

Effect of Gemfibrozil on the Metabolism of Brivaracetam In Vitro and in Human Subjects

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ABSTRACT:

Brivaracetam (BRV) is a new high-affinity synaptic vesicle protein 2A ligand in phase III for epilepsy. Initial studies suggested that the hydroxylation of BRV into BRV-OH is supported by CYP2C8. Other metabolic routes include hydrolysis into a carboxylic acid derivative (BRV-AC), which could be further oxidized into a hydroxy acid derivative (BRV-OHAC). The aim of the present study was to investigate the effect of gemfibrozil (CYP2C9 inhibitor) and its 1-O- β -glucuronide (CYP2C8 inhibitor) on BRV disposition both in vivo (healthy participants) and in vitro (human liver microsomes and hepatocytes). In a two-period randomized crossover study, 26 healthy male participants received a single oral dose of 150 mg of BRV alone or at steady state of gemfibrozil (600 mg b.i.d). Gemfi-

brozil did not modify plasma and urinary excreted BRV, BRV-OH, or BRV-AC. The only observed change was a modest decrease (approximately -40%) in plasma and urinary BRV-OHAC. In human hepatocytes and/or liver microsomes, gemfibrozil potently inhibited the hydroxylation of BRV-AC into BRV-OHAC (K_i 12 μ M) while having a marginal effect on BRV-OH formation ($K_i \geq 153$ μ M). Gemfibrozil-1-O- β -glucuronide had no relevant effect on either reaction ($K_i > 200$ μ M). In conclusion, gemfibrozil did not influence the pharmacokinetics of BRV and its hydroxylation into BRV-OH. Overall, in vitro and in vivo data suggest that CYP2C8 and CYP2C9 are not involved in BRV hydroxylation, whereas hydroxylation of BRV-AC to BRV-OHAC is likely to be mediated by CYP2C9.

Introduction

Brivaracetam [(2S)-2-[(4R)-2-oxo-4-propylpyrrolidinyl] butanamide] (BRV) is an investigational compound that displays high and selective affinity for SV2A, a protein involved in synaptic vesicle exocytosis and neurotransmitter release (Gillard et al., 2011). In addition, BRV has inhibitory effects on voltage-dependent sodium currents (Zona et al., 2010). In adequate well controlled trials completed to date, adjunctive BRV demonstrated efficacy and good tolerability in adults with focal epilepsy (van Paesschen et al., 2007; French et al., 2010; Werhahn et al., 2010).

BRV exhibits a linear and predictable pharmacokinetic profile with rapid and complete absorption after oral administration, low plasma protein binding, and a 7- to 8-h elimination half-life (Sargentini-Maier et al., 2007, 2008; Rolan et al., 2008). BRV is mainly cleared by metabolism. The major pathway involves hydrolysis of the acetamide side chain, giving rise to a carboxylic acid derivative BRV-AC. The latter reaction has wide tissue distribution and involves amidase rather than cytochrome P450 (P450). In addition, both BRV and BRV-AC can be hydroxylated to BRV-OH and BRV-OHAC, respectively. Up to 97% of an oral dose is eliminated in the urine, with 9, 16, 34, and 15% recovered as BRV, BRV-OH, BRV-AC, and BRV-OHAC, respectively (for complete structure determinations, see Sargentini-Maier et al., 2008).

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Initial in vitro phenotyping assays suggested that BRV hydroxylation into BRV-OH was primarily supported by CYP2C8 with some involvement of other isoforms (Whomsley et al., 2007). CYP2C8 accounts for 6% of total P450 (Rostami-Hodjegan and Tucker, 2007) and is involved in the metabolism of many endogenous substances (e.g., arachidonic acid and retinoid acid) and clinically important drugs. Its activity has wide interindividual variability probably resulting from genetic polymorphism and drug-mediated induction or inhibition (Lai et al., 2009). Altered pharmacokinetics of the CYP2C8 substrate cerivastatin was associated with potential fatal rhabdomyolysis, which led to the withdrawal of the drug from the market in 2001 (Farmer, 2001). This finding and other severe drug interactions focused attention on CYP2C8, which is now listed in the updated version of the U.S. Food and Drug Administration guidance document discussing drug interaction studies (Huang et al., 2008).

Gemfibrozil is a lipid-lowering agent found to be a potent in vitro inhibitor of CYP2C9 ($K_i = 5.8$ μ M) with a much weaker effect on the other isoforms (K_i values of 24, 69, 82, and >300 μ M for CYP2C19, CYP2C8, CYP1A2, and CYP3A4, respectively) (Wen et al., 2001; Hinton et al., 2008). Contrasting with the in vitro findings, gemfibrozil-mediated pharmacokinetic interactions in vivo are mostly reported with CYP2C8 substrate drugs as exemplified by cerivastatin (5.6-fold increase in parent drug AUC) (Backman et al., 2002), montelukast (4.5-fold) (Karonen et al., 2010), atorvastatin (1.4-fold) (Whitfield et al., 2011), pioglitazone (3.4-fold) (Deng et al., 2005), loperamide (2.9-fold) (Niemi et al., 2006), rosiglitazone (2.3-fold) (Niemi et al., 2003), and repaglinide (7.0-fold) (Tornio et al., 2008). In large part,

ABBREVIATIONS: BRV, brivaracetam; BRV-AC, carboxylic acid derivative of BRV; P450, cytochrome P450; BRV-OH, hydroxylated metabolite of BRV; BRV-OHAC, hydroxy acid metabolite of BRV; LC-MS/MS, liquid chromatography-tandem mass spectrometry; AUC, area under the plasma curve; CI, confidence interval; HLM, human liver microsomes; IS, internal standard.

