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Pharmacokinetics of Phytocannabinoid Acids and Anticonvulsant Effect of Cannabidiolic Acid in a Mouse Model of Dravet Syndrome

Lyndsey L. Anderson,[†,‡] Ivan K. Low,[†] Samuel D. Banister,[†,§] Iain S. McGregor,[†,⊥] and Jonathon C. Arnold*[,†,‡]

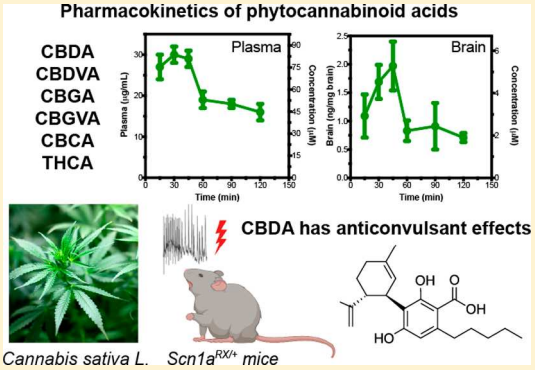
[†]Lambert Initiative for Cannabinoid Therapeutics, Brain and Mind Centre, The University of Sydney, Sydney, New South Wales 2050, Australia

[‡]Discipline of Pharmacology, Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales 2006, Australia

[§]School of Chemistry, Faculty of Science, The University of Sydney, Sydney, New South Wales 2006, Australia

[⊥]School of Psychology, Faculty of Science, The University of Sydney, Sydney, New South Wales 2006, Australia

ABSTRACT: *Cannabis sativa* produces a complex mixture of many bioactive molecules including terpenophenolic compounds known as phytocannabinoids. Phytocannabinoids come in neutral forms (e.g., Δ^9-tetrahydrocannabinol, THC; cannabidiol, CBD; etc.) or as acid precursors, which are dominant in the plant (e.g., Δ^9-tetrahydrocannabinolic acid, THCA; cannabidiolic acid, CBDA; etc.). There is increasing interest in unlocking the therapeutic applications of the phytocannabinoid acids; however, the present understanding of the basic pharmacology of phytocannabinoid acids is limited. Herein the brain and plasma pharmacokinetic profiles of CBDA, THCA, cannabichromenic acid (CBCA), cannabidivarinic acid (CBDVA), cannabigerolic acid (CBGA), and cannabigerovarinic acid (CBGVA) were examined following intraperitoneal administration in mice. Next it was examined whether CBDA was anticonvulsant in a mouse model of Dravet syndrome (*Scn1a*$^{RX/+}$ mice). All the phytocannabinoid acids investigated were rapidly absorbed with plasma t_{max} values of between 15 and 45 min and had relatively short half-lives (<4 h). The brain−plasma ratios for the acids were very low at ≤0.04. However, when CBDA was administered in an alternate Tween 80-based vehicle, it exhibited a brain−plasma ratio of 1.9. The anticonvulsant potential of CBDA was examined using this vehicle, and it was found that CBDA significantly increased the temperature threshold at which the *Scn1a*$^{RX/+}$ mice had a generalized tonic-clonic seizure.



Cannabis sativa L. (Cannabaceae) contains more than 500 potentially bioactive compounds including approximately 140 terpenophenolic molecules called phytocannabinoids. Phytocannabinoids come in neutral forms such as Δ^9-tetrahydrocannabinol, THC, and cannabidiol, CBD, or acid forms as Δ^9-tetrahydrocannabinolic acid, THCA, and cannabidiolic acid, CBDA.[1,2] The phytocannabinoid acids are formed enzymatically and are the most abundant in raw plant material. Neutral cannabinoids are formed via decarboxylation of these acid precursor molecules as a result of heat or light exposure.[2] The legalization of medicinal cannabis in many countries around the world has provoked great interest in the phytocannabinoid acids, as there is increasing use of cannabis extract formulations that are not heated, smoked, or vaporized. Since these formulations of cannabis are produced via cold extraction or "juicing" of raw cannabis material, they contain much higher concentrations of the acid forms of the phytocannabinoids.[2,3]

While research focusing on the therapeutic potential of phytocannabinoid acids is escalating, there is still a very limited understanding of the basic pharmacodynamic and pharmacokinetic nature of these abundant components. CBDA and THCA may have widespread therapeutic applications, as CBDA has anxiolytic, anti-inflammatory, and antihyperalgesic effects in animal models.[4,5] Moreover, both CBDA and THCA have antiemetic effects in shrews and rats, and THCA appears to be the key component mediating the anti-inflammatory actions of *C. sativa* extracts in cellular models of inflammatory bowel disease.[6−9] THCA exerts its anti-inflammatory and neuroprotective effects by reducing tumor necrosis factor alpha (TNF-α) levels modulated by peroxisome proliferator-activated receptor gamma (PPARγ).[10,11] It has been speculated that CBDA and THCA have anticonvulsant properties, as these phytocannabinoids are highly abundant in artisanal *C. sativa* oils being used in the community to treat intractable childhood epilepsies.[3,9] Indeed, it was reported in a patent that CBDA reduced seizure severity in a rat model of epilepsy; however, no study has yet documented anticonvulsant effects of this molecule in the peer-reviewed scientific literature.[12]

The capture and dissemination of pharmacokinetic data for the phytocannabinoid acids is an important contribution in the

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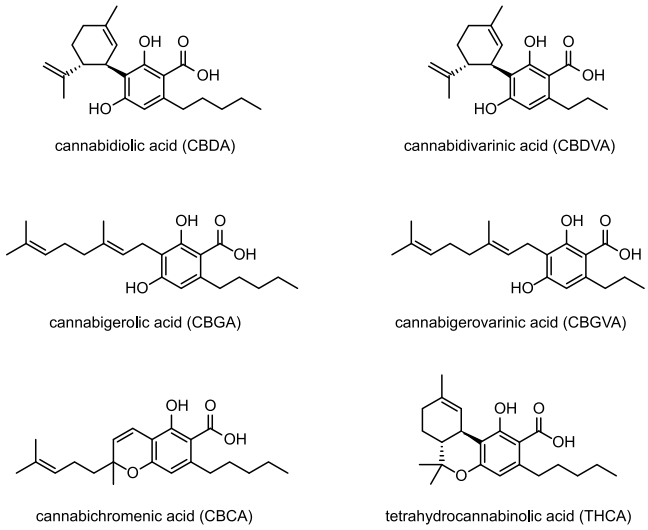

Figure 1. Chemical structures of phytocannabinoid acids used in this study.

search for novel therapeutic applications, revealing optimal timing of dosing for experiments involving in vivo disease models. The basic pharmacokinetics of the phytocannabinoid acids are unknown; thus, the present study characterized the pharmacokinetic profiles of CBDA, THCA, cannabichromenic acid (CBCA), cannabigerolic acid (CBGA), cannabigerovarinic acid (CBGVA), and cannabidivarinic acid (CBDVA) (Figure 1) in mouse plasma and brain.

