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
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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

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Key words:
Neurosteroid, efficacy, progesterone, allopregnanolone, refractory status epilepticus
Abstract

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

Objectives:
To study bioavailability of oral micronized progesterone that convert to allopregnanolone (an inhibitory neurosteroid) and determine its efficacy and safety in treating Thai patients with refractory status epilepticus (RSE).

Materials and Methods:
A prospective interventional study was conducted at Phramongkutklao Hospital. Patients diagnosed with RSE on midazolam infusion were assigned to receive oral progesterone 200 mg every 8 hours via nasogastric tube for 5 days. Standard treatments for RSE were maintained. Serum allopregnanolone levels were monitored. Outcomes including seizure terminations, hospital stays, mortality rates, and accumulated midazolam dosage were analyzed and compared with historical cohort.

Results:
Total of 6 patients receiving oral micronized progesterone (intervention group), and 6 patients in control group were compared. Demographic data between groups were not different, mean age of 63 and 62.3 years old, respectively. It was shown that oral micronized progesterone clearly converted to allopregnanolone. Allopregnanolone significantly shorten the duration of RSE, 25.5 hours vs. 58.4 hours, p-value 0.004. The average duration of hospital and ICU stays were 57 days, 28.7 days for intervention group and 58.7 days and 44.5 days for control group, p-value 0.929 and 0.399, respectively. The mortality rate of intervention group was lower in intervention group, 33.3% vs. 66.7%, p-value 0.513. The intervention group required lower total accumulated midazolam infusion dosage, 87.5 mg vs. 330.4 mg, p-value 0.394. No any clinical adverse events nor worsening of laboratory profiles were reported.

Conclusion:
Allopregnanolone, converted from oral micronized progesterone, demonstrated efficacy and safety in treating refractory status epilepticus.
Introduction

The mortality rate of status epilepticus (SE) in Thailand is approximately 12%, while the mortality rate of refractory status epilepticus (RSE) is significant higher, which is around 42%.\(^1\), \(^2\) Several antiepileptic drugs (AEDs) indicated for treatments of RSE, especially anesthetic agents, cause significant side effects, particularly hypotension, cardiac arrhythmias, prolonged intubation, long ICU stay and increase mortality rate.\(^3\), \(^4\)

From animal studies, allopregnanolone, the reduced progesterone metabolite or a positive allosteric modulator of \(\gamma\)-aminobutyric acid type A (GABA\(_A\)) receptor-mediated conduction, can reduce neuronal excitabilities and increase seizure thresholds.\(^5\), \(^6\)

Similarly, from previous human studies, it has been recognized that both oral and parenteral forms of progesterone can improve seizure control as well as suppress seizure discharges in electroencephalography (EEG).\(^7\), \(^8\) This indicates that progesterone contains antiepileptic property. Some studies using oral allopregnanolone added on with other AEDs improved seizure control among women with catamenial epilepsies.\(^9\) There are few studies reported of using intravenous allopregnanolone in pediatric and adult patients with refractory status epilepticus as an adjunctive treatment are beneficial.\(^10\), \(^11\) Moreover, allopregnanolone has been proposed as a safe medication.

Allopregnanolone, so called “a neurosteroid”, is not available in Thailand. Nevertheless, in vivo, this can be converted from micronized progesterone which can be administered as enteric or suppository route, Figure 1. The micronized progesterone is accessible in our country, mainly prescribed by physicians for treating various gynecological conditions. The micronized progesterone has higher bioavailability and more sustainability than other progesterone formulations.

According to the beneficial of allopregnanolone in seizures control been proved, we aimed to study for efficacy and safety of allopregnanolone as an adjunctive treatment among Thai patients with RSE. We also assessed bioavailability of allopregnanolone converting from oral micronized progesterone among these critically ill patients.

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Figure 1. Progesterone metabolisms
Objectives

To study pharmacokinetic parameters focusing on bioavailability of oral micronized progesterone to convert to allopregnanolone (a neurosteroid) and determine efficacy and safety of oral micronized progesterone as an adjunctive treatment in patients with RSE.

Materials and Methods

This study was a prospective open-label interventional study, phase IIa, studied from January 1st, to December 31st, 2019, at intensive care units, Phramongkutklao Hospital, Bangkok, Thailand.

