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# Acute and long-term effects of brivaracetam and brivaracetam–diazepam combinations in an experimental model of status epilepticus

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## SUMMARY

**Objective:** To evaluate acute and long-term effects of intravenous brivaracetam (BRV) and BRV + diazepam (DZP) combination treatment in a rat model of self-sustaining status epilepticus (SSSE).

**Methods:** Rats were treated with BRV (10 mg/kg) 10 min after initiation of perforant path stimulation (PPS) as early treatment; or BRV (10–300 mg/kg), DZP (1 mg/kg), or BRV (0.3–10 mg/kg) + DZP (1 mg/kg) 10 min after the end of PPS (established SSSE). Seizure activity was recorded electrographically for 24 h posttreatment (acute effects), and for 1 week at 6–8 weeks or 12 months' posttreatment (long-term effects). All treatments were compared with control rats using one-way analysis of variance (ANOVA) and Bonferroni's test, or Kruskal–Wallis and Dunn's multiple comparison tests, when appropriate.

**Results:** Treatment of established SSSE with BRV (10–300 mg/kg) resulted in dose-dependent reduction in SSSE duration and cumulative seizure time, achieving statistical significance at doses  $\geq 100$  mg/kg. Lower doses of BRV (0.3–10 mg/kg) + low-dose DZP (1 mg/kg) significantly reduced SSSE duration and number of seizures. All control rats developed spontaneous recurrent seizures (SRS) 6–8 weeks after SSSE, whereas seizure freedom was noted in 2/10, 5/10, and 6/10 rats treated with BRV 200 mg/kg, 300 mg/kg, and BRV 10 mg/kg + DZP, respectively. BRV (10–300 mg/kg) showed a dose-dependent trend toward reduction of SRS frequency, cumulative seizure time, and spike frequency, achieving statistical significance at 300 mg/kg. Combination of BRV (10 mg/kg) + DZP significantly reduced SRS frequency, cumulative seizure time, and spike frequency. In the 12-month follow-up study, BRV (0.3–10 mg/kg) + low-dose DZP markedly reduced SRS frequency, cumulative seizure time, and spike frequency, achieving statistical significance at some doses. Early treatment of SSSE with BRV 10 mg/kg significantly reduced long-term SRS frequency.

**Significance:** These findings support clinical evaluation of BRV for treatment of status epilepticus or acute repetitive seizures.

**KEY WORDS:** Epilepsy, Antiepileptic drug, Seizures, Neuronal injury, Perforant path stimulation.



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## KEY POINTS

- Intravenous brivaracetam (BRV) was evaluated in an experimental rat model of self-sustaining status epilepticus (SSSE)
- Acute treatment with BRV  $\geq 100$  mg/kg significantly reduced SSSE duration
- Lower doses of BRV (0.3–10 mg/kg) were effective in combination with low-dose diazepam (DZP) for acute treatment of SSSE
- SSSE induction resulted in delayed development of spontaneous recurrent seizures (SRS) in control rats
- Acute treatment of SSSE with BRV 300 mg/kg alone, or BRV 1–10 mg/kg in combination with DZP, prevented or reduced the frequency of SRS

Status epilepticus (SE) is a serious and severe medical emergency that is associated with high mortality and substantial morbidity among survivors.<sup>1–4</sup> Poor outcome is correlated with the duration of seizure activity, as well as patient age, diagnosis, and comorbidities.<sup>5</sup> Current practice and clinical guidelines thus recommend that treatment is initiated rapidly and continued until the seizures are controlled.<sup>4,6–8</sup> However, choice of treatment is limited by a lack of high-quality, randomized, controlled trials, particularly involving patients in the later stages of SE, due to methodologic concerns.<sup>5</sup> Effective management of SE thus remains a clinical challenge, and there is a recognized need for well-tolerated, fast-acting, and efficacious treatment options.

Brivaracetam (BRV) is a selective, high-affinity ligand for synaptic vesicle protein 2A (SV2A), which has been approved as adjunctive treatment for focal (partial-onset) seizures in adults with epilepsy. BRV is available as an intravenous formulation, but is not approved for use in patients with SE. However, the preclinical profile of BRV<sup>9</sup> indicates that it may have potential for the treatment of acute seizures. Levetiracetam (LEV), another SV2A ligand, was effective in a preclinical model of self-sustaining status epilepticus (SSSE).<sup>10</sup> BRV binds to SV2A with a 15- to 30-fold higher affinity than LEV<sup>11</sup> and has a differential interaction with SV2A.<sup>12</sup> BRV penetrates the brain faster than LEV in preclinical models.<sup>9</sup> BRV also had higher potency and showed more complete seizure suppression than LEV in a wide range of animal models of epilepsy.<sup>13</sup> BRV pre-treatment inhibited kindling acquisition in a corneal kindling model in mice at doses approximately 10 times lower than those of LEV,<sup>13</sup> suggesting potent antiepileptogenic activity, which may be beneficial in reducing the consequences of SE.