RESULTS AND DISCUSSION

Rapid absorption of CBDVA was observed following intraperitoneal (i.p.) administration with a plasma t_{max} of 15 min and a $t_{1/2}$ of 49 min (Figure 2A, Table 1). Absorption into the brain was slightly delayed, as the t_{max} was 30 min; however, the C_{max} was quite low (0.8 ± 0.1 ng/mg brain; Figure 2B) and elimination was rapid ($t_{1/2}$ 19 min). At 60 min, CBDVA was detected in brain tissue but was below the limit of quantification (LOQ), so a value of 1/2 LOQ (0.25 ng/mg brain) was used. Elimination was complete by 90 min. The brain−plasma ratio (0.02) suggests CBDVA exhibits poor brain penetration. The neutral form of CBDVA, cannabidivarin (CBDV), was not detected in the brain or plasma following injection of CBDVA, suggesting there is no significant decarboxylation of CBDVA to CBDV in vivo following i.p. injection.

Intraperitoneal administration of CBCA in an oil vehicle resulted in rapid absorption as the plasma t_{max} was 30 min with

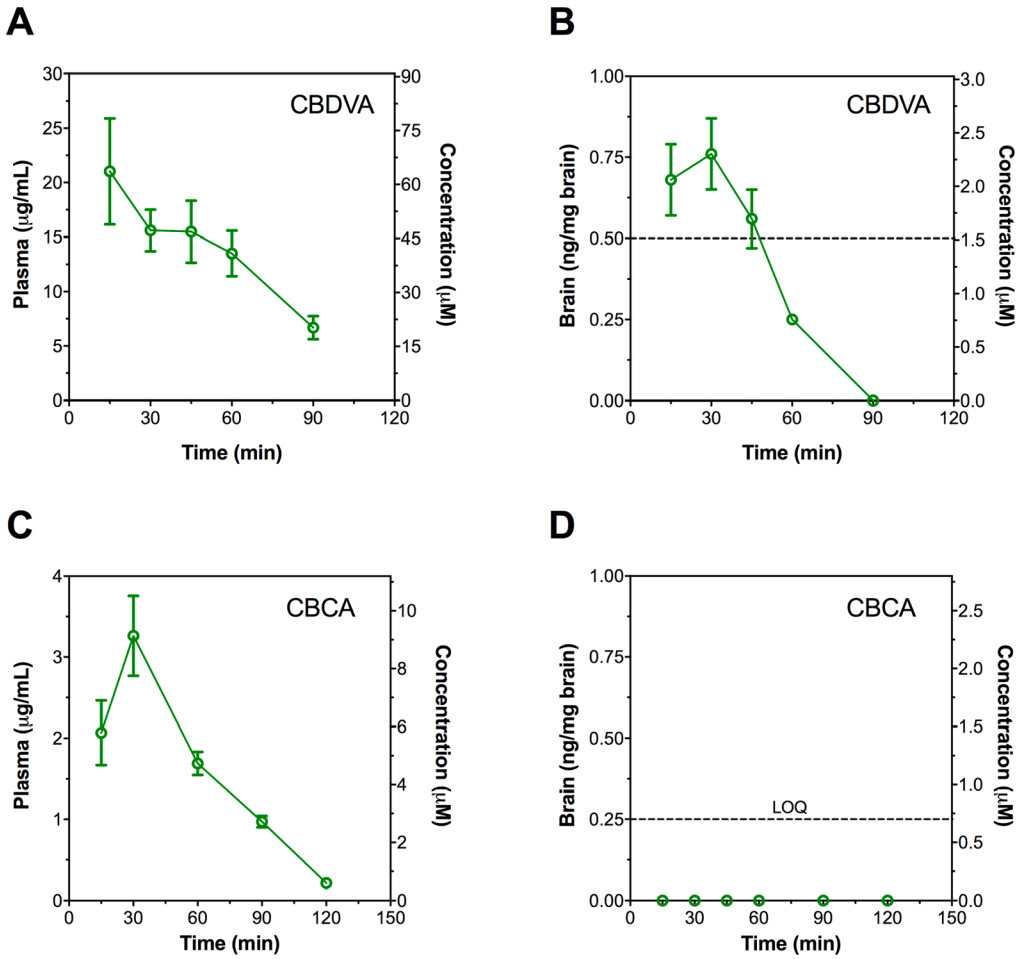

Figure 2. Pharmacokinetic analysis of CBDVA and CBCA in mouse plasma and brain samples. Concentration−time curves for CBDVA in (A) plasma and (B) brain and CBCA in (C) plasma and (D) brain following 5 mg/kg i.p. injections. Phytocannabinoids were administered in a vegetable oil vehicle. Phytocannabinoid concentrations are depicted as both mass concentrations (left *y*-axis) and molar concentrations (right *y*-axis). Dashed lines represent limit of quantification (LOQ). Data are expressed as means ± SEM, with n = 3−5 per time point.

