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RESEARCH ARTICLE

Efficacy and safety of cannabidivarin treatment of epilepsy in girls with Rett syndrome: A phase 1 clinical trial

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Summary

Objective: Rett syndrome (RTT), commonly caused by methyl-CpG-binding protein 2 (*MECP2*) pathogenic variants, has many comorbidities. Fifty to ninety percent of children with RTT have epilepsy, which is often drug-resistant. Cannabidivarin (CBDV), a non-hallucinogenic phytocannabinoid, has shown benefit in *MECP2* animal models. This phase 1 trial assessed the safety and tolerability of CBDV in female children with RTT and drug-resistant epilepsy, as well as the effect on mean monthly seizure frequency (MMSF), the electroencephalogram (EEG), and non-epilepsy comorbid symptoms.

Methods: Five female children with drug-resistant epilepsy and a pathogenic *MECP2* variant were enrolled. Baseline clinical and laboratory assessments, including monthly seizure frequency, were recorded. CBDV oral solution (50 mg/ml) was prescribed and titrated to 10 mg/kg/day. Data collected included pharmacokinetics, seizure type and frequency, adverse events, EEG, and responses to the Rett Syndrome Behaviour Questionnaire and Rett Syndrome Symptom Severity Index, and were compared to baseline data.

Results: All five children reached the maximum CBDV dose of 10 mg/kg/day and had a reduction in MMSF (median = 79% reduction). Three children had MMSF reduction > 75%. This corresponded to an overall reduction in seizure frequency from 32 to 7.2 seizures per month. Ninety-one percent of adverse events were mild or moderate, and none required drug withdrawal. Sixty-two percent were judged to be unrelated to CBDV. Thirty-one percent of adverse events were identified as possibly related, of which nearly all were mild, and the remainder were later assessed as RTT symptoms. Hypersomnolence and drooling were identified as related to CBDV. No serious adverse events reported were related to CBDV. No significant change was noted in EEG or non-epilepsy-related symptoms of RTT.

Significance: A dose of 10 mg/kg/day of CBDV is safe and well tolerated in a pediatric RTT cohort and suggests improved seizure control in children with *MECP2*-related RTT.

KEYWORDS

antiseizure, CBDV, epilepsy, pediatric, pharmacokinetics

1 | INTRODUCTION

Rett syndrome (RTT), an X-linked dominant neurodevelopmental condition, affects one in 10 000 female births.[1–3] In 90%–97% of classical RTT,[4,5] and 50%–70% of atypical RTT,[1,4,5] there are de novo, and rarely, familial variants[6] in the methyl-CpG-binding protein 2 (*MECP2*) gene. Diagnostic criteria for classical and atypical RTT have evolved over time.[1,6,7] Diagnosis of classical RTT relies on normal development during the first year of life followed by a period of motor and speech regression, then stabilization. Significant medical and behavioral comorbidities are described,[7,8] including breathing dysregulation, scoliosis, sleep disturbance, and autistic behaviors.[5] The symptomology of RTT has a large impact on a child and their family,[8,9] particularly as many comorbidities have no proven treatment strategies.[5]

Epilepsy is debilitating and is reported in 50%–90% of RTT.[3,6,10] Eighty percent present with epilepsy before 8 years of age, commonly in the 2–5-year group.[11] The genotype–phenotype correlation of epilepsy in RTT is controversial.[10] Large deletions, early truncation, and missense variants[11] of the *MECP2* gene are associated with early, severe epilepsy, whereas late truncating or C-terminal deletions demonstrate a protective effect.[6] Focal seizures are most commonly reported (47.6%), followed by generalized tonic–clonic seizures (GTCS; 18.3%),[11] and other seizure types. The electroencephalogram (EEG) in RTT is initially normal, with abnormality developing progressively in stages,[7,12] often preceding the onset of seizures.[13]

Approximately half of children with RTT and epilepsy are managed on monotherapy, although this falls to 33% in adolescents, and 32% are considered drug-resistant.[10,11] Sodium valproate, carbamazepine, and lamotrigine are reported to be the most efficacious antiseizure medications.[3,6] Other reported treatments include levetiracetam,[6] topiramate,[6] ketogenic diet,[6,14] and vagus nerve stimulation.[6]