Patients

Inclusion criteria

1. Adults, age 18 – 80 years old
2. Diagnosed with refractory status epilepticus (RSE), either convulsive or non-convulsive
3. Receiving standard antiepileptic drugs for RSE longer than 60 minutes, including intravenous midazolam infusion

Exclusion criteria

1. Pregnancy or lactation
2. Renal failure on dialysis, metastatic cancer, active thromboembolism, active bleeding, elevated transaminase enzymes ≥ 5 times of normal value (>160 U/L)
3. Blood pressure lower than 80/50 mmHg
4. Absolute NPO (as studied drug was an enteric tubal formulation)
5. Receiving drugs with potential major interaction with progesterone (e.g. estrogen, edoxaban and venetoclax)

Method

The patients diagnosed with RSE on intravenous midazolam infusion then received oral micronized progesterone (Utrogestan®) enteric feeding, dose 200 mg, every 8 hours, for 5 days. Each dose was combined with 10 mL of olive oil, an inert solute administered as a vehicle carrying the agent through nasogastric tube. Treatments for RSE, as standard guideline, using EEG as a guidance, had been continuing along the clinical trial period. Serum allopregnanolone levels were measured by radioimmunoassay (ELISA; ARBOR ASSAYS®). Each patients were assessed serum allopregnanolone level for 7 times, Figure 2.

- Baseline
  - Before progesterone administration (Point No.1)
- T_{max} or C_{peak} (Absorption period)
  - 2, 3, 4, 8 hours after progesterone administration (Point No.2, 3, 4, 5, respectively)
- Steady-state period, (Note: half-life for allopregnanolone is 8 hours)
  - After 5 times of half-life (index time = the 6th dose or at the day 3)
    - Peak level: 4 hours after the 6th dose (Point No.6)
    - Trough level: 8 hours after the 6th dose (Point No.7)

Figure 2 Time points to assess serum allopregnanolone level

Outcomes measurements

Primary outcome

- Serum allopregnanolone level (7 points) for pharmacokinetic study, performed over the 48 hours
- Timing of the first RSE termination, indicated by EEG, performed daily for 5 days
  - EEG SE termination based on burst suppression, background suppression, periodic epileptiform discharges which frequency<0.5 Hz

Secondary outcome

- Duration of hospital, ICU stays
- Total midazolam use until the first time of SE termination or until 5 days, and in hospital mortality rate, compared with RSE control patients using standard treatment and midazolam infusion, randomly selected, demographic-matched from our hospital cohort during 2015-2019, which their SE termination or death occur within 5 days, (n = 6, 1:1 ratio)
- Safety (adverse events)
- Vital signs, clinical adverse events (i.e. rashes, jaundice, hypotension)
- Abnormal laboratory values: complete blood count, liver function test, creatinine, coagulation test (before and day-5 after progesterone administration)

**Ethical aspects**

The people responsible read information sheet and signed consent form. This study was approved by Institutional Review Board (IRB), Royal Thai Army, Medical Department (R150h/61).

**Statistical analyses:**

Continuous data were presented as mean, standard deviation. Discrete data was assessed by number and percentage. The changes of laboratories for safety assessments were analyzed by paired t-test. The difference between groups (progesterone and control) were compared by independent sample t-test or Mann-Whitney U test (for non-parametric parameter) and Chi-square test or Fisher exact test were tested. Significance (p-value < 0.05) was indicated statistic significant. The analysis was performed using SPSS version 23 software (IBM Corp., Armonk, NY).

