# 3.479 Cannabidiol and Sodium Valproate Demonstrate Pharmacodynamic Synergism in an Acute Mouse Model of Generalised Seizures

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## BACKGROUND AND OBJECTIVE

- Plant-derived highly-purified CBD is anticonvulsive in preclinical models of seizure.[1-3] It has also shown antiseizure effects in clinical trials of childhood-onset intractable epilepsies.[4,5]
- The concomitant use of multiple antiseizure medications (ASMs), such as cannabidiol (CBD), clobazam (CLB) and sodium valproate (VPA), is common in the management of epilepsy, especially refractory epilepsies.
- An enhanced pharmacokinetic-pharmacodynamic (PK-PD) drug-drug interaction (DDI) between CBD and CLB has been reported[7] in *Scn1a*$^{+/-}$ mice. Additionally, we have previously reported an isobolographic analysis in the mouse maximal electroshock (MES) model of generalized seizure to reveal a synergistic PD DDI between CBD:CLB and showed that the active metabolite, 7-hydroxy-cannabidiol (7-OH-CBD) was 5-fold more potent than CBD.[8]
- Objective: Here, we employ a robust isobolographic DDI analysis, to assess the pharmacodynamic (PD) interactions between CBD and VPA in the mouse MES.

## METHODS

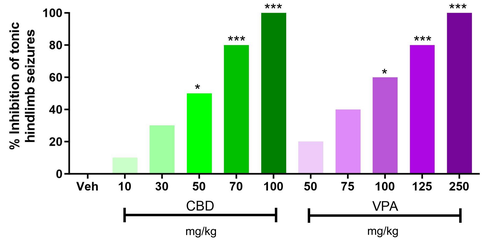
- The MES model of acute generalized seizure was used in male, C57BL/6J mice to evaluate anticonvulsive properties of each ASM administered alone and then in combination.
- ASMs were administered 60 min[9] (CBD; GW Research Ltd, UK) or 30 min[10] (VPA; Sigma Merck, UK) as per $T_{max}$, before the MES test at 1–250 mg/kg via the intraperitoneal (i.p.) route (n=10 per dosed group). Brain samples were collected immediately after MES test for bioanalysis.
- Based on effective brain exposures that produce 50% anticonvulsive effects ($EE_{50}$) for each ASM alone, mice were then treated with 3 fixed ratios of CBD and VPA (1:3, 1:1, and 3:1) calculated using Loewe's equation[11] that yield a theoretical additive effect ($EE_{50+ADD}$).
- The isobolographic method[10-12] for DDI analysis was then used to assess any synergism, additivity or antagonism between CBD and VPA using CalcuSyn[12] ver 2.0 (Biosoft):
  - Assessment of any DDI employed net exposures in the brain (the site of action), considering parent plus active metabolite exposures (normalized to parent potency).
- This approach was justified since the ASMs do not selectively compete for the same molecular targets or sites (CBD: GPR55, TRPV1, ENT1[13] and VPA:$GABA_A$[14]).
- Effects of CBD:VPA DDI and their active metabolites were assessed, which includes 7-OH-CBD, but not 4-ene-vaproic acid due to a lack of anticonvulsive activity.
- These studies were conducted with Epidiolex® active pharmaceutical ingredient and results do not apply to other CBD-containing products.

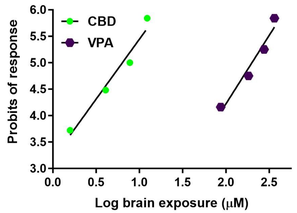
## SUMMARY AND CONCLUSION

- CBD or VPA, when administered alone, exerted independent and brain exposure-dependent anticonvulsive effects in a mouse model of acute generalized seizure.
- An isobolographic analysis using net brain exposures (parent and their active metabolite for CBD) revealed synergy at all three CBD and VPA ratios tested.
- Net PK effect on brain exposures was negligible for DDIs in these acute-dosed MES tests.
- Overall, we reveal a PD synergistic DDI between CBD and VPA at all three ($EE_{50+ADD}$ 1:3, 1:1 and 3:1) ratios tested based on the isobologram, combination index (CI) and exposure reduction index (ERI) values.

## RESULTS

### 1. CBD and VPA revealed dose- and brain exposure-dependent efficacy in the mouse MES

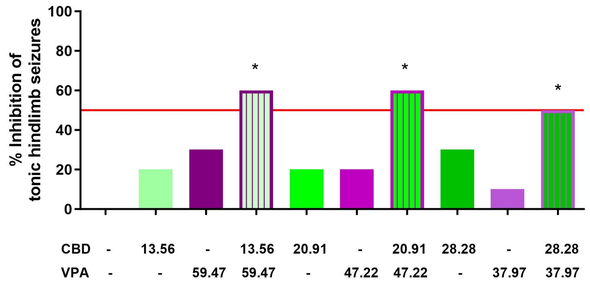



| ASM administered | Goodness of fit, $R^2$ values | Derived $EE_{50}$ (μm) |
|---|---|---|
| CBD | 0.96 | 6.4 |
| VPA | 0.95 | 199.4 |

