FULL-LENGTH ORIGINAL RESEARCH

Stereoselective anticonvulsant and pharmacokinetic analysis of valnoctamide, a CNS-active derivative of valproic acid with low teratogenic potential

*Tawfeeq Shekh-Ahmad, *Naama Hen, *†Boris Yagen, ‡John H. McDonough, §Richard H. Finnell, §Bogdan J. Wlodarczyk, and *†Meir Bialer

Epilepsia, 55(2):353–361, 2014
doi: 10.1111/epi.12480

Tawfeeq Shekh-Ahmad is currently a PhD student at the Hebrew University-School of Pharmacy. This paper is part of his PhD thesis.

Summary

Objective: **Valnoctamide (VCD), a central nervous system (CNS)–active chiral constitutional isomer of valpromide, the corresponding amide of valproic acid (VPA), is currently undergoing phase IIb clinical trials in acute mania. VCD exhibits stereoselective pharmacokinetics (PK) in animals and humans. The current study comparatively evaluated the pharmacodynamics (PD; anticonvulsant activity and teratogenicity) and PK of the four individual stereoisomers of VCD.**

Methods: **The anticonvulsant activity of VCD individual stereoisomers was evaluated in several rodent anticonvulsant models including maximal electroshock, 6 Hz psychomotor, subcutaneous metrazol, and the pilocarpine-induced and soman-induced status epilepticus (SE). The PK-PD (anticonvulsant activity) relationship of VCD stereoisomers was evaluated following intraperitoneal administration (70 mg/kg) to rats. Induction of neural tube defects (NTDs) by VCD stereoisomers was evaluated in a mouse strain that was highly susceptible to teratogen-induced NTDs.**

Results: **VCD had a stereoselective PK, with (2S,3S)-VCD exhibiting the lowest clearance, and consequently a twice-higher plasma exposure than all other stereoisomers. Nervertheless, there was less stereoselectivity in VCD anticonvulsant activity and each stereoisomer had similar median effective dose (ED_{50}) values in most models. VCD stereoisomers (258 or 389 mg/kg) did not cause NTDs. These doses are 3–12 times higher than VCD anticonvulsant ED_{50} values.**

Significance: **VCD displayed stereoselective PK that did not lead to significant stereoselective activity in various anticonvulsant rodent models. If VCD exerted its broad-spectrum anticonvulsant activity using a single mechanism of action (MOA), it is likely that it would exhibit a stereoselective PD. The fact that there was no significant difference between racemic VCD and its individual stereoisomers suggests that VCD's anticonvulsant activity is due to multiple MOAs.**

KEY WORDS: **New antiepileptic drugs, CNS drugs, Strereoselective pharmacokinetic and pharmacodynamics analysis, Chiral switch.**

Valnoctamide (VCD; Fig. 1) is a central nervous system (CNS)–active chiral constitutional isomer of valpromide (VPD; Fig. 1), the corresponding amide of valproic acid (VPA) that exhibits stereoselective pharmacokinetics (PK) in animals and humans.[1–3] Unlike VPD that acts as a prodrug to VPA in humans, VCD acts as a drug on its own with minimal biotransformation to its corresponding acid valnoctic acid (VCA).[1,4,5] VCD (racemate) was commercially available as an anxiolytic drug (Nirvanil, Sanofi-Midy

Accepted October 15, 2013; Early View publication December 6, 2013
*Faculty of Medicine, School of Pharmacy, Institute for Drug Research, The Hebrew University of Jerusalem, Jerusalem, Israel; †David R. Bloom Center for Pharmacy, The Hebrew University of Jerusalem, Jerusalem, Israel; ‡Pharmacology Branch, Research Division, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground,, Maryland, U.S.A.; and §Department of Nutritional Sciences, Dell Pediatric Research Institute, The University of Texas at Austin, Austin, Texas, U.S.A.

Address correspondence to Meir Bialer, Faculty of Medicine, School of Pharmacy, Institute for Drug Research, The Hebrew University of Jerusalem, PO Box 12065, Jerusalem 91120, Israel. E-mail: bialer@md.huji.ac.il

Wiley Periodicals, Inc.

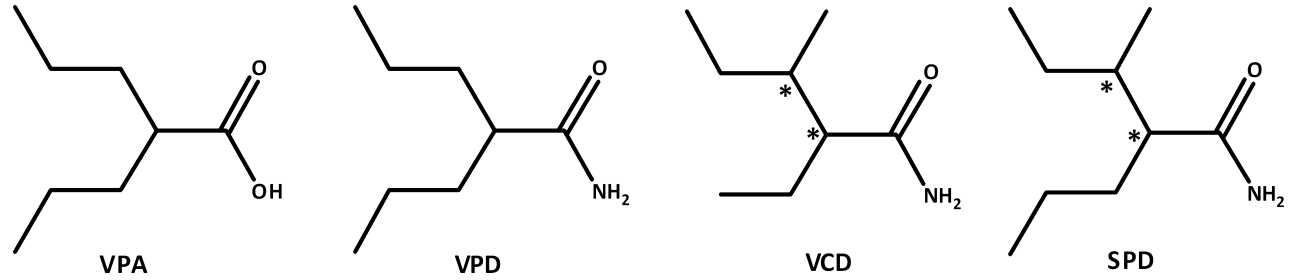

Figure 1.
Chemical structure of valproic acid (VPA), valpromide (VPD), valnoctamide (VCD), and *sec*-butyl-propylacetamide (SPD).
Epilepsia © ILAE

S.p.A., Milan, Italy) in several European countries from 1964 until as recently as 2005.[2,3,6] Two suicide attempts with VCD overdose led patients into coma; however, despite the significantly elevated VCD serum level, the patients survived without adverse consequences and recovery was rapid and complete.[7,8] VCD half-life ($t_{1/2}$) in this overdose case (15 h) was similar to its clinically relevant $t_{1/2}$ of 7–13 h.[4,5] The outcomes of these two suicide attempts show that racemic VCD is a safe compound even at high doses.

