Effect of Rifampin on the Disposition of Brivaracetam in Human Subjects: Further Insights into Brivaracetam Hydrolysis

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ABSTRACT

Brivaracetam (BRV) is a high-affinity synaptic vesicle protein 2A ligand developed for the treatment of uncontrolled partial-onset seizures. The present phase I, open-label, two-way crossover study was designed to assess the effect of rifampin on the pharmacokinetics of BRV and its hydroxy (BRV-OH), acid (BRV-AC), and hydroxy acid (BRV-OHAC) metabolites. Twenty-six healthy subjects received BRV (150-mg single oral dose) either alone or following 5 days of rifampin 600 mg/day. BRV and its metabolites were examined for their plasma profiles and urinary excretion. Pharmacokinetic modeling was developed to estimate the rate constants of the various metabolic routes. Parallel in vitro assays were conducted to characterize the hydrolysis of BRV to BRV-AC as well as to identify any potential effect of rifampin on the hydrolysis reaction. Rifampin did not significantly affect the maximum plasma concentration (Cmax) of BRV, but decreased its area under the curve (AUC) by 45%. In addition, rifampin significantly increased the AUC of BRV-OH (3.7-fold increase in the rate constant).

Introduction

Brivaracetam [(2S)-2-[(4R)-2-oxo-4-propylpyrrolidinyl] butanamide] (BRV) is a selective, high-affinity ligand for synaptic vesicle protein 2A (Gillard et al., 2011) that is effective in different animal models of epilepsy (Matagne et al., 2008). In patients with photosensitive epilepsy, BRV showed a dose-dependent effect in suppressing or attenuating the photoparoxysmal response (Kasteleijn-Nolst Trenite et al., 2007). Both phase II (French et al., 2010; Van Paeschen et al., 2013) and phase III (Biton et al., 2014; Ryvlin et al., 2014; Klein et al., 2015) placebo-controlled trials have provided evidence for the efficacy, safety, and tolerability of adjunctive BRV for adult patients with partial-onset seizures. BRV was recently approved as adjunctive therapy in the treatment of partial-onset (focal) seizures in patients 16 years of age and older with epilepsy.

BRV exhibits linear and dose-proportional pharmacokinetics with low interindividual variability (Sargentini-Maier et al., 2007; Rolan et al., 2008). Its distribution volume is close to total body water, and it binds weakly to plasma proteins (17.5%) (Sargentini-Maier et al., 2008). BRV is eliminated primarily by metabolism, with only 8.6% of the dose recovered as unchanged compound in excreta (Sargentini-Maier et al., 2008). The major metabolic pathway involves the hydrolysis of the acetylamide group, resulting in formation of an acid metabolite (BRV-AC; 34% of radiolabeled dose in urine) (Sargentini-Maier et al., 2008). Secondary pathways include CYP2C9-mediated hydroxylation of BRV to brivaracetam hydroxy metabolite (BRV-OH; 16% of dose in urine) (Sargentini-Maier et al., 2008; Stockis et al., 2014) and CYP2C9-mediated hydroxylation of BRV-AC to the hydroxy acid metabolite (BRV-OHAC; Fig. 1; 15% of dose in urine (Sargentini-Maier et al., 2008; Nicolas et al., 2012; Stockis et al., 2015). All three metabolites of BRV are pharmacologically inactive (UCB data on file).

BRV is likely to be coadministered with other antiepileptic drugs known to produce drug interactions through cytochrome P450 (P450) induction. The present clinical study was designed to evaluate the pharmacokinetics and metabolism of BRV when coadministered with rifampin, a potent pan-inducer of hepatic and intestinal P450 isoforms (CYP3A4, 2B6, 2C8, 2C9, and 2C19), with numerous clinically relevant interactions reported (Branch et al., 2000; Niemi et al., 2003; Nassr et al., 2009; Tapaninen et al., 2010). The present clinical interaction study also aimed to determine whether rifampin might affect the major metabolic pathway of BRV, namely, its hydrolysis into BRV-AC. Such an interaction could not be a priori ruled out, as rifampin had been reported to be a substrate (Nakajima et al., 2011), inducer (Sarma et al., 1986), or inhibitor (Hernandez et al., 1997) of some hydrolytic enzymes. Further, the enzyme responsible for BRV hydrolysis was unknown.

The present study investigated the effect of repeated administration of rifampin on the pharmacokinetics of BRV and its three circulating metabolites after a 150-mg single oral dose in healthy subjects.