Cannabidivarin (CBDV) is a propyl analogue of cannabidiol, a non-hallucinogenic phytocannabinoid.[15,16] CBDV has poor oral bioavailability but is liposoluble and easily crosses the blood–brain barrier.[15] CBDV has a weak affinity for CB_1 receptors and therefore appears to lack the psychotropic effects seen with tetrahydrocannabinol (THC).[17] The antiseizure mechanism of CBDV is inferred from in vitro receptor binding and functional studies, with limited in vivo testing in epilepsy models. CBDV may exert antagonistic effects on G-protein-coupled receptor 55 and its signaling via inhibition of lysophosphatidylinositol,[14,18] as well as agonistic but rapidly desensitizing effects on transient receptor potential channels, vanilloid subtype, members 1 and 2 and transient receptor potential cation channel, subfamily A, member 1 channels.[15,19–21] Together, the pharmacological actions of CBDV may lead to a reduction in intracellular calcium and reduced neuronal excitability. CBDV also has antagonistic effects on diacylglycerol lipase-alpha and thus 2-arachidononyl glycerol,[22,23] and transient receptor potential cation channel, subfamily M, member 8 channels, and may have a role in reducing γ-aminobutyric acid (GABA) current rundown through $GABA_A$ receptors.[14–16]

Key Points

- Cannabidivarin (CBDV) dosing at 10 mg/kg/day is safe and tolerated in a pediatric RTT population
- CBDV reduced mean monthly seizure frequency in females with RTT and drug-resistant epilepsy
- Hypersomnolence and drooling were the only identified drug-related adverse events
- Larger trials of CBDV in the pediatric population with drug-resistant epilepsy should be considered

Despite growing interest in CBDV in several conditions,[24–26] there remains limited clinical data on CBDV in epilepsy, particularly in the pediatric cohort. Animal models have demonstrated an antiseizure effect and a favorable safety profile.[19,27] *MECP2*-308 mouse models treated with CBDV demonstrated improved sociability, brain weight, and overall health and partial improvement in motor function.[25,28] In an adult focal epilepsy cohort, Brodie et al.[29] demonstrated safety in humans and observed a 41% reduction in seizure frequency with CBDV. However, a large reduction in the placebo arm (38%) limited the significance of these findings.

This phase 1 trial aimed to determine the safety and tolerability of CBDV in pediatric RTT patients and drug-resistant epilepsy. Secondary endpoints included the change in mean monthly seizure frequency (MMSF), effect on spike-wave changes on EEG, and change in RTT symptom scores.

2 | MATERIALS AND METHODS

This investigator-initiated phase 1 dose-finding escalation study was undertaken at the Sydney Children's Hospital, Randwick from February 2019 until August 2020. The CBDV, sourced from GW Pharmaceuticals, was a plant-based oil extract of *Cannabis sativa*, containing 50 mg/ml of CBDV and <0.2% wt/wt THC. Patients were recruited over a 6 month period, with the aim of enrolling six to 14 children.

Female children with a confirmed pathogenic variant of the *MECP2* gene and drug-resistant epilepsy, aged at least 6 years and with a minimum weight of 10 kg, were eligible for enrollment (Table 1). Drug-resistant epilepsy was defined as the failure of at least two standard antiseizure drugs,[30] and at least four quantifiable seizures in the 8 weeks prior to screening (with at least two in the baseline period). All patient medication and nonpharmacologic interventions needed to be stable for the 4 weeks prior to screening.

Exclusion criteria (Table 1) included coexisting neurological or non-neurological medical diagnosis that may compromise participation or interpretation of results. Significant abnormality in baseline screening investigations, the use of more than four antiseizure medications, felbamate use within 12 months, cannabis or cannabinoid product use in the past 3 months, pregnancy, and allergy to CBDV or its excipients were also exclusionary.

Patients underwent baseline screening with collection of medical history, medications, clinical examination, vital signs, hematology, biochemistry, urinalysis, urine/serum THC screen, pregnancy test, and an electrocardiogram (ECG). Patients meeting eligibility criteria underwent a 28-day observation period utilizing a paper patient diary recording seizure frequency, concomitant medications, and adverse events, with an EEG and repeat baseline investigations. Additionally, baseline parental self-reporting questionnaires were completed: Rett Syndrome Symptom Severity Index[31] and Rett Syndrome Behaviour Questionnaire (RSBQ).[32]

Patients were commenced on 2.5 mg/kg/day orally of CBDV delivered in bi-daily dosing and, if no adverse effects were reported, this was increased by 2.5 mg/kg/day every 7 days up to a maximum dose of 10 mg/kg/day, as GW Pharmaceuticals determined that doses greater than 10 mg/kg/day were associated with increased somnolence. During dose escalation, patients had weekly review and phone calls at days 2 and 4 after review