VCD possesses two chiral (stereogenic) centers in its chemical structure (Fig. S1). Racemic VCD has a wide spectrum of anticonvulsant activity and its median effective dose (ED_{50}) values are 2–16 times (depending on the model) more potent than those of VPA.[2] VCD (65 mg/kg, i.p.) also provided full protection in the pilocarpine-induced status epilepticus (SE) rat model when administered at seizure onset. However, in contrast to its one-carbon homolog *sec*-butyl-propylacetamide (SPD), VCD lost its behavioral SE protection when administered (80 mg/kg) 30 min after seizure onset,[9] but it did block pilocarpine-induced electrographic SE at a higher dose (180 mg/kg).[10]

Racemic VCD, its corresponding acid VCA, and two of its individual stereoisomers, (2R,3S)-VCD and (2S,3S)-VCD, all failed to exert any significant teratogenic effect in Swiss-Vancouver (SWV)/Finn mice. This is an inbred mouse strain that has previously been shown to be highly susceptible to VPA-induced teratogenicity, following a single dose (600 mg/kg, i.p.) at day 8.5 of gestation.[11,12] VCD also inhibited human brain *myo*-inositol-1-phosphate (MIP) synthase at VPA clinically relevant concentrations (0.5–1 mM), indicating its potential in bipolar disorder.[13] Recently, a successful double-blind controlled phase IIa clinical trial with racemic VCD in patients with mania was completed.[14,15] This study showed that VCD could be an important substitute for VPA in women of child-bearing age with bipolar disorder.

Following a successful phase IIa study in patients with mania funded by the Stanley Medical Research Institute (SMRI),[14] teratogenicity studies were conducted (by the SMRI) comparing racemic VCD to VPA (head-to head) in mice, rats, and rabbits at Covance Laboratories. In these additional studies, VCD in contrast to VPA failed to demonstrate teratogenic potential in mice and rabbits. In rats, a modest teratogenic signal was observed at plasma concentrations 15 times higher than VCD therapeutic plasma levels.[16] Consequently, VCD is currently undergoing a 3-week SMRI-funded phase IIb, randomized double-blind multicenter study in 300 patients with bipolar manic episodes. The study is a three-arm monotherapy parallel group trial in which patients are randomized to placebo (n = 120), VCD 1,500 mg/day (n = 120), and risperidone, up to 6 mg/day (n = 60). The study's major objective is to evaluate the efficacy of VCD compared to placebo in patients with acute manic or mixed episodes. Risperidone is included as an active control to ascertain the trial's assay validity.[16]

All studies so far investigated racemic VCD and two of its four individual stereoisomers (2S,3S)-VCD and (2R,3S)-VCD. The current study comparatively investigates the anticonvulsant activity, teratogenicity, and PK of the two remaining VCD strereoisomers, (2S,3R)-VCD and (2R,3R)-VCD. The study also aims to pinpoint the most potent VCD individual stereoisomer and to explore if an individual stereoisomer might be better than racemic VCD, and thus serves after a chiral switch as its follow-up compound.[17,18]

Materials and Methods

Chemicals, reagents and animals

See Supporting Information.

Effect of VCD stereoisomers on lithium-pilocarpine–induced SE

Seizures were induced by systemic administration of pilocarpine HCl (50 mg/kg, i.p.); LiCl (20 mg/kg, i.p.) was administered 24 h prior to the pilocarpine dosing. Pilocarpine induces behavioral seizures within a few minutes, and those animals showing no seizures after 45 min of pilocarpine were removed from the study. At the time of the first stage 3 or higher (Racine scale) seizure, rats were randomized into two groups: pilocarpine alone or pilocarpine + an individual VCD stereoisomer. The latter group received the

individual VCD stereoisomer or the racemate at 0 or 30 min after the first stage 3 seizure. Animals were observed and scored for seizure severity for 1.5 h before being returned to their home cages. All animals were given 1 ml of 0.9% saline (oral) to compensate for the fluid loss induced by excessive cholinergic activation. Each VCD stereoisomer was dissolved in multisol: a solution of propylene glycol, alcohol, and water for injection 5:1:4.

The ED_{50} for anticonvulsant activity for the various VCD stereoisomers was determined by probit analysis.[19]

Effect of VCD on soman-induced SE

An established rodent models of nerve agent–induced SE were used: a rat HI-6 pretreatment model.[20,21] The model utilized a pretreatment and adjunctive drugs that counter the acute immediate lethal effects of the nerve agent without inhibiting the development of SE. In this model the challenge dose of soman is sufficient to elicit SE in all animals 5–8 min following soman challenge.

Subjects: Male Sprague–Dawley rats (Crl:DCBR VAF/Plus) from Charles River Labs (Wilmington, DE, U.S.A.), weighing 250–300 g upon receipt, served as subjects. The animals were housed individually in temperature- (21 ± 2°C) and humidity- (50 ± 10%) controlled environments and maintained on a 12-h light-dark full-spectrum lighting cycle with lights on at 06:00. Laboratory rat chow and tap water were freely available.

Each animal was anesthetized with isoflorane (5% induction; 3–1.5% maintenance, with oxygen) and placed in a stereotaxic instrument. Two stainless steel screws were placed in the skull bilaterally midway between bregma and lamda and approximately 3 mm lateral to the midline. A third screw was placed over the cerebellum. The screws were connected to a miniature connector with wires and the screws; wires and connector were then anchored to the skull with dental cement. The incision was sutured; the animal was removed from the frame, given the analgesic buprenorphine HCl (0.03 mg/kg, s.c.), and placed on a warming pad for at least 30 min before being returned to the animal quarters. Approximately 7 days elapsed between surgery and experimentation.