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ABBREVIATIONS: AUC, area under the curve; AUCinf, area under the plasma concentration versus time curve extrapolated to infinity; AUCt, area under the plasma concentration versus time curve from 0 to the last measurable concentration; BRV, brivaracetam; BRV-AC, brivaracetam acid metabolite; BRV-OH, brivaracetam hydroxy metabolite; BRV-OHAC, brivaracetam hydroxy acid metabolite; CI, confidence interval; CL/F, apparent plasma clearance; FAAH, fatty acid amide hydrolase; LSM, least-squares mean; P450, cytochrome P450; ucb 30542, 2-(4-methyl-2-oxopyrrolidin-1-yl)butanamide.
Pharmacokinetic modeling was developed to estimate the rate constants of the various metabolic routes. Parallel in vitro assays were conducted to characterize the hydrolysis of BRV to BRV-AC and to confirm the assumptions used in the pharmacokinetic modeling.

Materials and Methods

Study Design and Participants. This was a single-center, open-label, randomized, two-sequence, two-period, two-treatment crossover study. The study enrolled 26 male adults aged 18–55 years, with a body mass index of 19–28 kg/m², who were in good health as determined by medical history, general examination, clinical laboratory tests, vital signs, and ECG. Participants with hepatic, renal, or gastrointestinal dysfunction, or any other significant medical condition were excluded from the study. Other exclusion criteria were history or presence of drug addiction; excessive use of alcohol (>28 units/week) or caffeine (>5 cups/day); grapefruit consumption within 2 weeks before study start; smoking; use of any prescription or over-the-counter medication within 2 weeks, or hepatic enzyme-inducing drugs within 2 months prior to start of treatment (apart from occasional acetaminophen ≥2 g/day, and ≥10 g in 2 weeks); and blood donation or participation in another clinical study during the 2 months prior to start of treatment.

Participants received the following treatments: A, a single dose of 150 mg of BRV (three 50-mg tablets; UCB Pharma SA, Brussels, Belgium); and B, 600 mg of rifampin every 24 hours (Erenefa 600; Fatao Arzneimittel GmbH, Schifflweiler, Germany), administered from day 1 to day 8, with a single dose of BRV (150 mg) given on day 5 of rifampin dosing. Rifampin was administered daily at 7 a.m., and BRV was administered at 8 a.m. A washout period of 4 weeks separated the two treatment arms. Participants were to remain in an upright position for ≥1 hour after BRV intake, and not eat for 4 hours after administration. All doses were taken after a fasting period of ≥10 hours. For treatment A, mandatory confinement at the study center was from the evening before the first administration until the morning of day 1, and from the evening of day 4 until the morning of day 8. During the confinement period, medication was given under supervision. Participants were instructed to take the remaining tablets at home. During the nonconfinement period, the investigator’s staff made phone calls to every participant at the appropriate times. The dates and times of intake were recorded in personal diaries and in the case record forms. Treatment-emergent adverse events, vital signs (predose, 1, 4, and 72 hours after BRV intake), physical examinations, ECGs, laboratory tests (hematology, chemistry, urinalysis, serology; at enrollment and discharge), drug screen, and alcohol breath test (at every admission in the unit) were assessed throughout the study.

Participants abstained from alcohol, grapefruit juice, and caffeine-containing preparations for at least 48 hours before any study drug administration, until the last pharmacokinetic plasma sampling of each treatment period; excessive food consumption and exercise were avoided.
(BRV alone) treatments and corresponding 90% confidence intervals (CIs) were estimated from this model and back-transformed to derive estimates of the geometric LSM ratios (test/reference) and the 90% CIs for these ratios. Lack of pharmacokinetic interaction of rifampin with BRV and its metabolites would be concluded if the 90% CIs for the geometric LSM ratios (test/reference) were fully contained within the 0.80–1.25 interval for \( C_{max} \), \( AUC_t \), and \( AUC_{inf} \). All statistical analyses were performed using SAS (version 9.1; SAS Institute, Cary, NC) or StaXAct (version 7.0; Cytel Software, Cambridge, MA).