TABLE 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Female	Comorbid significant neurological diagnosis (e.g., traumatic brain injury, metabolic condition, CNS infection)
≥6 years old	Comorbid significant nonneurological diagnosis (e.g., severe cardiac or respiratory disease)
≥10 kg	Significant abnormality of baseline blood tests[a,b]
Confirmed disease causing mutation in *MECP2* gene[c]	Clinically significant ECG abnormality[b,d]
Drug resistant epilepsy defined: 1. Failed adequate trial of at least two standard anticonvulsants[e] 2. At least four quantifiable seizures[f] 8 weeks prior to screening AND at least two quantifiable seizures in the baseline period	Current or previous use of recreational or medicinal cannabis, or cannabinoid-based medication within 3 months Pregnant Known allergy to CBDV component or any cannabinoid
Patient and guardian able to comply with trial requirements	Other significant disease or disorder that might put patient at risk, or influence trial results or patient's ability to comply
All medications and interventions for Rett syndrome-related symptoms stable 4 weeks prior to screening	Abnormal physical examination that would impact safety of patient undertaking trial Concomitant use of more than four anticonvulsant medications Felbamate use in the prior 12 months[g]

Abbreviations: CBDV, cannabidivarin; CNS, central nervous system; ECG, electrocardiogram.

[a]Complete blood picture (white blood cell count < 4.0 x 10^9/L, platelets < 60 000, absolute neutrophil count < 1.0 x 10^9/L), electrolytes, hepatic function, or enzymes (alanine aminotransferase or aspartate aminotransferase > 2 × upper limit normal).

[b]As assessed by investigator.

[c]Assessed by neurologist or medical geneticist.

[d]Corrected QT interval > 460 ms, PR interval > 0.2 s, QRS duration > 0.1 s.

[e]Assessed by the investigator/treating clinician.

[f]Easily counted by parents: generalized tonic–clonic, drop attacks, focal seizure with impaired awareness. Excludes simple staring and myoclonus.

[g]Due to high risk of side effects and difficulty establishing whether adverse events are secondary to CBDV or felbamate introduction.

for 28 days. The patients then had monthly visits with weekly phone calls for 90 days, then 3-monthly visits for the remainder of the trial. At each visit, data collected included medical history, physical examination, vital signs, seizure frequency, adverse events, medication history, blood tests, and questionnaires. All data was reviewed by the Data Safety Monitoring Board for safety of ongoing participation.

Seizure semiology was determined by parental interview and review of medical records and classified according to the International League Against Epilepsy classification (2017) as focal with or without impaired awareness, generalized, or unknown onset. Further description regarding motor or non-motor seizure was recorded. "Other" was applied to seizures that were unclassified (i.e., nonconvulsive status), those not designated a description in the diaries, or those that were witnessed by others (such as teachers) and not described adequately. At completion of the study, patients provided their seizure diary and questionnaires, and a final EEG was recorded.

Seizure frequency was documented from the patient diaries recorded daily during the 28-day baseline period and at each trial visit. Seizure outcome was determined by comparing the baseline monthly seizure frequency to that of the MMSF on treatment (total seizures averaged across the total time on treatment). This was represented as a percentage change, and true treatment responders were defined as those with >50% MMSF reduction. The interictal EEG at baseline and at study completion was assessed independently by two clinicians, primarily assessing four domains (state, symmetry, spike-wave frequency, and seizures) in the first 10 minutes of each recording.

Comorbid symptoms were assessed comparing baseline and end of study RSBQ and Rett Syndrome Symptom Severity Index and represented as a percentage change. Both evaluate RTT symptoms including breathing patterns, behavioral outbursts, hand movements, anxiety and mood disturbance, facial expression, purposeful movements, mobility, communication and interaction, and staring spells. The RSBQ includes 45 questions rated on a scale of 0–2, with a maximum score of 90 representing significant symptoms. The Rett Syndrome Symptom Severity Index scores individual RTT-related symptoms on a Likert scale from 0 to 9. In addition, for each domain, patients identify improvement, stabilization, or worsening.

At dose initiation, blood samples (EDTA) were taken for assessment of plasma concentrations of CBDV and metabolites at pre-dose (Time 0) and 30 min, 1 h, 2 h, and 4 h post-treatment as well as a sample approximately 12 h after the final dose of CBDV.

Plasma samples were analyzed using a validated liquid chromatography tandem mass spectrometry method for CBDV and the metabolites 7-hydroxy-cannabidivarin (7-OH-CBDV) and 7-carboxy-cannabidivarin (7-COOH-CBDV). The pharma-cokinetic parameters maximum plasma concentration (C_{max}), time of C_{max}, and area under the plasma concentration time curve (AUC) were calculated using GraphPad Prism.

Deidentified patient data were collected and entered into medical records and the Paediatric Trials Network Australia database. Given the small sample size, descriptive statistical analysis was used to summarize the data.