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Table 1. Pharmacokinetics of Phytocannabinoid Acids in Mouse Plasma and Brain

	CBDVA		CBCA		CBGVA		THCA		CBDA		CBGA	
	5 mg/kg		5 mg/kg		10 mg/kg		10 mg/kg		10 mg/kg		10 mg/kg	
	plasma	brain	plasma	brain	plasma	brain	plasma	brain	plasma	brain	plasma	brain
C_{max} (μg/mL; ng/mg brain)	21.0 ± 4.9	0.8 ± 0.1	3.3 ± 0.5	BLQ	34.4 ± 2.1	3.8 ± 0.5	2.8 ± 0.4	BLQ	29.6 ± 2.2	2.0 ± 0.4	63.5 ± 7.6	2.3 ± 0.4
t_{max} (min)	15	30	30	n/a	15	90	15	n/a	30	45	45	30
$t_{1/2}$ (min)	49	19	24	n/a	204	29	67	n/a	92	41	62	31
AUC (μg min/mL; ng min/mg brain)	1485	33	173	n/a	11 958	529	302	n/a	4699	183	5960	94
brain–plasma ratio	0.02		n/a		0.04		n/a		0.04		0.02	

a short half-life ($t_{1/2}$ = 24 min; Figure 2C, Table 1). However, CBCA was not detectable in brain tissue (Figure 2D). The decarboxylation product of CBCA, cannabichromene (CBC), was not detectable in the brain or plasma following injection of CBCA.

A high maximal plasma concentration (C_{max} 34.4 ± 2.1 μg/mL; Figure 3A, Table 1) of CBGVA was achieved 15 min post i.p. injection in an oil vehicle. CBGVA exhibited a long half-life ($t_{1/2}$ = 204 min). Distribution into brain tissue was delayed, not reaching t_{max} until 90 min (Figure 3B). The half-life of CBGVA in brain, though, was short ($t_{1/2}$ = 29 min). Concentrations and overall drug exposure (AUC) of CBGVA in brain were significantly lower than plasma values, resulting in a brain–plasma ratio of 0.04. Cannabigerivarin (CBGV) was not detected in the brain following the i.p. injection of CBGVA but was present in the plasma (~0.2% of CBGVA concentrations; the CBGVA administered was 97% pure).

THCA was rapidly absorbed (t_{max} 15 min) and had a relatively short half-life ($t_{1/2}$ = 67 min) in plasma (Figure 3C, Table 1) in an oil vehicle. Notably, there was a general absence of THCA in brain tissue (Figure 3D). While THCA was detectable in brain tissue at some time points, it was below the LOQ (a value of 1/2 LOQ 0.25 ng/mg brain was used). Despite not being able to accurately calculate pharmacokinetic parameters in brain tissue, it can be concluded that THCA has poor brain penetration. On comparing the LOQ (0.5 ng/mg brain) of THCA in the brain to the C_{max} (2.8 ± 0.4 μg/mL) in the plasma, at best the brain–plasma ratio is 0.15. THC was not detected in the brain following i.p. injection of THCA but was found in the plasma (~1% of plasma THCA concentrations; the THCA administered was 95% pure with 5% THC content).

Similarly, CBDA was rapidly absorbed following i.p. administration with a plasma t_{max} of 30 min and C_{max} of 29.6 ± 2.2 μg/mL (Figure 4A, Table 1). The half-life of CBDA was quite long ($t_{1/2}$ = 92 min). Absorption into the brain was slightly delayed, as the t_{max} was 45 min (Figure 4B); however, the elimination was faster ($t_{1/2}$ = 41 min). Total exposure, represented by AUC values, of CBDA in brain tissue was a fraction of total plasma exposure. The brain–plasma ratio (0.04) suggests poor brain penetration by CBDA in an oil vehicle. CBD was not detected in the brain following i.p. injection of CBDA but was found in plasma at ~0.5% of the plasma CBDA concentration.

CBGA absorption peaked at 45 min post i.p. injection with a high maximal concentration (C_{max} 63.5 ± 7.6 μg/mL; Figure 4C, Table 1). Interestingly, absorption into brain tissue (t_{max} 30 min) occurred before the plasma t_{max} (Figure 4D). The $t_{1/2}$ of CBGA in the plasma (62 min) was double the $t_{1/2}$ in the brain (31 min). While distribution into brain tissue was rapid, the maximal concentration was low (2.3 ± 0.4 ng/mg brain) compared to the plasma. Overall, the brain concentrations and AUC were lower than plasma values, resulting in a brain–plasma ratio of 0.02. Like the other acidic phytocannabinoids, CBGA exhibits poor brain penetration in an oil vehicle. The decarboxylated form of CBGA, cannabigerol (CBG), was not present in brain or plasma following i.p. injection of CBGA.

Brain Uptake of CBDA Is Increased with a Tween-Based Vehicle. Here vegetable oil was used as a vehicle for the phytocannabinoid acids because solubility is substantially increased compared to a standard ethanol–Tween 80–saline (ratio of 1:1:18) vehicle. Since neutral cannabinoids, such as

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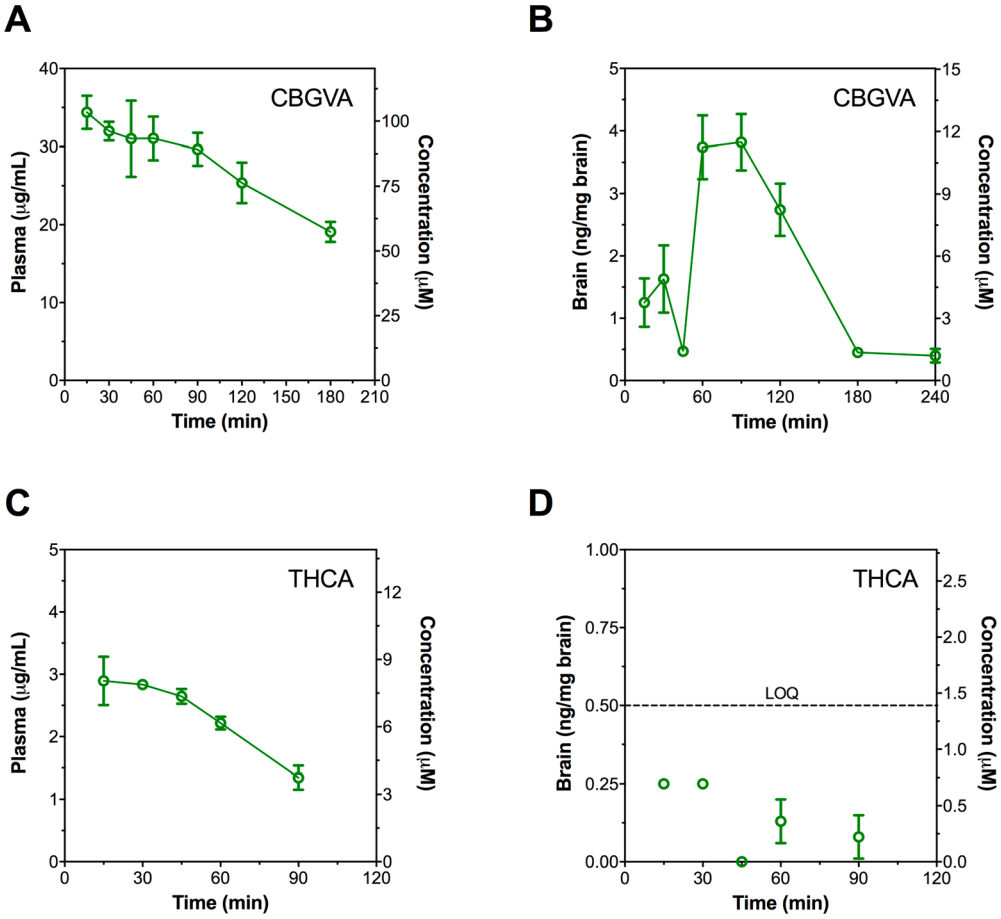