**BRV In Vitro Hydrolysis by Human Recombinant Hydrolytic Enzymes.** The human recombinant hydrolytic enzymes tested for BRV hydrolysis included acetylcholinesterase (EC 3.1.1.7; Sigma-Aldrich, St. Louis, MO), amidase (EC 3.5.1.4; Sigma-Aldrich), butyrylcholinesterase (EC 3.1.1.8; MyBioSource, San Diego, CA), and carboxylesterase 1 and 2 (EC 3.1.1.1; Cypex, Dundee, UK).

Incubations were performed according to the manufacturer’s instructions. In brief, the hydrolysis enzymes (1, 10, and 100 \( \mu \)g/mL) were incubated at 37°C (butyrylcholinesterase and acetylcholinesterase) or 30°C (amidase and carboxylesterase) with BRV (10, 100, and 1000 \( \mu \)M). Phosphate buffer (50 mM) was used with some modifications, i.e., pH 7.4 for amidase and carboxylesterase incubations, pH 8.0 for acetylcholinesterase, and pH 8.0 plus 0.15 M NaCl for butyrylcholinesterase. The reaction was stopped after 60-minute incubation by adding an equivalent of acetonitrile. Incubates were centrifuged at 10,000 g for 10 minutes at 4°C, and the supernatants were collected for analysis. Control incubates were performed without adding substrate or protein. The amount of BRV–AC formed (pmol/min/mg protein) was determined by liquid chromatography/tandem mass spectrometry. The high-performance liquid chromatography system consisted of a UPLC Acquity instrument coupled with a Quattro Premier Mass spectrometer (Waters). The analytical column was an Acuity UPLC HSS T3 (50 × 2.1 mm, 1.8 \( \mu \)m; Waters) operated at 30°C.

Analyses were performed with a gradient method. Eluant A was water containing 0.1% formic acid, and eluant B was acetonitrile containing 0.1% formic acid. Electrospray ionization in positive ion mode was used, with multiple reaction monitoring. Data acquisition and determination of the analytical parameters, including the selection of ions for each compound, were performed with the application software, MassLynx 4.1 (Waters). The m/z values of precursor and product ions were 214.10–168.00 for BRV–AC and 220.10–174.10 for d6-BRV–AC (internal standard).

**In Vitro Induction and Inhibitory Effects of Rifampin on Amidase.** Of interest, amidase (EC 3.5.1.4) can be regarded as the same enzyme as fatty acid amide hydrolase (FAAH; EC 3.5.1.99) since both enzymes are encoded by the same gene (NG_012195.1 Refseq gene) and have the same protein sequence (UniProtKB - O00519). The potential of rifampin to induce P450/amidase was shown if the 90% CIs for the geometric LSM ratios (test/reference) were fully contained within the 0.80–1.25 interval for \( C_{max} \), \( AUC_t \), and \( AUC_{inf} \). Rifampin inhibition parameters for an oral dose of 600 mg (\( F_e = 1, I_{max} = 20 \mu \text{g/mL}, f_{ext} = 0.15, K_e = 0.54 \text{ hour}^{-1} \) were obtained from literature data (Baneix et al., 2014; Einolf et al., 2014). Hepatic blood flow (\( Q_R \)) was 97 l/h; \( f_{ext} \), the fraction of BRV metabolized by hydrolysis, was set at 0.56 (Table 3); the inhibition constant (\( K_i \)) was set at 23.5 \( \mu \)M (i.e., \( I_{50/2} \)), representing a worst-case scenario.

**Pharmacokinetic Modeling.** Based on the known pharmacokinetic profile of BRV, a one-compartment model was developed with apparent first-order absorption and elimination processes. The rate constants for each pathway are shown in Fig. 1: \( k_{10} \) through \( k_{60} \) for urinary excretion of (1) BRV, (2) BRV–AC, (3) BRV–OH, and (4) BRV–OHAC, respectively; \( k_{12} \) and \( k_{13} \) for the hydrolysis and hydroxylation of BRV, respectively; and \( k_{32} \) and \( k_{34} \) for the formation of BRV–OHAC by hydrolysis of BRV–AC or by hydrolysis of BRV–OH, respectively. Plasma and urinary pharmacokinetic parameters of BRV and its metabolites with and without rifampin were used to calculate the relative contributions of the hydrolysis and hydroxylation biotransformation pathways. The following assumptions were made: 1) the fraction of the dose remaining to be excreted in the urine as unchanged BRV and metabolites at the end of the 72-hour collection interval was evenly distributed along the four elimination pathways; 2) the volume of distribution (\( V_d \)) of each metabolite was identical to that of BRV (i.e., total body water); and 3) the hydrolysis rate constants \( k_{12} \) and \( k_{34} \) were not affected by rifampin, as demonstrated in vitro. To determine the four elimination rate constants (\( k_{10}, k_{20}, k_{30}, \) and \( k_{60} \)) (Fig. 1), the following calculations were made.