**Results:**

There were 6 patients receiving oral micronized progesterone (intervention group), and 6 patients in control group. Among the intervention group: mean age was 63 years (SD 10.1), range 55-80. Most common etiology of RSE was cerebrovascular disease, 2/6 patients (33.3%). Average duration of status epilepticus before recruitment was 7.0 hours (SD 0.84). Four commonest AEDs use during RSE were 1) levetiracetam (6/6 patients, 100%), 2) phenytoin (5/6 patients, 83%), 3) lacosamide (4/6 patients, 67%), and 4) valproate (3/6 patients, 50%). Only one patient was previously diagnosed with epilepsy (17%). Five of six patients (83%) had earlier convulsion (convulsive SE) then turned to non-convulsive SE at the entry of the study. Most of demographic characteristics between groups were not statistically significant difference. Status Epilepticus Severity Score (STESS) was calculated. The intervention group had relatively higher average STESS value, indicating more severe, than control group, however, there was no statistic significant difference (3.83 versus 2.67, p-value 0.143). The demographic details were shown in Table 1.
Table 1. Baseline demographic characteristics

<table>
<thead>
<tr>
<th></th>
<th>Progesterone (n=6)</th>
<th>Control (n=6)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-year Mean (SD)</td>
<td>63.0 (10.1)</td>
<td>62.3 (5.5)</td>
<td>0.890</td>
</tr>
<tr>
<td>Range</td>
<td>55-80</td>
<td>55-71</td>
<td></td>
</tr>
<tr>
<td>Female-no. (%)</td>
<td>2 (33)</td>
<td>3 (50)</td>
<td>0.558</td>
</tr>
<tr>
<td>Etiology of RSE-no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>2 (33)</td>
<td>2 (33)</td>
<td></td>
</tr>
<tr>
<td>Autoimmune</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>0.849</td>
</tr>
<tr>
<td>CNS infection</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td></td>
</tr>
<tr>
<td>Trauma</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td></td>
</tr>
<tr>
<td>Metabolic</td>
<td>1 (17)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>0</td>
<td>1 (17)</td>
<td></td>
</tr>
<tr>
<td>SE duration-hours Mean (SD)</td>
<td>7 (0.8)</td>
<td>10.33 (9.0)</td>
<td>0.388</td>
</tr>
<tr>
<td>Range</td>
<td>5.5-8</td>
<td>1-24</td>
<td></td>
</tr>
<tr>
<td>STESS score- 0-6 points</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>3.83 (1.3)</td>
<td>2.67 (2.2)</td>
<td>0.143</td>
</tr>
<tr>
<td>Range</td>
<td>3-6</td>
<td>2-5</td>
<td></td>
</tr>
<tr>
<td>AEDs use-no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenytoin</td>
<td>5 (83)</td>
<td>6 (100)</td>
<td>0.500</td>
</tr>
<tr>
<td>Valproate</td>
<td>3 (50)</td>
<td>5 (83)</td>
<td>0.221</td>
</tr>
<tr>
<td>Levetiracitam</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>1.000</td>
</tr>
<tr>
<td>Lacosamide</td>
<td>4 (67)</td>
<td>0</td>
<td>0.030*</td>
</tr>
<tr>
<td>Topiramate</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>1.000</td>
</tr>
<tr>
<td>Perampanel</td>
<td>1 (17)</td>
<td>0</td>
<td>0.500</td>
</tr>
<tr>
<td>Convulsive seizure type-no. (%)</td>
<td>5 (83)</td>
<td>5 (83)</td>
<td>1.000</td>
</tr>
<tr>
<td>Epilepsy history-no. (%)</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

The pharmacokinetic aspect focusing on bioavailability or the conversion from oral micronized progesterone to allopregnanolone been measured in 5 patients was described in Table 2, Figure 3. It was shown that oral micronized progesterone clearly converted to allopregnanolone. Average baseline (natural endogenous) allopregnanolone level was 584.5 pmol/mL and T_{max} at 4-hour was 2,883.3 pmol/mL. At steady state, average C_{trough} was 2,694.4 pmol/mL, and average C_{peak} was 3,255.3 pmol/mL.
### Table 2. Individual and average allopregnanolone levels (pmol/mL)