Figure 3. Pharmacokinetic analysis of CBGVA and THCA in mouse plasma and brain samples. Concentration−time curves for CBGVA in (A) plasma and (B) brain and THCA in (C) plasma and (D) brain following 10 mg/kg i.p. injections. Phytocannabinoids were administered in a vegetable oil vehicle. Phytocannabinoid concentrations are depicted as both mass concentrations (left *y*-axis) and molar concentrations (right *y*-axis). Dashed lines represent limit of quantification (LOQ). Data are expressed as means ± SEM, with n = 3−5 per time point.

CBD and THC, are often administered in a Tween-based vehicle, the pharmacokinetic parameters of CBDA and THCA in this alternate vehicle were investigated.

Administration of CBDA in the Tween-based vehicle was rapidly absorbed in plasma (t_{max} 15 min; Figure 5A, Table 2); however, the C_{max} (17.6 ± 1.7 μg/mL) was significantly less than that achieved with the vegetable oil vehicle (C_{max} 29.6 ± 2.2 μg/mL). Additionally, elimination from the plasma was faster in the Tween-based vehicle ($t_{1/2}$ = 20 min) than in oil ($t_{1/2}$ = 92 min). Elimination from the brain, however, was slower in the Tween-based vehicle ($t_{1/2}$ = 55 min; Figure 5B), which led to a greater AUC value compared to the plasma. Total exposure of CBDA in brain tissue was nearly double that of the plasma (brain−plasma ratio 1.9), suggesting that the brain uptake of CBDA is improved using a Tween-based vehicle.

Injection of THCA in a Tween-based vehicle resulted in rapid absorption in the plasma (t_{max} 15 min; Figure 5E, Table 2). Interestingly, the C_{max} (6.9 ± 0.6 μg/mL) was significantly greater than that achieved with the vegetable oil vehicle (C_{max} 2.8 ± 0.4 μg/mL). However, elimination from the plasma was faster in the Tween-based vehicle ($t_{1/2}$ = 33 min) than in vegetable oil ($t_{1/2}$ = 67 min), resulting in an equivalent plasma exposure of THCA between vehicles. While THCA was detectable in brain tissue at some time points, it was once again below the LOQ (a value of 1/2 LOQ 0.25 ng/mg brain was used), so the pharmacokinetic parameters in brain tissue could not be calculated (Figure 5F, Table 2). It can be concluded that with both vehicles THCA has very poor brain penetration.

CBDA Is Anticonvulsant against Hyperthermia-Induced Seizures in *Scn1a*$^{RX/+}$ Mice. Children with Dravet syndrome often exhibit seizures that are provoked by fever. *Scn1a*$^{RX/+}$ mice recapitulate this phenotype and have generalized tonic-clonic seizures (GTCS) in response to elevated body temperature. The effects of CBDA were evaluated against hyperthermia-induced seizures in *Scn1a*$^{RX/+}$ mice since CBDA had excellent brain penetration in the Tween-based vehicle. Moreover, it is one of the most prevalent cannabinoids, after CBD, in *C. sativa* extracts being used to treat childhood epilepsies, including Dravet syndrome.[3] Between postnatal days 14 and 16, *Scn1a*$^{RX/+}$ mice were treated with a single i.p. injection of vehicle or CBDA and subjected to a thermal challenge. CBDA was anticonvulsant against hyperthermia-induced seizures (Figure 6). A significant increase in GTCS temperature threshold was observed with 10 and 30 mg/kg CBDA (p = 0.0100 and 0.0112, respectively).

Herein are the first published pharmacokinetic data for the phytocannabinoid acids CBCA, CBDA, CBDVA, CBGA, CBGVA, and THCA. All the phytocannabinoid acids were rapidly absorbed following an i.p. injection with plasma t_{max} values of between 15 and 45 min. The rapid absorption of the phytocannabinoid acids is consistent with absorption of the