Preclinical studies also indicate that BRV has a rapid onset of action, a key attribute for the treatment of acute

seizures. BRV penetrates the blood–brain barrier (BBB) faster than LEV due to its higher lipophilicity, leading to a faster onset of action in audiogenic mice.<sup>14</sup> Physiology-based pharmacokinetic modeling has predicted that BBB penetration by BRV would also be rapid in humans, with brain concentrations peaking within min of intravenous administration.<sup>14</sup> In support of this, a recent post hoc analysis of phase III data has indicated that oral BRV has an early, sustained onset of action in a subset of responders.<sup>15</sup>

We previously developed an experimental model of SSSE induced by electrical perforant path stimulation (PPS) in adult rats.<sup>16,17</sup> Intermittent stimulation of excitatory pathways above afterdischarge threshold for 30 min creates a reverberating limbic circuit in which seizures are self-sustaining and continue for many hours in the absence of further stimulation. Once established, this process is refractory to diazepam (DZP) and phenytoin.<sup>16</sup> This model can be used to study the effects of acute treatment given either before or after the seizures have become self-sustaining. Long-term effects of acute treatment can also be evaluated, since most rats subjected to SSSE develop chronic epilepsy characterized by quantifiable spontaneous recurrent seizures (SRS).

The objective of the studies reported here was to investigate the acute and long-term effects of treatment with BRV in this SSSE model. Because benzodiazepines are well established as the initial treatment of choice for SE,<sup>18,19</sup> the role of a new medication is likely to be as a second drug to be administered simultaneously with a benzodiazepine or immediately after failure of a benzodiazepine. Therefore, we examined the effect on SSSE of BRV alone or in combination with DZP.

## METHODS

### Animals

Male Wistar rats (Simonsen Laboratories, Gilroy, CA, U.S.A.) aged 8–10 weeks and weighing 260–280 g at the beginning of the study, were used. All studies were conducted in accordance with the protocol approved by the Animal Care Committee of the Greater Los Angeles Veterans Administration Health Care System.

### Surgical implantation of stimulating and recording electrodes

Using previously described methodology,<sup>17</sup> rats anesthetized with ketamine (60 mg/kg; Hospira, San Jose, CA, U.S.A.) and xylazine (15 mg/kg; Baxter, Deerfield, IL, U.S.A.) were implanted with a bipolar stimulating electrode into the angular bundle of the perforant path, and a bipolar recording electrode into the granule cell layer of the ipsilateral dentate gyrus. The depth of the electrodes was optimized by finding the maximal population spike evoked from the dentate gyrus by PPS.

### Induction of self-sustaining status epilepticus

SSSE was induced in the awake state by PPS delivered for 30 min with the following parameters: 10 s 20 Hz trains of 1 msec, 20 V pulses delivered every minute together with continuous 2 Hz stimulation using the same parameters, as described previously.<sup>16,17</sup> Electroencephalography (EEG) and behavioral observations were made for 10 min after the end of PPS to verify the presence of SSSE before any treatment was initiated. Rats with population spike amplitude <2 mV and those that did not develop SSSE were excluded from the studies. Acute effects were evaluated by EEG recordings made during the 24 h after PPS, whereas long-term effects were investigated by continuous 1-week EEG/video recordings made 6–8 weeks or 1 year later. Behavioral effects of acute treatment were evaluated qualitatively by observation.

### Administration of study treatments

Rats were randomly assigned to treatment groups that were matched by body weight. A commercial preparation of DZP in 0.9% saline (Hospira) was used. BRV was dissolved in 0.9% saline (Baxter). Treatments (0.5–0.8 ml) were infused at 0.20–0.25 ml/min into a tail vein. Combination treatments were administered as close to simultaneously as possible. Control rats treated with saline were included in all studies.

The effect of early treatment was investigated by administration of BRV 10 mg/kg 10 min after the initiation of PPS. Long-term outcomes were evaluated 6–8 weeks after induction of SSSE.

The effect of treatment on established SSSE was investigated by administration of treatments 10 min after the end of PPS. Acute effects were evaluated after treatment with BRV 10, 20, 30, 100, 200, and 300 mg/kg; and BRV 10 mg/kg, DZP 1 mg/kg, and BRV + DZP combinations: 0.3 + 1, 1 + 1, 3 + 1, and 10 + 1 mg/kg. Long-term effects were evaluated 6–8 weeks after treatment with BRV 10, 20, 30, 100, 200, and 300 mg/kg, DZP 1 mg/kg, and BRV + DZP 10 + 1 mg/kg; and 12 months after BRV 10 mg/kg, DZP 1 mg/kg, and BRV + DZP combinations: 0.3 + 1, 1 + 1, 3 + 1, and 10 + 1 mg/kg. Each treatment group included 3–10 rats.