The trial was approved by the Sydney Children's Hospital Network Human Research Ethics committee (HREC/18/SCHN/68). Written parental consent was obtained prior to study commencement. The trial was conducted in accordance with International Council for Harmonisation Good Clinical Practice guidelines and the Australian Code for Responsible Conduct of Research, 2018.[33]

3 | RESULTS

Six children met the study inclusion criteria. One patient withdrew prior to the baseline visit due to parental inability to commit to the trial requirements (an inclusion criterion); therefore, they had no data collected and are considered a screen failure. We therefore include only the data of the remaining five patients, for whom baseline characteristics are presented in Table 2. The cohort had a median age of 12.6 years (range = 6.0–13.7 years). The median number of antiseizure medications taken at baseline was three. A range of seizure semiology was reported at baseline including GTCS, focal seizures with impaired awareness with and without motor onset, and generalized seizures with motor onset. The patients had a median baseline monthly seizure frequency of 32 (range = 6–371) seizures per month.

3.1 | CBDV Dose

All five patients commenced 2.5 mg/kg/day of CBDV as per study protocol with dose titration to a final mean dose of 9.9 mg/kg/day (range = 9.6–10.1 mg/kg/day). Three patients had their doses reduced for brief periods due to concerns about reported adverse events, but all were able to increase to their previous dose without ongoing issues.

3.2 | Seizure frequency

The median relative reduction in MMSF after commencement of CBDV was 82% (range = 7%–98%), with all

TABLE 2 Baseline characteristics

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age, years	11.5	13.7	12.6	13.4	6
Sex	F	F	F	F	F
Antiseizure medications, *n*	4	4	2	2	3
Antiseizure medications	Primidone Carbamazepine Zonisamide Perampanel	Phenobarbitone Lamotrigine Levetiracetam Sodium valproate	Levetiracetam Clobazam	Sodium valproate Clobazam	Sodium valproate Clobazam Levetiracetam
Seizure frequency, *n*	32	35	6	7	371
Seizure semiology, baseline period	Focal IA (motor), $n = 32$	Focal IA (non-motor), $n = 32$ Other, $n = 3$	Focal IA (motor), $n = 1$ Generalized (motor, other), $n = 5$	Focal IA (motor), $n = 3$ Generalized (motor, other), $n = 4$	GTCS, $n = 25$ Generalized (motor, other), $n = 18$ Unknown onset (other), $n = 328$
All semiology experienced	GTCS Focal IA (motor) Other (generalized motor)	Focal IA (non-motor) Other	Focal IA (motor) Generalized (motor, other) Other (generalized motor)	Focal IA (motor) Other (generalized motor)	GTCS Generalized (motor, other) Unknown onset (motor)

Abbreviations: GTCS, generalized tonic–clonic seizures; IA, impaired awareness.

patients reporting a reduction in MMSF. Four of the five children demonstrated a MMSF reduction of >50%, and of these, three reported a reduction of >75%. The cohort had a reported post-treatment median of 6.3 (range = 0.6–14.4) seizures per month.

The most common seizure types were motor seizure (non-tonic–clonic) with unknown onset (34%) and focal motor seizure with impaired awareness (33%). Other seizures reported were focal seizures with impaired awareness and non-motor onset (11%), GTCS (7%), generalized motor (non-tonic–clonic) seizures (7%), other (4%), unknown onset tonic–clonic seizures (1%), non-motor seizures (1%), and focal to bilateral tonic–clonic seizures (1%). At baseline, motor seizure (non-tonic–clonic) with unknown onset was the commonest (73%), exclusively occurring in one patient (Patient 5), and post-treatment, focal seizure with impaired awareness and motor onset was the commonest (56%; Table 3 and Table S1).

Four patients reduced their baseline antiseizure medications after CBDV initiation (Table S4). Patients 1, 3, and 5 each ceased one baseline antiseizure medication, and Patient 4 was on a reduced dose of valproate at the end of trial. Patient 2 maintained baseline dose of antiseizure medications at the end of the trial.

3.3 | Electroencephalogram

A baseline EEG was recorded in four of the five patients, and all five patients had an EEG performed after completion of the study period. At baseline, of the four available EEGs, all were symmetric, and all five were symmetric post-treatment. Spike-wave frequency varied between patients. Patient 1 had a 73% reduction in spike-wave frequency, and Patient 4 had no epileptiform discharges in either recording. Spike-wave activity doubled in Patient 3 and increased by >500 times in Patient 5. Both assessors identified a likely ictal rhythm during the post-treatment EEG of Patient 5. A comparison could not be made for Patient 2, as a baseline EEG was not available. Other features included generalized often rhythmic delta–theta background slow activity. The runs of delta–theta activity were qualitatively reduced between the baseline and post-treatment EEG in Patients 1, 3, and 4. This activity was not quantified in the study design (Table 4).