<table>
<thead>
<tr>
<th>Patient</th>
<th>#1 Baseline</th>
<th>#2 2-hour</th>
<th>#3 3-hour</th>
<th>#4 4-hour</th>
<th>#5 8-hour</th>
<th>#6 C_peak</th>
<th>#7 C_trough</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>704.3</td>
<td>2,488.0</td>
<td>2,055.0</td>
<td>2,093.8</td>
<td>5,740.0</td>
<td>1,777.0</td>
<td>2,450.5</td>
</tr>
<tr>
<td>2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>2,795.0</td>
<td>1,301.8</td>
</tr>
<tr>
<td>3</td>
<td>409.3</td>
<td>2,630.0</td>
<td>2,240.0</td>
<td>4,375.0</td>
<td>3,405.0</td>
<td>7,280.0</td>
<td>5,597.5</td>
</tr>
<tr>
<td>4</td>
<td>294.8</td>
<td>3,950.0</td>
<td>3,510.0</td>
<td>3,555.0</td>
<td>1,342.8</td>
<td>3,135.0</td>
<td>3,445.0</td>
</tr>
<tr>
<td>5</td>
<td>929.8</td>
<td>2,178.3</td>
<td>1,768.5</td>
<td>1,509.3</td>
<td>590.3</td>
<td>1,289.3</td>
<td>677.0</td>
</tr>
<tr>
<td>Average</td>
<td>584.5</td>
<td>2,811.9</td>
<td>2,370.9</td>
<td>2,883.3</td>
<td>2,769.5</td>
<td>3,255.3</td>
<td>2,694.4</td>
</tr>
</tbody>
</table>

NA: not applicable

### Figure 3. Serum Allopregnanolone level
The average duration of RSE after treatment with midazolam plus progesterone (intervention group) and midazolam without progesterone (control), until SE termination, were 25.5 and 58.4 hours, respectively, *p*-value 0.004, Table 3. The finding indicated that allopregnanolone, in vivo, significantly shorten the RSE duration.

The average duration of ICU and hospital stay were shorter in progesterone group than control group, Table 3. The average duration of hospital stay were 57 days for intervention group and 58.7 days for control group, *p*-value 0.929. Also the average duration of ICU stay were 28.7 days for intervention group and 44.5 days for controls group, *p*-value 0.399.

The mortality rate of intervention group was half of control group (33.3% versus 66.7%), *p*-value 0.513. Median value of total accumulated midazolam infusion until SE termination or death within 5-days were 87.5 mg for progesterone group and 330.4 mg for control group, *p*-value 0.394, Table 3.

**Table 3. Clinical outcomes of refractory status epilepticus (RSE)**

<table>
<thead>
<tr>
<th></th>
<th>Progesterone (n=6) mean (SD)</th>
<th>Control (n=6) mean (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of RSE (hour)</td>
<td>25.5 (12.8)</td>
<td>58.4 (17.1)</td>
<td>0.004*</td>
</tr>
<tr>
<td>Duration of hospital stay (day)</td>
<td>57.0 (21.8)</td>
<td>58.7 (39.0)</td>
<td>0.929</td>
</tr>
<tr>
<td>Duration of ICU stay (day)</td>
<td>28.7 (12.2)</td>
<td>44.5 (42.3)</td>
<td>0.399</td>
</tr>
<tr>
<td>Death: no. (%)</td>
<td>2 (33.3)</td>
<td>4 (66.7)</td>
<td>0.513</td>
</tr>
<tr>
<td>Total accumulated midazolam use (mg): median†</td>
<td>87.5</td>
<td>330.4</td>
<td>0.394≡</td>
</tr>
</tbody>
</table>

*p*-value<0.05, † Non-parametric data, ≡ Mann Whitney U test

There was no clinical adverse events (i.e. rashes, anaphylactic reactions) in both groups. There was no hypotension or cardiac arrhythmias reported in intervention group, while, 5/6 patients (83.3%) in control group developed hypotension, requiring inotropic agents.

Changes of laboratory findings between baseline and day-5 after administrating progesterone were compared in Table 4. It was shown that there was no worsening of laboratory parameters.
### Table 4. Laboratory findings comparing pre- and post-treatments

