral doses of amifampridine phosphate salt (20 mg API) were considered to be well tolerated when administered in healthy male and female subjects in both the fed and fasted conditions.

Amifampridine has a narrow therapeutic index in which a relatively small increase in exposure at the intended dose (20 mg) can increase the risk for seizure.2 The potentials for higher exposure and overdose are increased in subjects who display slow acetylator phenotypes,12 and/or who are taking amifampridine under fasting conditions. Thus, with subjects of unknown slow acetylator phenotype in a fasted state taking a compounded form of 3,4-DAP of unknown strength, a “perfect storm” could result, in which unintended maximal exposures are achieved. Adverse effects observed with amifampridine dosing appear to be related to a greater C\text{max} in those with slow acetylator phenotypes.12 Based on the findings presented herein, amifampridine exposures are moderated when amifampridine is taken with food and are maximized when amifampridine is taken in a fasted state. Therefore, in the absence of individual acetylator status information, amifampridine phosphate salt tablets should be taken with or without food in a consistent manner to avoid inadvertent variable exposure, and on the initiation of treatment, the dose should be increased gradually to determine a well-tolerated and effective level. If the desired effect is not observed, dosing in the fed or fasted state may be changed slowly to ensure well-tolerated dosing up to an effective level.

**CONCLUSIONS**

In this randomized, open-labeled, crossover trial in healthy male and female subjects, the ingestion of

<table>
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<tr>
<th>Parameter</th>
<th>Fed (n = 46)</th>
<th>Fasted (n = 45)</th>
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<tr>
<td>Amifampridine</td>
<td>Mean (SD) 19.2 (12.8)</td>
<td>18.8 (11.8)</td>
</tr>
<tr>
<td>Range 0.940–42.2</td>
<td>2.34–36.3</td>
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</tr>
<tr>
<td>3-N-acetyl amifampridine metabolite</td>
<td>Mean (SD) 74.0 (18.3)</td>
<td>81.7 (15.3)</td>
</tr>
<tr>
<td>Range 26.9–104</td>
<td>42.7–122</td>
<td></td>
</tr>
<tr>
<td>Total (amifampridine + 3-N-acetyl amifampridine metabolite)</td>
<td>Mean (SD) 93.2 (12.4)</td>
<td>100 (11.7)</td>
</tr>
<tr>
<td>Range 54.6–121</td>
<td>69.8–126</td>
<td></td>
</tr>
</tbody>
</table>

Table III. Effects of food intake on urinary excretion of amifampridine and 3-N-acetyl amifampridine metabolite with the administration of amifampridine 20 mg in healthy subjects. Data are given as % of total dose excreted in urine.
amifampridine phosphate salt with a high-fat meal was associated with a decrease in exposure, based on mean C_{max} (44%) and AUC (~20%) and an approximate 2-fold decrease in absorption rate based on T_{max}, compared with the fasting state. The major amifampridine metabolite, 3-N-acetyl-amifampridine, was identified and quantified in this study for the first time in humans. Plasma concentrations of the metabolite were observed to have been greater than those of amifampridine in all subjects at all time points in both the fed and fasted states. The results from this study and from a previous Phase I PK study suggest that amifampridine phosphate salt PK properties can be affected by both a subject’s acetylation status and whether the drug is administered under fed or fasted conditions.

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CONFLICTS OF INTEREST
P.E. Haroldsen, D.G. Musson, B. Hanson, A. Quartel, and C.A. O’Neill are employees of BioMarin Pharmaceutical Inc, and may own stock and/or stock options in BioMarin Pharmaceutical Inc.

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