these interactions observed *in vivo* are not caused by gemfibrozil itself but by its 1-*O*- β -glucuronide metabolite, which is a specific CYP2C8 mechanism-based inhibitor with a K_i of 20 to 52 μM and a k_{inact} of 0.21 min^{-1} (Ogilvie et al., 2006; Jenkins et al., 2011). The glucuronide provides plasma peak concentrations close to those of the parent drug (Tornio et al., 2008), shows lower plasma protein binding (88.5 and 99.4%, respectively) (Shitara et al., 2004), and is reported to accumulate in the liver (Sabordo et al., 1999). Considering all these properties, the average unbound hepatic concentrations for unchanged gemfibrozil and for its glucuronide after a standard 600-mg gemfibrozil dose were estimated to be 3 and 89 μM , respectively (Hinton et al., 2008). These figures together with the *in vitro* inhibitory constants reported above predict massive inhibition of CYP2C8-mediated reactions (via the glucuronide) with a more modest effect on CYP2C9 (via the parent drug). The U.S. Food and Drug Administration has recommended using gemfibrozil as a model CYP2C8 inhibitor for *in vivo* drug interaction studies (Huang et al., 2007).

The present study investigated the *in vitro* and *in vivo* effects of gemfibrozil on the pharmacokinetics of BRV and its biotransformation into BRV-OH, BRV-AC, and BRV-OHAC.

Materials and Methods

Pharmacokinetic Interaction Study in Healthy Participants. *Study design and participants.* This was a single-center, open-label, randomized, two-sequence, two-period, two-treatment crossover study in 26 male healthy participants, who received the following two treatments: A) a single dose of 150 mg of BRV (as three 50-mg tablets; UCB Pharma SA, Brussels, Belgium); and B) 600 mg of gemfibrozil every 12 h for 7 days (Gevilon; Pfizer Pharma GmbH, Berlin, Germany), with a single dose of 150 mg of BRV given on the 4th day of gemfibrozil dosing. There were at least 14 days between the end of dosing for the first period and the start of dosing for the second period. Gemfibrozil was administered daily at 7:00 AM and 7:00 PM, and BRV was administered at 8:00 AM. Meals were served 1/2 h after gemfibrozil intake except on the morning of BRV administration when fasting was maintained until 4 h after BRV dosing.

Mandatory confinement was from the evening before the first administration until the morning of day 4 for treatment A; for treatment B it was from the evening before the first administration until the morning of day 1 and from the evening of day 3 until the morning of day 7, and any additional confinement was at the discretion of the investigator or at the request of the individual subject. The first morning dose of gemfibrozil (day 1) was taken at the clinical center, and the subjects were instructed to take the remaining tablets until the morning tablet of day 3 at home. During the nonconfinement period, the investigator's staff gave phone calls to every subject at the appropriate times. The dates and times of intake were recorded in personal diaries and in the case record forms. From the evening of day 3 until release from the unit in the morning of day 8, subjects were confined again, and medication was given under supervision.

Inclusion and exclusion criteria. To enter the study, individuals were required to have a body mass index between 18 and 28 kg/m^2 and were required to be in good health, as determined by medical history, physical examination, vital signs, ECG, and clinical laboratory measurements. All subjects were men aged 18 to 55 years. Subjects with hepatic, renal, or gastrointestinal dysfunction or any other significant medical condition were excluded from the study. Subjects were also excluded if they had donated blood or participated in a drug study during the preceding 2 months. The consumption of grapefruit and grapefruit-derived products was prohibited from 14 days before the study. Alcohol, xanthine-containing preparations (coffee, chocolate, tea, and cola), and herbal therapies (including St. John's wort) were also prohibited from 48 h before each drug treatment period.

The final protocol and informed consent documentation were reviewed and approved by the Medical Ethics Committee of the Faculty of Medicine of the University of Liege (Liege, Belgium) before the start of any study procedures. The study was performed in accordance with Good Clinical Practice regulations and the Declaration of Helsinki.

Blood sampling. Venous blood samples for pharmacokinetic analysis were drawn at the following times on the days that BRV was administered: predose

and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 6, 9, 12, 24, 36, 48, and 72 h postdose. Blood samples were collected in tubes containing lithium heparin, and the plasma was separated in a refrigerated centrifuge during 10 min at 1500g within 30 min after sampling. The samples were stored at -20°C before analysis.

Blood samples were taken for determination of gemfibrozil trough levels before the morning dose on days 4, 5, 6, and 7. The corresponding plasma samples were stored at -80°C before analysis.

All urine emissions were collected in the following time intervals: 0 to 12, 12 to 24, 24 to 48, and 48 to 72 h after each BRV administration. They were stored in separate, labeled vessels and were kept refrigerated at approximately $4-8^\circ\text{C}$ until each fraction was completed. The total urine collected in each time interval was mixed thoroughly, and the total volume was recorded. Aliquots were stored at -20°C before analysis.

Determination of analyte concentrations. BRV and its three metabolites (BRV-OH, BRV-AC, and BRV-OHAC) were determined in plasma and urine using a validated LC-MS/MS method (Sargentini-Maier et al., 2012). The quantification range was 10 to 2000 ng/ml in plasma and 100 to 20,000 ng/ml in urine for the parent compound, and 2 to 2000 ng/ml in plasma and 20 to 20,000 ng/ml in urine for the metabolites. Metabolite concentrations were expressed in microgram equivalents of brivaracetam. Intermediate precision was $\leq 13\%$, and relative error was 8% or less for all analytes. Gemfibrozil was determined by Parexel International (Poitiers, France) using a proprietary high-performance liquid chromatography method with fluorescence detection, with a lower quantification limit of 100 ng/ml.