The amount of compound eliminated by each pathway (\( A_{ei} \)) was calculated based on the first assumption:

\[
A_{ei} = A_n + \frac{D - A_{i1} - A_{i2} - A_{i3} - A_{i4}}{4}
\]

where \( A_n \) is the amount of compound i excreted in urine, and \( D \) is the dose of BRV (150 mg). Oral bioavailability of BRV is considered 100% (Sargentini-Maier et al., 2008). The four elimination rate constants could then be calculated using the known \( V_d \) of 0.6 l/kg or 42 l (Sargentini-Maier et al., 2007), based on the second assumption:

\[
V_d \cdot k_0 = \frac{A_n}{AUC_t}
\]

In a single-compartment pharmacokinetic model, the exposure to a metabolite is:

\[
AUC_t = F_{met}t \cdot \frac{D}{k_0 \cdot V_d}
\]

where \( F_{met} \) and \( k_0 \) are the fraction of dose transformed in the metabolite and the metabolite elimination rate constant, respectively. Under the second assumption, the AUCs of BRV and the three metabolites can be expressed as a function of the respective rate constants:

\[
AUC_1 = \frac{D}{(k_{10} + k_{12} + k_{13}) \cdot V_d}
\]

\[
AUC_2 = \frac{k_{12}}{k_{10} + k_{12} + k_{13}} \cdot \frac{D}{(k_{30} + k_{32} + k_{34}) \cdot V_d}
\]

\[
AUC_3 = \frac{k_{13}}{k_{10} + k_{12} + k_{13}} \cdot \frac{D}{k_{24} \cdot V_d}
\]

\[
AUC_4 = \frac{k_{32}}{k_{30} + k_{32} + k_{34}} \cdot \frac{D}{k_{20} + k_{24} + k_{30} \cdot V_d}
\]

Assuming there is no other significant excretion product [\( A_{i1} + A_{i2} + A_{i3} + A_{i4} = D \) (150 mg)], the system can be rewritten for each rate constant:
Using the third assumption, the following equation is obtained:
\[ k_{34}^* = k_{34}^* = \frac{(A_{20} + A_{40}) \cdot AUC_2 - AUC_1 \cdot (A_{20} + A_{40})}{(AUC_1 \cdot AUC_3 - AUC_1 \cdot AUC_3)} \cdot \frac{1}{V_d} \]
(17)

where the superscript * indicates the induced state (BRV plus rifampin). Based on the respective plasma AUCs and cumulative urinary excretions, the eight rate constants were determined, with and without rifampin.

Finally, the proportions of BRV-OHAC (4) generated by hydroxylation of BRV-AC (2) and by hydrolysis of BRV-OH (3) were calculated as:
\[ F_{2 \rightarrow 4} = \frac{k_{24} \cdot AUC_2}{k_{24} \cdot AUC_2 + k_{34} \cdot AUC_3} \]
(18)

and
\[ F_{3 \rightarrow 4} = \frac{k_{34} \cdot AUC_3}{k_{24} \cdot AUC_2 + k_{34} \cdot AUC_3} \]
(19)

Participants

The 26 participants (25 Caucasian, one black) had a mean (range) age of 30 (20–56) years, body weight of 76 (55–90) kg, height of 177 (167–189) cm, and body mass index of 24.3 (19–28) kg/m². All 26 participants completed the study. Adverse events (mild or moderate dizziness and fatigue) were of short duration and resolved by the end of the study. No serious adverse events or deaths were reported, and there were no discontinuations. There were no clinically relevant changes in vital signs, physical examinations, ECGs, or laboratory tests.

Pharmacokinetics of BRV and Its Metabolites

The geometric mean plasma concentration versus time profiles for BRV and its three metabolites, with and without rifampin, are presented in Fig. 2. Noncompartmental pharmacokinetic parameters are listed in Table 1, together with the point estimates of LSM ratios (BRV plus rifampin versus BRV alone) and their 90% CIs. Urinary excretions of BRV and its metabolites appeared to be complete 48 hours after the intake of BRV in both treatment groups (Fig. 3). The effect of the interaction with rifampin was predominantly seen for BRV, BRV-OH, and BRV-AC; the effect was less pronounced for BRV-OHAC (Table 1).

