

The Pharmacokinetic Study of Progesterone and Allopregnanolone in Refractory Epilepsy : Phase II Study

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Abstract

Background: Progesterone belongs to a class of neurosteroids used for the reduction of seizure frequency in patients with refractory epilepsy. However, the pharmacokinetics of progesterone and its active derivative, allopregnanolone, have never been studied in these patients.

Objectives: This study was aim to explore the pharmacokinetic parameters of progesterone 400 mg every 12 h, for 3 months, in patients with refractory epilepsy as an add-on therapy to control seizures. Phoenix® WinNonlin® was used to analyse the pharmacokinetic parameters.

Results: Twelve patients were recruited. From a therapeutic drug monitoring, the serum progesterone and allopregnanolone levels after taking the first dose of progesterone were characterised by a time to maximum concentration (T_{max}) median of 1 and 2.5 h, a maximum concentration (C_{max}) median of 274.97 and 3.81 ng/mL, and a minimum concentration (C_{min}) 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: t_{1/2} of 2.4 and 2.0 h, C_{max} of 964.35 and 8.92 ng/mL, and C_{min} of 64.67 and 1.86 ng/mL, respectively. By examining the relationship between the progesterone or allopregnanolone concentrations with seizure-controlling ability, we could identify a responder patient group with 6- to 7-fold higher serum concentrations of progesterone and allopregnanolone than the non-responders.

Conclusions: We could establish higher serum levels of both progesterone and allopregnanolone, which could consequently relate to lowering the seizure frequency in patients with refractory epilepsy. The suggested progesterone dose was 400 mg orally every 12 h against refractory epilepsy

Trial registration: This study has been registered on the Thai Clinical Trials Registry (No. TCTR20200710005, 10 July 2020)

Background

Refractory epilepsy is described as a form of epilepsy not responding to treatment with at least two types of antiepileptic drugs that are appropriately dosed as such.[1] Refractory epilepsy is characterised by a higher repeated seizure frequency and has been 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 internalisation of gamma-aminobutyric acid A (GABA_A) receptors.[2] As a result, patients with refractory epilepsy respond poorly to antiepileptic drugs.[3, 4]

Brexanolone and ganaxolone, two neurosteroids activating the binding with the GABA_A receptors by benzodiazepines, are currently in use, and studies on their use by patients with refractory epilepsy already exist.[5–7] However, these drugs have not been registered for use in Thailand. Hence, we searched for

neurosteroids or other available drugs in Thailand that could provide an active metabolite that could act as a neurosteroid. As a result, and for the purpose of this study, we decided to choose progesterone.

Progesterone is a hormone that is activated in the central nervous system and has been classified as a neurosteroid. When progesterone enters the body, it is metabolised into allopregnanolone, which is an active metabolite also known as 3 α -hydroxy-5 α -pregn-20-one.[8] Allopregnanolone activates the GABA_A receptors in a manner similar to that of benzodiazepines, but it differs with regard to the activation of the extrasynaptic GABA_A receptors in that it does not allow for the internalisation of GABA_A receptors.[9, 10] As a result, neurosteroids can be effective in patients with refractory epilepsy. Previous studies have shown that progesterone (administered at doses of 600 mg/day) can be utilised to control seizures in patients with catamenial epilepsy, as part of an add-on therapy.[11–13] These studies have also suggested that a progesterone blood concentration between 5 and 25 ng/mL can be effective in reducing the seizure frequency.[11] Incidentally, the pharmacokinetic studies of progesterone are limited, whereas no pharmacokinetic studies in patients with refractory epilepsy are available.

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. However, the pharmacokinetics of progesterone as part of an add-on therapy to control seizures in patients with epilepsy have not been studied directly. There is only one study of the pharmacokinetics of allopregnanolone injections in patients with Alzheimer's disease[14] and one of brexanolone injections in postpartum depression.[15–17] To date and to our knowledge, no study has examined the drug levels and the pharmacokinetics of allopregnanolone after receiving progesterone in patients with refractory epilepsy.

Only one study has assessed the dosage of progesterone at 600 mg/day in catamenial epilepsy,[12] however, that was not a study of the pharmacokinetics of progesterone and allopregnanolone. The importance of a pharmacokinetic study is that it can analyse and predict the appropriate dosage able to exert a specific response to treatment. We decided to assess the pharmacokinetics of progesterone in patients with refractory epilepsy by using a higher dosage of progesterone (800 mg/day) than the aforementioned study.

This study aimed to record the pharmacokinetic parameters of progesterone and allopregnanolone in patients with refractory epilepsy who received progesterone as an add-on therapy at doses of 800 mg/day (in the form of 400 mg every 12 h) for 3 months. We proceeded to predict the serum levels of progesterone and allopregnanolone required to control seizures. Moreover, we studied the relationships between the serum progesterone or allopregnanolone levels and the treatment response. To the best of our knowledge, this is the first pharmacokinetic study on the use of progesterone in patients with refractory epilepsy.