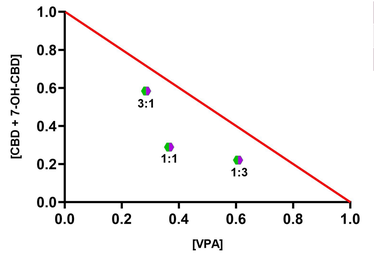
- CBD and VPA, when administered alone, exerted dose-dependent efficacy (n=10). *p<0.05 and ***p<0.001 denote significant inhibition of tonic hindlimb seizures versus vehicle group (Fisher's exact test).
- CBD and VPA alone, exerted independent and brain exposure-based efficacy with $EE_{50}$ values of 6.4 μM and 199.4 μM, respectively.
- Overall effect was derived for [CBD+7-OH-CBD] after normalization of metabolite to parent potency, before conducting the isobolographic analysis of DDI.

### 2. CBD and VPA (1:3, 1:1, and 3:1) exerted significant inhibition of tonic hindlimb seizures




- CBD and VPA combinations (1:3, 1:1 and 3:1) ) calculated using Loewe's equation[11] to yield a theoretical additive effect ($EE_{50+ADD}$) exerted significant (*p<0.05) inhibition of tonic hindlimb seizures versus vehicle group (Fisher's exact test).

### 3. Isobolographic analysis of CBD and VPA net brain exposures reveal a synergistic DDI

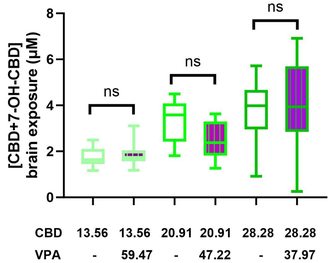


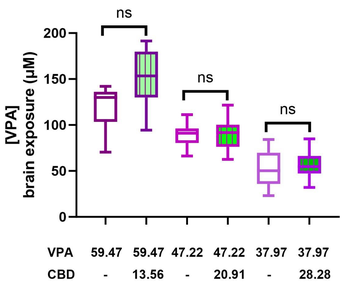

| CBD:VPA ($EE_{50+ADD}$) ratio | Combination Index (CI) | CI Description |
|---|---|---|
| 1:3 | 0.822 | Moderate Synergism |
| 1:1 | 0.648 | Synergism |
| 3:1 | 0.874 | Slight Synergism |

| | Exposure Reduction Index | |
|---|---|---|
| CBD:VPA ($EE_{50+ADD}$) ratio | [CBD+7-OH-CBD] exposure reduction | [VPA] exposure reduction |
| 1:3 | 4.641 | 1.648 |
| 1:1 | 3.542 | 2.735 |
| 3:1 | 1.699 | 3.509 |

- Isobolographic analysis of DDI using net brain exposures against efficacy, revealed synergy at all CBD:VPA ($EE_{50+ADD}$) 1:3, 1:1 and 3:1 ratios tested.
- All combination data points lie under the theoretical line of additivity (red) in the isobologram and each combination index (CI) value was indicative of synergy (CI<1).
- Coadministration of CBD and VPA resulted in a 1.6- to 4.6-fold reduction in net brain exposures to produce anticonvulsive effect, depicting synergy by the exposure reduction index (ERI>1).

### 4. No PK effects observed on net brain exposures with/out CBD:VPA coadministration




- No PK DDI was observed on net [CBD+7-OH-CBD] and [VPA] brain exposures at all CBD:VPA ($EE_{50+ADD}$) 1:3, 1:1 and 3:1 ratios (Kruskal-Wallis and Dunn's comparison tests; data (n=10) presented as box (median/interquartile range) and whisker (minimum and maximum) plots).

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**References:** **1.** Patra et al. *Epilepsia*. 2019; 60: 3. **2.** Jones et al. *Seizure*. 2012; 21(5): 344-52. **3.** Rosenberg et al. *Epilepsy Behav*. 2017; 70: 319-327. **4.** Devinsky et al. *N Engl J Med*. 2017; 376(21): 2011-2020. **5.** Thiele et al. *Lancet*. 2018; 391(10125): 1085-1096. **6.** Morrison et al. *Clin Pharmacol Drug Dev*. 2019: 1-23. **7.** Anderson et al. *Epilepsia*. 2019: 1-11. **8.** Rana et al. *Experimental Neurology*. 2023; 360 (114286): 1-7. **9.** Deiana et al. *Psychopharmacology*. 2012; 219: 859-873. **10.** Andres-Mach et al. *Pharmacology*. 2014; 93(3-4):172-7. **11.** Luszczki et al. *Epilepsy Res*. 2019; 90: 188-198. **12.** Chou et al. *Pharmacol Rev*. 2006; 58: 621-681. **13.** Gray and Whalley. *Epileptic Disoders*. 2020: 22; S10-15. **14.** Rosenberg. *Cell Mol Life Sci*. 2007: 64(16): 2090-103.

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