**BRV.** Mean BRV plasma concentration declined more rapidly after C\(_{\text{max}}\) when BRV was administered at rifampin steady state, compared with BRV alone (Fig. 2). BRV CL/F was increased by 80% for BRV plus rifampin compared with BRV alone (geometric mean CL/F: 1.45 vs. 0.80 ml/min/kg, respectively); AUC_t, AUC_inf, and t\(_{1/2}\) were decreased by approximately half, and the cumulative urinary excretion of BRV was decreased by 36% (Fig. 3; Table 1).

**BRV-OH.** Mean BRV-OH plasma concentration up to 36 hours, in particular around t\(_{\text{max}}\), was significantly higher with BRV plus rifampin...
Effect of Rifampin on the Metabolism of BRV

Values of plasma AUC and cumulative urinary excretion determined for each participant with and without coadministration of rifampin were used in the pharmacokinetic model to compute rate constants for each of the key metabolic steps. The variability of the calculated rate constants was low to moderate (−10–50%), apart from the hydrolysis of BRV-AC (K_{\text{AC}}^\text{H} \sim 120\%)

TABLE 1
Pharmacokinetic parameters of BRV, BRV-OH, BRV-AC, and BRV-OHAC after a single dose of brivaracetam (150 mg) with and without rifampin administration

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Brivaracetam (Reference) N = 26</th>
<th>Brivaracetam Plus Rifampin (Test) N = 26</th>
<th>Test/Reference (90% CI)</th>
<th>Residual CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_{\text{max}}, \mu g/ml</td>
<td>4.22 (21.2)</td>
<td>3.74 (27.1)</td>
<td>0.89 (0.83–0.95)</td>
<td>14.1</td>
</tr>
<tr>
<td>t_{\text{max}}, h</td>
<td>0.75 (0.25–3)</td>
<td>0.63 (0.25–2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-\infty}, \mu g h/ml</td>
<td>41.1 (14.6)</td>
<td>22.8 (23.4)</td>
<td>0.56 (0.53–0.58)</td>
<td>8.31</td>
</tr>
<tr>
<td>CL/F, ml/min/kg</td>
<td>13.6 (67.3)</td>
<td>29.5 (49.7)</td>
<td>2.16 (1.96–2.38)</td>
<td>20.8</td>
</tr>
<tr>
<td><strong>BRV-OH</strong></td>
<td></td>
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</tr>
<tr>
<td>C_{\text{max}}, \mu g eq/ml</td>
<td>0.14 (67.3)</td>
<td>0.42 (34.3)</td>
<td>3.06 (2.68–3.51)</td>
<td>29.2</td>
</tr>
<tr>
<td>t_{\text{max}}, h</td>
<td>12 (5.5–12)</td>
<td>6 (6–9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-\infty}, \mu g eq h/ml</td>
<td>3.7 (63.5)</td>
<td>7.7 (41.5)</td>
<td>2.09 (1.94–2.25)</td>
<td>16.1</td>
</tr>
<tr>
<td><strong>BRV-AC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_{\text{max}}, \mu g eq/ml</td>
<td>0.24 (21.2)</td>
<td>0.18 (28.8)</td>
<td>0.76 (0.71–0.82)</td>
<td>15.3</td>
</tr>
<tr>
<td>t_{\text{max}}, h</td>
<td>3 (2–6)</td>
<td>2 (1.5–3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-\infty}, \mu g eq h/ml</td>
<td>3.2 (22.7)</td>
<td>1.5 (29.9)</td>
<td>0.47 (0.45–0.49)</td>
<td>10.6</td>
</tr>
<tr>
<td><strong>BRV-OHAC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_{\text{max}}, \mu g eq/ml</td>
<td>0.053 (21.5)</td>
<td>0.052 (24.5)</td>
<td>0.97 (0.92–1.03)</td>
<td>12.7</td>
</tr>
<tr>
<td>t_{\text{max}}, h</td>
<td>6 (6–12)</td>
<td>6 (3–6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-\infty}, \mu g eq h/ml</td>
<td>0.98 (17.5)</td>
<td>0.85 (22.9)</td>
<td>0.87 (0.84–0.92)</td>
<td>9.56</td>
</tr>
<tr>
<td><strong>Rate-Constant Calculations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k_{\text{OH}}</td>
<td>3.51 (10.7)</td>
<td>0.49 (10.7)</td>
<td>0.49 (10.6)</td>
<td></td>
</tr>
<tr>
<td>k_{\text{AC}}</td>
<td>2.25 (16.1)</td>
<td>0.58 (10.4)</td>
<td>0.58 (10.3)</td>
<td></td>
</tr>
<tr>
<td>k_{\text{AC}}^\text{H}</td>
<td>3.8 (62.1)</td>
<td>7.8 (40.7)</td>
<td>2.07 (1.92–2.23)</td>
<td>15.7</td>
</tr>
<tr>
<td>k_{\text{AC}}^\text{H}^\text{H}</td>
<td>5.1 (49.7)</td>
<td>14.0 (34.7)</td>
<td>0.43 (0.40–0.47)</td>
<td>17.3</td>
</tr>
</tbody>
</table>