Methods

Participants

For this study, 12 patients were recruited. We selected these patients from the Outpatient Department of the Phramongkutklao Hospital between 1 December 2019 and 28 February 2021. The inclusion criteria were patients (i) aged over 12 years, suffering from epilepsy and receiving more than two types of antiepileptic drugs for at least 2 weeks, without being able to control seizures, (ii) experiencing seizures more than five times per month and (iii) giving informed consent to participate to this 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 with a renal function test of <30 mL/min, (vi) presenting with a CHA₂DS₂-VASc score ≥ 3 , (vii) presenting with a serum AST or ALT level increase ≥ 3 -fold when compared to baseline within 3 months prior to recruitment, (viii) being treated with antibiotics, (ix) diagnosed with any type of cancer and (x) with a history of abnormal vaginal bleeding (Figure 1).

Interventions

The drug used in this study came in the form of micronised progesterone soft gelatin capsules (UtrogestanTM; Besins Healthcare) at 200 mg; in these capsules, the particle size was: ≤ 10 μm at 95–100%, 2–4 μm at 30–55% and ≤ 2 μm at 20–60% (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.[18]

All participants received the 200-mg progesterone soft gelatin capsules at a dose of two capsules every 12 h as part of their add-on therapy, for 3 months. We 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 progesterone (at 400 mg) right before taking the medication and 1, 3, 4, 6 and 8 h after taking the medication (first dose). A month later, blood samples were collected from patients right before taking the next medication and 2, 4 and 8 h after taking the medication (steady state).

Procedures

Blood samples (3 mL) were collected according to the timeframe set in the trial protocol. Subsequently, they were left to coagulate at room temperature for 1 h and were centrifuged at 1,500 rpm for 10 min at room temperature. The separated serum was frozen at -80°C until the progesterone and allopregnanolone levels were analysed.

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

Assay protocols

After acquiring a blood sample, we analysed the progesterone and allopregnanolone levels using ELISA; we used the NovaTec™ (NVTDN006) and the ArborAssay® DetectX® test kits (ABAK061-H5), respectively.

Progesterone assay

Serum progesterone levels were analysed 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 to 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 450 nm with a 96-well plate reader within 5 min, and a standard curve was generated by a 4-Parameter Logistic (4-PL) that allowed for the determination of the progesterone concentration in the serum samples.[19]

Allopregnanolone assay

The serum allopregnanolone levels were analysed by extracting the serum with ethyl acetate and vortexing it for 2 min. Subsequently, the mixture was allowed to stand for 5 min for the complete separation of the phases. The mixture was frozen by placing it in a dry ice bath. Then, the top solution was collected in clean tubes. The mixture was then re-extracted to maintain as much allopregnanolone in the mixture as possible.

To analyse the allopregnanolone levels, both the DetectX® allopregnanolone conjugate and the DetectX® allopregnanolone antibody were added to the extracted samples at a volume of 50+50 µL each well. The plates were incubated on a shaker at 700–900 rpm for 2 h, at room temperature. Subsequently, the TMB substrate and the stop solution were added. The absorbance was measured at 450 nm by a 96-well plate reader within 5 min. A standard curve was generated by a 4-PL that allowed for the determination of the concentration of allopregnanolone in the serum samples.[20]

Outcomes

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

Results

Study population

There were 12 patients with refractory epilepsy in this study. Of these, seven were male (58.3%) and five were female (41.7%). The median interquartile (IQR) age of the participants was 33 (23–45) years. Of the 12 participants in this study, nine (75%) had been diagnosed with refractory epilepsy and three (25%) had been diagnosed with Lennox–Gastaut syndrome. All patients had a history of receiving more than two types of antiepileptic drugs. The median (IQR) number of drugs that the patients were receiving at the time of the study was 4 (2–7) items. Three of the items that these patients were most likely to be receiving were topiramate, brivaracetam and lamotrigine (Table 1).

Table 1. Baseline characteristics of the subjects recruited.

General information		Data range	Median (IQR)
Sex	Male	n = 7 (58.3%)	-
	Female	n = 5 (41.7%)	-
Age (years)		13-54	33 (23-45)
Body weight (kg)		22.5-81.0	60.0 (53.0-71.8)
Height (cm)		145-177	163.5 (156-170)
BMI (kg/m ²)		10.70-30.86	23.56 (19.08-24.36)
Diagnosis	Lennox–Gastaut syndrome	n = 3 (25%)	-
	Refractory epilepsy	n = 9 (75%)	-
AED(s) usage		2 - 9	4 (2-7)
	1-2 item(s)	n = 3 (25%)	-
	3 items	n = 9 (75%)	-
Current AED(s)	Topiramate	n = 5 (41.67%)	
	Brivaracetam	n = 4 (33.33%)	
	Clonazepam	n = 4 (33.33%)	
	Lamotrigine	n = 4 (33.33%)	
	Levetiracetam	n = 4 (33.33%)	
	Valproic acid	n = 4 (33.33%)	
	Carbamazepine	n = 3 (25%)	
	Lacosamide	n = 3 (25%)	
	Phenytoin	n = 3 (25%)	
	Clobazam	n = 2 (25%)	
	Gabapentin	n = 2 (25%)	
	Perampanel	n = 2 (25%)	
	Zonisamide	n = 2 (25%)	
Past AED(s) usage	1-2 item(s)	n = 0 (0%)	-
	3 items	n = 12 (100%)	-