The animals were typically tested in groups of eight and were randomized among treatment cohorts each test day. The animals were weighed, placed in individual recording chambers, and connected to the recording apparatus. Electroencephalography (EEG) signals were recorded using CDE 1902 amplifiers and displayed on a computer running Spike2 software (Cambridge Electronic Design Ltd., Cambridge, United Kingdom). Baseline EEG was recorded for at least 20 min. The animals were then pretreated with 125 mg/kg, i.p., of the oxime HI-6 to prevent the rapid lethal effects of the soman challenge. Thirty minutes after pretreatment, the rats were challenged with 180 μg/kg, s.c., soman (1.6 × median lethal dose [LD_{50}]) and 1 min later treated with 2.0 mg/kg, i.m., atropine methyl nitrate to inhibit peripheral secretions. The rats were then closely monitored both visually and on EEG for seizure onset. Seizure onset was operationally defined as the appearance of >10 s of continuous rhythmic high amplitude spikes or sharp waves that were at least twice the baseline amplitude accompanied by a rhythmic bilateral flicking of the ears, facial clonus, and possibly forepaw clonus. The rats received standard medical countermeasures (0.1 mg/kg atropine sulfate + 25 mg/kg 2-pyridine aldoxime methyl chloride (2-PAM Cl) admixed to deliver 0.5 ml/kg, i.m., and 0.4 mg/kg im diazepam) at 5, 20, or 40 min after seizure onset and then were immediately given a dose (10–165 mg/kg, i.p.) of VCD dissolved in multisol (a solution of propylene glycol, alcohol, and water for injection 5:1:4). These standard medical countermeasures (atropine, 2-PAM, and in the rat model, diazepam), at the doses and times used, are insufficient by themselves to terminate soman-induced seizures in this model. The rats were monitored for at least 5 h after exposure and then returned to the animal housing room. Twenty-four hours after the exposure, surviving animals were weighed and the EEG was again recorded for at least 30 min. Evaluation and categorization of the EEG response by an individual animal to treatment were performed by a technician and investigator, both well-experienced with the appearance of nerve agent-induced EEG seizure activity. The overall rating and timing of different events required consensus between both individuals, who were aware of the treatment conditions of an individual animal. To be rated as having the seizure terminated, all spiking and/or rhythmic waves had to stop and the EEG had to remain normal for at least 60 min. For each animal in which the seizure was terminated, the latency to seizure termination was measured as the time from when the animal received VCD to the last observable epileptiform event in the EEG.

Teratogenic investigations of susceptibility to the induction of neural tube defect (NTD)

For this study, the highly inbred SWV/Fnn mouse strain with a known susceptibility to antiepileptic drug (AED)–induced NTDs[12,22] was used, according to the previously published procedure.[22] Dams were allowed to mate overnight with male mice, and were examined on the following morning for the presence of vaginal plugs. The onset of gestation was set at 10 p.m. of the previous night. On gestational day 8.5, pregnant females received a single intraperitoneal injection (10 μl per gram body weight) of sodium valproate at doses of2.7 or 1.8 mmol/kg, or VCD (racemate or its individual stereoisomers) at equimolar doses of 2.7 or 1.8 mmol/kg. A 25% Cremophor EL (purchased from Fluka, St. Louis MO, U.S.A.) water solution was injected intraperitoneally to dams constituting the control group. On gestation day 8.5, the dams were sacrificed by CO_2 asphyxiation, the abdomen opened, and the gravid uteri removed. The locations of all viable, dead, and resorbed fetuses were recorded, and the fetuses were grossly examined for the presence of exencephaly.

Pharmacokinetic studies

Analysis of VCD stereoisomers in plasma and calculation of pharmacokinetic (PK) parameters

See Supporting Information.

Results

Chemistry

The general synthesis of two individual stereoisomers (2R,3R)-VCD and (2S,3R)-VCD is depicted in Scheme S1 and detailed in the Supporting Information. The synthesized products were purified by crystallization. ^{1}H nuclear magnetic resonance (NMR) spectra of the synthesized compounds were measured in dimethyl sulfoxide using tetramethylsilane as an internal standard. Elemental analyses were performed for all the synthesized compounds.

Time to peak effects (TPEs) of VCD stereoisomers and determination of their median effective dose (ED_{50}) or behavioral toxic dose (TD_{50})

All quantitative in vivo anticonvulsant/behavioral toxicity studies were conducted at time to peak effect (TPE) previously determined in a qualitative analysis. The TPE of VCD strereoisomers was 0.25–0.5 and 0.5–2 h following intraperitoneal and oral administration, respectively. Groups of four to eight mice or rats were tested with various doses of VCD stereoisomers until at least two points were established between the limits of 100% protection or minimal toxicity and 0% protection or minimal toxicity. The dose of drug required to produce the desired end point in 50% of animals (ED_{50} or TD_{50}) in each test; the 95% confidence interval (CI), the slope of the regression line, and the standard error of the mean (SEM) of the slope were then calculated by a computer program based on the method described by Finney.[23]

Anticonvulsant efficacy of VCD stereoisomers in seizure and epilepsy rodent models

The anticonvulsant activity of the individual VCD stereoisomers (in comparison to racemic VCD) is depicted in Table 1. In mice the four VCD individual stereoisomers exhibited similar anticonvulsant activity to one another as well as to racemic VCD in the subcutaneous Metrazol-induced seizure test (scMet) and 6 Hz tests, whereas at the MES test (2R,3R)-VCD was more potent than its enantiomer (2S,3S)-VCD and diastereoisomer (2S,3R)-VCD. In rats (p.o.) racemic VCD and its four individual stereoisomers exhibited similar anticonvulsant activity in the MES test, whereas (2R,3S)-VCD was the most potent compound evaluated by the scMet test with an ED_{50} value 5 times more potent than the racemate.