Pharmacokinetics. The following pharmacokinetic parameters were derived for BRV and each metabolite: maximum observed plasma concentration (C_{max}), time to C_{max} (t_{max}), AUC from time 0 to the last quantifiable concentration [AUC $_{(0-t)}$], AUC from time 0 extrapolated to infinity (AUC $_{\text{inf}}$), apparent terminal $t_{1/2}$, and percentage of the dose excreted in the urine from time 0 to 72 h (f_e). Oral plasma clearance (CL/F) was derived for BRV only. Values for pharmacokinetic parameters were determined using WinNonlin 4.0.1 (Pharsight, Mountain View, CA) with the linear trapezoidal method for calculation of AUC values. AUC $_{\text{inf}}$ was calculated as AUC $_{(0-t)}$ + C_t/λ_z , where C_t is the last quantifiable concentration (observed) and λ_z is the slope of linear regression of the natural logarithm (ln) of concentration against time during the terminal phase of the concentration-time profile. Apparent terminal $t_{1/2}$ was calculated as $\ln(2)/\lambda_z$. CL/F was obtained as the ratio of dose to AUC $_{\text{inf}}$. Actual sample collection times were used for the pharmacokinetic analysis. Concentrations below the limit of quantification were not used. For gemfibrozil, the morning trough levels on days 4, 5, 6, and 7 were reported.

Statistical analysis. To assess the effects of gemfibrozil on the pharmacokinetics of BRV and its metabolites, natural log-transformed C_{max} , AUC $_{(0-t)}$, AUC $_{\text{inf}}$, $t_{1/2}$, and A $_{\text{e}(0-72 \text{ h})}$ values were analyzed with a crossover analysis of variance model consisting of subject, period, and treatment as fixed effects and

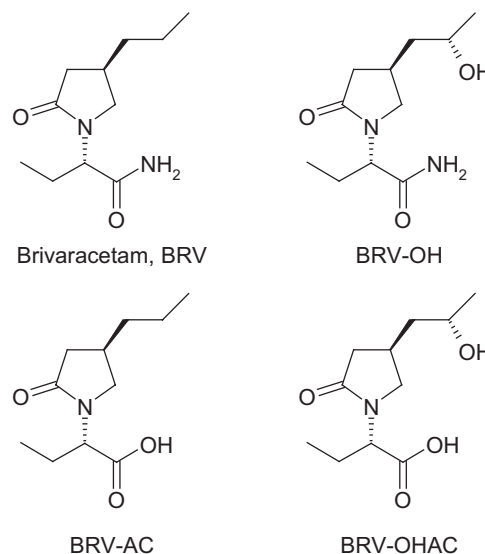


FIG. 1. Chemical structures of brivaracetam and its hydroxy (BRV-OH), acid (BRV-AC), and hydroxy acid (BRV-OHAC) metabolites.

subject within sequence as a random effect. Least-squares mean differences between test (BRV with gemfibrozil) and reference (BRV alone) treatments and corresponding 90% confidence intervals (CI) were estimated from this model and back-transformed to derive estimates of the mean ratios (test/reference) of least-squares geometric means and the 90% CIs for these ratios. Lack of pharmacokinetic interaction of gemfibrozil on BRV and its metabolites would be concluded if the 90% CIs for the mean ratios (test/reference) of least-squares geometric means were fully contained within the 80 to 125% interval for the primary parameters C_{max} , $AUC_{(0-t)}$, and AUC_{inf} .

The sample size calculation was based on a type I error of 5% and on an intrasubject coefficient of variation of 23%, on the basis of previous studies. It was estimated that 24 subjects would provide at least 90% power for the ratio of pharmacokinetic parameters to be within the range of 0.80 to 1.25. To account for dropouts, 26 subjects were enrolled.

All statistical analyses were performed using SAS (version 9.1; SAS Institute, Cary, NC) or StatXact (version 7.0; Cytel Software, Cambridge, MA).

Chemicals and Reagents. BRV and its three metabolites BRV-OH, BRV-AC, and BRV-OHAC (Fig. 1) were synthesized at UCB Pharma (Braine-l'Alleud, Belgium). Gemfibrozil was obtained from Sigma-Aldrich (Bornem, Belgium) and gemfibrozil-1-*O*- β -glucuronide from Toronto Research Chemicals, Inc. (North York, ON, Canada). A NADPH-regenerating system was obtained from BD Gentest (Woburn, MA). All other chemical reagents were of analytical grade. Human liver microsomes (HLM) (pool of 50 donors) were purchased from XenoTech, LLC (Lenexa, KS). Cryopreserved human hepatocytes (pool of 10 donors) were obtained from CellZ-Direct (Durham, UK).

BRV and BRV-AC were added to the in vitro incubates as a solution in potassium phosphate buffer to which gemfibrozil and gemfibrozil-1-*O*- β -

glucuronide were added as a solution in water-acetonitrile (50:50, v/v) or dimethyl sulfoxide, respectively. The final solvent concentration in incubates was ≤ 1 and 0.2% for acetonitrile and dimethyl sulfoxide, respectively.