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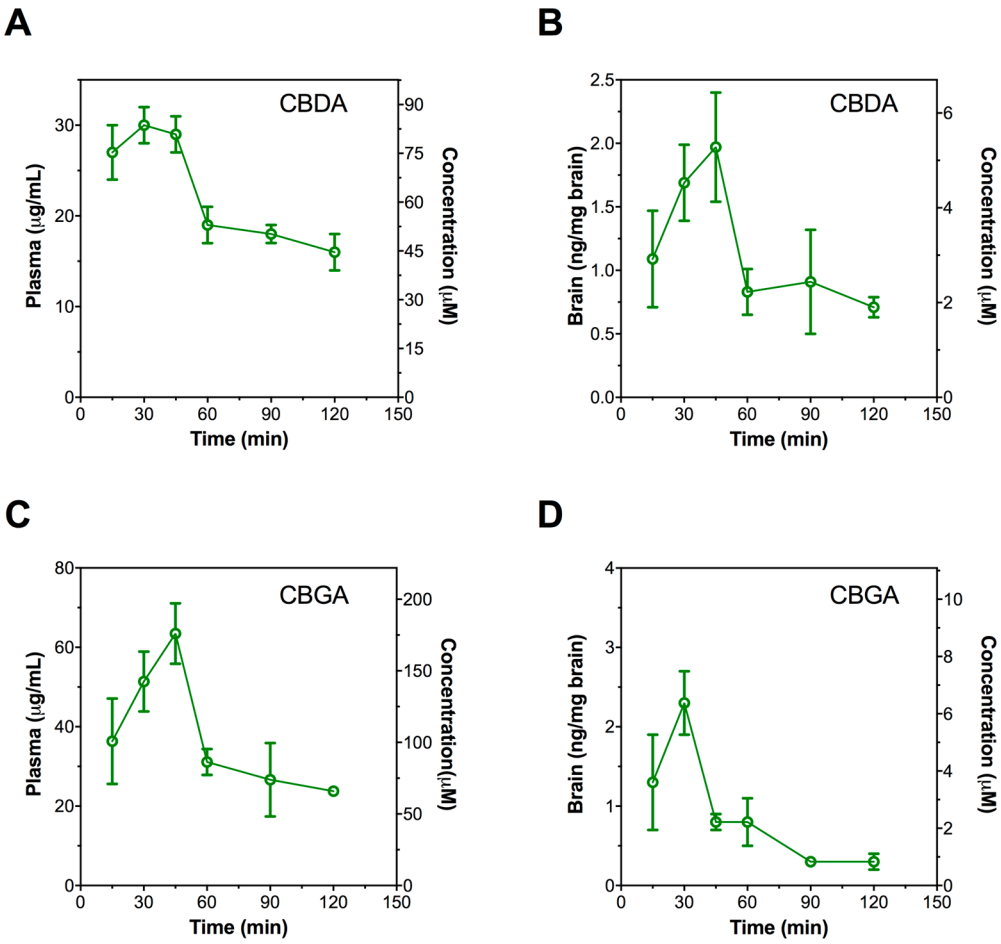

Figure 4. Pharmacokinetic analysis of CBDA and CBGA in mouse plasma and brain samples. Concentration−time curves for CBDA in (A) plasma and (B) brain and CBGA in (C) plasma and (D) brain following 10 mg/kg i.p. injections. Phytocannabinoids were administered in a vegetable oil vehicle. Phytocannabinoid concentrations are depicted as both mass concentrations (left *y*-axis) and molar concentrations (right *y*-axis). Data are expressed as means ± SEM, with n = 3−5 per time point.

neutral phytocannabinoids (CBD, CBDV, CBG), which have t_{max} values between 30 min and 2 h.[13] In contrast to the neutral phytocannabinoids, the acids, except CBGVA, had relatively short plasma half-lives (20−90 min). Half-lives, however, might be predicted to be longer in humans, since mice typically exhibit vastly higher metabolic rates.[14] For example, the elimination half-life of CBD in mice (4.5 h) is much shorter than that observed in humans (24 h).[13,15] However, the half-lives in mice and humans may not necessarily be different; in the case of THCA, it has a $t_{1/2}$ of 102 min in humans, which is comparable to the $t_{1/2}$ of 67 min observed here in mice.[16]

In general, the phytocannabinoid acids exhibited poor brain penetration. This is not surprising, as drugs with a carboxylic acid moiety are negatively charged at physiological pH, with ionization impeding passive permeation across the blood−brain barrier (BBB).[17] Indeed, nonsedative antihistamines were developed through the addition of a carboxylic acid functional group with the specific purpose to restrict these agents peripherally. Physiochemical parameters predictive of successful central nervous system (CNS) drugs include (a) lipophilicity, partition coefficient (log P) less than 2.5; (b) pK_a between 7.5 and 10.5; (c) molecular weight ≤ 310; (d) rotatable bonds < 5; (e) topological polar surface area (TPSA) < 60−70 Å; (f) CNS multiparameter optimization (MPO) score ≥ 4.[17,18] If one examines these basic physiochemical properties of the phytocannabinoid acids (Figure 7, Table 3), limited brain penetration might be expected. Phytocannabinoid acids do not have optimal lipophilicity or ionic strength for CNS penetration, with log P values ranging from 5.7 to 7.3 and pK_a values of approximately 2.9. The large number of rotatable bonds (5−10) in phytocannabinoid acids leads to substantial flexibility, which is also not ideal for CNS penetration. Despite being within the TPSA range for CNS drugs, the phytocannabinoid acids fall near the upper limit. Indeed, these physiochemical properties lead to the phytocannabinoid acids having low CNS MPO scores (3.22−4.12), which would not predict extensive brain penetration. Interestingly, the neutral phytocannabinoids CBD, CBDV, and THC also exhibit high log P values and low CNS MPO scores but have excellent brain penetration (60−160%), which could be due to small TPSA values and more rigid structures resulting from fewer rotatable bonds.[13,19]

A major finding was that the brain−plasma ratio of CBDA was substantially increased when administered in a Tween-based vehicle compared to a vegetable oil vehicle. In oil, CBDA was poorly brain-penetrant (brain−plasma ratio = 0.04), which is consistent with its suboptimal lipophilicity and a low CNS MPO score (Figure 7). However, the Tween-based vehicle increased the brain−plasma ratio of CBDA to 1.9. While this result was initially surprising, closer scrutiny of the literature revealed several reports of nonionic surfactants, including Tween 80, increasing biomembrane permeability and altering