General information		Data range	Median (IQR)
Renal function	Serum creatinine (mL/min)	52.7-137.13	0.78 (0.63-0.98)
	eGFR (mL/min/1.73m ²)	52.70-137.13	115.30 (80.02-120.89)
Liver function	AST (U/L)	18.80-47.00	22.00 (19.10-28.00)
	ALT (U/L)	12.40-49.00	21.60 (19.00-38.80)

Abbreviations: IQR, interquartile range; AED(s), antiepileptic drug(s); eGFR, estimated glomerular filtration rate; AST, aspartate aminotransferase; ALT, alanine transaminase.

Pharmacokinetic study pre–post the first dose

From the pharmacokinetic study of patients with refractory epilepsy pre–post the first dose (n = 12), we could identify that the median progesterone serum level before the drug administration (C0) was 0.13 ng/mL. After taking the medicine, the median values of their progesterone serum levels at 1, 3, 4, 6 and 8 h (C1, C3, C4, C6 and C8) were 64.14, 69.90, 120.14, 73.23 and 56.93 ng/mL, respectively (Table 2). The drug levels in the patients' blood at all six examined timepoints were analysed by using the pharmacokinetic analysis software (Phoenix® WinNonlin® version 8.3). The median values of the analysed pharmacokinetic parameters were as follows: time to maximum concentration (Tmax) = 1 h, maximum concentration (Cmax) = 274.97 ng/mL, t_{1/2} = 2.6 h and area under the curve (AUC_{last}) = 694.99 h·ng/mL (Table 3).

Table 2. The median (and IQR) of progesterone and allopregnanolone levels in the blood, pre–post receiving the first progesterone dose.

Serum neurosteroid levels pre–post the first dose of progesterone		
Sampling time	Progesterone (ng/mL)	Allopregnanolone (ng/mL)
C0	0.133 (0.000 - 0.410)	0.805 (0.233 – 1.017)
C1	64.136 (9.918 - 349.534)	1.965 (1.447 – 4.738)
C3	69.896 (1272.876 - 272.823)	1.935 (0.986 – 4.335)
C4	120.138 (25.818 - 198.717)	2.382 (1.178 – 3.274)
C6	73.232 (17.471 - 229.506)	1.881 (0.897 – 2.449)
C8	56.93 (18.270 - 117.359)	1.058 (0.499 – 2.138)

Abbreviations: C0, before taking the first dose of progesterone; C1, 1 h after taking the first dose of progesterone; C3, 3 h after taking the first dose of progesterone; C4, 4 h after taking the first dose of progesterone; C6, 6 h after taking the first dose of progesterone; C8, 8 h after taking the first dose of progesterone.

Table 3. Pharmacokinetic parameters of progesterone and allopregnanolone after receiving the first dose of progesterone.

First dose of progesterone on the neurosteroid pharmacokinetic parameters (expressed as median and IQR)		
Parameter	Progesterone	Allopregnanolone
t1/2 (h)	2.552 (1.799 - 4.495)	3.306 (2.217 - 3.306)
Tmax (h)	1.000 (1.000 - 4.000)	2.500 (1.000 - 4.000)
Cmax (ng/mL)	274.973 (92.442 - 468.162)	3.805 (2.282 - 6.187)
AUC (h·ng/mL)	694.989 (272.690 - 1666.734)	18.007 (10.519 - 25.474)

Abbreviations: t1/2, half-life; Tmax, time to maximum concentration; Cmax, maximum concentration; AUC, area under the concentration–time curve.

From the pharmacokinetic study of patients with refractory epilepsy pre–post the first dose ($n = 12$), we could identify that the median allopregnanolone serum level before the drug administration (C_0) was 0.81 ng/mL. After taking the medicine, the median values of their allopregnanolone serum levels at 1, 3, 4, 6 and 8 h (C_1, C_3, C_4, C_6 and C_8) were 1.97, 1.94, 2.38, 1.88 and 1.06 ng/mL, respectively (Table 2). When the blood drug levels were analysed at all six timepoints by using the pharmacokinetic analysis software (Phoenix® WinNonlin® version 8.3), we could identify the following medians: $T_{max} = 2.5$ h, $C_{max}=3.81$ ng/mL, $t_{1/2} = 3.3$ h, and $AUC_{last} = 18.01$ h·ng/mL (Table 3).