In Vitro Assays. Preliminary assays were conducted in HLM and hepatocytes to determine kinetic parameters of BRV and BRV-AC hydroxylation reactions. These were followed by a first inhibition assay to quantify the IC_{50} of gemfibrozil on both reactions. All these data (not shown) were combined to set up the substrate and inhibitor concentration range to explore for proper K_i determination.

Incubations with human liver microsomes. All incubations with HLM were performed in polypropylene containers at approximately 37°C in a shaking water bath with 50 mM potassium phosphate buffer (pH 7.4) containing a NADPH-regenerating system [NADP (1.3 mM), glucose 6-phosphate (3.3 mM), magnesium chloride (3.3 mM), and glucose-6-phosphate dehydrogenase (0.4 U/ml)]. Incubations were performed in triplicate.

HLM concentration and incubation times were selected so that initial rate conditions for BRV and BRV-AC hydroxylation were respected (HLM concentrations of 0.5 and 0.2 mg/ml and incubation times of 20 and 30 min, for BRV and BRV-AC, respectively). Both reactions were determined using varying concentrations of substrate (from 0.5 to 6 mM) and of gemfibrozil (0–750 μ M) to allow K_i determination.

Gemfibrozil-1-*O*- β -glucuronide was examined for its potential to act as a metabolism-dependent inhibitor of BRV and BRV-AC hydroxylation. Gemfibrozil-1-*O*- β -glucuronide (200 μ M) was preincubated in triplicate with HLM and a NADPH-regenerating system for 30 min. After this 30-min preincubation, BRV and BRV-AC (2 and 1.5 mM, respectively) were added, and the incubation was resumed to measure residual activity. Incubations were also

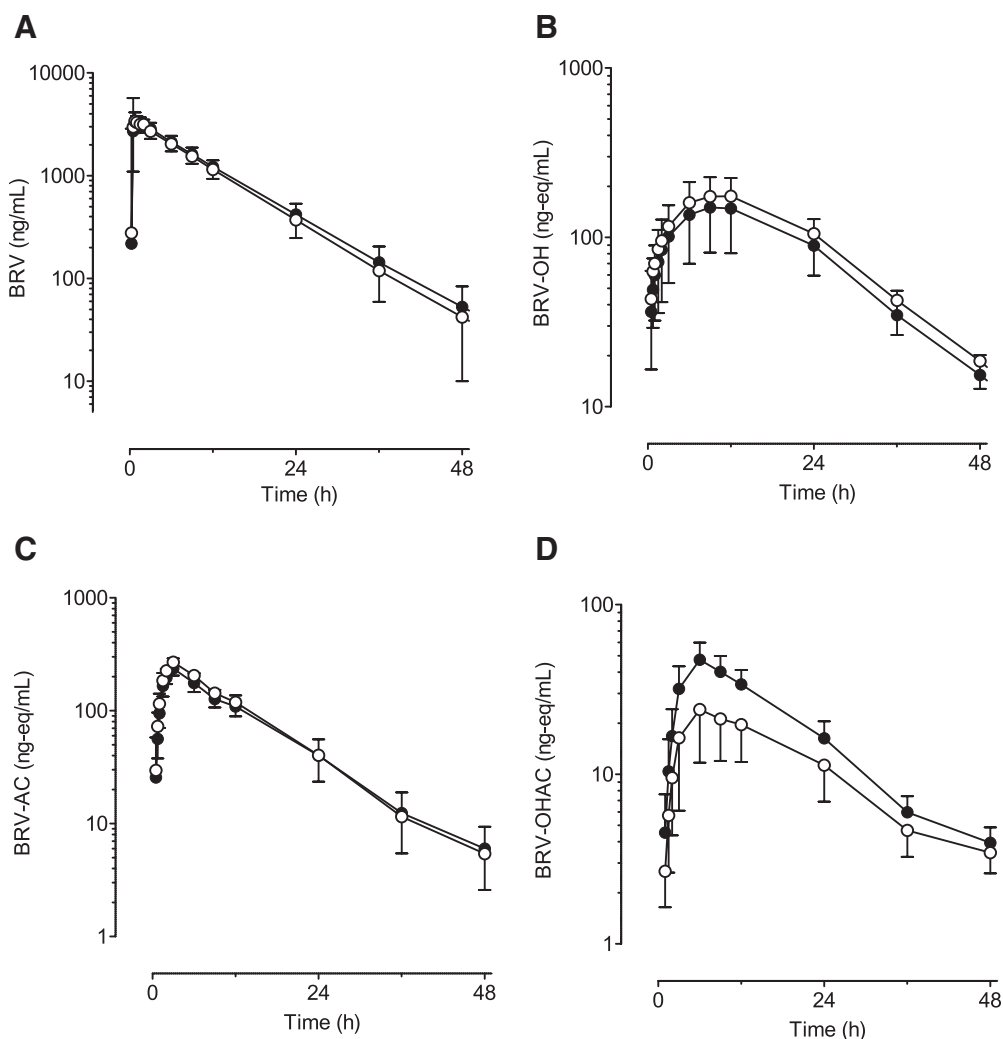


FIG. 2. Geometric mean (SD) plasma concentrations of BRV (A), BRV-OH (B), BRV-AC (C), and BRV-OHAC (D) in 26 healthy volunteers after a single oral of 150 mg of BRV given alone (●) or with gemfibrozil 600 mg twice daily (○).

performed without preincubation to evaluate the direct inhibitory potential of gemfibrozil-1-*O*- β -glucuronide.