### Seizure monitoring and quantification

Electrographic activity from the dentate gyrus was recorded using the Monitor 8.1 computer program (Stellate Systems, Montreal, QC, Canada). Seizures and spikes were detected and counted off-line using Harmonie Software (Stellate Systems) as described previously.<sup>20</sup> Events were recognized as seizures if the mean amplitude was 2.7 times baseline amplitude, minimum frequency was 3 Hz, maximum coefficient of variation was 70, and duration was at least 3 s. Events were recognized as spikes if the mean amplitude was 4 times

baseline amplitude. Seizure authenticity was verified by manual review and video analysis.

End points for acute effects were the following: SSSE duration (min) measured from treatment initiation to the end of the last electrographic seizure; number of seizures recorded from treatment initiation to 24 h post-treatment; cumulative seizure time (min) defined as the sum of all individual seizure durations; mean duration of individual seizures (s); and spike frequency (Hz).

End points for long-term effects were the following: SRS frequency per week; cumulative seizure time (min per week); mean duration of individual seizures (s); spike frequency (Hz); and seizure freedom. Calculation of mean cumulative seizure time included rats with no observed seizures (i.e., seizure time = 0).

### Statistical analyses

Data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's test, or if data were not normally distributed, ANOVA on ranks (Kruskal-Wallis test) followed by Dunn's multiple comparison test using Sigma-Stat (Systat Software Inc, San Jose, CA, U.S.A.);  $p < 0.05$  was regarded as statistically significant.

## RESULTS

### Evolution of self-sustaining status epilepticus

Intermittent PPS for 30 min induced SSSE in all control rats. Behavioral limbic seizures were accompanied by high-frequency spikes, which were recognized as seizures by the software. Between software-recognized seizures, rats displayed less severe seizure behavior accompanied by spikes.

### Behavioral effects of treatment

Rats injected with BRV 0.3–30 mg/kg showed no obvious behavioral changes. A dose of 100 mg/kg resulted in muscle relaxation and sedation lasting 10–15 min, with intact corneal reflex and tail pinch response. Following administration of 200–300 mg/kg, rats became unresponsive with loss of corneal reflex and tail pinch response, but showed progressive recovery over 1 h. Injection of DZP 1 mg/kg induced mild ataxia, drowsiness, and muscle relaxation, which lasted for a few min. The behavioral effects of combinations of BRV 0.3–10 mg/kg with DZP 1 mg/kg were similar to those observed with DZP alone.

### Effect of early treatment with brivaracetam

All 10 control rats developed SRS after 6–8 weeks, compared with 7/10 rats who were treated with BRV 10 mg/kg administered 10 min after initiation of PPS. Early treatment with BRV 10 mg/kg significantly reduced SRS frequency and cumulative seizure time versus control, but had no effect on spike frequency (Table 1).

**Table 1. SRS frequency, cumulative seizure time, spike frequency, and seizure freedom rates 6–8 weeks after treatment with BRV 10 mg/kg administered before the establishment of SSSE**

	Mean (SEM)	
	Control (n = 10)	BRV 10 mg/kg (n = 10)
SRS frequency/week	8.4 (3.0)	1.4* (0.4)
Cumulative seizure time (min/week)	4.62 (1.18)	0.87* (0.31)
Spike frequency (Hz)	0.64 (0.12)	0.45 (0.13)
Seizure freedom, n (%)	0	3 (30)

ANOVA, analysis of variance; BRV, brivaracetam; SEM, standard error of mean; SRS, spontaneous recurrent seizure; SSSE, self-sustaining status epilepticus.  
\* $p < 0.05$ , ANOVA on ranks and Dunn's multiple comparison test.

