Original Article

The Pharmacokinetic Study of Progesterone and Allopregnanolone in Patients with Drug-Resistant Epilepsy: A Phase II Study

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Background: Progesterone, one of a class of neurosteroids, has been linked to seizure control. However, the pharmacokinetics of progesterone and its active metabolite, allopregnanolone, have never been investigated among patients with drug-resistant epilepsy (DRE).

Objective: To investigate the pharmacokinetics of progesterone, 400 mg micronized progesterone per oral every 12 hours for three months, among patients with DRE as an add-on therapy for seizure control. The pharmacokinetic parameters were examined using Phoenix WinNonlin® Software.

Results: Twelve patients were recruited. The biochemical measurements of the serum progesterone and allopregnanolone levels after taking the first dose of micronized progesterone were characterized by a time to maximum concentration (Tmax) median of 1.0 and 2.5 hours, a maximum concentration (Cmax) median of 274.97 and 3.81 ng/mL and a minimum concentration (Cmin) median of 56.93 and 1.06 ng/mL, respectively. The median values of the pharmacokinetic parameters of progesterone and allopregnanolone during the steady state were as follows: half-life (t1/2) of 2.4 and 2.1 hours, a Cmax of 964.35 and 8.92 ng/mL and a Cmin of 64.67 and 1.86 ng/mL, respectively. By examining the relationship between progesterone or allopregnanolone concentrations with seizure-controlling ability, the authors were able to identify a responder patient group with 5- to 7-fold higher serum concentrations of progesterone and 2- to 4-fold in allopregnanolone than the non-responders.

Conclusion: The present study revealed the pharmacokinetic parameters of progesterone and allopregnanolone. Moreover, the authors could establish higher serum levels of both progesterone and allopregnanolone, which could consequently relate to lowering the seizure frequency among patients with DRE.

Keywords: Neurosteroids; Dose regimens; Epilepsy; Pharmacokinetics; Drug-resistant epilepsy

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Drug-resistant epilepsy (DRE) is described as a form of epilepsy not responding to treatment with at least two antiepileptic drugs and dose regimens that are appropriately dosed as such[1]. It has been characterized by multiple seizures and attributed to (i) an overexpression of the P-glycoprotein efflux transporters that can reduce the concentration of antiepileptic drugs, (ii) changes in the sensitivity of voltage-gated sodium channels, and (iii) changes in the internalization of gamma-aminobutyric acid A (GABAA) receptors[2]. These cause patients with DRE to respond poorly to antiepileptic drugs[3,4].

Brexanolone, the aqueous form of allopregnanolone, and ganaxolone, the synthetic allopregnanolone, two neurosteroids that bind to GABAA receptors and enhance benzodiazepine effect[5-8], are now in use, and have been investigated concerning their use among individuals with

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DRE[6-8]. However, these medications have not been approved for use in Thailand. The authors searched for neurosteroids or other medications in Thailand that may provide an active metabolite that could operate as a neurosteroid. From the authors' knowledge, they opted to use micronized progesterone for the purposes of the present study.

Progesterone is a neurosteroid. It constitutes as a hormone activated in the central nervous system. Progesterone can be absorbed by 10% to 15% and reaches its maximal concentration in 1.5 to 2.3 hours after it enters the body. Progesterone metabolizes to allopregnanolone, an active metabolite also known as 3α-hydroxy-5α-pregnan-20-one, and has a protein binding rate of 96% to 99%[9]. Allopregnanolone stimulates GABAA receptors in a similar way to benzodiazepines but differs in the activation of extrasynaptic GABAA receptors because it does not enable GABAA receptors to be internalized[10,11]. A few studies have used allopregnanolone for refractory status epilepticus treatment to achieve seizure control[12]. Moreover, neurosteroids may be beneficial in patients with DRE. Related research has indicated that progesterone, at 600 mg/day, can be used as an add-on medication to manage seizures among patients with catamenial epilepsy[13-15]. Progesterone serum concentrations of 5 to 25 ng/mL have also been shown to be beneficial in lowering seizure frequency in these investigations[13]. In addition, progesterone pharmacokinetic studies are sparse, and no pharmacokinetic studies have been conducted among individuals with DRE.

At present, only the pharmacokinetics of progesterone at doses ranging from 200 to 600 mg/day as part of a hormone replacement therapy have been thoroughly studied[16-27]. However, the pharmacokinetics of progesterone as part of an add-on therapy to control seizures among patients with epilepsy have not been studied directly. Only one study had been conducted of the pharmacokinetics of allopregnanolone injections among patients with Alzheimer's disease[28] and one of brexanolone injections in postpartum depression[29-31]. To date, and to the authors' knowledge, no study has examined the drug levels and pharmacokinetics of allopregnanolone after receiving progesterone among patients with DRE.