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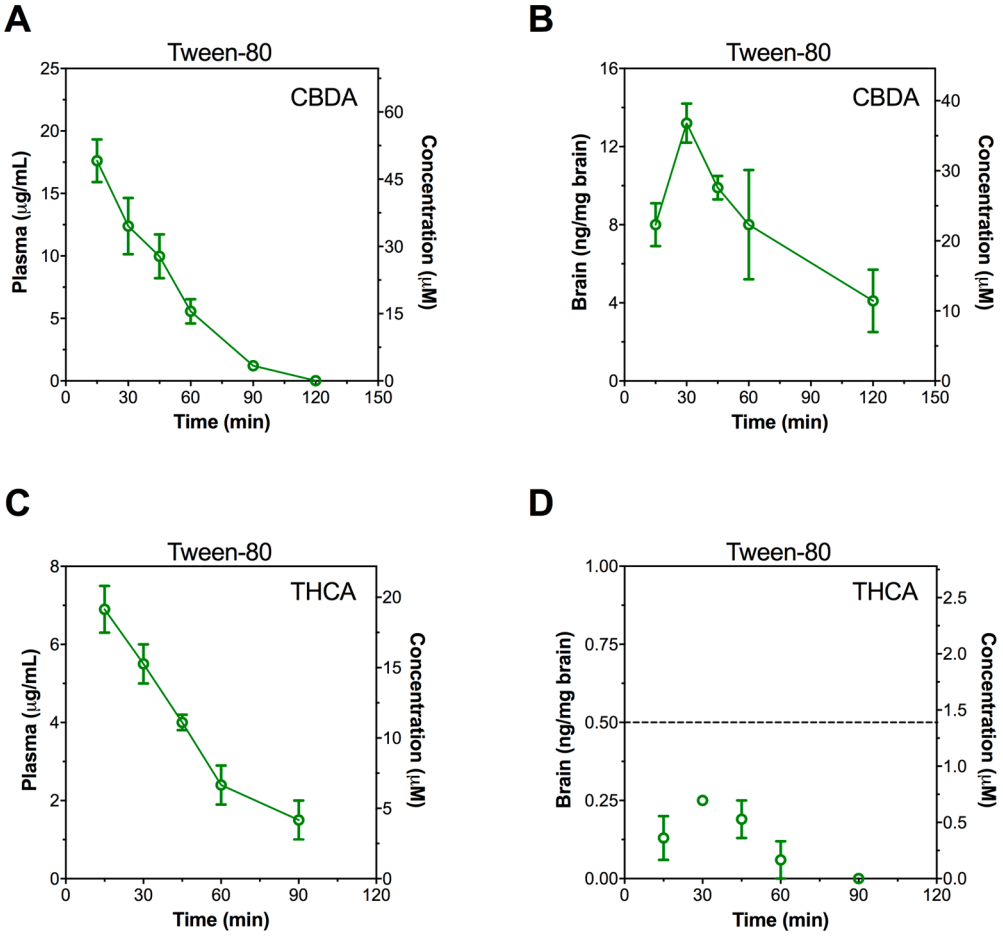

Figure 5. Pharmacokinetic analysis of CBDA and THCA when administered in a Tween-based vehicle. Concentration−time curves for CBDA in (A) plasma and (B) brain following a 10 mg/kg i.p. injection. Concentration−time curves for THCA in (C) plasma and (D) brain following a 10 mg/kg i.p. injection. Phytocannabinoid concentrations are depicted as both mass concentrations (left *y*-axis) and molar concentrations (right *y*-axis). Data are expressed as means ± SEM, with n = 3−5 per time point.

Table 2. Pharmacokinetics of CBDA and THCA in Mouse Plasma and Brain Following Administration in Different Vehicles

	CBDA, oil		CBDA, Tween-80		THCA, oil		THCA, Tween-80	
	10 mg/kg		10 mg/kg		10 mg/kg		10 mg/kg	
	plasma	brain	plasma	brain	plasma	brain	plasma	brain
C_{max} (μg/mL; ng/mg brain)	29.6 ± 2.2	2.0 ± 0.4	17.6 ± 1.7	13.2 ± 1.0	2.8 ± 0.4	BLQ	6.9 ± 0.6	BLQ
t_{max} (min)	30	45	15	30	15	n/a	15	n/a
$t_{1/2}$ (min)	92	41	20	55	67	n/a	33	n/a
AUC (μg min/mL; ng min/mg brain)	4699	183	598	1142	302	n/a	338	n/a
brain−plasma ratio	0.04		1.9		n/a		n/ai	

pharmacokinetics of drugs.[20−23] Indeed, Azmin et al.[20] reported that Tween 80 (polysorbate 80) increased brain concentrations of methotrexate. It is hypothesized that nonionic surfactants, which encapsulate drugs in micellar-like structures, disrupt interendothelial cell tight junctions.[23,24] Therefore, one possibility is that Tween 80 increases the brain uptake of CBDA via such a mechanism. However, if this were true, one might anticipate that the Tween-based vehicle would similarly increase the brain uptake of THCA. The Tween-based vehicle had no effect on the brain penetration of THCA, suggesting other mechanisms might be involved. Nonionic surfactants inhibit P-glycoprotein (P-gp)-mediated transport.[25] As some cannabinoids appear to be P-gp substrates,[19,26,27] it is possible that CBDA is a P-gp substrate and its efflux from the brain is inhibited by Tween 80. Further studies could explore whether CBDA and the phytocannabinoids are substrates for efflux transporters.

In parallel to the pharmacokinetic studies, the effect of CBDA was examined on hyperthermia-induced seizures in *Scn1a*$^{RX/+}$ mice. CBDA significantly increased the temperature threshold of GTCS. The anticonvulsant action of CBDA in the *Scn1a*$^{RX/+}$ mouse model of Dravet syndrome is consistent with a report in the patent literature showing CBDA was anticonvulsant against pentylenetetrazole-induced seizures.[12] CBDA was anticonvulsant at 10 and 30 mg/kg, which is lower than the effective dose of CBD (100 mg/kg) shown in our hands and in another study, suggesting CBDA may be more potent than CBD.[28,29] The effective anticonvulsant dose of

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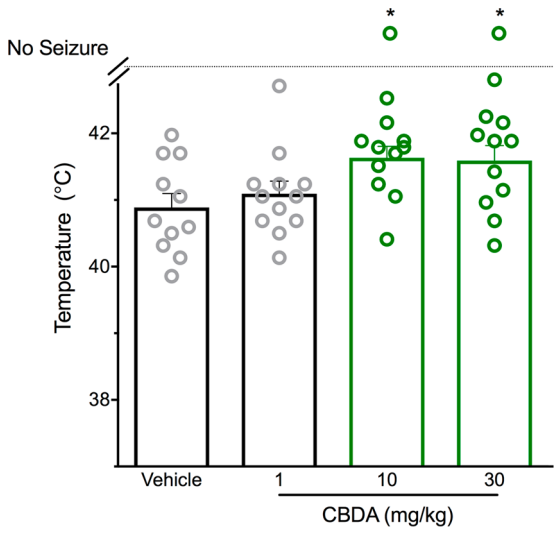