3.4 | Safety and adverse events

Overall, 77 adverse events were reported, the most common being infective (22.1%), central nervous system (20.8%), general (14.3%), and skin (13%) disorders. Fifty-eight percent of the infective events were respiratory.

TABLE 3 Mean monthly seizure frequency

		Total	Treatment period, months	MMSF	Monthly seizure burden reduction, %
Patient 1	Baseline	32	15.5	32	55
	After CBDV	223		14.4	
Patient 2	Baseline	35	14.75	35	82
	After CBDV	93		6.3	
Patient 3	Baseline	6	14	6	88
	After CBDV	10		0.7	
Patient 4	Baseline	7	14.75	7	7
	After CBDV	96		6.5	
Patient 5	Baseline	371	13.75	371	98
	After CBDV	87		6.3	

Abbreviations: CBDV, cannabidivarin; MMSF, mean monthly seizure frequency.

TABLE 4 EEG data

		State	Background symmetry	Spike-wave frequency	Seizure	Other features
Patient 1	Baseline EEG	Awake Sleep	Symmetric	39.5	0	Runs of rhythmic delta +++ Photic noncontributory[a]
	Post-treatment	Awake Drowsy Sleep	Symmetric	10.5	0	Intermittent rhythmic delta + Frontal beta[a] Photic noncontributory[a]
Patient 2	Baseline EEG	NA	NA	NA	NA	NA
	Post-treatment	Awake	Symmetric	0	0	Focal rhythmic theta ++
Patient 3	Baseline EEG	Awake	Symmetric	26	0	Prominent generalized rhythmic theta
	Post-treatment	Awake	Symmetric	55	0	Spikes seen were focal
Patient 4	Baseline EEG	Awake	Symmetric	0	0	Rhythmic theta ~80%–85% Slow background[a]
	Post-treatment	Awake	Symmetric	0	0	Runs rhythmic theta >50%, with breath-holding Slow posteriorly
Patient 5	Baseline EEG	Awake Drowsy	Symmetric	1	0	Focal rhythmic theta Posterior bursts (1–5 s) slow and fast[a]
	Post-treatment[b]	Awake Drowsy	Symmetric	605	Ictal pattern	NA

Abbreviations: EEG, electroencephalogram; NA, not available.

[a]Only identified by one investigator.

[b]Given consistency and severity of changes, only 5 min of spike-wave frequency was assessed and doubled.

Nervous system adverse effects included hypersomnolence (9.1%), increased seizures (3.9%), staring episodes (2.6%), abnormal hand positioning (1.3%), and tremor (1.3%). The most commonly reported general system events were a lateral truncal tilt and sleep disturbance (Table 5).

Three events (3.9%) were identified as definitely related to CBDV: two episodes of hypersomnolence and one episode of drooling. Twenty-four events (31.2%) were identified as possibly related, all of which were mild except for three events in Patient 4: a forward lean, tremor, and hand contortion. Forty-eight events (62.3%) were identified as unrelated and two were not specified. Twenty-three adverse events (29.9%) were identified as expected and included hypersomnolence, drooling, and diarrhea.

The severity of adverse events was graded as mild in 60 cases (77.9%), moderate in 10 cases (13%), and severe in four cases (5.2%), and was not reported in three cases. All four severe events were reported in Patient

TABLE 5 Adverse events

Adverse event	*n*	Severity, *n*				SAEs, *n*	Relation to CBDV, *n*			
		Mild	Moderate	Severe	Unknown		Unrelated	Possibly	Definitely	Unknown
Blood system	1	1					1			
Epistaxis	1	1					1			
Dental	4	4					3	1		
Loose teeth	2	2					1	1		
Lost teeth	2	2					2[a]			
Endocrine	1	1					1			
Precocious puberty	1	1					1			
Gastrointestinal	7	6		1			2	4	1	
Vomiting	1	1					1			
Drooling	2	2						1	1[b]	
Anorexia	1	1						1		
Diarrhea	2	2						2[b]		
Reduced motility	1			1		1[a]	1[a]			
General	11	9	2				2	8		1
Body tilt or lean	4	3	1			2		3		1
Altered sleeping pattern	4	4					2 (1[a])	2		
Forward lean	1		1			1		1		
Agitation	1	1						1		
Breath-holding	1	1						1		
Infectious	17	8	3	3	3		16	1		
Respiratory	10	3	3	1	3	4[c]	9	1		
Gastrointestinal	3	2		1		2[d]	3			
ENT	2	2					2			
Genitourinary	1			1		1[e]	1			
Skin	1	1					1			
Injury	6	4	2				6			
Fall (± concussion)	3	2	1			1[f]	3 (2[a])			
Bruise	2	2				1[g]	2			
Foot injury	1		1				1			
Investigations	1	1						1		
Raised transaminases	1	1						1		
Nervous system	16	14	2				5	8	2	1
Increased seizure frequency	3	3					2[a]	1[a]		
Somnolence	7	7				1[b]	2	2[b]	2[b]	1
Increased postictal period	1	1					1[a]			
Facial twitch	1	1						1[a]		
Dazed/staring	2	2						2		
Abnormal hand positioning	1		1			1		1		
Tremor	1		1			1		1		
Respiratory	3	3					2	1		
Cough	2	2					1	1		