Reactions were stopped by ice-cold acetonitrile. The tubes were then thoroughly mixed using a vortex mixer and centrifuged (10,000g at 4°C) for 10 min. Clear supernatants were analyzed by LC-MS/MS.

Incubations with human hepatocyte suspensions. Cryopreserved hepatocytes were thawed according to the provider's information. Viability was assessed by trypan blue exclusion (>90% viability). Incubations (approximately 0.5×10^6 cells/ml) were performed in polypropylene containers placed on a rotor agitator, at 37°C, with Hanks' balanced salt solution buffer. BRV (1.5–12 mM) was incubated for 30 min in the presence of 0 to 2.5 mM gemfibrozil. BRV-AC (0.25–2 mM) was incubated for 60 min in the presence of 0 to 30 μ M gemfibrozil.

Reactions were stopped by the addition of ice-cold acetonitrile. The tubes were then thoroughly mixed using a vortex mixer and centrifuged (14,000g at 4°C) for 10 min.

In vitro sample analysis. The amount of BRV-OH and BRV-OHAC formed was determined by LC-MS/MS. The high-performance liquid chromatography system used consisted of an UPLC Acquity instrument (Waters, Milford, MA) coupled with a Quattro Premier mass spectrometer. The analytical column was an Acquity UPLC HSS T3 (50×2.1 mm, 1.8 μ m), operated at 30°C. Analyses were performed with a gradient method. Eluent A was water containing 0.1% formic acid, and eluent B was acetonitrile containing 0.1% formic acid. Multiple reaction monitoring in positive ion mode was used for all analytes with electrospray ionization. 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 Ltd., Hertfordshire, UK). Deuterated (d_6) internal standards (ISs) were used for the quantification of both analytes. The *m/z* of precursor ions and product ions for each compound was identified as follows: *m/z* 229.10 \rightarrow 184.00 for BRV-OH, *m/z* 235.27 \rightarrow 190.03 for d_6 -BRV-OH (IS), *m/z* 230.00 \rightarrow 184.00 for BRV-OHAC, and *m/z* 236.05 \rightarrow 190.06 for d_6 -BRV-OHAC (IS). The concentrations were determined by the peak area ratio method.

In vitro data analysis. K_i values were determined by nonlinear regression analysis using Galileo (version 3.0.0.01; Thermo Fisher Scientific, Waltham, MA).

Results

Pharmacokinetic Interaction Study in Healthy Participants.

Trough plasma levels of gemfibrozil were in the range of 0.8 to 1.2 μ g/ml, in line with literature data (Karonen et al., 2011).

The plasma-concentration time curves (Fig. 2) and pharmacokinetic parameters (Table 1) for BRV, BRV-OH, and BRV-AC were similar whether BRV was given alone or in combination with gemfibrozil at steady state (i.e., <20% changes). The only significant gemfibrozil-induced changes were restricted to the BRV-OHAC metabolite. Gemfibrozil decreased BRV-OHAC C_{max} (–48%), AUC_{inf} (–34%), and fraction excreted in urine (–40%) and prolonged its elimination half-life (1.4-fold). All these effects were significant because the corresponding 90% confidence intervals on the ratios were entirely outside of the no effect 80 to 125% boundaries.

Treatment-emergent adverse events (mild or moderate dizziness and fatigue) were consistent with the known safety profile of BRV. There were no clinically relevant changes in laboratory tests, vital signs, physical examination, and ECG data. Gemfibrozil was discontinued in one participant on the 6th day of intake during the first period because of the appearance of an erythematous pruritic rash. Recovery was uneventful. All other participants completed both treatment periods.

Inhibitory Effects of Gemfibrozil on the In Vitro Metabolism of BRV. The two hydroxylation reactions, i.e., BRV into BRV-OH and BRV-AC into BRV-OHAC, were monitored in human liver microsomes and in hepatocytes using various substrate and gemfibrozil concentrations (Fig. 3). Gemfibrozil weakly inhibited the hydroxylation of BRV into BRV-OH, in either human liver micro-

TABLE 1

Pharmacokinetic parameters of BRV and its three major metabolites in healthy participants after a single oral dose of 150 mg of BRV given alone or with 600 mg of gemfibrozil twice daily

Variable	BRV Alone (n = 25)	BRV + Gemfibrozil (n = 26)	Mean Ratio % (90% CI)
Data are geometric means (coefficient of variation %).			
BRV			
C_{max} , μ g/ml	4.15 (24.3)	4.16 (26.2)	101 (94–108)
$AUC_{(0-t)}$, μ g \cdot h/ml	41.3 (14.7)	39.1 (16.6)	95 (93–97)
AUC_{inf} , μ g \cdot h/ml	41.4 (14.8)	39.2 (16.6)	95 (93–97)
CL/F, l/h	3.62 (14.8)	3.83 (16.6)	
$t_{1/2}$, h	8.09 (13.4)	7.64 (14.8)	94 (93–96)
f_e , %	8.33 (28.8)	8.47 (20.8)	103 (95–112)
BRV-OH			
C_{max} , μ g/ml	0.154 (52.3)	0.183 (49.0)	119 (113–124)
$AUC_{(0-t)}$, μ g \cdot h/ml	4.12 (44.9)	4.87 (47.0)	118 (114–122)
AUC_{inf} , μ g \cdot h/ml	4.18 (44.6)	4.94 (45.4)	118 (114–122)
$t_{1/2}$, h	9.58 (13.4)	9.25 (15.5)	97 (94–99)
f_e , %	12.9 (43.1)	14.6 (46.4)	113 (106–120)
BRV-AC			
C_{max} , μ g/ml	0.244 (20.1)	0.285 (21.8)	117 (108–127)
$AUC_{(0-t)}$, μ g \cdot h/ml	3.23 (22.3)	3.55 (22.0)	111 (106–116)
AUC_{inf} , μ g \cdot h/ml	3.29 (22.5)	3.61 (22.0)	110 (105–116)
$t_{1/2}$, h	8.59 (17.9)	7.99 (16.8)	93 (90–96)
f_e (%)	27.2 (20.6)	30.0 (26.3)	111 (102–121)
BRV-OHAC			
C_{max} , μ g/ml	0.0476 (25.9)	0.0247 (44.3)	52 (46–58)
$AUC_{(0-t)}$, μ g \cdot h/ml	0.905 (22.9)	0.553 (42.0)	61 (55–67)
AUC_{inf} , μ g \cdot h/ml	0.966 (21.8)	0.634 (33.3)	66 (61–71)
$t_{1/2}$, h	11.2 (9.2)	15.7 (31.1)	139 (125–155)
f_e , %	14.2 (19.6)	8.55 (41.9)	60 (53–67)