Only one study had assessed the dose of progesterone at 600 mg/day in catamenial epilepsy[14]. However, that was not a study of the pharmacokinetics of progesterone and allopregnanolone. The importance of a pharmacokinetic study is that it can analyze and predict the appropriate dose able to exert a specific response to treatment. The authors decided to assess the pharmacokinetics of progesterone among patients with DRE using a higher dose of progesterone, at micronized progesterone 800 mg/day, than in the aforementioned study.

The goal of the present study was to evaluate progesterone and allopregnanolone pharmacokinetic characteristics among patients with DRE receiving micronized progesterone as an add-on medication at a dose of 800 mg/day, in the form of 400 mg every 12 hours, for three months. The authors then went on to calculate the progesterone and allopregnanolone serum levels needed to keep seizures under control. Furthermore, the relationship between serum progesterone or allopregnanolone levels and the treatment response was investigated.

Materials and Methods

Ethics approval and consent to participate

All patients in the present study provided their informed consent to participate under the declaration of Helsinki. Approval from the Institutional Review Board of the Royal Thai Army Medical Department was provided (Project number R150h/62). The present study was registered on the Thai Clinical Trials Registry (No. TCTR20200710005 first registration 10/07/2020).

Participants

For the present study, 12 patients were recruited. The patients were from the outpatient Department of Medicine and Pediatrics at Phramongkutklao Hospital between December 1, 2019 and February 28, 2021. The inclusion criteria were patients (i) aged over 12, experiencing epilepsy and receiving more than two types of antiepileptic drugs for at least two weeks without being able to control seizures, (ii) experiencing seizures more than five times monthly, and (iii) providing informed consent to participate to the present study. The exclusion criteria were patients (i) allergic to progesterone, (ii) using all kinds of hormones, (iii) who were pregnant, (iv) with a history of stroke or myocardial infarction within the previous year, (v) presenting a renal function test of less than 30 mL/minute, (vi) presenting a CHA_2DS_2-VASc score of 3 or more, (vii) presenting a serum AST or ALT level increase of 3-fold or more when compared with baseline within three months before recruitment, (viii) being treated with antibiotics, (ix) diagnosed with any type of cancer, (x) with a history of abnormal vaginal bleeding, and (xi) any

antiepileptics dose adjustment.

Interventions

The drug used in the present study was micronized progesterone soft gelatin capsules (Utrogestan™; Besins Healthcare) at 200 mg, in these capsules, the particle size was 10 μm or less at 95% to 100%, 2 to 4 μm at 30% to 55%, and 2 μm or less at 20% to 60% (Lot No. 0449 and 0453 filled in the opaque white capsule). The drug consisted of sunflower oil, soy lecithin, gelatin, glycerol, and titanium dioxide in a blister pack[32].

All participants received the 200 mg micronized progesterone soft gelatin capsules at a dose of two capsules every 12 hours as part of their add-on therapy, for three months. The authors monitored the progesterone and allopregnanolone serum levels in two treatment periods, pre-post the first dose and during the steady state (SS). Blood samples were collected from patients on the first day of receiving micronized progesterone at 400 mg, right before taking the medication (C0) and 1, 3, 4, 6, and 8 hours after taking the first dose of the medication (C1, C3, C4, C6, and C8 consecutively). Three months later, blood samples were collected from patients right before taking the next dose of medication (SS0) and 2, 4, and 8 hours after taking the medication as steady state (SS2, SS4 and SS8, consecutively).

Procedures

Blood samples of 3 mL were collected according to the timeframe set in the trial protocol. Subsequently, they were left to coagulate at room temperature for one hour and centrifuged at 1,500 rpm for ten minutes at room temperature. The separated serum was frozen at –80°C until the progesterone and allopregnanolone levels were analyzed.

Throughout the present study, patients were monitored for compliance through history taking, pill counts, and seizure frequency monitoring, recorded in a seizure diary. Researchers performed at least one telephone call with each patient, as well as a patient follow-up during the first to third months of the treatment period.

Assay protocols

The authors analyzed the progesterone and allopregnanolone levels using enzyme-linked immunosorbent assay (ELISA) by using the NovaTec™ (NVTDNOV006) and the ArborAssay® DetectX® test kits (ABAK061-H5), respectively.