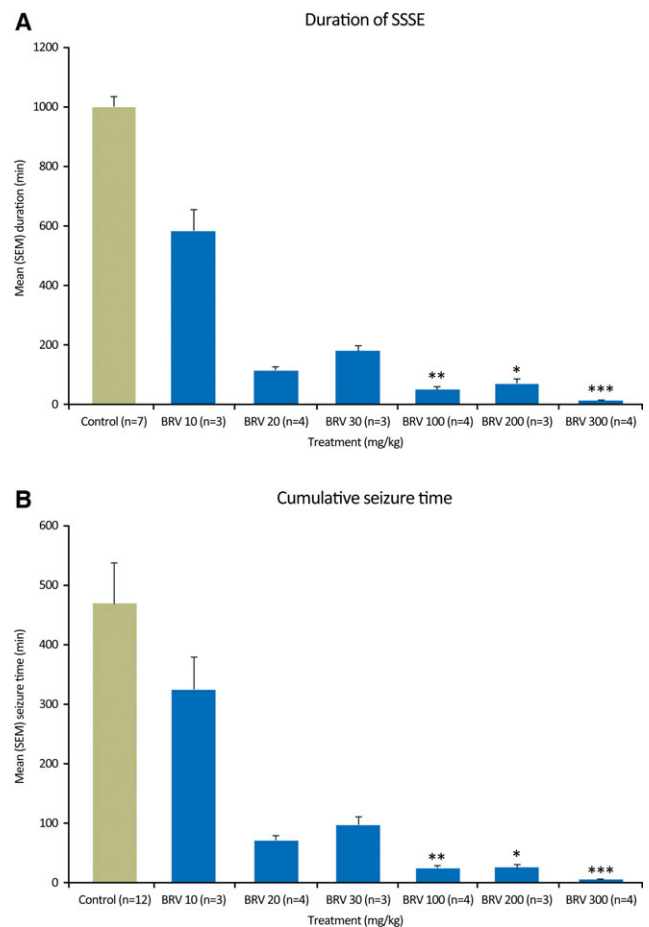
## Effect of treatment on established self-sustaining status epilepticus

### Acute effects of brivaracetam

BRV 10–300 mg/kg administered 10 min after the end of PPS produced a dose-dependent reduction in both duration of SSSE (Fig. 1A) and cumulative seizure time (Fig. 1B), which was statistically significant versus control at doses  $\geq 100$  mg/kg. Mean (standard error of mean [SEM]) SSSE duration was reduced from 999 (34) min in control rats to 583 (72) at BRV 10 mg/kg, 116 (13) at 20 mg/kg, 180 (17) at 30 mg/kg, 50 (9) at 100 mg/kg, 72 (14) at 200 mg/kg, and 13 (1) min at 300 mg/kg. Cumulative seizure time was reduced from 467 (68) min in control rats to 323 (55) at BRV 10 mg/kg, 71 (7) at 20 mg/kg, 97 (13) at 30 mg/kg, 24 (5) at 100 mg/kg, 25 (6) at 200 mg/kg, and 5 (1) min at 300 mg/kg.

### Acute effects of brivaracetam–diazepam combinations

Combination of BRV (0.3–10 mg/kg) with 1 mg/kg DZP markedly and significantly reduced SSSE duration (Fig. 2A) and number of seizures (Fig. 2B) versus control rats. SSSE lasted for 1,213 (94) min in control rats versus 897 (250) min with BRV 10 mg/kg alone, 628 (50) min with DZP 1 mg/kg alone, and 97 (55) min following combination of these two treatments. The number of seizures per 24 h was reduced from 72.4 (6.4) for control rats to 49.4 (2.0) with BRV 10 mg/kg, 55.0 (6.6) for DZP 1 mg/kg, and 6.8 (2.2) for combination treatment. The lowest dose of BRV (0.3 mg/kg) in combination with DZP 1 mg/kg reduced SSSE duration to 358 (194) min and number of seizures to 15.0 (6.0). All BRV–DZP combinations acted more rapidly than either drug alone (Fig. 3). Reappearance of seizure activity was observed 3–7 h posttreatment in rats treated with DZP alone, but was not seen after combination therapy. None of the treatments had a marked effect on spike frequency or mean duration of individual seizures (Table S1).



**Figure 1.**

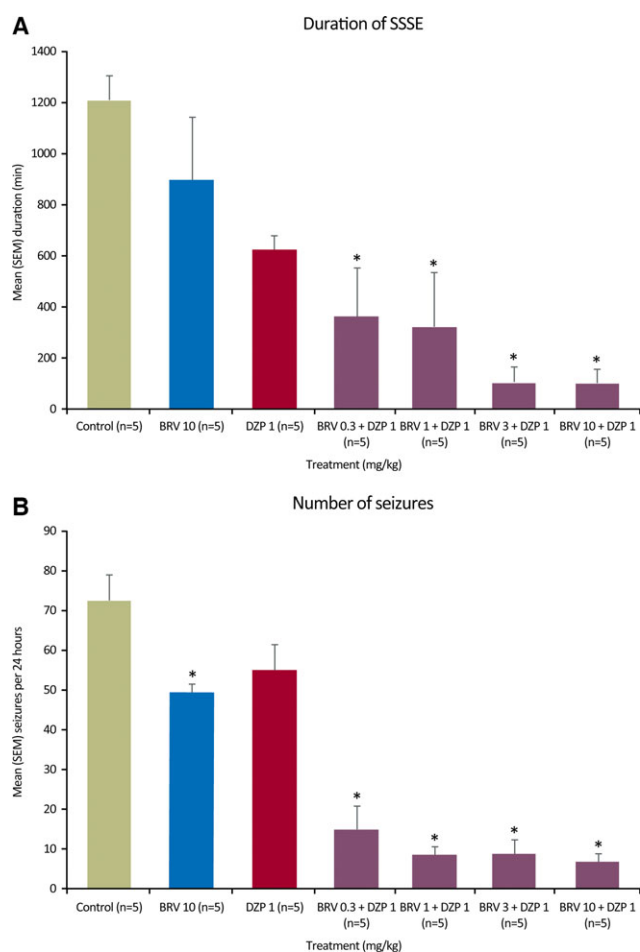
Duration of SSSE (A) and cumulative seizure time (B) during the 24 h following treatment of established SSSE with BRV 10–300 mg/kg. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. control, ANOVA on ranks and Dunn's multiple comparison test. ANOVA, analysis of variance; BRV, brivaracetam; SEM, standard error of mean; SSSE, self-sustaining status epilepticus.