TABLE 5 (Continued)

Adverse event	*n*	Severity, *n*				SAEs, *n*	Relation to CBDV, *n*			
		Mild	Moderate	Severe	Unknown		Unrelated	Possibly	Definitely	Unknown
Wheeze	1	1					1			
Skin	10	9	1				10			
Pressure sore	4	3	1				4[a]			
Extravasation of IVC	2	2					2			
Rash	2	2					2 (1[a])			
Skin mark	1	1					1			
Blisters	1	1					1[a]			
Total	77	60	10	4	3	16	48	24	3	2

Abbreviations: CBDV, cannabidivarin; ENT, ear/nose/throat; ESBL, extended spectrum beta-lactamase; IVC, intravenous catheter; SAE, serious adverse event.

[a]Expected as related to disease, development, epilepsy, or secondary illness.

[b]Expected CBDV side effect.

[c]ESBL pneumonia, rhinovirus pneumonia, community acquired pneumonia, and influenza B infection.

[d]Severe oral thrush and *Clostridium difficile*.

[e]ESBL urinary tract infection.

[f]Fall with concussion.

[g]Sacroiliac bruising presumed from scoliosis surgery rod.

2 during two hospital admissions and were identified as unrelated to CBDV. One was for reduced gastrointestinal motility during an orthopedic admission, and three were during an admission for pneumonia complicated by extended spectrum beta-lactamase (ESBL) urinary tract infection, secondary lower respiratory tract infection, and *Clostridium difficile* infection. Of the moderate events, six were identified as serious adverse events (SAEs): ESBL pneumonia in Patient 2; forward lean, hand contortion, tremor, and fall with concussion in Patient 4; and pneumonia and influenza B infection in Patient 5. A transient rise in hepatic transaminases (<1.5 × upper limit of normal) was seen in one patient with brief reduction of the CBDV dose, which was subsequently increased without further issue. Apart from this and changes expected due to intercurrent illness, there were no reported side effects related to hematology, biochemistry, vital signs, or ECG.

Sixteen SAEs were identified throughout the study period, none of which was thought to be definitely related to CBDV. Five SAEs (6.5%) were defined as possibly related and were described in Patient 4: tremor, forward lean, hand contortion, and two reports of a lateral truncal tilt to the right.

3.5 | RTT symptoms

RSBQ data were not available at baseline for Patients 3 and 5. For the remainder, the values had a median of 34 (range = 24-46) at baseline and 33 (range = 30-66) at end of study. Patients 1 and 4 demonstrated minimal change from baseline with mild improvement (3% and 5%, respectively), whereas Patient 2 had a 20% increase in reported symptomology (Tables S1, S2).

Rett Syndrome Symptom Severity Index was not completed at baseline by the family of Patient 5. Overall, most domains were identified as stable, with a trend toward no change (0) or mildly improved (+1) as the median score post-treatment. Mental alertness demonstrated improvement (+1 to +2) in all but Patient 4, who reported a mild worsening (−1); however, overall, the scores suggested a reasonable level of mental alertness (median = 6, range = 4–8). Other domains reporting a median mild improvement (+1) included attention span, eye contact, facial expression, and speech. Physical activity was scored as no change or mildly improved in all cases, with a wide variability in ability (median = 4, range = 0–6).