VCD stereoisomers block convulsive seizures induced by the cholinergic agonist, pilocarpine

Administration of lithium-pilocarpine induces SE characterized by convulsive and nonconvulsive seizures that can last for several hours. From a behavioral perspective, the number and severity of the observed convulsive seizures following pilocarpine administration were similar in the two treatment groups (pilocarpine alone and pilocarpine + VCD racemate/stereoisomer). The first con-

Table 1. Anticonvulsant activity and neurotoxicity of VCD (racemate) and its four individual stereoisomers following intraperitoneal administration to mice and intraperitoneal or oral administration to rats

	ED_{50} (95% confidence interval) (mg/kg)				
Anticonvulsant test	VCD (racemate)	(2R,3S)-VCD	(2S,3S)-VCD	(2S,3R)-VCD	(2R,3R)-VCD
Mice-i.p. administration					
Maximal electroshock seizure (MES)	125 (102–143)	119 (98–147)	132 (117–149)	144 (138–151)	105 (101–112)
Metrazol-induced seizure (scMet)	66 (50–74)	67 (60–74)	69 (63–72)	79 (63–94)	71 (63–81)
6 Hz – 22 mA		19 (15–25)	25 (15–40)		
6 Hz – 32 mA	37 (26–50)	48 (32–62)	33 (23–45)	40 (27–60)	59 (36–81)
6 Hz – 44 mA	67 (61–72)	67 (54–79)	80 (62–105)		64 (58–74)
Neurotoxicity (TD_{50})	164 (149–180)	127 (113–143)	128 (108–155)	170 (164–176) 152 (0.25 h)	143 (129–164) 164 (164–186)
Rats-i.p. administration					
Pilocarpine-induced status (0 min)	40 (30–65) ED_{97} = 86 (55–214)	39 (31–80) ED_{97} = 59 (48–131)	<65	82 (55–110) ED_{97} = 201 (131–1, 144)	68 (23–84) ED_{97} = 148 (109–998)
Pilocarpine-induced status (30 min)	No protection at 80 mg/kg	No protection at 75 mg/kg	No protection at 75 mg/kg	No protection at 100 mg/kg	
Rats-p.o. administration					
Maximal electroshock seizure (MES)	29 (19–38)	34 (14–79)	64 (43–90)	95 (56–163)	48 (29–87)
Metrazol-induced seizure (scMet)	54 (46–63)	11 (4–28)	33 (28–39)	27 (16–40)	41 (29–53)
Neurotoxicity (TD_{50})	58 (47–66)	123 (102–234)	194 (165–239)	92 (79–104)	75 (57–96)

vulsive stage 3 (marking the SE onset) or greater seizure was observed 12 min after pilocarpine administration and lasted for approximately 60 s. Within the succeeding 30 min, rats were observed to have 4.9 ± 0.2 seizures, with an interseizure interval of 3–5 min.[9] Racemic VCD, as well as (2R,3S)-VCD and (2S,3S)-VCD, administered at seizure onset blocked the SE with similar ED_{50} values of about 40 mg/kg. However racemic VCD and three of its individual strereoisomers lost this anti-SE activity when given (75–100 mg/kg) 30 min after seizure onset (Table 1).

VCD blocks electrographic and convulsive seizures induced by the organophosphate soman

Racemic VCD dissolved in multisol was administered at various doses along with the standard medical countermeasures at treatment delays of 5, 20, and 40 min after the onset of soman-induced seizures to determine effective dose for termination of electrographic seizures as described previously for racemic SPD.[9] Racemic VCD (dissolved in multisol) administered with the standard medical countermeasures at treatment delays of 5, 20, and 40 min after seizure onset was capable of stopping soman-induced SE seizures with ED_{50} values of 26, 60, and 62 mg/kg, respectively (Fig. 2). Following administration of VCD the average latency (seconds) for electrographic seizure termination (mean ± SEM) at 5, 20, and 40 min was: 115 ± 15, 497 ± 15, and 1,336 ± 318, respectively (Fig. 3). VCD is one of the few drugs effective at 40 min delay in the soman-induced SE model.

Pharmacokinetics of VCD stereoisomers in rats

The PK of VCD stereoisomers was studied following intraperitoneal administration (70 mg/kg) to rats. The dose was chosen for the PK study was the intermediate dose among the various ED_{50} values of VCD stereoisomers. The plasma concentration–time plots of VCD individual stereoisomers are presented in Figure 4. The PK parameters of VCD four individual stereoisomers, calculated by noncompartmental analysis, are summarized in Table S1. VCD had a stereoselective PK, with (2S,3S)-VCD exhibiting the lowest clearance, and consequently a twice-higher plasma exposure (area under the curve, AUC) than all other stereoisomers. The apparent volume of distribution and half-life of VCD's four individual stereoisomers were similar and ranged between 1–1.6 L/kg and 2.1–3 h, respectively (Table S1).

Teratogenicity

The teratogenic potential of racemic VCD and its four individual stereoisomers was assessed for their ability to induce gross morphologic defects in the SWV/Fnn mice that are highly susceptible to VPA-induced exencephaly. VPA, at a dose of 2.7 mmol/kg was embryotoxic and teratogenic, causing an almost twofold increase in the resorption rate compared to the control group (11.9% vs. 6.3%, respectively) and NTDs in 29.1% of live fetuses. At a lower dose of 1.8 mmol/kg VPA was still embryotoxic (13.6% of resorptions), but the number of fetuses with exencephaly (2) was not statistically significant. In contrast to VPA, racemic VCD and its four individual stereoisomers did not cause a statistically significant increase of NTDs at doses of 257 or 389 mg/kg (Table 2). These doses are 3–12 times higher than VCD anticonvulsant ED_{50} values. (2S,3S)-VCD, (2R,3S)-VCD, and racemic-VCD were embryotoxic and induced resorptions in 16%, 23%, and 21% of conceptions, respectively, when tested at the higher 2.7 mmol/kg dose (Table 2).