Pharmacokinetic study during the steady state

When the patients were receiving progesterone at 400 mg, every 12 h, for 3 months ($n = 6$), their median serum progesterone level before the next dose (SS_0) was 64.67 ng/mL. As we performed therapeutic drug monitoring at three timepoints after taking the last dose of progesterone, the median values of their serum progesterone levels at 2, 4 and 8 h (SS_2, SS_4 and SS_8) were 211.89, 694.41 and 306.83 ng/mL, respectively (Table 4). The drug levels in the patients' blood at all four examined timepoints were analysed by using the pharmacokinetic analysis software (Phoenix® WinNonlin® version 8.3). The median values of the analysed pharmacokinetic parameters were as follows: $T_{max} = 4.0$ h, $C_{max} = 964.35$ ng/mL, $t_{1/2} = 2.4$ hour, and $AUC_{last} = 3,093.870$ h·ng/mL (Table 5).

Table 4. The median (and IQR) of the progesterone and allopregnanolone levels in the blood pre–post receiving a progesterone dose during the steady state (1st–3rd month) at different timepoints.

Serum neurosteroid steady state levels		
Sampling time	Progesterone (ng/mL)	Allopregnanolone (ng/mL)
SS0	64.668 (24.309 - 212.815)	1.861 (0.884 - 9.264)
SS2	211.887 (75.301 - 330.380)	4.959 (1.526 - 8.300)
SS4	694.412 (208.152 - 1499.971)	6.298 (3.441 - 9.624)
SS8	306.826 (56.435 - 411.934)	2.072 (1.196 - 9.746)

Abbreviations: SS0, before taking the next dose of progesterone; SS2, 1 h after taking the dose of progesterone; SS4, 4 h after taking the dose of progesterone; SS8, 8 h after taking the dose of progesterone.

Table 5. Pharmacokinetic parameters regarding progesterone and allopregnanolone after receiving a progesterone dose during the steady state (1st-3rd month.)

Steady state pharmacokinetic parameters (expressed as median and IQR)		
Parameter	Progesterone	Allopregnanolone
t _{1/2} (h)	2.350 (1.600 - 3.100)	2.049 (1.696 - 10.630)
Tmax (h)	4.000 (2.375 - 4.000)	4.000 (2.500 - 6.625)
Cmax (ng/mL)	964.346 (92.442 - 468.162)	8.922 (4.780 - 10.970)
AUC (h·ng/mL)	3093.870 (1640.807 - 25474.125)	33.021 (26.342 - 70.372)

Abbreviations: t_{1/2}, half-life; Tmax, time to maximum concentration; Cmax, maximum concentration; AUC, area under the concentration–time curve.

When the patients were receiving progesterone at 400 mg, every 12 h, for 3 months (n=6), their median serum allopregnanolone level before the next dose (SS0) was 1.86 ng/mL. As we performed therapeutic drug monitoring at three timepoints after taking the last dose of progesterone, the median values of their serum allopregnanolone levels at 2, 4 and 8 h (SS2, SS4 and SS8) were 4.96, 6.30 and 2.07 ng/mL, respectively (Table 4). When the blood drug levels were analysed at all four timepoints by using the pharmacokinetic analysis software (Phoenix® WinNonlin® version 8.3), we could identify the following medians: Tmax = 4.0 h, Cmax = 8.92 ng/mL, t_{1/2} = 2.0 h and AUC_{last} = 33.02 h·ng/mL (Table 5).

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

In this study, the researchers divided the patients into two groups based on the seizure-controlling ability of the achieved progesterone and allopregnanolone levels. The first group consisted of the responders; these were patients who experienced a reduction in their seizure frequency by ≥50% compared to that

before the drug administration. The second group consisted of the non-responders; these were patients who experienced a reduction in their seizure frequency by <50% compared to that before the drug administration.

The results of the seizure-controlling ability study were extracted during the steady state. From their analyses, we ascertained that in the responder group, the median serum progesterone level before taking the next dose (SS0) was 198.86 ng/mL, whereas the median values at 4 and 8 h after taking the next dose (SS4 and SS8) were 1,215.95 and 311.00 ng/mL, respectively. The serum progesterone levels in this group were, of course, higher than those of the non-responder group, by 6–7 times (Figure 3).

Concurrently, the median of the serum allopregnanolone level in the responder group before taking the next dose (SS0) was 5.96 ng/mL, whereas the median values at 4 and 8 h after taking the next dose (SS4 and SS8) were 8.77 and 6.33 ng/mL, respectively. The serum allopregnanolone levels in this group were also higher than those of the non-responder group, by 2–6 times (Figure 4).

Discussion

Our study is the first to report on pharmacokinetic parameters pre–post the first dose of progesterone and during the steady state, as part of an add-on administration for epilepsy. We monitored the serum progesterone and allopregnanolone levels in patients with refractory epilepsy receiving progesterone at a dose of 400 mg, every 12 h, for 3 months as part of an add-on therapy aiming to control seizures.

We found that the median serum allopregnanolone level 4 h after taking the medicine is 8.6 ng/mL. As a result, patients with similar serum allopregnanolone levels tend to have a decrease in their seizure frequency. Furthermore, our results agree with those of Herzog *et al.*,[13] suggesting that the serum allopregnanolone levels 4 h after taking the medicine were approximately 5 ng/mL. At these serum allopregnanolone levels, the responder group exhibited a decrease in their seizure frequency that was higher than 50%.