3.6 | Pharmacokinetics

Table 6 summarizes the pharmacokinetic parameters for the five patients in this study. Plasma C_{max} was reached between 1 and 2 h following administration of the first dose of CBDV (2.5 mg/kg). The plasma C_{max} ranged 3.6–14.1 ng/ml for CBDV, 4.7–106 ng/ml for 7-OH-CBDV, and 850–2948 ng/ml for 7-COOH-CBDV. The plasma AUC_{0-4} was 140% of C_{max} for CBDV, 130% for 7-OH-CBDV, and 240% for 7-COOH-CBDV. Patient trough samples at the end of

TABLE 6 CBDV and metabolite pharmacokinetics

Analyte	Patient 1	2	3	4	5	Mean ± SD
CBDV						
C_{max}, ng/ml	5.3		6.5	3.6	14.1	7.4 ± 4.02
T_{max}, h	0.78		2.2	2.18	1.18	1.6 ± .62
AUC_{0-4}, ng/ml*h	7.71		11.4	7.5	16[a]	10.7 ± 3.45
EOT, ng/ml	6	11.1	5.1	5.7	4.3	5.3 ± .65
7-OH-CBDV						
C_{max}, ng/ml	7.3		8.8	4.7	106	31.6 ± 42.7
T_{max}, h	0.78		2.2	2.18	1.18	1.6 ± .62
AUC_{0-4}, ng/ml*h	13.7		18.6	12.1	120[a]	41 ± 45.6
EOT, ng/ml	6.2	29.2	4.0	7.3	20	9.4 ± 6.25
7-COOH-CBDV						
C_{max}, ng/ml	1777		2417	850	2948	1998 ± 782
T_{max}, h	0.78		2.2	4.13	1.18	2.07 ± 1.30
AUC_{0-4}, ng/ml*h	5036		5364	2310	4776[a]	4372 ± 1208
EOT, ng/ml	1176	5330	1657	11109	5193	4784 ± 3968

Abbreviations: 7-COOH-CBDV, 7-carboxy-cannabidivarin; 7-OH-CBDV, 7-hydroxy-cannabidivarin; AUC, area under the curve; CBDV, cannabidivarin; C_{max}, maximum plasma concentration; EOT, end of treatment; T_{max}, time of C_{max}.

[a]Time point of 2.35 h instead of 4 h.

the study were taken 12 h after final dose (10 mg/kg/day) and ranged 4.3–11.1 ng/ml for CBDV, 4.0-29.2 ng/ml for 7-OH-CBDV, and 1176–11 109 ng/ml for 7-COOH-CBDV.

4 | DISCUSSION

Epilepsy in RTT is common, debilitating, and often drug-resistant.[10,11] Despite the use of CBDV in animal models[28] and adult epilepsy clinical trials,[29] this study presents the first data in a pediatric epilepsy cohort. In our small patient cohort of children with drug-resistant epilepsy and *MECP2*-related RTT, CBDV treatment was well tolerated, and an overall trend toward reduction in mean monthly seizure frequency was observed.

Consistent with the heterogeneity of epilepsy in RTT, baseline monthly seizure frequency was variable, with multiple seizure types reported. Three patients demonstrated baseline seizure frequency of >30 seizures in a 1-month period. This represents a significant burden on the lives of the patients and their families. Four of the five patients were considered true treatment responders to CBDV, with three showing a >75% reduction in MMSF. The reduction was nonlinear, with seizure frequency fluctuation noted between visits, a well understood concept in epilepsy. One patient had only a small reduction in seizure frequency (MMSF reduction = 7%); however, they had a low baseline monthly seizure count of 7. Notably, this patient had no spike-wave activity identified on either EEG, highlighting the discordance of EEG abnormalities and seizures in RTT. Baseline antiseizure medications were reduced or ceased in four patients and maintained in one patient. This suggests CBDV is responsible for the MMSF reduction and may allow reduction in baseline antiseizure medications.

The EEG data was consistent with that known in RTT. All were abnormal but showed variability in the frequency and severity of spike-wave activity and delta–theta slowing. The intermittent rhythmic background slow activity was qualitatively observed to reduce in frequency after CBDV treatment in three patients, although our sample size is small, and this was not quantitatively measured.

Overall, no positive or negative conclusion can be deduced regarding spike-wave frequency. One patient had a reduction in, one patient maintained, and two patients demonstrated significant increases in spike-wave frequency post-treatment. Given the patients' ages and the short duration of the trial, it is unlikely that this increase in activity can be attributed to the expected EEG evolution in RTT. The post-treatment EEG of Patients 3 and 5 demonstrated increased spike-wave frequency despite a reported decrease in seizures in this period. This may suggest that background spike-wave activity in RTT does not correlate with clinical seizure activity. The post-treatment EEG of Patient 5, the youngest, demonstrated

a likely ictal rhythm with no clinical correlate, explaining the unexpectedly large increased spike-wave frequency. This is inconsistent with the improvement in EEG seen in a general epilepsy cohort of adults and children treated with other cannabinoids.[34] These data need to be interpreted acknowledging the unpredictable and fluctuant nature of epilepsy, dependent on external provoking factors such as puberty and sleep deprivation.[35–37] A limitation of the EEG data is that there may be sampling bias, as only a short epoch of each recording was considered, and sleep was rarely captured.