Figure 6. Dose−effect screening of CBDA against hyperthermia-induced seizures in *Scn1a*$^{RX/+}$ mice. Threshold temperatures of individual mice for generalized tonic-clonic seizure (GTCS) induced by hyperthermia following acute treatment with vehicle or varying doses of CBDA. CBDA significantly increased the temperature threshold for GTCS, resulting in greater resistance to thermal seizure induction (green, open symbols). The average temperature of GTCS is depicted by the bar. Error bars represent SEM, with n = 11−15 mice per group (*p < 0.05, logrank Mantel−Cox).

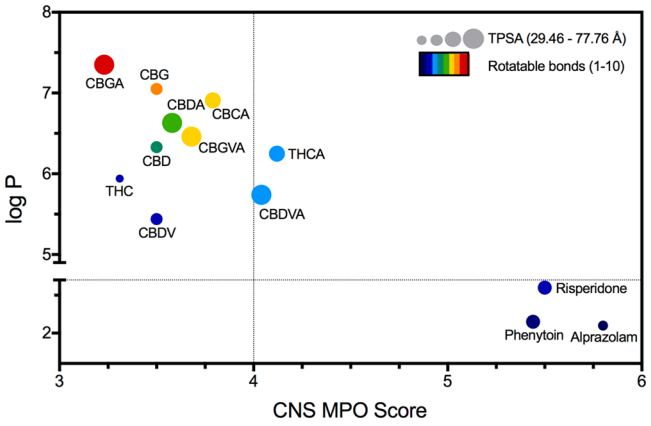

Figure 7. Physiochemical properties of phytocannabinoids. Plot displaying the distribution of partition coefficients (log P) vs CNS MPO scores for compounds (phytocannabinoid acids, neutral phytocannabinoids, and drugs). Symbol size represents the TPSA value, and color corresponds to number of rotatable bonds. Dotted lines represent the generally accepted cutoffs for penetration of the CNS, with log P < 2.5 and CNS MPO score ≥4 considered desirable for successful CNS drugs (lower right quadrant). For comparison, CNS drugs alprazolam, phenytoin, and risperidone have been included. Physiochemical properties of compounds were collated using Collaborative Drug Discovery Vault. Abbreviations: CNS = central nervous system; MPO = multiparameter optimization score; TPSA = topological polar surface area.

CBDA observed here in mice equates to approximately 50 and 150 mg doses, respectively, in a 60 kg human (FDA interspecies conversion). These doses approximate CBDA doses administered to childhood epilepsy patients in the form of artisanal *C. sativa* oils (average oral dose of 33 mg with a maximum dose of 540 mg).[3] As CBDA and CBD are often coadministered in artisanal medicinal *C. sativa* oils used by childhood epilepsy patients, it would be interesting to investigate whether there is any anticonvulsant synergy.[3] Future studies are required to elucidate the anticonvulsant mode(s) of action of CBDA, which may involve various epilepsy-relevant targets such as 5-HT1A, GPR55, and TRPV1 receptors.[30−32]

Concluding Remarks. The brain and plasma pharmacokinetic profiles of the cannabinoid acids presented herein provides useful information for future investigations of the in vivo pharmacological effects of these compounds in mice. The profound effects of different vehicle preparations on the pharmacokinetic parameters (absorption, elimination, and penetration) of the cannabinoids, as illustrated here with CBDA, highlight the importance of vehicle choice when designing experiments. Here, CBDA was highly brain penetrant when administered in a Tween-based vehicle and exhibited significant anticonvulsant properties in the *Scn1a*$^{RX/+}$ mouse model of Dravet syndrome. This provides further evidence that constituents in the *C. sativa* plant beyond CBD are anticonvulsant.

EXPERIMENTAL SECTION

General Experimental Procedures. CBDA, CBGA, and THCA were purchased from THC Pharm GmbH (Frankfurt, Germany). CBCA, CBDVA, and CBGVA were synthesized by Professor Michael Kassiou at the University of Sydney. The purity of all the cannabinoids used were CBCA (>99%), CBDA (>99%), CBDVA (99%), CBGA (98%), CBGVA (97%), and THCA (95%). Drugs were freshly prepared as solutions in vegetable oil (95% canola oil, 5% sunflower oil). CBDA and THCA were also prepared in ethanol−Tween 80−saline (1:1:18 ratio) (Sigma-Aldrich, St. Louis, MO, USA). When available, analytical standards were purchased from Cerilliant Corporation (Round Rock, TX, USA).

Animals. All animal care and experimental procedures were approved by the University of Sydney Animal Ethics Committee (protocols 2015/828 and 2016/1035), and all procedures were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. All cannabinoids were administered as a single i.p. injection in a volume of 10 mL/kg. C57BL/6J (Australian Resources Centre, stock 000664) and F1 mice generated by breeding 129S1/SvlmJ (Jackson Laboratory, stock 002448) or 129S6/SvEvTacAusb (Australian BioResources) with C57BL/6J mice were used for pharmacokinetic studies. *Scn1a*$^{RX/+}$ mice were generously provided by Professor Kazuhiro Yamakawa (RIKEN Brain Science Institute, Japan) by way of Professor Steven Petrou (Florey Institute, Australia). The mice were generated as previously described and were maintained as a congenic line on the 129S1/SvlmJ (129.*Scn1a*$^{RX/+}$) background.[33,34] For hyperthermia-induced seizure experiments, F1 mice were generated by breeding 129.*Scn1a*$^{RX/+}$ mice with wild-type C57BL/6J mice. The *Scn1a* genotype was determined as previously described.[33,34]

Pharmacokinetic Studies. Male and female mice 21−28 days postnatal received a single i.p. injection of 5 mg/kg of CBCA and CBDVA or 10 mg/kg of CBDA, CBGA, CBGVA, and THCA. These are nonsaturating doses that overlap with human equivalent doses of THCA and CBDA administered in *C. sativa* oils used by childhood epilepsy patients.[3] At selected time points (15−240 min), mice were anesthetized with isoflurane, and whole blood was collected by cardiac puncture. Plasma was isolated by centrifugation (9000*g* for 10 min, 4 °C) and stored at −80 °C until assayed. Whole brain was also collected and stored at −80 °C.