The median serum allopregnanolone level at minimum concentration (C_{min}) in our study was 2 ng/mL. This result agrees with that of the study of Ruttanajirundorn *et al.*,[21] reporting an allopregnanolone level at C_{min} of approximately 1 ng/mL. By using an electroencephalogram (EEG), Ruttanajirandorn *et al.* found that these patients also had a significantly decreased seizure latency (p-value = 0.004). Although our study did not monitor EEG, it did monitor the rates of the recorded seizures.

When considering the relationships between the serum progesterone or allopregnanolone levels with the treatment response during the steady state in the responder group compared to the non-responder group, we found that the serum progesterone and allopregnanolone levels in the responder group are higher than those of the non-responder group by 6–10 and 2–6 times, respectively. This result seems to be justified by the fact that allopregnanolone binds to the GABAergic receptors, and this binding acts in an antiepileptic manner.[8] In fact, the higher the serum allopregnanolone levels, the greater the seizure frequency decrease, according to an *in vivo* study conducted by Lucchi *et al.*[22] The latter can be simply

interpreted as a proof that the decrease in seizure frequency relates directly to the higher circulating levels of progesterone and allopregnanolone.

Our pharmacokinetic study also found that the t_{1/2} values of progesterone and allopregnanolone after the first dose and during the steady state are different. Our study is the first to report these medians. Although the T_{max} values of both substances are similar to those of previous studies,[23-30] the C_{max} recorded by our study is higher than that of other studies. A reason for that might be the fact that the progesterone administration scheme employed by our study is higher in terms of dosage than that employed by the previous studies. Finally, we found that the AUC values of progesterone and allopregnanolone are higher than those reported in the study conducted by Andreen *et al.*,[24] probably because the higher dosage used in the latter. Similarly, the studies of Wang *et al.*[23] and McAuley *et al.* [25] focused on menopausal women by giving them progesterone at doses of 200 and 300 mg, respectively; as a result, their reported AUC values are 1.7 times lower than those of our study.

When considering drug administration in patients with refractory epilepsy, the serum allopregnanolone level seems to be relatively 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.* [31] examined the levels of neurosteroids in the cerebrospinal fluid and the serum, and found that the levels of progesterone and allopregnanolone in the brain are actually 7 and 300 times higher than those in the blood, respectively. Therefore, it is understood that although the serum allopregnanolone level (at C_{min}) in our study was 2 ng/mL, the level of allopregnanolone in the brain should be around 600 ng/mL. Nevertheless, no studies thus far have shown that the serum level of allopregnanolone can control seizures in practice.

After our patients took the first progesterone dose, we discovered that the serum progesterone and allopregnanolone levels reached their T_{max} at 4 h. Following that point, the levels decreased back to C_{min} (Figure 2). As far as the serum allopregnanolone levels are concerned, we could identify two T_{max} points, at 1 and at 4 h after taking the medicine. When compared to previous study, allopregnanolone[14] and other neurosteroids, such as ganaxolone,[32] pregnanolone[33] and alfaxolone,[34] were also reported to exhibit their drug distribution in a compartmental (double-picked) manner. In this study, we employed a non-compartmental analysis (NCA) model) based on the practice adopted by older pharmacokinetic studies of progesterone.[23, 33] When using this same NCA model, no differences regarding the T_{max} were identified.

Conclusion

Our study is the first to focus on the pharmacokinetics of progesterone at a dose of 400 mg, every 12 h, for 3 months, in patients with refractory epilepsy. It is also the first to conduct an analysis of progesterone's pharmacokinetics pre–post the first dose and during the steady state. Although our study is conducted on a specific group of patients, the data and ideas derived from this study could form a guide for the undertaking of similar research in the future.

A limitation of this study is its small sample size. Prospective research should consider an increase in the number of participants and should utilise the results of this study in further adjusting the drug levels, to control seizures more effectively in the future.

Abbreviations

4-PL; 4-Parameter Logistic

AUC_{last}; area under the curve

C₀; serum progesterone or allopregnanolone level after taking the first dose progesterone

C₁; serum progesterone or allopregnanolone level after taking the first dose progesterone for 1 hour

C₃; serum progesterone or allopregnanolone level after taking the first dose progesterone for 3 hours

C₄; serum progesterone or allopregnanolone level after taking the first dose progesterone for 4 hours C₆;
serum progesterone or allopregnanolone level after taking the first dose progesterone for 6 hours

C₈; serum progesterone or allopregnanolone level after taking the first dose progesterone for 8 hours

C_{max}; a maximum concentration

C_{min}; a minimum concentration

EEG; electroencephalogram

ELISA;

GABA_A receptors; gamma-aminobutyric acid A receptors

IQR; interquartile

progesterone-HRP; progesterone-horseradish protein

SS; steady state

SS₀; serum progesterone or allopregnanolone level at steady state before the next dose progesterone

SS₂; serum progesterone or allopregnanolone level at steady state after the progesterone for 2 hours

SS₄; serum progesterone or allopregnanolone level at steady state after the progesterone for 4 hours