somes [K_i (S.D.) = 153 ± 33 μ M] or human hepatocytes (K_i = 284 ± 74 μ M) (Table 2). Gemfibrozil showed a more pronounced effect on the hydroxylation of BRV-AC into BRV-OHAC with K_i of 11.8 ± 1.8 and 11.5 ± 0.7 μ M in microsomes and hepatocytes, respectively.

Nonlinear regression analysis resulted in better goodness-of-fit parameters when different mechanisms of inhibition (mixed, competitive, or noncompetitive) were used for the two reactions and test systems. However, these observed differences were considered minor and were not further explored.

As explained earlier, the in vivo effects of gemfibrozil on CYP2C8 are mediated by a reactive form of its 1-*O*- β -glucuronide. Thus, in a subsequent assay, gemfibrozil 1-*O*- β -glucuronide was tested for its potential to inhibit BRV metabolism, either directly or after prior incubation to allow metabolism-based inhibition (Table 3). The two hydroxylation reactions remained poorly affected by gemfibrozil 1-*O*- β -glucuronide, irrespective of the incubation protocol ($\leq 25\%$ inhibition at 200 μ M).

Discussion

CYP2C8 was previously identified as the major P450 isoform responsible for the hydroxylation of BRV into BRV-OH. Other contributors (i.e., 2C19, 2B6, and 3A4) were identified but were thought to play a minor role (Whomsley et al., 2007). Only approximately 16% of an oral dose of BRV is excreted as BRV-OH (Sargentini-Maier et al., 2008). As a consequence, total inhibition of that pathway would result in a <20% increase of parent AUC. However, this figure might be underestimated because BRV-OH could possibly be transformed into BRV-OHAC or other minor metabolites. The purpose of the present study was to investigate the effects of gemfibrozil, a potent and well recognized 2C8 inhibitor, on the pharmacokinetics and metabolism of BRV.