Progesterone assay

Serum progesterone levels were analyzed by adjusting the temperatures of the sample and the test kits to room temperature. Next, 20 μL of the serum samples were placed in the test plates. The progesterone in the sample serum bound and reacted to the addition of progesterone-horseradish protein (progesterone-HRP) and reacted at 37°C to the addition of the progesterone-HRP. Subsequently, the progesterone attached to the bottom of the well of a 96-well plate and reacted with the tetramethylbenzidine (TMB) substrate. The absorbance was measured at wavelength of 450 nm with a 96-well plate reader within five minutes, and a standard curve was generated by a 4-Parameter Logistic (4-PL) Curve that determined the progesterone concentration in the serum samples[33].

Allopregnanolone assay

By extracting the serum with ethyl acetate and vortexing for two minutes, the levels of allopregnanolone in the blood were determined. After that, the mixture was let to stand for five minutes to allow the stages to completely separate. The mixture was placed in a dry ice bath until frozen. The top solution was then collected in clean tubes. To save as much allopregnanolone as feasible, the mixture was re-extracted.

To assess allopregnanolone levels, the extracted samples were mixed with the DetectX® allopregnanolone conjugate and the DetectX® allopregnanolone antibody in a volume of 50+50 μL for each well. The plates were shaken for two hours at room temperature at 700 to 900 rpm. After that, stop solution and TMB substrate were added. A 96-well plate reader was used to test the absorbance at wavelength of 450 nm in under five minutes. The concentration of allopregnanolone in serum samples were evaluated using 4-PL regression to produce a standard curve[34].

Outcomes

The authors monitored the progesterone and allopregnanolone levels pre-post the first dose and during the steady state, along with their pharmacokinetic parameters, using Phoenix WinNonlin® Software, version 8.3 (Certara USA, Inc., Princeton, NJ). The authors then examined the relationships between the progesterone or allopregnanolone serum levels and the patients' treatment responses. The patients were divided in two groups: responders and non-responders.

Table 1. Baseline characteristics of the recruited subjects (n=12)

No.	Sex	Age (years)	BMI (kg/m^2)	Diagnosis	Current AEDs									CYP 2C9 inhibitors	CYP 2C19 inducers			eGFR (mL/minute/ 1.73 m^2)	AST (U/L)	ALT (U/L)
					BRI	CLB	LCM	LEV	LTG	PER	RUF	TPM	ZNS	VPA	PB	PHT	CBZ			
1	Male	13	10.70	LGS			/	/				/						84.20	18.80	12.40
2	Male	23	19.23	LGS			/		/			/		/				137.13	23.20	37.60
3	Male	31	23.23	LGS	/			/	/	/	/	/					/	116.70	22.00	49.00
4	Male	23	24.26	RE											/	/		120.89	N/A	N/A
5	Female	35	19.03	RE	/	/	/	/									/	115.39	23.60	19.70
6	Male	54	24.39	RE	/								/	/				70.26	29.60	19.30
7	Female	45	20.20	RE				/	/				/					N/A	19.00	19.00
8	Female	29	27.56	RE		/						/				/		124.96	28.00	38.80
9	Male	43	30.86	RE	/												/	80.02	19.10	35.50
10	Female	51	16.65	RE														52.70	47.00	49.00
11	Male	24	23.91	RE					/	/		/		/				105.39	19.50	13.50
12	Female	43	23.88	RE										/		/		115.30	20.20	21.60
Median (IQR)		33 (23 to 45)	23.56 (19.08 to 24.36)															115.30 (80.02 to 120.89)	22.00 (19.10 to 28.00)	21.60 (19.00 to 38.80)

AEDs=antiepileptic drugs; ALT=alanine transaminase; AST=aspartate aminotransferase; BMI=body mass index; BRI=brivaracetam; CBZ=carbamazepine; CLB=clobazam; CYP=Cytochrome P450; eGFR=estimated glomerular filtration rate in CKD-EPI formular; IQR=interquartile range; LCM=lacosamide; LEV=levetiracetam; LGS=Lennox-Gastaut syndrome; LTG=lamotrigine; N/A=not available data; PB=phenobarbital; PER=perampanel; PHT=phenytoin; RE=refactory epilepsy; RUF=rufinamide; TPM=topiramate; VPA=valproic acid; ZNS=zonisamide

Results

Study population

Twelve patients with DRE met the criteria of the present study. Of these, seven were male (58.3%). The median (interquartile, IQR) age of the participants was 33 with a range of 23 to 45 years. Of the 12 participants, nine (75%) had received a diagnosis of adult DRE and three (25%) with Lennox-Gastaut syndrome. All patients had a history of receiving more than two types of antiepileptic drugs. The median (IQR) number of drugs the patients received at the time of the present study was four (two to seven) items. Three items that these patients were most likely to receive were topiramate (41.67%), brivaracetam (33.33%) and lamotrigine (33.33%) (Table 1). One patient dropped out to adjust antiepileptic drug dose, and four for adverse effects.