SS₈; serum progesterone or allopregnanolone level at steady state after the progesterone for 8 hours

T_{max}; a time to maximum concentration

Declarations

Ethics approval and consent to participate

All patients had given their informed consent to participate and performed under the declaration of Helsinki in this study. Approval from the institutional review board of the Royal Thai Army Medical Department was provided (Project number R150h/62). This study has been registered on the Thai Clinical Trials Registry (No. TCTR20200710005 first registration 10/07/2020)

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

JS was a major contributor in writing the manuscript. PS contributed to the conception and design of the study. PM and CD were main in patients enrollment and collected data. MS analyzed and interpreted the pharmacokinetic analyses. WS was recheck and confirm the result of pharmacokinetic analyses. All authors critical revision of the manuscript for important intellectual content and approved the final manuscript.

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Disclosure

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

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Figures

Figure 1. Algorithm of the subject recruitment.

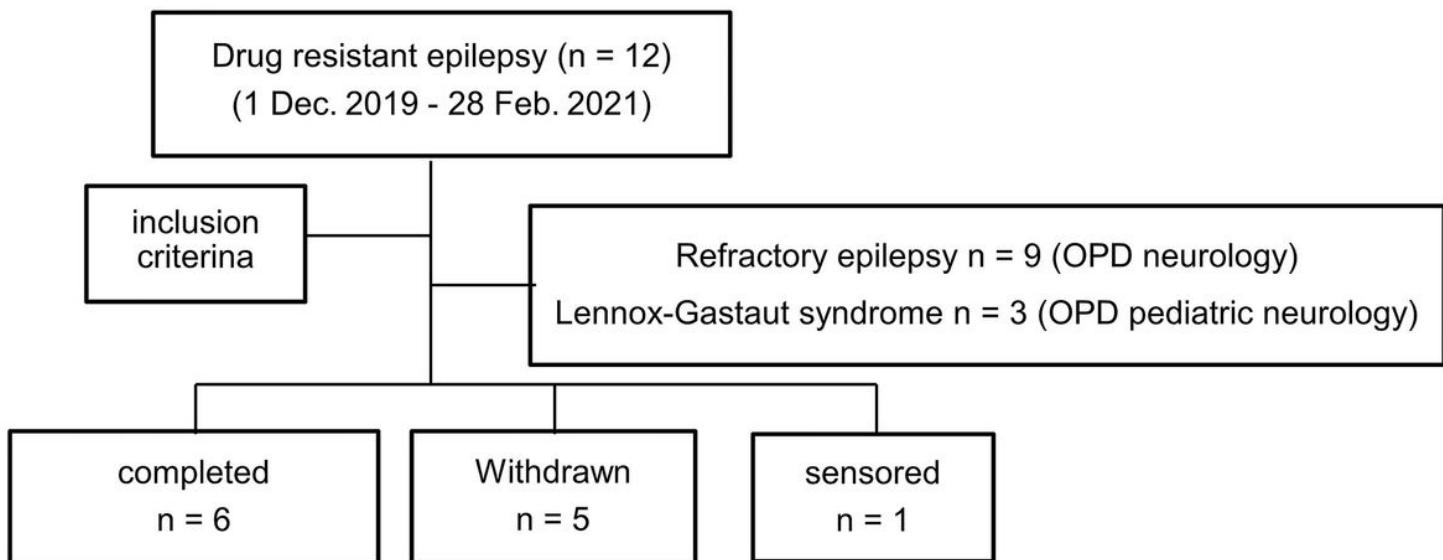
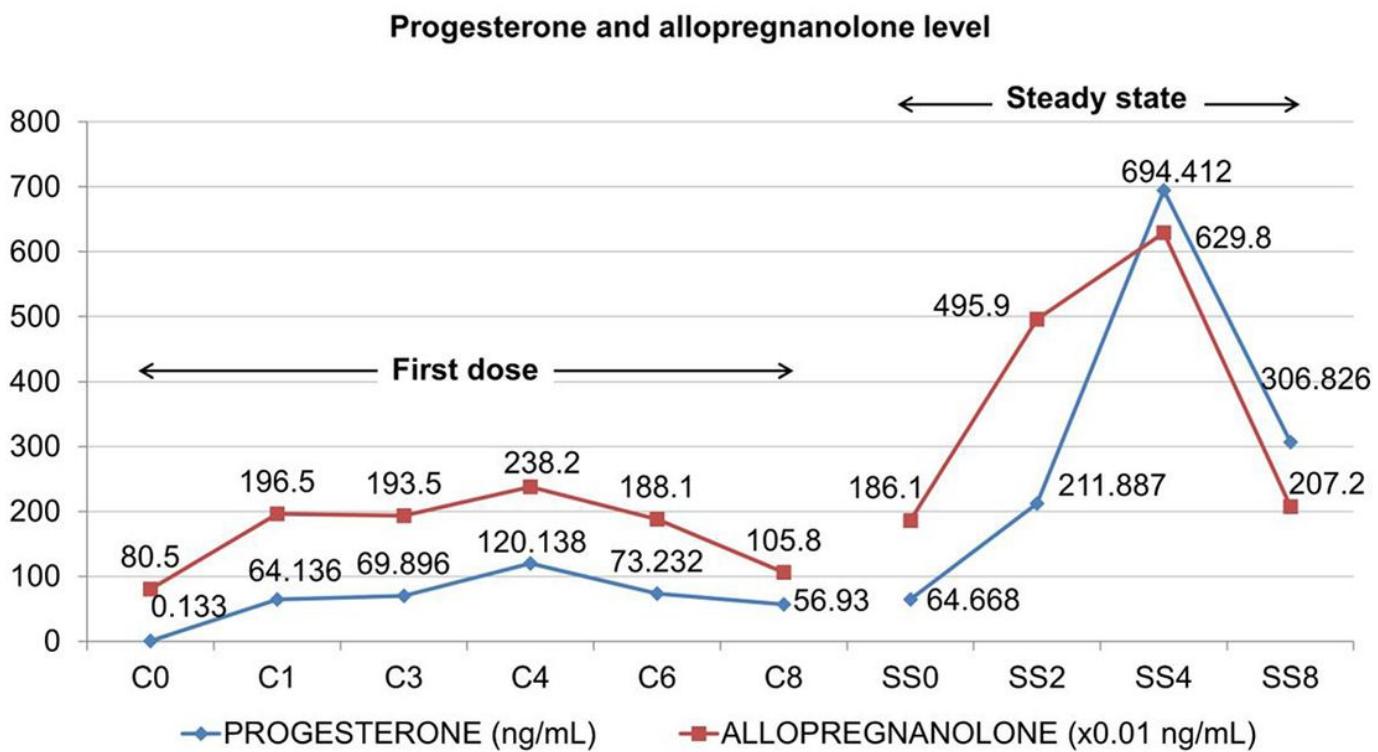