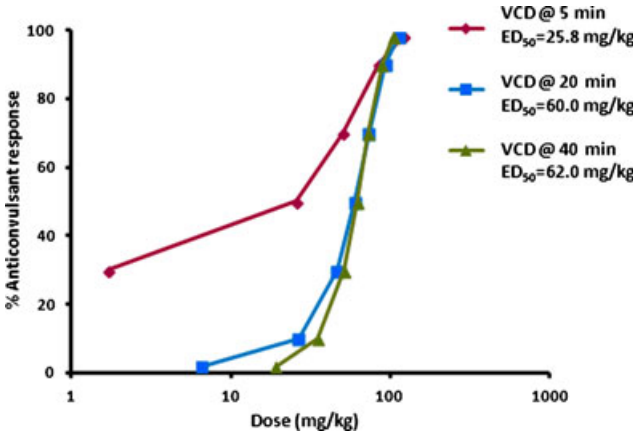

Figure 2.
Anticonvulsant dose–response curve of VCD (racemate) administered at 5, 20, and 40 min after onset of soman-induced (electrographic) status epilepticus (SE) in rats.
Epilepsia © ILAE

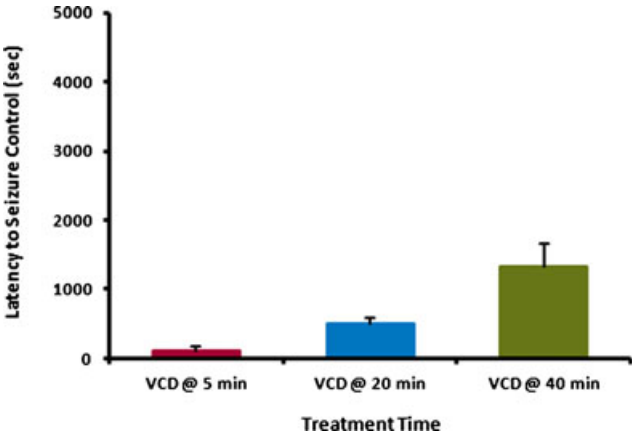

Figure 3.
Latency (mean and SEM) for seizure control —the time from when racemic VCD was administered to rats 5, 20, and 40 min after seizure onset until the last epileptiform event could be detected on the EEG record.
Epilepsia © ILAE

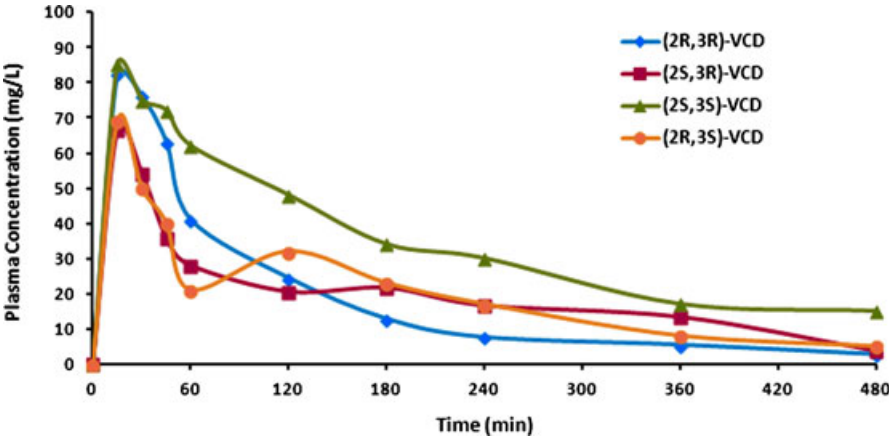

Figure 4.
Plasma concentration–time plots of the four VCD individual stereoisomers obtained following intraperitoneal administration of 70 mg/kg (of each compound) to rats. [The data on (2R,3S)-VCD and (2S,3S)-VCD are taken from Kaufmann et al.[11]].
Epilepsia © ILAE

Table 2. Teratogenic effect in the SWV mouse model of VCD and its stereoisomers

Compound	Dose mg/kg (mmol/kg)	No. of litters	No. of implants	No. of resorptions (%)	No. of live fetuses (%)	No. of normal fetuses (%)	No. of fetuses with NTDs (%)
Control	25% CEL	14	207	13 (6.3)	194 (93.7)	194 (100)	0
Na-VPA[a]	452 (2.7)	13	160	19 (11.9)[b]	141 (88.1)	100 (70.9)	41 (29.1)[b]
Na-VPA[a]	301 (1.8)	12	154	21 (13.6)[b]	133 (86.4)	131 (98.5)	2 (1.5)
VCD[a]	389 (2.7)	9	119	25 (21.0)[b]	94 (79.0)	94 (100.0)	0[c]
VCD[a]	257 (1.8)	10	132	5 (3.8)[c]	127 (96.2)	126 (99.2)	1 (0.8)
(2S,3S)-VCD	389 (2.7)	22	301	50 (16.6)[b]	251 (83.4)	248 (98.8)	3 (1.2)[c]
(2S,3S)-VCD	257 (1.8)	19	244	15 (6.1)[c]	229 (93.9)	229 (100.0)	0
(2R,3S)-VCD[a]	389 (2.7)	10	124	29 (23.4)[b,c]	95 (76.6)	95 (100.0)	0[c]
(2R,3S)-VCD[a]	257 (1.8)	9	113	8 (7.1)	105 (92.9)	104 (99.0)	1 (1.0)
(2S,3R)-VCD	387 (2.7)	10	148	12 (8.1)	136 (91.9)	136 (100)	0[c]
(2S,3R)-VCD	258 (1.8)	10	141	10 (7.1)	131 (92.9)	131 (100)	0
(2R,3R)-VCD	387 (2.7)	10	137	12 (8.8)	125 (91.2)	124 (99.2)	1 (0.8)[c]
(2R,3R)-VCD	258 (1.8)	12	167	12 (7.2)	155 (92.8)	155 (100)	0

[a]Results from Kaufmann et al. [11].
[b]Significantly different when compared to the control group.
[c]Significantly different when compared to group treated with equimolar dose of VPA.

Discussion

VCD stereoisomers activity in anticonvulsant animal models

In the search for novel AEDs, the present study describes the broad-spectrum anticonvulsant activity of VCD four stereoisomers. All four VCD stereoisomers possess a similar broad-spectrum anticonvulsant profile. The anticonvulsant activity of VCD stereoisomers was equivalent to that of SPD stereoisomers in mice (i.p.) and rats (p.o.).[24] VCD (racemate) and its individual stereoisomers were 4–16 times more potent than VPA in a wide array of anticonvulsant animal models.[25]

At present, little can be said about the molecular mechanism through which VCD stereoisomers exert their anticonvulsant activity. These results would support the conclusion that VCD exerts its effects through an ability to prevent seizure spread and elevate seizure threshold. This conclusion is based on the marked effect exerted by VCD stereoisomers in the rat-MES test (seizure spread) and its ability to elevate seizure threshold in the scMet seizure model. VPA is a major AED with multiple mechanisms of action (MOAs) that contribute to its activity in bipolar disorder.[26,27] As an amide derivative of VPA it is likely that VCD has multiple MOAs.