All participants had self-reported measures of medication compliance. The adherence rate was estimated at 95%.

Pharmacokinetic study pre-post the first dose

The median progesterone blood level before medication administration (C0) was 0.13 ng/mL, according to a pharmacokinetic study of individuals with DRE pre-post the first dose of micronized progesterone (n=12). After taking the medication, their progesterone serum levels were 64.14, 69.90, 120.14, 73.23, and 56.93 ng/mL after 1, 3, 4, 6, and 8 hours (C1, C3, C4, C6, and C8), respectively (Figure 1, Table 2).

The median allopregnanolone serum level before medication administration (C0) was 0.81 ng/mL, according to a pharmacokinetic study of individuals with DRE pre-post the first dose of micronized progesterone (n=12). After taking the medication, their allopregnanolone serum levels were 1.97, 1.94, 2.38, 1.88, and 1.06 ng/mL at 1, 3, 4, 6, and 8 hours (C1, C3, C4, C6, and C8), respectively (Figure 1, Table 2).

Pharmacokinetic study during the steady state

Patients receiving 400 mg micronized progesterone every 12 hours until steady state was reached (n=8) presented a median serum progesterone level of 64.67 ng/mL before the following dose (SS0). The median values of their serum progesterone levels after 2, 4, and 8 hours (SS2, SS4, and SS8) were 211.89, 694.41, and 306.83 ng/mL, respectively, as the authors performed biochemical assays to monitor therapeutic medication at three timepoints after taking the last dose of micronized progesterone (Figure 1, Table 3).

For determination of allopregnanolone concentrations, eight participants taking 400 mg micronized progesterone every 12 hours at steady state had a median serum allopregnanolone level of 1.86 ng/mL before the following dose (SS0). The median values of their serum allopregnanolone levels after 2, 4, and 8 hours (SS2, SS4, and SS8) were 4.96, 6.30, and 2.07 ng/mL, respectively (as shown in Figure 1, Table 3).

Table 2. Pharmacokinetic parameters of progesterone and allopregnanolone after receiving the first dose of micronized progesterone (n=12)

Parameter	First dose pharmacokinetic parameters; median (IQR)	
	Progesterone	Allopregnanolone
Cmin (ng/mL)	56.93 (18.27 to 117.36)	1.06 (0.50 to 2.14)
t1/2 (hour)	2.55 (1.80 to 4.50)	3.31 (2.22 to 3.31)
Tmax (hour)	1.00 (1.00 to 4.00)	2.50 (1.00 to 4.00)
Cmax (ng/mL)	274.97 (92.44 to 468.16)	3.81 (2.28 to 6.19)
AUC_{last} (hours·ng/mL)	694.99 (272.69 to 1,666.73)	18.01 (10.52 to 25.47)

Cmin=minimum concentration; t1/2=half-life; Tmax=time to maximum concentration; Cmax=maximum concentration; AUC_{last}=area under the concentration-time curve; IQR=interquartile range

Table 3. Pharmacokinetic parameters regarding progesterone and allopregnanolone after receiving a micronized progesterone dose during the steady state (1st to 3rd month) (n=8)

Parameter	Steady state pharmacokinetic parameters; median (IQR)	
	Progesterone	Allopregnanolone
Cmin (ng/mL)	64.67 (24.31 to 212.82)	1.86 (0.88 to 9.26)
t1/2 (hour)	2.35 (1.60 to 3.10)	2.05 (1.70 to 10.63)
Tmax (hour)	4.00 (2.38 to 4.00)	4.00 (2.50 to 6.63)
Cmax (ng/mL)	964.35 (92.44 to 468.16)	8.92 (4.78 to 10.97)
AUC_{last} (hours·ng/mL)	3,093.87 (1,640.81 to 25,474.13)	33.02 (26.34 to 70.37)

Cmin=minimum concentration; t1/2=half-life; Tmax=time to maximum concentration; Cmax=maximum concentration; AUC_{last}=area under the concentration-time curve; IQR=interquartile range

Relationship between serum progesterone or allopregnanolone levels and their seizure-controlling ability

The seizure-controlling capacity of attained progesterone and allopregnanolone levels was used to split the patients in two groups in the present study. The responders comprised of patients experiencing a 50% reduction or more in seizure frequency after taking the micronized progesterone. Non-responders made up the second group. These were individuals having less than 50% drop in seizure frequency after taking the medicine.