CV%, coefficient of variation (%).

*aValues are given as geometric mean (CV%); for t_{\text{max}}, median (min–max).

*bPoint estimate and 90\% CI for the test/reference geometric LSM ratio derived from analysis of variance.

*cResidual coefficient of variation representing within-subject variability, derived from analysis of variance.

*dN = 23 (λc could not be accurately estimated in three subjects).

Comparison of BRV and BRV-OH: The pharmacokinetic parameters were significantly higher for BRV plus rifampin compared with BRV alone: C_{\text{max}} (3-fold), AUC_{0-\infty} (2-fold), and AUC_{\text{inf}} (2-fold) (Table 1). The peak time of BRV-OH was achieved earlier, and the half-life was shorter when BRV was administered with rifampin (by 50 and 30\%, respectively) because of the faster elimination of parent drug. The cumulative urinary excretion of BRV-OH was more than doubled when BRV was coadministered with rifampin (Fig. 3; Table 1).

**BRV-AC** Changes in mean plasma concentration versus time profiles of BRV-AC were similar to those observed for BRV. The slope of the elimination phase of BRV-AC was similar to that of BRV when BRV was administered with or without rifampin (Fig. 2). Coadministration of rifampin significantly decreased AUC_{0-\infty}, AUC_{\text{inf}}, and t_{\frac{1}{2}} by approximately 50\% compared with BRV alone (Table 1). The cumulative urinary excretion of BRV-AC was lower for BRV plus rifampin compared with BRV alone (Fig. 3; Table 1).

**BRV-OHAC** Mean plasma concentration versus time profiles of BRV-OHAC were similar when BRV was administered with and without rifampin (Fig. 2). Geometric LSM ratios for C_{\text{max}}, AUC_{0-\infty}, and AUC_{\text{inf}} were within the 80–125\% range, whereas t_{\frac{1}{2}} was marginally increased (+21\%), and fe was marginally decreased (−17\%) for BRV plus rifampin compared with BRV alone (Fig. 2; Table 1).

In Vitro Investigations

Among the recombinant hydrolytic enzymes tested, only amidase EC 3.5.1.4 was found to hydrolyze BRV, not acetylcholinesterase, butyrylcholinesterase, or carboxylesterase 1 and 2. The reaction rate did not saturate in the tested substrate concentration range, indicating a K_{m} > 1 mM. Subsequent in vitro assays showed that rifampin (10 \mu M) does not induce amidase at the gene expression level (less than 1.2-fold mRNA increase over control in three different donors), whereas CYP3A4 mRNA levels were increased 19- to 55-fold among the three donors (Fig. 4A). However, rifampin dose-dependently inhibited the
amidase enzyme, with an IC₅₀ of 47 μM (Fig. 4B). Such an in vitro inhibitory concentration largely exceeds the unbound Cₘₐₓ of rifampin following 600-mg/day dosing, i.e., 3 μM (Woo et al., 1996; Baneyx et al., 2014). Similarly, the mechanistic static model predicted that 600 mg/day rifampin should translate into an unbound portal vein concentration of 3.6 μM, which was predicted to have no effect on BRV disposition (AUC ratio = 1.08).