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### Long-term effects of brivaracetam and brivaracetam–diazepam combinations

**Follow-up at 6–8 weeks.** All control rats developed chronic epilepsy 6–8 weeks after induction of SSSE, with a mean (SEM) SRS frequency of 8.4 (3.0) seizures per week, cumulative seizure time of 4.6 (1.2) min per week, and spike frequency of 0.64 (0.12) Hz. Seizure freedom was noted in 2/10 and 5/10 rats who were treated with BRV alone at doses of 200 and 300 mg/kg, respectively, and 6/10 rats who received combination therapy with BRV 10 mg/kg + DZP 1 mg/kg (Fig. 4D). There were no rats who were seizure-free in the other treatment groups.

In rats treated with BRV, there was a trend toward dose-dependent reduction of SRS frequency (Fig. 4A), cumulative seizure time (Fig. 4B), and spike frequency (Fig. 4C), which was statistically significant at the highest dose of BRV (300 mg/kg), with mean (SEM) SRS frequency of 1.6



**Figure 2.** Duration of SSSE (**A**) and number of seizures (**B**) during the 24 h following treatment of established SSSE with BRV 10 mg/kg, DZP 1 mg/kg, and BRV–DZP combinations. \* $p < 0.05$  vs. control, ANOVA and Bonferroni's test. ANOVA, analysis of variance; BRV, brivaracetam; DZP, diazepam; SEM, standard error of mean; SSSE, self-sustaining status epilepticus. *Epilepsia* © ILAE

(0.6) seizures per week, cumulative seizure time of 1.0 (0.4) min per week, and spike frequency of 0.14 (0.02) Hz, versus control values of 8.4 [3.0], 4.6 [1.2], and 0.64 [0.12], respectively. BRV had no effect on duration of individual seizures (Table S2).

Treatment with BRV 10 mg/kg alone or DZP 1 mg/kg alone had no significant effect on mean (SEM) SRS frequency (8.3 [2.3] and 7.9 [1.9] seizures per week), cumulative seizure time (5.2 [1.4] and 5.6 [1.7] min/week), or spike frequency (0.33 [0.06] and 0.39 [0.07] Hz). However, the combination of BRV 10 mg/kg and DZP 1 mg/kg significantly (vs. control) reduced SRS frequency to 1.0 (0.5) seizures per week, cumulative seizure time to 0.5 (0.3) min per week, and spike frequency to 0.14 (0.03) Hz.

*Spontaneous recurrent seizures at 12 months.* In the 12-month follow-up study, large variations in computer-

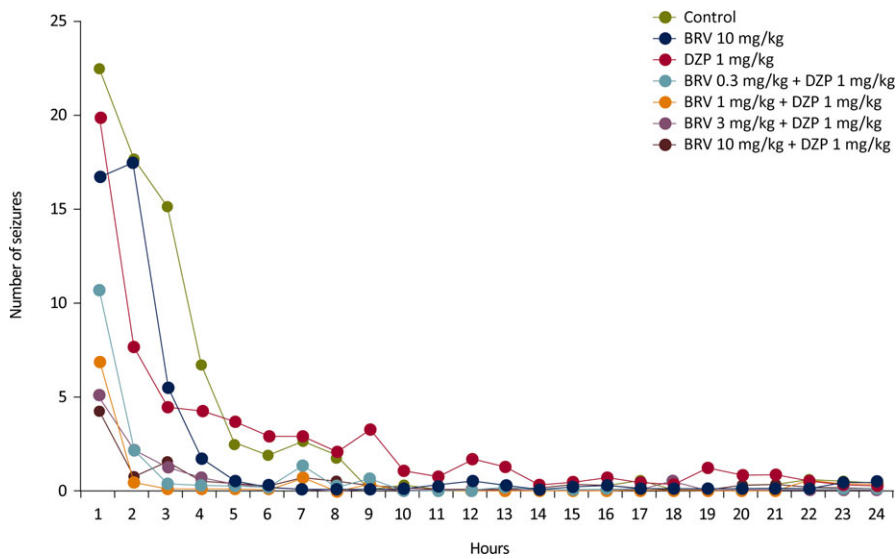
detected seizures within and between groups were noted; consequently, the record was analyzed manually. BRV 10 mg/kg and DZP 1 mg/kg alone had no significant effect on any parameter compared with control rats, whereas the combination of BRV (0.3–10 mg/kg) with DZP 1 mg/kg markedly reduced SRS frequency, cumulative seizure time, and spike frequency, achieving statistical significance at some doses (Table S3). Seizure freedom was noted at 12 months in 2/4 and 3/5 rats that received combination therapy with BRV 1 mg/kg and DZP 1 mg/kg, and BRV 10 mg/kg and DZP 1 mg/kg, respectively. None of the rats treated with BRV 10 mg/kg alone, DZP 1 mg/kg alone, or other combinations of BRV and DZP were seizure-free at 12 months.