During the steady state, the findings of the seizure-controlling abilities research were retrieved. According to their findings, the median serum progesterone level in the responder group before the next dose (SS0) was 142.68 ng/mL, but the median levels after four and eight hours following the next dose (SS4 and SS8) were 1,283.08 and 365.37 ng/mL, respectively. Interestingly, this group's serum progesterone levels were six to seven times greater than those of the non-responder group. Additionally, the median serum allopregnanolone level in the responder group before taking the next dose (SS0) was 4.51 ng/mL, with median levels of 9.37 and 2.30 ng/mL at four and eight hours after taking the following dose (SS4 and SS8), respectively. This group's serum allopregnanolone levels were similarly two to four times greater than those of the non-responder group (Table 4).

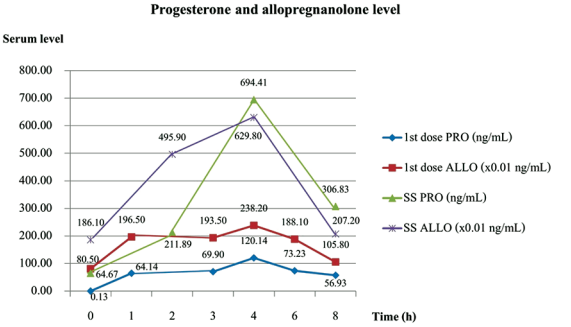

Figure 1. Progesterone and allopregnanolone levels.

1st dose=the first dose of micronized progesterone; PRO=progesterone; ALLO=allopregnanolone; SS=steady state

Adverse effects

Adverse effects in the present study, particularly somnolence (n=3) was the most common, and one patient reported depression with an unknown cause. No additional serious side effects were found (Table 4).

Discussion

The present study constitutes to firstly report on pharmacokinetic parameters of pre-post the first dose of micronized progesterone and during the steady state, as part of an add-on administration against epilepsy. The authors found the median serum allopregnanolone level four hours after taking the medicine was 6 ng/mL. This made patients with similar serum allopregnanolone levels tend to decrease their seizure frequency. Furthermore, the present study results agreed with those of Herzog et al[15], who studied 294 intractable seizure subjects randomized 2:1 to oral progesterone 200 mg lozenges TID at days 14 to 25 of each menstrual cycle or placebo for three menstrual cycles. Their findings suggested that the serum allopregnanolone levels four hours after taking the medicine was approximately 5 ng/mL. At these serum allopregnanolone levels, the responder group exhibited a decrease in their seizure frequencies that was higher than 50%. Moreover, the present study comprises the first finding on clinical outcome of high dose progesterone in DRE but only three patients (3/8, 37.5%) maintained their seizures.

Table 4. Percentage of reduction in seizure frequency of responders (n=3) and non-responders (n=5) during the steady state (1st to 3rd month)

No. of participants		Serum level (ng/mL)				% reduction in seizure frequency	Adverse effect
		SS0	SS2	SS4	SS8		
Responders							
6	PRO	366.14	347.49	1572.27	365.37	–50*	None
	ALLO	10.85	7.77	7.83	11.39		
8	PRO	31.58	54.27	859.64	256.64	–100*	None
	ALLO	1.06	0.71	9.71	1.27		
10	PRO	142.68	246.13	1,283.08	470.35	–84*	Somnolence
	ALLO	4.51	5.74	9.37	2.30		
Non-responders							
4	PRO	29.85	1,069.05	186.58	54.59	100	None
	ALLO	0.70	8.48	3.96	1.17		
7	PRO	97.76	279.06	1,572.27	357.01	3.3	Somnolence
	ALLO	10.85	7.77	7.83	11.39		
9	PRO	22.46	177.65	272.88	61.97	–20*	None
	ALLO	1.27	4.18	4.77	1.85		
11	PRO	236.19	138.38	529.19	427.46	–6.7*	Somnolence
	ALLO	12.38	14.84	16.74	15.46		
12	PRO	0.30	0.16	0.05	0.07	–14.3*	None
	ALLO	0.82	0.80	0.67	0.06		

SS0=serum progesterone or allopregnanolone level at steady state before the next dose of micronized progesterone; SS2=serum progesterone or allopregnanolone level at steady state after the next dose of micronized progesterone for 2 hours; SS4=serum progesterone or allopregnanolone level at steady state after the next dose of micronized progesterone for 4 hours; SS8=serum progesterone or allopregnanolone level at steady state after the next dose of micronized progesterone for 8 hours; PRO=progesterone; ALLO=allopregnanolone

* Minus described as reduction in seizure frequency

The median serum allopregnanolone level at minimum concentration (Cmin) in the present study was 2 ng/mL. This result agreed with that of the study by Ruttanajirundorn et al[12], six refractory status epilepticus subjects with oral micronized progesterone 200 mg every eight hours for five days, reported an allopregnanolone level at Cmin of approximately 1 ng/mL. By using an electroencephalogram (EEG), Ruttanajirandorn et al[12] found that these patients also had a significantly decreased seizure latency (p=0.004). Although the present study did not monitor EEG, it did monitor the rates of the recorded seizures.