<table>
<thead>
<tr>
<th>Laboratory investigations</th>
<th>Baseline Mean (SD)</th>
<th>Day 5 Mean (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>28.5 (2.5)</td>
<td>28.4 (5.7)</td>
<td>0.970</td>
</tr>
<tr>
<td>White cell count (cells/mm³)</td>
<td>12,040 (3,232)</td>
<td>8,940 (3,402)</td>
<td>0.261</td>
</tr>
<tr>
<td>Platelet (cells/mm³)</td>
<td>351,200 (35,604.8)</td>
<td>352,600 (201,978.5)</td>
<td>0.987</td>
</tr>
<tr>
<td>PTT (second)</td>
<td>20.70 (3.11)</td>
<td>26.95 (5.59)</td>
<td>0.495</td>
</tr>
<tr>
<td>INR</td>
<td>0.97 (0.01)</td>
<td>1.11 (0.25)</td>
<td>0.577</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.92 (0.81)</td>
<td>0.93 (0.78)</td>
<td>0.683</td>
</tr>
<tr>
<td>Serum Sodium (mmol/L)</td>
<td>140.14 (3.25)</td>
<td>141.32 (5.09)</td>
<td>0.686</td>
</tr>
<tr>
<td>Serum Potassium (mmol/L)</td>
<td>3.39 (0.26)</td>
<td>3.72 (0.20)</td>
<td>0.134</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dL)</td>
<td>0.41 (0.07)</td>
<td>0.29 (0.07)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Direct Bilirubin (mg/dL)</td>
<td>0.26 (0.09)</td>
<td>0.20 (0.05)</td>
<td>0.102</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>79.0 (64.8)</td>
<td>30.3 (20.8)</td>
<td>0.374</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>86.6 (58.0)</td>
<td>40.3 (37.3)</td>
<td>0.342</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>149.0 (51.2)</td>
<td>131.3 (76.2)</td>
<td>0.381</td>
</tr>
</tbody>
</table>

INR, international normalized ratio; PTT, partial thromboplastin time; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase, *p-value*<0.05

### Discussion:

Natural progesterone has poor gastrointestinal absorption property and short biologic half-life,\(^ {15,16}\), therefore, progesterone in other formulations, particularly injection form and oral and suppository micronized forms, have been developed in order to improve and stabilize plasma levels.

From our pharmacokinetic study, it was shown that allopregnanolone concentration peaked at 3-4 hours (T\(_{\text{max}}\)), then declined within 4-6 hours after oral micronized progesterone administration. The findings on absorption and metabolism were similar to a recent study.\(^ {17}\) This supported that oral micronized progesterone should be administered every 8 hours to maintain serum allopregnanolone level.

At steady state (half-life, T\(_{1/2}\)), peak level (C\(_{\text{peak}}\)) was 3,255.26 pmol/mL and trough level (C\(_{\text{trough}}\)) was 2,694.36 pmol/mL. From Herzog G, et al study\(^ {7}\), using progesterone 200 mg lozenges formulation 3 times daily in female patients with catamenial epilepsy, found C\(_{\text{peak}}\) 5,240 pmol/mL. The lower C\(_{\text{peak}}\) in our study, indicating lower bioavailability, would be due to 1) formulation differences or 2) absorption problems or drug interactions among critically ill patients and 3) studied population variation.

Allopregnanolone is one of several agents so called neurosteroids. Few neurosteroids, including allopregnanolone, tetrahydrodeoxycorticosterone (THDOC), and 3-alpha androstanediol are inhibitory neurosteroids, augmenting GABAergic neurons.\(^ {18}\) Only
allopregnanolone has been proved, both in vitro and in vivo, its ability on seizures controls, reported in women with catamani allopregnanolone epilepsy and adult and pediatric with RSE.\textsuperscript{7,8,11}

Allopregnanolone in our study showed an improvement on SE controls since it significantly shorten RSE duration, \( p \)-value 0.004. It was also noticed that average ICU and hospital stay were relatively shorter than control group as well as mortality rate was reduced. Lower midazolam dosage was noticeable among RSE patients using allopregnanolone than controls. It was quite safe to use in RSE, as there was no report of adverse events or serious adverse events, clinically and laboratorily. These efficacy and safety findings were concordant with other previous international studies.\textsuperscript{11,19}

This study was the first clinical research using a neurosteroid in controlling Thai patients with RSE. However, it was only a phase IIa study, therefore, the authors suggests for further studies to improve methodology i.e. larger sample size, multi-center, longer duration, and double-blind study.

\textbf{Conclusion:}

Oral micronized progesterone can be converted to allopregnanolone and demonstrated its efficacy and safety in treating refractory status epilepticus.

\textbf{Funding}

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\textbf{Conflicts of Interests}

None
References