## DISCUSSION

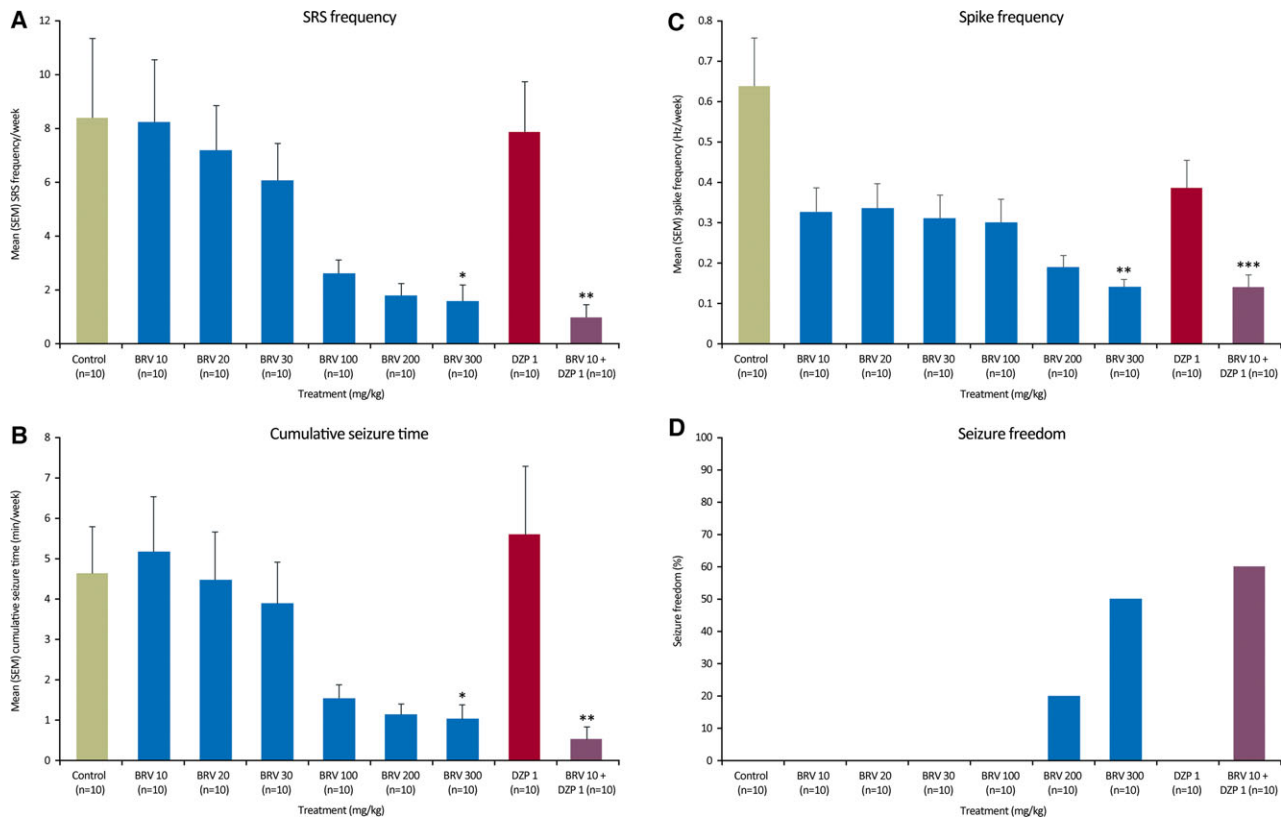
The findings of this study in an experimental rodent model of established SSSE showed that high-dose BRV, and the BRV and DZP combination, acted rapidly with minimal adverse effects and reduced SSSE duration at least ninefold compared to untreated rats.