The authors considered the relationships between serum progesterone or allopregnanolone levels with the treatment response during the steady state in the responder group compared with the non-responder group. The authors found that serum progesterone and allopregnanolone levels in the responder group were higher than those in the non-responder group by five to seven and two to four times, respectively. This result seemed to be justified by the fact that allopregnanolone binds to the GABAergic receptors and that this binding acts in an antiepileptic manner[9]. In fact, the higher the serum allopregnanolone levels, the greater the seizure frequency decreased, according to an in vivo study conducted by Lucchi et al[35]. The latter can be simply interpreted as proof that the decrease in seizure frequency relates directly to the higher circulating levels of progesterone and allopregnanolone.

The present pharmacokinetic study also found that the t1/2 values of progesterone and allopregnanolone after the first dose and during the steady state differed. The present study constitutes the first to report these medians. Although the Tmax values of both substances are similar to those of related studies[16-23], the Cmax recorded by the present study was higher than that of other studies. One reason for that might be the fact that the progesterone administration scheme employed by the present study was higher in terms of dose than that employed by related studies. Finally, the authors found the AUC_{last} values of progesterone and allopregnanolone were higher than those reported in the study conducted by Andreen et al[17], in which 36 postmenopausal women with climacteric symptoms were treated with add-on 400 or 800 mg vaginal progesterone daily or placebo for

14 days per cycle, probably because of the higher dose used in the latter. Similarly, the studies of Wang et al[16] and McAuley et al[18] focused on menopausal women by administering progesterone at doses of 200 and 300 mg, respectively. Their reported AUC values were 1.7 times lower than those of the present study. Following the volume of distribution (Vd) could not be investigated in the present study because the number of participants was limited.

When considering drug administration among patients with DRE, the serum allopregnanolone level seemed to be low. However, one should not neglect that the results refer to the drug level in the blood, whereas the antiepileptic drug levels frequently refer to drug levels in the brain. Kancheva et al[36] examined the levels of neurosteroids in the cerebrospinal fluid and the serum, and found that the levels of progesterone and allopregnanolone in the brain were actually 7 and 300 times higher than those in the serum, respectively. Therefore, it could be understood that although the serum allopregnanolone level (at Cmin) in the present study was 2 ng/mL, the level of allopregnanolone in the brain should be around 600 ng/mL. Nevertheless, no studies have shown that the serum level of allopregnanolone can control seizures in practice.

After the present study patients took the first micronized progesterone dose, the authors discovered the serum progesterone and allopregnanolone levels reached their Tmax at four hours. Following that point, the Tmax of progesterone was similar to related studies[16-27]. When compared with related studies, allopregnanolone[28] and other neurosteroids, such as ganaxolone[37], pregnanolone[38], and alfaxolone[39], were also reported to exhibit their drug distribution in a compartmental, double-picked, manner. In the present study, the authors employed a non-compartmental analysis (NCA) model based on the practice adopted by older pharmacokinetic studies of progesterone[16,38]. When using this same NCA model, no differences were identified regarding the Tmax of progesterone.

The present study encountered limitations. The participants varied widely in age. In addition, the study enrolled a small sample size. Prospective research should examine an increased number of participants to expand the study's findings of efficacy and safety as well as dose optimization to better manage seizures in the future.

Conclusion

The present study constitutes the first to focus on the pharmacokinetics of progesterone at a dose of 400 mg every 12 hours for three months among patients with DRE. It also comprises the first to conduct an analysis of progesterone's pharmacokinetics pre-post the first dose and during the steady state. Although the present study was conducted on a specific group of patients, the data and ideas derived from the present study could form the basis for undertaking similar research in the future.

What is already known on this topic?

Previously, neurosteroids, brexanolone, and ganaxolone showed benefits among individual patients with DRE but no studies have investigated progesterone.

What this study adds?

This study presented new knowledge about progesterone and its derivatives, allopregnanolone, pharmacokinetics, and add-on benefits when used with other antiepileptic drugs among patients with DRE.

Acknowledgement

The authors genuinely appreciate the dedication of all patients participating in the present research.