Anticonvulsant effects of VCD in animal models of SE

SE is initially treated with a benzodiazepines such as diazepam or lorazepam; these are effective when given early after SE onset. However, the benzodiazepines lose their efficacy when given >20 min after SE onset, and animals that experience prolonged SE quickly develop pharmacoresistant SE if treatment is not initiated within a short period after seizure onset.[28]

Racemic VCD and two VCD stereoisomers, (2S,3S)-VCD and (2R,3S)-VCD, were found to be a highly effective antiseizure drugs in the lithium-pilocarpine–induced SE model when given at seizure onset. However, in contrast to

SPD, which has an ED_{50} value of 84 mg/kg,[9] racemic VCD (80 mg/kg) and three of its individual stereoisomers lost their activity in behavioral SE when administered 30 min after pilocarpine-induced SE onset. At electrographic SE (ESE), racemic VCD was found to be less potent than SPD. VCD (180 mg/kg) stopped ESE when given 30 min after seizure onset, whereas SPD (180 mg/kg) stopped ESE at 60 min. At 30 min, SPD stopped ESE at a lower dose of 130 mg/kg.[10]

In contrast to the pilocarpine-induced SE model, racemic VCD exhibited a potent activity in the soman-induced SE model when administered at 20 and 40 min after onset of electrographic seizures in rats (Figs. 2 and 3). This unique activity in eliminating ESE is not shared by benzodiazepines or other AEDs. In this soman-induced SE model, VCD activity (ED_{50} = 60 mg/kg and ED_{50} = 62 mg/kg at 20 and 40 min, respectively) was equipotent to that of SPD (ED_{50} = 71 mg/kg and ED_{50} = 72 mg/kg at 20 and 40 min, respectively).[9,25] Unlike VCD, which is active only at the soman-induced SE model, SPD (racemate and its individual stereoisomers) is active in both the pilocarpine-induced and soman-induced SE models.[24] VCD and SPD are two of the few drugs effective at 40 min delay in the soman-induced SE model.

PK analysis of VCD stereoisomers

VCD had a stereoselective PK, with (2S,3S)-VCD exhibiting the lowest clearance and consequently twofold elevated plasma exposure (AUC) than all other stereoisomers. Nervertheless, there was no stereoselectivity in VCD anticonvulsant activity and each stereoisomer had similar ED_{50} values as did the racemic VCD.

VCD is primarily eliminated by metabolism and its blood-to-plasma ratio is 1 (unity).[29,30] Rat liver blood flow (Q) is 60–70 ml/min/kg.[31] Assuming that VCD metabolism is mainly hepatic, then the liver extraction ratio (E = CL/Q = CLm/Q) of the various VCD stereoisomers ranges between 5% [(2S,3S)-VCD] and 12% [(2S,3R)-VCD]. A similar E-value (E = 5%) was previously calculated for racemic VCD.[30] If these rat data can be extrapolated to humans, it is suggestive that each VCD individual stereoisomer will not be susceptible to hepatic first-pass effect after oral dosing, which was the case when racemic VCD was given orally to humans.[1,4] The clearance (CL/F) of the VCD individual stereoisomers was 6–10 times lower than those of SPD stereoisomers (Fig. 1). Therefore, the addition of one carbon to the VCD molecule has a profound effect on the clearance of the two homologous compounds VCD and SPD.[24]

Similar to the current rat study, in humans following oral administration of racemic VCD (400 mg) (2S,3R)-VCD had a clearance (CL/F) value twice higher that of all other VCD individual stereoisomers.[1] Stereoselective PK analysis across species of racemic VCD showed that following intravenous administration of racemic VCD to dogs (20 mg/kg) and rats (74 mg/kg) or mice (300 mg/kg, i.p.) there was a nonsignificant difference in the CL values of VCD individual stereoisomers.[32] Clearance (CL) values of VCD stereoisomers following intravenous administration to rats were similar to those found in the current study, indicating that VCD is completely absorbed following intraperitoneal administration to rats.

Lack of teratogenicity of VCD and its individual stereoisomers

Because most if not all commercially available AEDs are teratogenic, it is essential to develop new AEDs that are nonteratogenic.[33] VCD (racemate) and its four individual stereoisomers did not induce statistically significant increases in neural tube defects at doses 3–12 times higher than their anticonvulsant ED_{50} values (Tables 1 and 2). The lack of teratogenicity of racemic VCD, (2S,3S)-VCD, and (2R,3S)-VCD was previously reported.[11] Herein we demonstrate that all VCD stereoisomers failed to induce NTD in SWV mice at doses (2.7 mmol/kg = 389 mg/kg) that were higher than the nonteratogenic dose (1.8 mmol/kg) of SPD and its stereoisomers.[24] In contrast to VPA its constitutional isomer (and VCD corresponding acid) VCA was nonteratogenic and had a profile similar to that of VCD.[11] A similar nonteratogenic profile was observed with VCD's constitutional isomer propylisopropylacetamide (PID) and its two individual enantiomers.[34] Therefore, VCD and its stereoisomers are superior to VPA not only by exhibiting a more potent anticonvulsant activity but also by their reproductive safety.