**Discussion**

The most prominent metabolic routes of BRV involve the hydrolysis to BRV-AC and, to a lesser extent, the CYP2C19-mediated hydroxylation to BRV-OH and the CYP2C9-mediated hydroxylation of BRV-AC to the hydroxy acid derivative BRV-OHAC. The present study was aimed at investigating whether coadministration with the potent P450 inducer rifampin could affect BRV pharmacokinetics and metabolism. For this purpose, 26 healthy subjects received BRV (150 mg single oral dose), either alone or following 5 days of rifampin (600 mg/day). BRV and its metabolites were then measured in plasma and urine samples. This clinical trial was complemented by in vitro assays to further characterize BRV hydrolytic reaction. Of note, the ability of rifampin to induce BRV metabolic pathways could not be examined in vitro using hepatocytes; indeed, only BRV-OH was formed, whereas neither BRV-AC nor BRV-OHAC was detected.

At the time of the clinical study, little was known about the enzyme(s) involved in BRV hydrolysis. Although they play an important role in drug disposition, hydrolysis reactions are not as well characterized as the oxidative P450-mediated reactions. Various enzymes have been identified for their role in drug hydrolysis, including carboxylesterase, arylacetamide deacetylase, acetylcholinesterase, butyrylcholinesterase (Fukami and Yokoi, 2012), and, more recently, aldehyde oxidase (Sodhi et al., 2015). The methods for phenotyping hydrolytic reactions are

**TABLE 2**

Calculated rate constants for the disposition of brivaracetam and its metabolites, with and without rifampin

<table>
<thead>
<tr>
<th>Rate Constant (1/h)</th>
<th>Brivaracetam (Reference)</th>
<th>Brivaracetam Plus Rifampin (Test)</th>
<th>Ratio (Test/Reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{10} ) (BRV urinary excretion)</td>
<td>0.013 (25.1)</td>
<td>0.022 (28.6)</td>
<td>1.82</td>
</tr>
<tr>
<td>( k_{20} ) (BRV-OHAC urinary excretion)</td>
<td>0.433 (19.1)</td>
<td>0.532 (23.3)</td>
<td>1.23</td>
</tr>
<tr>
<td>( k_{30} ) (BRV-OH urinary excretion)</td>
<td>0.204 (57.3)</td>
<td>0.177 (19.3)</td>
<td>0.95</td>
</tr>
<tr>
<td>( k_{40} ) (BRV-AC urinary excretion)</td>
<td>0.851 (12.6)</td>
<td>0.919 (19.4)</td>
<td>1.08</td>
</tr>
<tr>
<td>( k_{12} ) (BRV → BRV-AC)</td>
<td>0.049 (13.6)</td>
<td>0.049 (13.6)</td>
<td>1.00^b</td>
</tr>
<tr>
<td>( k_{13} ) (BRV → BRV-OH)</td>
<td>0.025 (44.5)</td>
<td>0.089 (34.0)</td>
<td>3.67</td>
</tr>
<tr>
<td>( k_{32} ) (BRV-AC → BRV-OHAC)</td>
<td>0.192 (26.6)</td>
<td>0.210 (51.1)</td>
<td>1.12</td>
</tr>
<tr>
<td>( k_{34} ) (BRV-OH → BRV-OHAC)</td>
<td>0.081 (119.7)</td>
<td>0.081 (119.7)</td>
<td>1.00^b</td>
</tr>
</tbody>
</table>

^aRate constants are given as mean (coefficient of variation percentage).
^bFixed.

**Fig. 3.** Geometric mean (S.D.) cumulative urinary excretion of BRV (A), BRV-OH (B), BRV-AC (C), and BRV-OHAC (D) after a single dose of brivaracetam (150 mg) alone (solid circles) and with rifampin coadministration (open circles).
usually limited to the use of chemical inhibitors. A comprehensive list of the various hydrolases and their corresponding inhibitors is still needed. Also, most of the existing hydrolase inhibitors exhibit poor selectivity. Preliminary assays using chemical inhibitors failed to identify the enzyme responsible for BRV-AC formation (UCB data on file). However, data pointed to a B-esterase, typically inhibited by the organophosphate compound, paraoxon. Involvement of aldehyde oxidase was ruled out, considering that BRV-AC and BRV-OHAC were also formed in dog, a species deficient in aldehyde oxidase activity (Sanoh et al., 2015). In the present work, incubations with recombinant enzymes did allow the identification of amidase EC 3.5.1.4 as the enzyme possibly involved in BRV hydrolysis. To the best of our knowledge, this is one of the first reports of hydrolytic reaction phenotyping using a panel of recombinant enzymes. So far, the use of recombinant hydrolytic enzymes in reaction phenotyping has been mostly restricted to carboxylesterase isoform discrimination (e.g., Hatfield et al., 2010; Wang et al., 2011; Chanteux et al., 2014; Thomsen et al., 2014).