Trial registration

This study has been registered with the Thai Clinical Trials Registry (No. TCTR20200710005, 10 July 2020).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

MS and JS were a major contributor in writing the manuscript. JS and PS contributed to the conception and design of the study, patient screening as well as reading and approving the manuscript. PM and CD participated in patient enrollment and collecting data. MS and JS analyzed and interpreted the pharmacokinetic data. JS and WS rechecked and confirmed the results of the pharmacokinetic analyses. All authors critically revised the manuscript for important intellectual content and approved the final manuscript.

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Conflicts of interest

The authors report no conflicts of interest in undertaking the research presented herein, the authorship or the publication of this article.

References

1. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, et al. ILAE official report: a practical clinical definition of epilepsy. Epilepsia 2014;55:475-82.
2. Tang F, Hartz AMS, Bauer B. Drug-resistant epilepsy: Multiple hypotheses, few answers. Front Neurol 2017;8:301.
3. Chen Z, Brodie MJ, Liew D, Kwan P. Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic drugs: A 30-year longitudinal cohort study. JAMA Neurol 2018;75:279-86.
4. Brodie MJ, Barry SJ, Bamagous GA, Norrie JD, Kwan P. Patterns of treatment response in newly diagnosed epilepsy. Neurology 2012;78:1548-54.
5. Ahboucha S, Coyne L, Hirakawa R, Butterworth RF, Halliwell RF. An interaction between benzodiazepines and neuroactive steroids at GABA A receptors in cultured hippocampal neurons. Neurochem Int 2006;48:703-7.
6. Rosenthal ES, Claassen J, Wainwright MS, Husain AM, Vaitkevicius H, Raines S, et al. Brexanolone as adjunctive therapy in super-refractory status epilepticus. Ann Neurol 2017;82:342-52.
7. Kerrigan JF, Shields WD, Nelson TY, Bluestone DL, Dodson WE, Bourgeois BF, et al. Ganaxolone for treating intractable infantile spasms: a multicenter, open-label, add-on trial. Epilepsy Res 2000;42:133-9.
8. Pieribone VA, Tsai J, Soufflet C, Rey E, Shaw K, Giller E, et al. Clinical evaluation of ganaxolone in pediatric and adolescent patients with refractory epilepsy. Epilepsia 2007;48:1870-4.
9. Saporito MS, Gruner JA, DiCamillo A, Hinchliffe R, Barker-Haliski M, White HS. Intravenously administered ganaxolone blocks diazepam-resistant lithium-pilocarpine-induced status epilepticus in rats: Comparison with allopregnanolone. J Pharmacol Exp Ther 2019;368:326-37.
10. Tsutsui K, Haraguchi S. Chapter 96 - Neurosteroids. In: Takei Y, Ando H, Tsutsui K, editors. Handbook of hormones. San Diego: Academic Press; 2016. p. 537-e96-12.
11. Herzog AG. Hormonal therapies: progesterone. Neurotherapeutics 2009;6:383-91.
12. Ruttanajirundorn P, Samchai S, Suphanklang J, Sithinamsuwan P, Chinvarun Y. Role of inhibitory neurosteroid as an adjunctive treatment in refractory status epilepticus (Phase IIa study): efficacy, safety, and bioavailability of allopregnanolone converting from oral micronized progesterone. Thai J Neurol 2021;37:23-31.
13. Herzog AG. Reproductive endocrine considerations and hormonal therapy for women with epilepsy. Epilepsia 1991;32 Suppl 6:S27-33.
14. Herzog AG. Progesterone therapy in women with complex partial and secondary generalized seizures. Neurology 1995;45:1660-2.
15. Herzog AG, Frye CA. Allopregnanolone levels and seizure frequency in progesterone-treated women with epilepsy. Neurology 2014;83:345-8.
16. Wang H, Liu M, Fu Q, Deng C. Pharmacokinetics of hard micronized progesterone capsules via vaginal or oral route compared with soft micronized capsules in healthy postmenopausal women: a randomized open-label clinical study. Drug Des Devel Ther 2019;13:2475-82.
17. Andréen L, Sundström-Poromaa I, Bixo M, Andersson A, Nyberg S, Bäckström T. Relationship between allopregnanolone and negative mood in postmenopausal women taking sequential hormone replacement therapy with vaginal progesterone. Psychoneuroendocrinology 2005;30:212-24.
18. McAuley JW, Kroboth FJ, Kroboth PD. Oral administration of micronized progesterone: a review and more experience. Pharmacotherapy 1996;16:453-7.
19. Simon JA, Robinson DE, Andrews MC, Hildebrand JR 3rd, Rocci ML Jr, Blake RE, et al. The absorption of oral micronized progesterone: the effect of food, dose proportionality, and comparison with intramuscular progesterone. Fertil Steril 1993;60:26-33.
20. Freeman EW, Weinstock L, Rickels K, Sondheimer SJ, Coutifaris C. A placebo-controlled study of effects of oral progesterone on performance and mood. Br J Clin Pharmacol 1992;33:293-8.
21. Hargrove JT, Maxson WS, Wentz AC. Absorption of oral progesterone is influenced by vehicle and particle size. Am J Obstet Gynecol 1989;161:948-51.
22. Nahoul K, Dehennin L, Scholler R. Radioimmunoassay of plasma progesterone after oral administration of micronized progesterone. J Steroid Biochem 1987;26:241-9.
23. Maxson WS, Hargrove JT. Bioavailability of oral micronized progesterone. Fertil Steril 1985;44:622-6.
24. Arafat ES, Hargrove JT, Maxson WS, Desiderio DM, Wentz AC, Andersen RN. Sedative and hypnotic effects of oral administration of micronized progesterone may be mediated through its metabolites. Am J Obstet Gynecol 1988;159:1203-9.
25. Chakmakjian ZH, Zachariah NY. Bioavailability of progesterone with different modes of administration. J Reprod Med 1987;32:443-8.