These data coupled with a head-to-head comparison of the teratogenicity between VCD (racemate) and VPA conducted following multiple dosing in mice, rats, and rabbits[16] show that in contrast to VPA, VCD has a low teratogenic potential. Therefore, unlike VPA, which is a pregnancy category D, VCD may receive category B.[35] This is also acknowledged in a recent VCD phase IIa paper entitled: "Valnoctamide as a valproate substitute with low teratogenic potential in mania: …."[14]

Stereoselective pharmacokinetics (PK) and pharmacodynamics (PD)

Binding of a racemic drug (e.g., VCD, SPD) to the molecular targets that lead to its CNS activity (e.g., ion channel, a receptor, or an enzyme) may be stereospecific, and consequently individual stereoisomers that may display distinguished PK behavior could lead to stereoselective PD.[17,18,36] Consequently, consideration of chirality should be implemented into PK and PD studies.

The FDA's 1992 policy "Statement for Development of the New Stereoisomeric Drugs" triggered the development of single individual stereoisomers instead of racemates.[37] This policy coupled with marketing incentives of further profitability as a "line extension" has encouraged companies to look for chiral switches of established chiral drugs

that were first introduced to the market as racemic mixtures.[17,18]

In the current study some stereoselectivity was observed in the mice-MES test, where (2R,3R)-VCD was more potent than (2R,3S)-VCD and (2S,3S)-VCD. In the rat-MES test, (2R,3S)-VCD had a more potent ED_{50} value (34 mg/kg) than its diastereoisomer (2S,3S)-VCD (64 mg/kg).[11] In the rat-scMet test, (2R,3S)-VCD was the most potent stereoisomer.

Bialer et al.[38] demonstrated a correlation between the anticonvulsant-ED_{50} values in mice and rats of various AEDs and their dose and therapeutic average steady-state plasma concentrations in patients with epilepsy. This analysis shows that VPA is the least potent AED in anticonvulsant tests and thus VCD stereoisomers that are 4–16 times more potent than VPA in various animal models[25] may be more potent in patients. The comparative analysis among various AEDs may also be useful for estimating target concentration range in humans of new AED candidates.[38]

Conclusions

VCD had a stereoselective PK, with (2S,3S)-VCD exhibiting the lowest clearance, and consequently a twofold higher plasma exposure than all other stereoisomers. Nevertheless, there was less stereoselectivity in VCD anticonvulsant activity and each stereoisomer had similar ED_{50} values in most models. VCD stereoisomers (258 or 389 mg/kg) did not cause NTD at doses that are 3–12 times higher than VCD anticonvulsant ED_{50} values.

If VCD would exert its broad-spectrum anticonvulsant activity due to a single MOA, it is likely that it would exhibit stereoselective PD. The fact that there was no significant difference between racemic VCD and its individual stereoisomers in most of the anticonvulsant rodent models (except the rat-scMet test) may indicate that VCD anticonvulsant activity is due to multiple MOAs. The choice for further drug development between racemic VCD and one of its individual stereoisomers will be based on comparative toxicologic analysis and additional anticonvulsant testing that will discriminate between these five CNS-active compounds.

Acknowledgment

This study was supported by an unrestricted research grant (#039-4489 and #039-4576) from Israel Ministry of Defense, Medical Branch, Nuclear Biological Chemical (NBC) Protection Division and by an Inter-Agency Agreement between NIH/NINDS (Y1-O6-9613-01) and USAMRICD (A120-B.P2009-2). This work is abstracted from the PhD thesis of Mr. Tawfeeq Shekh-Ahmad in a partial fulfillment for the requirements of a PhD degree at The Hebrew University of Jerusalem. The authors wish to thank Drs. John H. Kehne and Tracy Chen of the NIH–NINDS-Anticonvulsant Screening Program (ASP) for testing the compounds in the ASP. The authors also wish to acknowledge the technical assistance of the faculty and staff at the University of Utah Anticonvulsant Drug Development (ADD) Program who conducted the in vivo anticonvulsant testing, pilocarpine SE, and in vitro slice studies described in this manuscript. The anticonvulsant studies were supported by NINDS, NIH Contract No. NO1-NS-4-2359. The views expressed in this report are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the U.S. Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996) and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

Disclosure of Conflict of Interest

Dr. Meir Bialer has received in the last 3 years speakers' or consultancy fees from Bial, CTS Chemicals, Desitin, Janssen-Cilag, Johnson & Johnson, Medgenics, Rekah, Sepracor, Teva, UCB Pharma, and Upsher-Smith. Dr. Bialer has been involved in the design and development of new antiepileptics and CNS drugs as well as new formulations of existing drugs. None of the other authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

1. Barel S, Yagen B, Schurig V, et al. Stereoselective pharmacokinetic analysis of valnoctamide in healthy subjects and in patients with epilepsy. *Clin Pharmacol Ther* 1997;61:442–449.
2. Bialer M, Yagen B. Valproic acid–second generation. *Neurotherapeutics* 2007;4:130–137.
3. Bialer M. Chemical properties of antiepileptic drugs (AEDs). *Adv Drug Deliv Rev* 2012;64:887–895.
4. Bialer M, Haj-Yehia A, Barzaghi N, et al. Pharmacokinetics of a valpromide isomer, valnoctamide, in healthy subjects. *Eur J Clin Pharmacol* 1990;38:289–291.
5. Bialer M. Clinical pharmacology of valpromide. *Clin Pharmacokinet* 1991;20:114–122.
6. Bialer M, White HS. Key factors in the discovery and development of new antiepileptic drugs (AEDs). *Nat Rev Drug Discov* 2010;9:68–83.
7. Melissant CF, Vinks AATM, Sleeboom HP. Coma due to overdose of valnoctamide. *Ned Tijdschr Geneeskd [Dutch Med J]* 1992;132:793–794.
8. Stepansky W. A clinical study in the use of valmethamide, an anxiety-reducing drug. *Curr Ther Res* 1960;2:144–147.
9. White HS, Alex AB, Pollock A, et al. A new derivative of valproic acid amide possesses a broad-spectrum antiseizure profile and unique activity against status epilepticus and organophosphate neuronal damage. *Epilepsia* 2012;53:134–146.
10. Pouliot W, Bialer M, Hen N, et al. Electrographic analysis of the effect of sec-butyl-propylacetamide on pharmacoresistant status epilepticus. *Neuroscience* 2013;231:145–156.
11. Kaufmann D, Yagen B, Minert A, et al. Evaluation of the antiallodynic, teratogenic and pharmacokinetic profile of stereoisomers of valnoctamide, an amide derivative of a chiral isomer of valproic acid. *Neuropharmacology* 2010;52:1228–1236.
12. Finnell RH, Bennett GD, Karras SB, et al. Common hierarchies of susceptibility to the induction of neural tube defects in mouse embryos by valproic acid and its 4-propyl-4-pentenoic acid metabolite. *Teratology* 1988;38:313–320.
13. Shalitiel G, Mark S, Kofman O, et al. Effect of valproate derivatives on human brain-myo-inositol-1-phosphate (MIP) synthase activity and amphetamine-induced rearing. *Pharmacol Rep* 2007;59:402–407.
14. Bersudsky Y, Applebaum J, Gaiduk Y, et al. Valnoctamide as valproate substitute with low teratogenic potential in mania: double blind controlled clinical trial. *Bipolar Disord* 2010;12:376–382.
15. Bialer M, Johannessen SI, Levy RH, et al. Progress report on new antiepileptic drugs: a summary of the Tenth Eilat Conference (EILAT X). *Epilepsy Res* 2010;92:89–124.
16. Bialer M, Johannessen SI, Levy RH, et al. Progress report on new antiepileptic drugs: a summary of the Eleventh Eilat Conference (EILAT XI). *Epilepsy Res* 2013;103:2–30.