Figure 1

See image above for figure legend

Figure 2. Progesterone and allopregnanolone levels.

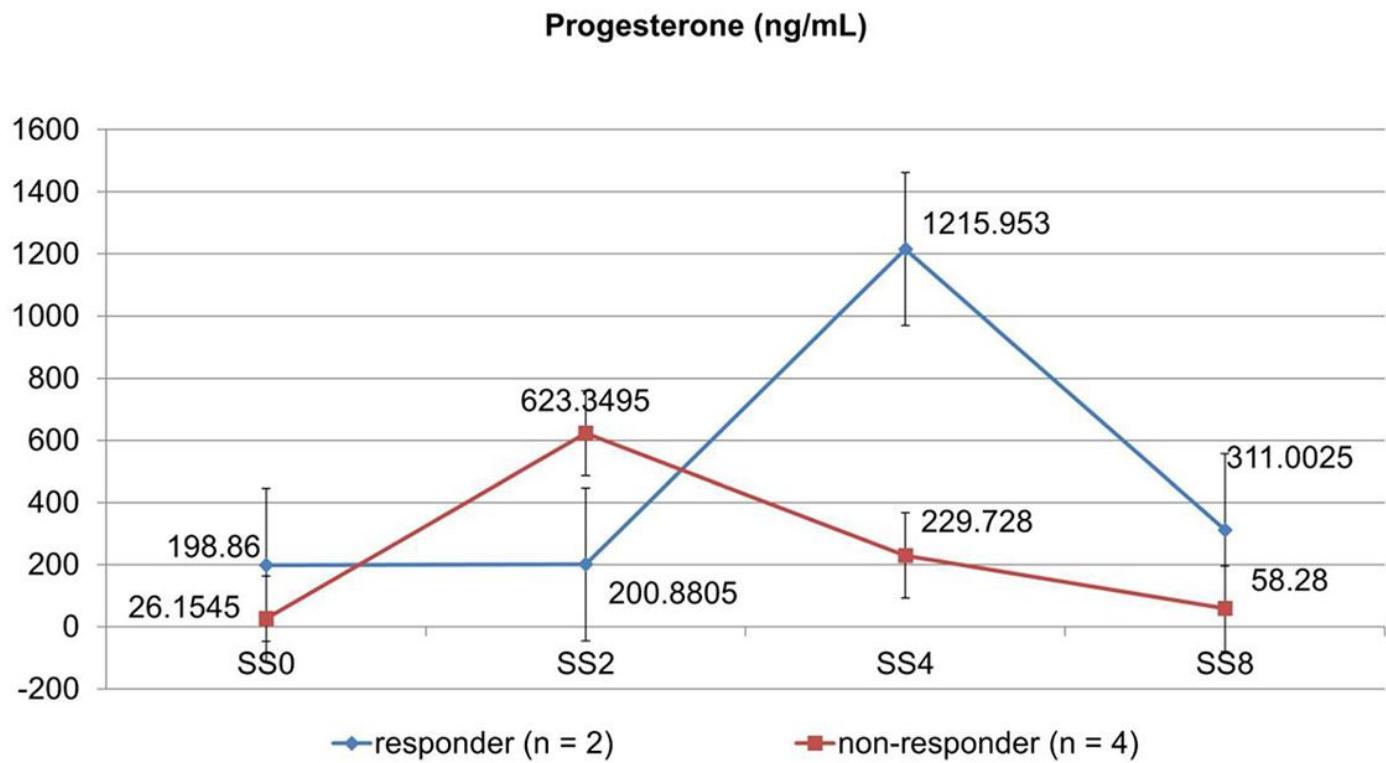


Abbreviations: C0, before taking the first dose of progesterone; C1, 1 h after taking the first dose of progesterone; C3, 3 h after taking the first dose of progesterone; C4, 4 h after taking the first dose of progesterone; C6, 6 h after taking the first dose of progesterone; C8, 8 h after taking the first dose of progesterone; SS0, before taking the next dose of progesterone; SS2, 1 h after taking the dose of progesterone; SS4, 4 h after taking the dose of progesterone; SS8, 8 h after taking the dose of progesterone.

Figure 2

See image above for figure legend