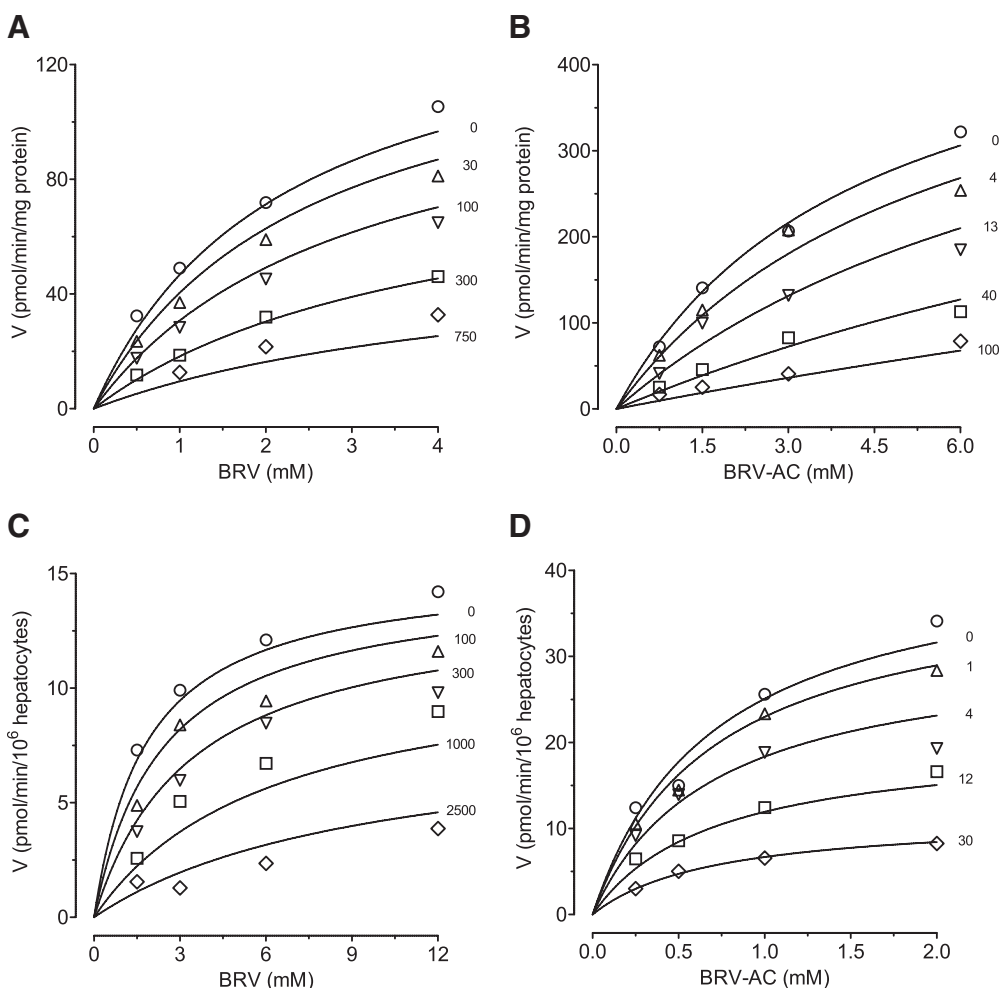


FIG. 3. Inhibitory effect of gemfibrozil on the hydroxylation of BRV to BRV-OH (A and C) and of BRV-AC to BRV-OHAC (B and D), in human liver microsomes (A and B) and in human hepatocytes (C and D). Experimental data are shown as means of triplicate determinations. Curves were obtained by simultaneous nonlinear regression. Gemfibrozil concentrations (micromolar) are given for each inhibition curve.

In healthy participants, 600 mg of gemfibrozil twice daily for 7 days did not affect the formation of BRV-OH (i.e., there was no change in its AUC, C_{\max} , or f_e). Likewise, the pharmacokinetics of parent BRV remained unchanged (i.e., no effect on its AUC, C_{\max} , $t_{1/2}$, or f_e). Although encouraging for the program, these findings were mostly unexpected. Indeed, after such a dosing regimen of gemfibrozil, the hepatic free concentration of gemfibrozil 1-*O*- β -glucuronide is estimated to be 89 μM (Hinton et al., 2008). The latter value combined with published values of K_i (20 μM), maximal rate of inactivation (k_{inact}) (0.21 min^{-1}), and rate constant describing enzyme degradation (k_e) (0.0008 min^{-1}) predict an almost complete inhibition of CYP2C8-mediated reactions. The impact on the AUC of parent drug can be derived using the following equation (Ogilvie et al., 2006):

$$\frac{\text{AUC}_i}{\text{AUC}} = \frac{1}{\left(\frac{f_m}{1 + [k_{\text{inact}}/K_i \times I/k_e]} \right) + (1 - f_m)}$$

where I is the hepatic free gemfibrozil 1-*O*- β -glucuronide concentration and f_m is the fraction of BRV dose eliminated by CYP2C8. On the basis of this simulation, gemfibrozil was predicted to inhibit more than 99% of BRV-OH formation with a minimum 19% increase in the AUC of BRV, which contradicts the actual data.

The effect of gemfibrozil on BRV hydroxylation was thus explored in vitro using both NADPH-fortified human liver microsomes and intact hepatocytes. Indeed, the inhibitory potential of gemfibrozil toward CYP2C8 was reported to vary according to the test system used. The inhibition is weaker in microsomes compared with that in hepatocytes (IC_{50} of 120–150 versus 2–64 μM , respectively) (Par-

TABLE 2

In vitro inhibitory effect of gemfibrozil on brivaracetam metabolic pathways in human liver microsomes and hepatocytes

Fitted K_i values are expressed \pm S.E.

Pathway	Human Liver	K_i	Mechanism
		μM	
BRV hydroxylation into BRV-OH	Microsomes	153 \pm 33	Mixed ($\alpha = 2.95$)
	Hepatocytes	284 \pm 74	Mixed ($\alpha = 10.5$)
BRV-AC hydroxylation into BRV-OHAC	Microsomes	11.8 \pm 1.8	Competitive
	Hepatocytes	11.5 \pm 0.7	Noncompetitive

TABLE 3

In vitro inhibitory effect of gemfibrozil 1-*O*-glucuronide on brivaracetam metabolic pathways in human liver microsomes

Results expressed as mean \pm S.D.

Pathway	Test System	% Inhibition at 200 μM
BRV hydroxylation into BRV-OH	Direct	23 \pm 2
	With preincubation	25 \pm 2
BRV-AC hydroxylation into BRV-OHAC	Direct	14 \pm 3
	With preincubation	16 \pm 8

kinson et al., 2010). This difference is due to the ability of hepatocytes to activate gemfibrozil to gemfibrozil 1-*O*- β -glucuronide through a UGT2B7-mediated reaction (Mano et al., 2007). The glucuronide metabolite acts as a potent irreversible metabolism-dependent CYP2C8 inhibitor, a property not shared by gemfibrozil itself. In the present work, gemfibrozil inhibited BRV-OH formation with the same, if not lower, potency in hepatocytes than in microsomes. In addition, the measured inhibition in hepatocytes appeared at gemfibrozil concentrations approximately 10-fold higher than those reported to inhibit 2C8 in the same model. A subsequent *in vitro* assay showed that gemfibrozil 1-*O*- β -glucuronide was not inhibitory against BRV-OH formation ($\leq 25\%$ inhibition at 200 μM), even after preincubation to allow metabolism-based inhibition to occur.