Sample Preparation. Plasma samples (50 μL) were spiked with internal standard (diazepam or Δ^9-THC-d_3), and protein precipitation was achieved by vortex-mixing with a 4× volume of acetonitrile. The organic layer was isolated by centrifugation (4000*g* for 10 min) and evaporated to dryness with N_2. Samples were reconstituted in methanol and 0.1% formic acid in water (150:250, v/

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Table 3. Physiochemical Properties of Phytocannabinoid Acids

phytocannabinoid	molecular weight	pK_a	log P	TPSA (Å)	rotatable bonds	CNS MPO score
CBCA	358.4	2.87	6.91	66.76	8	3.79
CBDA	358.5	2.91	6.63	77.76	7	3.59
CBDVA	330.4	2.91	5.94	77.76	5	4.04
CBGA	360.5	2.92	7.35	77.76	10	3.23
CBGVA	332.4	2.92	6.46	77.76	8	3.68
THCA	358.4	2.89	6.25	66.76	5	4.12

Table 4. Parameters for LC-MS/MS Detection of Cannabinoids

compound	molecular weight	parent > daughter ions (m/z)
CBCA	358.4	359.10 > 341.25
		359.10 > 316.00
CBDA	358.5	359.30 > 341.25
CBDVA	330.42	331.10 > 191.05
		331.10 > 233.15
CBGA	360.49	361.20 > 343.10
		361.30 > 219.10
CBGVA	332.4	331.10 > 193.05
		333.10 > 235.15
THCA	358.47	357.20 > 245.35
		357.20 > 191.30

v) for supported-liquid extraction with methyl *tert*-butyl ether (MTBE) using Biotage Isolute SLE+ columns (Uppsala, Sweden). Samples were evaporated to dryness with N_2 and reconstituted in acetonitrile and 0.1% formic acid in water (1:1.5, v/v) for analysis. Brain samples were prepared as described above with minor modifications. Briefly, each half brain was homogenized in phosphate-buffered saline solution (15×, w/v). Homogenates were centrifuged (20000*g* for 30 min, 4 °C), and brain supernatant (67 mg brain tissue/mL) was spiked with diazepam as an internal standard. Extraction was achieved by vortex-mixing with a 3× volume of ice-cold acetonitrile. The organic layer was isolated by centrifugation (20000*g* for 15 min, 4 °C) and evaporated to dryness with N_2. Samples were reconstituted with ice-cold acetonitrile (2 mL) and centrifuged (14000*g* for 30 min, 4 °C). The supernatant was evaporated to dryness with N_2. Samples were reconstituted in methanol and 0.1% formic acid in water for supported-liquid extraction as above. Samples were evaporated to dryness with N_2 and reconstituted in acetonitrile and 0.1% formic acid (1:1, v/v) for analysis.

Analytical Methods. Cannabinoid concentrations in plasma and brain samples were assayed by liquid chromatography−tandem mass spectrometry (LC-MS/MS) using a Shimadzu Nexera ultra-high-performance liquid chromatograph coupled to a Shimadzu 8030 triple quadrupole mass spectrometer (Shimadzu Corp., Kyoto, Japan), as previously described.[3,35] Briefly, LC separation was achieved on a Zorbax XDB-C_{18} (100 × 2.1 mm, 3.5 μm) reverse-phase column with a Zorbax C_8 guard column (Agilent, Santa Clara, CA, USA). A gradient elution with 0.1% formic acid in water and acetonitrile at a flow rate of 0.3 mL/min was used. The mass spectrometer was operated in a positive electrospray ionization mode (negative for THCA) with multiple reaction monitoring. Details regarding MS conditions for each cannabinoid are provided in Table 4. Quantification of cannabinoids in plasma and brain samples was performed by comparing samples to standards prepared with known amounts of drug.

Pharmacokinetic Calculations and Physiochemical Properties. Plasma and brain cannabinoid concentrations at each time point were averaged, and pharmacokinetic parameters were calculated by noncompartmental analysis. Each elimination rate constant (k_e) was determined by linear regression of the terminal component of the concentration−time curve using GraphPad Prism 7.0 (La Jolla, CA, USA). The log−linear trapezoidal method was used to calculate total drug exposure (area under concentration−time curve) using equations previously described.[36] Physiochemical properties of phytocannabinoids were determined using Collaborative Drug Discovery Vault (Burlingame, CA, USA).

Hyperthermia-Induced Seizures. Hyperthermia-induced seizure experiments were conducted on male and female *Scn1a*$^{RX/+}$ mice at age postnatal days 14−16 using a rodent temperature regulator (TCAT-2DF, Physitemp Instruments, Inc., Clifton, NJ, USA) reconfigured with a Partlow 1160+ controller (West Control Solutions, Brighton, UK) connected to a heat lamp and RET-3 rectal temperature probe. Mice acclimated to the temperature probe for 5 min prior to the induction of the hyperthermia protocol. The mouse core body temperature was elevated 0.5 °C every two minutes until the onset of the first clonic convulsion with loss of posture or until 42.5 °C was reached. Mice that reached 42.5 °C were held at that temperature for 3 min and were considered seizure free if no seizure occurred during the hold period. Threshold temperatures were compared using logrank Mantel-Cox with GraphPad Prism, and $p < 0.05$ was considered statistically significant. CBDA (1−30 mg/kg) or vehicle was administered after the acclimation period, immediately before the hyperthermia protocol, which is based on time-to-peak plasma concentration.

AUTHOR INFORMATION

Corresponding Author

*E-mail: jonathon.arnold@sydney.edu.au. Tel: +61293510812.

ORCID

Lyndsey L. Anderson: 0000-0002-0037-0891

Samuel D. Banister: 0000-0002-4690-4318

Notes

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