Figure 3. Progesterone levels in responders and non-responders.

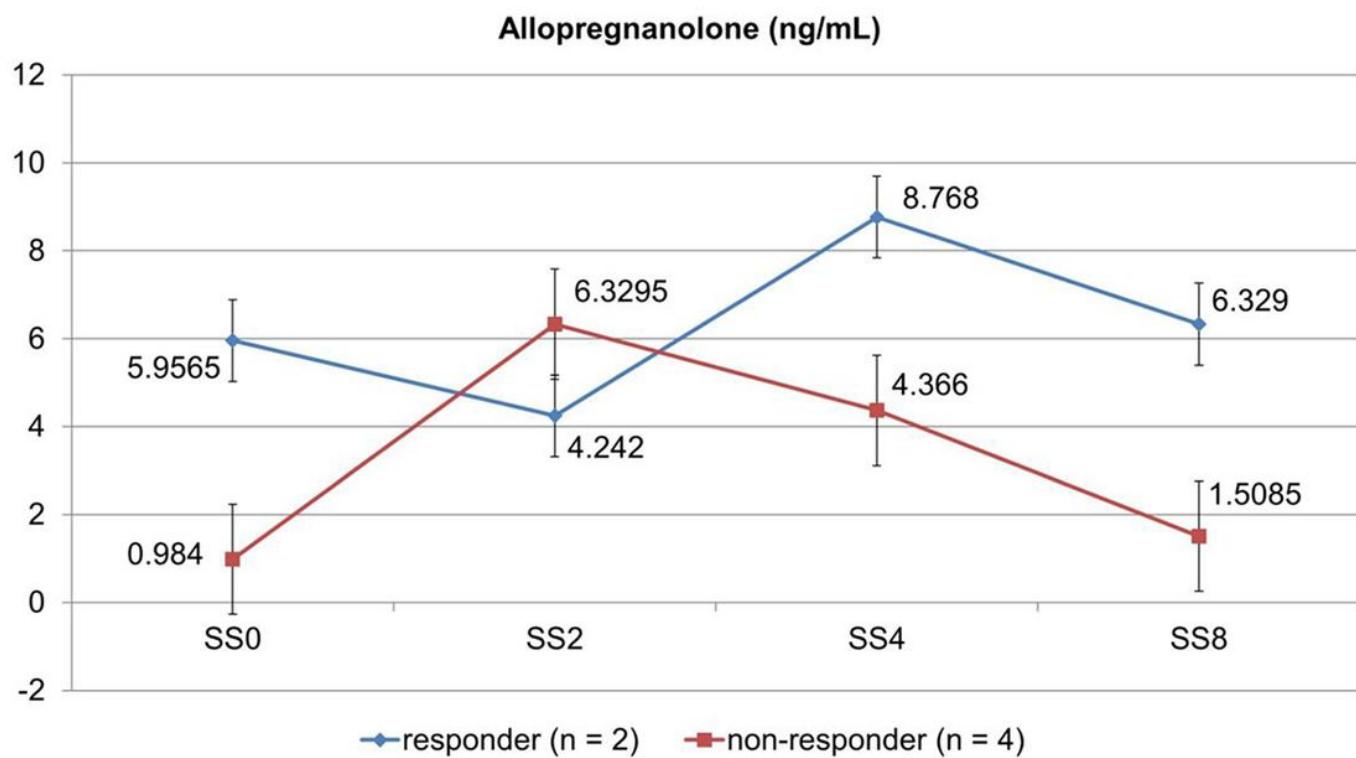


Abbreviations: SS0, before taking the next dose of progesterone; SS2, 1 h after taking the dose of progesterone; SS4, 4 h after taking the dose of progesterone; SS8, 8 h after taking the dose of progesterone.

Figure 3

See image above for figure legend

Figure 4. Allopregnanolone levels in responders and non-responders.



Abbreviations: SS0, before taking the next dose of progesterone; SS2, 1 h after taking the dose of progesterone; SS4, 4 h after taking the dose of progesterone; SS8, 8 h after taking the dose of progesterone.

Figure 4

See image above for figure legend