17. Agranat I, Caner H, Caldwell J. Putting chirality to work: the strategy of chiral switches. *Nat Rev Drug Discov* 2002;1:753–768.
18. Tucker GT. Chiral switches. *Lancet* 2003;55:1085–1087.
19. Bliss C. The statistics of bioassay with special reference to the vitamins. In Gyorgy P (Ed.) *Vitamin methods*. New York: Academic Press, 1952:445–628.
20. McDonough JH, Shih TM. Pharmacological modulation of soman-induced seizures. *Neurosci Biobehav Rev* 1991;17:203–215.
21. Shih TM, McDonough JH. Neurochemical mechanisms in soman-induced seizures. *J Appl Toxicol* 1991;17:255–264.
22. Finnell RH, Bielec B, Nau H. Anticonvulsant drugs: mechanisms and pathogenesis of teratogenicity. In Kavlock R, Daston G (Eds.) *Handbook of experimental pharmacology 124 (drug toxicity in embryonic development II)*. Berlin: Springer-Verlag, 1997:121–159.
23. Finney DJ. Statistical logic in the monitoring of reactions to therapeutic drugs. *Methods Inf Med* 1971;10:237–245.
24. Hen N, Shekh-Ahmad T, Yagen B, et al. Stereoselective pharmacoydnamic and pharmacokinetic analysis of sec-propyl-butylacetamide (SPD), a new CNS-active derivative of valproic acid with unique activity against status epilepticus. *J Med Chem* 2013;56:6467–6477.
25. Shekh-Ahmad T, Hen N, Yagen B, et al. Valnoctamide and sec-propyl-butylacetamide (SPD) for acute seizures and status epielpticus. *Epilepsia* 2013;54(Suppl. 5):98–101.
26. Nalivaeva NN, Belyaev NCD, Turner AJ. Sodium valproate: an old drugs with new roles. *Trends Pharmacol Sci* 2009;30:509–514.
27. Bialer M. Why are antiepileptic drugs used for nonepileptic conditions? *Epilepsia* 2012;50(Suppl. 7):26–33.
28. Jones DM, Esmaeil N, Maren S, et al. Characterization of pharmacoresistance to benzodiazepines in the rat Li-pilocarpine model of status epilepticus. *Epilepsy Res* 2002;50:301–312.
29. Haj-Yehia A, Bialer M. Pharmacokinetics of a valpromide isomer, valnoctamide, in dogs. *J Pharm Sci* 1988;77:831–834.
30. Blotnik S, Bergman F, Bialer M. The disposition of valpromide, valproic acid and valnoctamide in brain, liver, plasma and urine of rats. *Drug Metab Dispos* 1996;24:560–564.
31. Altman PL, Dittmer DS. *Biology data book*. 2nd Ed., Vol. 3. Bethesda, MD: Federation of American Societies for Experimental Biology; 1974:1702–1710.
32. Spiegelman O, Yagen B, Bennett GD, et al. Strereoselective pharmacokinetics of valnoctamide, a CNS-active chiral amide analogue of valproic acid in dogs, rats and mice. *Ther Drug Monit* 2000;22:574–581.
33. Meador KJ. Effect of in-utero antiepileptic drug exposure. *Epilepsy Curr* 2009;8:144–147.
34. Spiegelman O, Yagen B, Levy RH, et al. Strereoselective pharmacokinetics and pharmacodynamics of propylisopropyl acetamide - a CNS-active chiral amide derivative of valproic acid. *Pharm Res* 1999;16:1582–1588.
35. FDA-Federal Register. Content and format of labeling for human prescription drug and biological products: requirements for pregnancy and lactation labeling. *Fed Regist* 2008;73:30381–30837.
36. Levy RH, Boddy AV. Stereoselectivity in pharmacokinetics: a general theory. *Pharm Res* 1991;8:551–555.
37. Hubbard WK. FDA policy on period of marketing exclusivity for newly approved drug products with enantiomer active ingredients; request for comments. *Fed Regist* 1997;62:2167–2169.
38. Bialer M, Twyman RE, White HS. Correlation analysis between anticonvulsant ED_{50} values of antiepileptic drugs in mice and rats and their therapeutic doses and plasma levels. *Epilepsy Behav* 2004;5:866–872.

Supporting Information

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

Figure S1. Chemical structures of VCD and its four individual stereoisomers.

Scheme S1. Total stereoselective synthesis of (2R,3R)-VCD and (2S,3R)-VCD.

Table S1. PK parameters of VCD stereoisomers calculated following i.p. administration (70 mg/kg) of each individual stereoisomer to rats.