Drug dosages in humans and rodents can be compared by expressing them per body surface area.<sup>21,22</sup> A supratherapeutic BRV single dose of 200 mg (approximately 2.86 mg/kg) is equivalent to a rodent dose of approximately 18.6 mg/kg. Therefore, BRV doses used in rodents in this study are not far above the “human equivalent dose,” since BRV 20 mg/kg reduced the mean duration of SSSE to 116 min and cumulative seizure time to 71 min, compared with 999 and 467 min, respectively, in control rats.

Lower doses of BRV were markedly more effective when administered in combination with DZP than either drug administered alone. SSSE was terminated within approximately 1.5 h by the combination of BRV 10 mg/kg and DZP 1 mg/kg, compared with a mean duration of >20 h in control rats. Low doses of BRV (0.3–3 mg/kg) also significantly reduced SSSE duration in a dose-dependent manner when administered in combination with DZP. The fact that two treatments that had no action when given alone were so effective in combination suggests the possibility of a synergistic interaction. Isobolographic analyses could have provided a more thorough assessment of any synergistic interaction, but were beyond the scope of these studies. Because BRV is a selective, high-affinity ligand for SV2A<sup>11</sup> and DZP acts as a positive allosteric modulator at the  $\gamma$ -aminobutyric acid (GABA)<sub>A</sub> receptor,<sup>23</sup> the molecular mechanism of this interaction remains to be explained. In humans, BRV weakly inhibits cytochrome P450 (CYP) 2C19<sup>24</sup> and may thus increase plasma concentrations of drugs metabolized by CYP2C19, such as DZP. Although DZP is metabolized in rodents by other rodent-specific isoforms, for example, CYP2D1, studies in mice have shown that BRV 14.7 mg/kg intraperitoneal produced a threefold increase in DZP concentrations in both plasma and brain

**Figure 3.**

Time course of status epilepticus over 1 h intervals in rats with established self-sustaining status epilepticus (SSSE) treated with BRV 10 mg/kg, DZP 1 mg/kg, and BRV–DZP combinations. BRV, brivaracetam; DZP, diazepam. *Epilepsia* © ILAE

**Figure 4.**

SRS frequency (A), cumulative seizure time (B), spike frequency (C), and seizure freedom rates (D) 6–8 weeks after treatment of established SSSE with BRV 10–300 mg/kg, DZP 1 mg/kg, and BRV–DZP combination treatment. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. control, ANOVA on ranks and Dunn's multiple comparison test. ANOVA, analysis of variance; BRV, brivaracetam; DZP, diazepam; SEM, standard error of mean; SRS, spontaneous recurrent seizure; SSSE, self-sustaining status epilepticus.

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(UCB, data on file). Currently available evidence indicates that BRV has the same disposition in rats and mice (UCB, data on file). It is thus possible that a pharmacokinetic drug–

drug interaction contributed to the observed greater effectiveness of DZP when administered in combination with BRV in the current study. However, since plasma

concentrations of BRV and DZP were not measured in this study, the extent and influence of any pharmacokinetic drug–drug interaction are not known.

The dose of DZP administered to rats in these studies (1 mg/kg) can be related to the human equivalent dose when expressed per body surface area and is the equivalent of 11 mg DZP for a 70-kg human. This is lower than the dose used to treat SE in humans (0.25–0.4 mg/kg),<sup>6</sup> and was ineffective when given alone in this model. The dose of BRV that may be effective in clinical SE is not known. The human BRV dose equivalent to that successfully used in rats (0.3–10 mg/kg) in combination with DZP is 0.46–1.54 mg/kg, or 33–108 mg for a 70-kg human.<sup>21,22</sup> This is close to the recommended BRV dose range for adjunctive treatment of focal seizures, which is 50–200 mg/day administered in two equally divided doses (i.e., 25–100 mg twice daily). However, this study was conducted in an experimental model in rats, and caution is required in extrapolation of the dose findings to the clinical situation. The small number of rats in some treatment groups is a further study limitation.

The mean duration of each seizure was unaffected by treatment; this was expected given that this parameter is frequently unchanged by the administration of antiepileptic drugs (AEDs). LEV has previously been reported to be effective in the acute treatment of DZP-resistant, established SSSE in the model used in this study.<sup>10</sup> Compared with the effective doses of BRV reported here ( $\geq 100$  mg/kg), markedly higher, supratherapeutic doses of LEV were required to significantly diminish (200 mg/kg) or abort (500–1,000 mg/kg) seizures. This finding is consistent with the higher potency and efficacy of BRV compared with LEV seen in other animal models of epilepsy and seizures.<sup>13</sup> Although the combination of BRV + DZP has not been evaluated in other animal models of seizures and epilepsy, previous preclinical studies have confirmed that LEV may act synergistically with the benzodiazepines, clonazepam (CLZ), and DZP. Combination of LEV with CLZ markedly enhanced its anticonvulsant potency in audiogenic susceptible mice without any concurrent effect on adverse effect potential or brain and plasma concentrations of either drug.<sup>25</sup> Further studies showed that LEV markedly enhanced seizure suppression by CLZ in amygdala-kindled rats,<sup>26</sup> and by DZP in SSSE induced by PPS.<sup>10</sup>