Taken together, the above *in vivo* and *in vitro* findings strongly suggest that BRV-OH formation is not primarily supported by CYP2C8. Several similar reports have been published in which *in vitro* P450 phenotyping data did not match clinical findings, especially within the CYP2C family. The antidiabetic agent glibenclamide is primarily transformed *in vitro* by CYP3A4 with some minor contribution of CYP2C19 (van Giersbergen et al., 2002; Naritomi et al., 2004; Zhou et al., 2010). This finding contrasts with clinical data showing that glibenclamide *in vivo* clearance is primarily mediated by CYP2C9 (Niemi et al., 2002; Yin et al., 2005). Another example is chlorpropamide, which is oxidized *in vitro* by both CYP2C9 and CYP2C19, whereas its *in vivo* disposition is influenced by CYP2C9 but not by CYP2C19 genetic polymorphism (Shon et al., 2005). Overall, it has been suggested that P450 phenotyping might be particularly difficult for low-turnover compounds with multiple P450s involved (Zhang et al., 2007).

The initial identification of CYP2C8 as the main isoform supporting BRV-OH formation was primarily driven by chemical inhibition assays (Whomsley et al., 2007). The reaction was most strongly inhibited by quercetin, a frequently used reference CYP2C8 inhibitor (Nebot et al., 2010). Retrospective literature analysis suggested that quercetin is not strictly specific to CYP2C8 and could also inhibit CYP2C9 and CYP2C19 with IC_{50} values in the low micromolar range (Zou et al., 2002). The interpretation of the above-mentioned chemical inhibition assay was further complicated by the large effect observed with the vehicle alone (approximately 50% inhibition with methanol 1% v/v). Immunoinhibition assays and incubations with recombinant P450s were performed in parallel and revealed the simultaneous involvement of several isoforms, i.e., CYP2C8, 2C9, 2C19, and 3A4. Furthermore, data obtained with recombinant P450s were not adjusted for relative P450 abundance nor for any other correction factors (e.g., intersystem extrapolation factor). It can be tentatively hypothesized that taken together these technical difficulties led to wrong P450 assignment of BRV hydroxylation.

A standard gemfibrozil dosing regimen is also expected to affect CYP2C9-mediated pathways, although to a much lower extent than with CYP2C8. The hepatic free concentration of unchanged gemfibrozil was estimated to reach 3 μM , close to its K_i on CYP2C9 (5.8 μM) (Hinton et al., 2008). *In vitro/in vivo* extrapolation would predict a maximal 34% decrease in a 2C9-mediated reaction $[1/(1 + I/K_i)]$, with much less effect, if any, on the other isoforms. Consistent with these predictions, gemfibrozil increases the AUC of some CYP2C9 substrates such as nateglinide (+47%); gemfibrozil coadministered with itraconazole (Niemi et al., 2005), glimepiride (+23%) (Niemi et al., 2001), and rosuvastatin (+88%) (Schneck et al., 2004). The larger effect on rosuvastatin is thought to be due to additional non-P450-related mechanisms (i.e., inhibition of organic anion-transporting polypeptide 1B1-mediated hepatic uptake). The absence of any effect of gemfibrozil on BRV-OH formation in human participants suggests

that the reaction does not primarily involve CYP2C9. In support of this hypothesis, gemfibrozil has a very marginal inhibitory effect on BRV-OH formation in human liver microsomes (K_i of 153 μM where 5.8 μM is reported for CYP2C9-mediated reactions) (Hinton et al., 2008).

In the present study, the effect of gemfibrozil was restricted to a modest *in vitro* and *in vivo* inhibition of a secondary metabolic pathway of BRV, i.e., its hydroxylation into BRV-OHAC. The reaction does not appear to be supported by CYP2C8 because it is not inhibited *in vitro* by gemfibrozil 1-*O*-glucuronide. On the other hand, the measured K_i of gemfibrozil (12 μM) on that pathway is in the same range as the published value describing its inhibitory effect on CYP2C9 (5.8 μM), suggesting involvement of this latter isoform in the reaction. In addition, the amplitude of the interaction observed *in vivo* (i.e., 30–40% reduction in BRV-OHAC AUC and excretion) is consistent with the expected effect on a CYP2C9 reaction (34%, see above).

In conclusion, in healthy participants, gemfibrozil had no effect on the hydroxylation of BRV into BRV-OH. Together with the *in vitro* inhibition assays, these findings suggest that neither CYP2C8 nor CYP2C9 is involved in the reaction, which contradicts the previously reported *in vitro* P450 phenotyping data. The reasons underlying this discrepancy remain to be investigated. Of interest, a recently reported study in healthy Japanese participants suggested the involvement of CYP2C19 in the hydroxylation of BRV. Plasma exposure and excretion of BRV-OH was indeed decreased 10-fold in subjects with the CYP2C19 poor metabolizer genotype, whereas the clearance of parent BRV was merely decreased by 30% (Stockis et al., 2011).

In the present clinical interaction study, the only measurable effect of gemfibrozil was a modest but significant decrease in a secondary metabolic route, i.e., hydroxylation of BRV-AC into BRV-OHAC. On the basis of the measured *in vitro* inhibitory constants, CYP2C9 is possibly involved in that reaction. In any case, gemfibrozil did not affect the pharmacokinetic parameters of unchanged BRV, suggesting that BRV can be safely coadministered with CYP2C8 or CYP2C9 inhibitors.

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Authorship Contributions

Participated in research design: Nicolas, Chanteux, Rosa, Watanabe, and Stockis.

Conducted experiments: Chanteux, Rosa, and Watanabe.

Contributed new reagents or analytic tools: Chanteux and Rosa.

Performed data analysis: Nicolas, Chanteux, Rosa, Watanabe, and Stockis.

Wrote or contributed to the writing of the manuscript: Nicolas, Chanteux, and Stockis.

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