Amidase EC 3.5.1.4 is a ubiquitous enzyme in the living world, but its role in drug biotransformation is not yet fully elucidated (Fournand and Arnaud, 2001). There are no published case studies in which that particular enzyme was formally designated as responsible for the hydrolysis of a given drug. Similarly, there are no reports of its potential involvement in drug-drug interactions. In the absence of literature precedent, a potential effect of rifampin on amidase activity and, thus, on BRV hydrolysis could not be ruled out. The present in vitro data showed that rifampin does not significantly induce or inhibit the hydrolytic enzyme. This contrasts with the ability of rifampin to induce the enzyme involved in isoniazid hydrolysis (Sarma et al., 1986) or to inhibit those enzymes involved in the hydrolysis of phenylacetate and paraoxon (Hernandez et al., 1997).

Additional work is required to better understand the distribution of BRV hydrolysis and its sensitivity to inducers and inhibitors. However, the clinical data accumulated so far suggest that the reaction is insensitive to pharmacokinetic interaction (Chanteux et al., 2015).

In the present study, rifampin was found to increase the fraction of the dose recovered as hydroxylated metabolite BRV-OH (2.16-fold or +116%) while decreasing the hydrolysis by-product BRV-AC (−57%) and having a limited effect on the hydroxy acid BRV-OHAC (−17%). The interplay between the various metabolic routes complicated the data interpretation. Thus, a modeling approach was developed to compute the rate constants and better characterize rifampin effects on each individual reaction. The model was built on the assumption that rifampin should not affect the BRV hydrolysis pathway, which was confirmed in parallel in vitro assays. Modeling showed that rifampin significantly induced the CYP2C19-mediated formation rate of BRV-OH (3.7-fold), whereas the CYP2C9-mediated hydroxylation rate of BRV-AC to BRV-OHAC was barely affected (12% increase). According to the model, the induction of the hydroxylation of BRV to BRV-OH accounts for the observed decrease in the acid BRV-AC formation. The higher induction effect of rifampin on the CYP2C19-mediated reaction over CYP2C9 is consistent with previous in vitro hepatocyte data (Raucy et al., 2002; Madan et al., 2003; Paris et al., 2009), as well as reported clinical interaction studies (Inui et al., 2013). Of interest, when tested in vitro, rifampin induces both CYP2C9 and CYP2C19 mRNA, with a somewhat larger effect on the former (Yajima et al., 2014).

The induction of the CYP2C19-mediated reaction by the potent P450 inducer rifampin translated into a 45% decrease in BRV plasma exposure. A previous clinical interaction study showed that carbamazepine, another pan-inducer of P450s, increased the oxidative metabolism of BRV, with a modest 29% decrease in the parent drug exposure (Stockis et al., 2015).

Finally, rifampin was reported to interact with organic anion–transporting polypeptide transporters (OATP) as a substrate, inhibitor, and inducer (Kalliokoski and Niemi, 2009). Rifampin is also known for inducing P-glycoprotein efflux transporter (Kim et al., 2008). The effects mediated by rifampin in the present clinical trial are unlikely to involve transporters. Indeed, BRV is not a substrate of drug transporters (Chanteux et al., 2015), and rifampin did not affect BRV metabolite

\[
\begin{align*}
\text{CV\%} & \quad \text{coefficient of variation (\%)}.
\end{align*}
\]
elimination constants $k_{20}$, $k_{30}$, and $k_{40}$ (which might account for active transport of metabolite).

In conclusion, coadministration with the strong enzyme inducer rifampin (600 mg once daily during 5 days) decreased BRV exposure by 45%, mostly through induction of the CYP2C19 pathway. Prescribers should consider increasing the BRV dose while patients are on treatment with rifampin. This work has helped elucidate the relative contributions of the various metabolic pathways in the disposition of BRV. Finally, hydrolysis to BRV-AC was confirmed to be the major metabolic pathway, possibly involving EC 3.5.1.4.

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Authorship Contributions

Participated in research design: Stockis, Nicolas, Chanteux.
Conducted experiments: Scheen, Rosa, Gerin.
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Wrote or contributed to the writing of the manuscript: Stockis, Nicolas, Chanteux.

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