Data reported here support the clinical evaluation in a randomized clinical trial of the addition of low doses of BRV to standard benzodiazepine treatment for SE. Benzodiazepines are recommended as emergency first-line treatment, with the goal of terminating seizure activity as soon as possible, but limitations of this approach include significant adverse effects<sup>4</sup> and the rapid development of benzodiazepine pharmacoresistance during SE.<sup>6,27</sup> In contrast, BRV 50–200 mg/day administered orally is effective and has a

good tolerability profile in adults with focal seizures,<sup>28–30</sup> and intravenous BRV is well tolerated when given by bolus or infusion, either initiated as a new treatment or switched from oral BRV.<sup>31</sup>

Low-dose BRV monotherapy (10 mg/kg) was effective in reducing the long-term effects of SSSE when administered early, but showed no therapeutic benefit once SE had become self-sustaining. Loss of potency with delayed administration has also been reported in studies of DZP and phenytoin,<sup>16</sup> LEV,<sup>10</sup> and lacosamide.<sup>32</sup> Clinically, early treatment is more effective than delayed treatment,<sup>6,18</sup> and a 1 h delay in AED treatment is associated with poorer outcome.<sup>3</sup> Efficacy of early treatment is of limited clinical relevance, since treatment of established SSSE more closely mimics the typical clinical situation. Treatment is usually initiated when the patient arrives in critical care, at which point the seizures may have been present for min or hours.<sup>33</sup> However, these observations support potential consideration of BRV as an early treatment option that could be administered in a pre-hospital setting.

All control rats demonstrated SRS in EEG recordings conducted continuously for 1 week after a latent phase, indicating that SSSE had resulted in the development of chronic epilepsy. Acute treatment of established SSSE with a high dose of BRV (300 mg/kg) administered alone, or lower BRV doses (1–10 mg/kg) given simultaneously with DZP, reduced the frequency of SRS. Some of the rats treated with BRV at doses of 200 or 300 mg/kg, and half of those who received BRV 10 mg/kg with DZP, showed no evidence of SRS at 6 weeks. Seizure freedom was also noted at 12 months in some rats who received BRV 1 or 10 mg/kg with DZP. However, it is not possible to conclude whether the observed seizure freedom resulted from a true antiepileptogenic effect, from the reduction in duration and severity of SSSE resulting in less brain damage and synaptic reorganization, or from a combination of both factors. In addition, the rats were only monitored for 1 week, which may not have been sufficient to reliably detect a lowering of seizure frequency. A further study limitation is the lack of rotarod test data for the combination of BRV and DZP, and consequent need for caution in assessing the safety of treatment. Although these observations indicate that BRV may be effective in reducing the long-term consequences of SE when administered as an acute treatment, particularly in combination with a benzodiazepine, more studies are required to determine the clinical significance.

In conclusion, the high efficacy of BRV observed in this study in an experimental model of SSSE, together with the rapid brain penetration coupled with fast onset of action observed in preclinical studies, and significant efficacy and good tolerability profile in clinical studies, support further evaluation of BRV as a potential treatment for SE or acute repetitive seizures.



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## DISCLOSURE OF CONFLICT OF INTEREST

Jerome Niquet, Lucie Suchomelova, and Kerry Thompson have declared no conflicts of interest. Henrik Klitgaard and Alain Matagne are employees of UCB Pharma. Claude Wasterlain received a research grant and travel support from UCB Pharma to present these results at neurology meetings, and has received research grants from The Cho Foundation, NIH/NINDS, and the University of California Institute for Mexico and the United States. We confirm that we have read the Journal's position on issues involved with ethical publication and affirm that this report is consistent with those guidelines.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Mean individual seizure duration and spike frequency during the 24 h following treatment of established SSSE with BRV 10 mg/kg, DZP 1 mg/kg, and BRV–DZP combinations.

**Table S2.** Mean individual SRS duration 6–8 weeks after treatment of established SSSE with BRV 10–300 mg/kg, DZP 1 mg/kg, and BRV–DZP combination treatment.

**Table S3.** SRS frequency, cumulative seizure time, mean

individual seizure duration, spike frequency, and seizure freedom rates 12 months after treatment of established SSSE with BRV 10 mg/kg, DZP 1 mg/kg, and BRV–DZP combinations.