26. Erny R, Pigne A, Prouvost C, Gamerre M, Malet C, Serment H, et al. The effects of oral administration of progesterone for premature labor. Am J Obstet Gynecol 1986;154:525-9.
27. Ottoson UB, Carlstrom K, Damber JE, von Schoultz B. Serum levels of progesterone and some of its metabolites including deoxycorticosterone after oral and parenteral administration. Br J Obstet Gynaecol 1984;91:1111-9.
28. Hernandez GD, Solinsky CM, Mack WJ, Kono N, Rodgers KE, Wu CY, et al. Safety, tolerability, and pharmacokinetics of allopregnanolone as a regenerative therapeutic for Alzheimer's disease: A single and multiple ascending dose phase 1b/2a clinical trial. Alzheimers Dement (N Y) 2020;6:e12107.
29. Kanes SJ, Colquhoun H, Doherty J, Raines S, Hoffmann E, Rubinow DR, et al. Open-label, proof-of-concept study of brexanolone in the treatment of severe postpartum depression. Hum Psychopharmacol 2017;32:e2576.
30. Kanes S, Colquhoun H, Gunduz-Bruce H, Raines S, Arnold R, Schacterle A, et al. Brexanolone (SAGE-547 injection) in post-partum depression: a randomised controlled trial. Lancet 2017;390:480-9.
31. Meltzer-Brody S, Colquhoun H, Riesenberg R, Epperson CN, Deligiannidis KM, Rubinow DR, et al. Brexanolone injection in post-partum depression: two multicentre, double-blind, randomised, placebo-controlled, phase 3 trials. Lancet 2018;392:1058-70.
32. Utrogestan 200 mg, soft capsule product information [Internet]. Besins Healthcare Australia; 2017 [cited 2022 Apr 8]. Available from: https://www.tga.gov.au/sites/default/files/auspar-progesterone-170601-pi-02.pdf.
33. NovaTec Immundiagnostica GmbH. Progesterone enzyme immunoassay for the quantitative determination of Progesterone in human serum or plasma only for in-vitro diagnostic use product leaflets. Dietzenbach, Germany; 2022.
34. Arbor Assays. DetectX allopregnanolone enzyme immunoassay kit monoclonal antibody based. Ann Arbor, MI: Arbor Assays; 2016.
35. Lucchi C, Costa AM, Rustichelli C, Biagini G. Allopregnanolone and pregnanolone are reduced in the hippocampus of epileptic rats, but only allopregnanolone correlates with seizure frequency. Neuroendocrinology 2021;111:536-41.
36. Kancheva R, Hill M, Novák Z, Chrastina J, Kancheva L, Stárka L. Neuroactive steroids in periphery and cerebrospinal fluid. Neuroscience 2011;191:22-7.
37. Zolkowska D, Wu CY, Rogawski MA. Intramuscular allopregnanolone and ganaxolone in a mouse model of treatment-resistant status epilepticus. Epilepsia 2018;59 Suppl 2:220-7.
38. Andréen L, Spigset O, Andersson A, Nyberg S, Bäckström T. Pharmacokinetics of progesterone and its metabolites allopregnanolone and pregnanolone after oral administration of low-dose progesterone. Maturitas 2006;54:238-44.
39. Goodchild CS, Serrao JM, Sear JW, Anderson BJ. Pharmacokinetic and pharmacodynamic analysis of alfaxalone administered as a bolus intravenous injection of phaxan in a Phase 1 randomized trial. Anesth Analg 2020;130:704-14.