Consistent with other oral cannabinoids, plasma C_{max} was reached 1–2 h following administration of CBDV. The plasma C_{max} ranged fourfold for CBDV and the metabolites, similar to the wide variability seen with other cannabinoid medicines. Patient trough samples at the end of treatment ranged fourfold for the parent and up to 10-fold for the metabolites, suggesting variable liver enzyme activity in addition to the prehepatic variables, causing a wide range of concentrations.

Although a large number of adverse events were reported, in line with previously reported data, CBDV was well tolerated,[29] with the majority of reported adverse events mild (77.9%) and many expected (29.9%). The only adverse events identified as definitely related to CBDV were hypersomnolence and increased drooling. Somnolence has been previously identified.[29] Most other expected adverse events were related to underlying RTT symptoms (sleep disturbance, increased seizure frequency, or increased postictal period), development (loose teeth) or secondary illness (fall following seizure, facial twitch, delayed gastric motility, pressure area from orthopedic splinting), or were previously reported,[29] including diarrhea. No expected event required CBDV dose alteration. The commonest reported events were infectious, particularly respiratory infections, which are commonly seen in children.[38] A large portion of the adverse events were reported in Patient 2, including seven of the severe adverse events and seven of the SAEs. Most of the severe events occurred during an intensive care unit admission for a severe chest infection, unrelated to CBDV. This admission highlights the high complexity and health care burden of this patient cohort.

Although there were 16 SAEs reported (20.8%), a higher percentage than reported in an adult cohort by Brodie et al.,[29] none were assessed as definitely related to CBDV. Three independent RTT experts reviewed these, and of the five possibly related SAEs, tremor and abnormal hand posturing were felt to be reflective of underlying RTT diagnosis. Additionally, a forward lean was identified as menses-related, and two episodes of lateral truncal tilt, which briefly prompted medication reduction, were ultimately assessed as stable despite subsequent dose increase, thus the link to CBDV is unlikely. CBDV had no definite drug-related severe outcomes, and most SAEs initially identified as possibly related to CBDV were later identified as RTT-related. Due to the relatively short study duration and multiple exclusionary criteria, there is an acknowledged limitation in extrapolation of SAE data to chronic use in a larger population.

The change in RSBQ scoring was incomplete in two patients, making it difficult to draw meaningful conclusions in the small cohort. The three remaining patients' scores were variable. Definitive conclusions cannot be drawn from the Rett Syndrome Symptom Severity Index data, due to the wide variability of symptom domain severity experienced in each patient. This demonstrates the individual but pervasive symptom burden experienced by children with RTT. Most domains demonstrated no change. A mild improvement in the median raw score values was seen following CBDV in some of the cognitive domains: mental alertness, attention, eye contact, facial expression, and speech. Improved cognition with improved seizure control is a well understood concept in most epileptic syndromes.[39,40]

The lack of substantial change in the questionnaire data, despite a considerable change in seizure burden, is likely due to the questionnaires almost exclusively assessing nonepileptic symptoms of RTT. This is supported when comparing to MMSF; Patient 3, who had the least end of trial monthly recorded seizures, had the highest RSBQ raw value, and Patient 5, who had the most end of trial monthly recorded seizures, had an RSBQ score comparable to the other patients.

This phase 1 clinical trial reports a small cohort of patients with *MECP2*-related RTT and drug-resistant epilepsy treated with 10 mg/kg/day of CBDV. Related adverse events included hypersomnolence and drooling. No patients required medication withdrawal, as no SAEs were identified as definitely CBDV-related. We anticipate a reduction in MMSF would result in a reduction in the burden of care of these patients for families and the health care system.

We acknowledge that using seizure diaries accepts an element of human error with recording seizures; however, they are a commonly used tool in epilepsy literature. Additionally, more accurate capture with continuous video recorded EEG is impractical in a trial with this patient cohort and duration. We also understand the small sample size and lack of a control (nonblinded, nonrandomized) make it impossible to determine statistical significance. Despite these limitations, the results are promising regarding the use and safety of CBDV in children with RTT-related epilepsy. This extends current data to confirm tolerability in a pediatric population and to support trials investigating the use of CBDV in other neurodevelopmental conditions associated with drug-resistant epilepsy. A larger phase 2 trial would be required to test the validity of these results.

4.1 | Statistical methods

This is a phase 1 trial and therefore has a small sample size, with descriptive statistics reported.

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CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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