Add-On Pregnenolone with L-Theanine to Antipsychotic Therapy Relieves Negative and Anxiety Symptoms of Schizophrenia: An 8-Week, Randomized, Double-Blind, Placebo-Controlled Trial

Adasa Kardashev [1], Yael Ratner [1, 2], Michael S. Ritsner [1, 2]

Abstract

Aims: Pregnenolone (PREG) and L-theanine (LT) have shown ameliorative effects on various schizophrenia symptoms. This is the first study to evaluate the efficacy and safety of augmentation of antipsychotic treatment among patients with chronic schizophrenia or schizoaffective disorder with PREG-LT. **Methods:** Double-blind, placebo-controlled trial of PREG-LT or placebo augmentation was conducted for eight weeks with 40 chronic *DSM-IV* schizophrenia and schizoaffective disorder patients with suboptimal response to antipsychotics. Oral PREG (50 mg/day) with LT (400 mg/day) or placebo were added to a stable regimen of antipsychotic medication from March 2011 to October 2013. The participants were rated using the Scale for the Assessment of Negative Symptoms (SANS), the Hamilton Scale for Anxiety (HAM-A), and the Positive and Negative Syndrome Scale (PANSS) scales bi-weekly. The decrease of SANS and HAM-A scores were the co-primary outcomes. Secondary outcomes included assessments of general functioning and side effects. **Results:** Negative symptoms such as blunted affect, alogia, and anhedonia (SANS) were found to be significantly improved with moderate effect sizes among patients who received PREG-LT, in comparison with the placebo group. Add-on PREG-LT also significantly associated with a reduction of anxiety scores such as anxious mood, tension, and cardiovascular symptoms (HAM-A), and elevation of general functioning (GAF). Positive symptoms, antipsychotic agents, concomitant drugs, and illness duration did not associate significantly with effect of PREG-LT augmentation. PREG-LT was well-tolerated. **Conclusions:** Pregnenolone with L-theanine augmentation may offer a new therapeutic strategy for treatment of negative and anxiety symptoms in schizophrenia and schizoaffective disorder. Further studies are warranted. ***Trial Registration:*** clinicaltrials.gov Identifier: NCT01831986.

Key Words: Schizophrenia, Pregnenolone, L-Theanine, Clinical Trial

Introduction

Despite the large number of psychotropic medications currently available, effective management of schizophrenia continues to be a challenging task. Indeed, antipsychotics are only partially effective (20%–45%) (1, 2), and about 5%–10% of patients derive no benefit at all (3). There is evidence that anxiety is a frequent symptom of schizophrenia (23.8%) (4), which is highly associated with an increased risk of relapse and suicidal behavior (5). Furthermore, the common practice of prescribing benzodiazepines remains unsatisfactory (6). Consequently, clinicians increase the antipsychotic dosage, switch the antipsychotic compound, and introduce polypharmacy or augmentation strategies (7).

Applying various psychopharmacological combinations and augmentation strategies in schizophrenia is common clinical practice (8, 9). There is growing interest in the use of neuroprotective agents for targeting negative, anxiety, cognitive and other symptoms in schizophrenia (10-12).

[1]*Sha'ar Menashe Mental Health Center, Hadera, Israel*
[2]*The Rappaport Faculty of Medicine, Technion, Haifa, Israel*

Address for correspondence: Michael S. Ritsner, MD, PhD, Director, Acute Department, Sha'ar Menashe Mental Health Center, Mobile Post Hefer 38814, Hadera, Israel
Phone: +972-4-6278750; Fax: +972-4-6278045;
E-mail: ritsner@sm.health.gov.il; ritsnerm@gmail.com

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Clinical Implications

The present study with add-on pregnenolone with L-theanine (PREG-LT) to antipsychotic therapy confirms an ameliorative effect of PREG on negative symptoms and a beneficial effect of LT on anxiety and general psychopathology, but not on positive symptoms or extrapyramidal side effects. However, this assertion in the present design might not be sustained because there is no just PREG arm or no just LT arm; therefore, we cannot say which particular substance contributed to the improvement. Furthermore, PREG-LT augmentation indicated significant improvement in general functioning (GAF), which was reported in previous trials. Thus, augmentation with PREG-LT not only replicated the beneficial effect of PREG on negative symptoms and LT on anxiety, but the combined treatment demonstrated a possible interplay effect, such as improvement in general functioning of patients. Further replication of this finding is warranted.

Pilot clinical trials have demonstrated a promising role of pregnenolone (13-15) and L-theanine (12) in concomitant therapy.

Pregnenolone (PREG), its sulphate (PREGS), and its downstream products (dehydroepiandrosterone [DHEA], its sulfate [DHEAS] and others) are neurosteroids that show multiple pronounced neuroprotective properties (16, 17). These neurosteroids also regulate neuronal function by affecting neuronal excitability through prominent modulatory effects on the γ-aminobutyric acid type A ($GABA_A$), N-methyl-D-aspartate (NMDA/glutamate), sigma-1 (18, 19), and dopamine systems (20). There is evidence that PREG and its metabolites may be involved in the pathophysiology of schizophrenia and mood disorders (21, 22).

L-theanine (LT; gamma-ethylamino-L-glutamic acid), an amino acid found in green tea, is an analog to glutamine and glutamate (23). LT is rapidly absorbed after intake of 50–200 mg via capsules and seems to be hydrolyzed to ethylamine and glutamic acid (24). It readily crosses the blood-brain barrier (25), and exerts a variety of neurophysiological and pharmacological effects that are neuroprotective (26, 27), and anxiolytic (28-30), mood-enhancing and relaxation effects (27, 31), owing to its possible modulation of dopamine (DA), and serotonin (5-hydroxytryptamine, or 5-HT), GABA, and glutamate (32-35). Administration of LT is safe (400 mg/d) (36) and has been granted "generally recognized as safe" status by the U.S. Food and Drug Administration (http://www.accessdata.fda.gov/scripts/fcn/gras_notices/615880A.pdf).

Three 8-week add-on trials with PREG and one study with LT in schizophrenia have been published (11-13, 37, 38). One study demonstrated marginally significant improvement among 9 patients who received PREG (fixed escalating doses to 500 mg/day) on Scale for the Assessment of Negative Symptoms (SANS) scores compared with 9 patients who received placebo (13). Another study reported that, compared with placebo, 30 mg/day PREG administration was associated with significant reduction in positive symptom scores, extrapyramidal side effects, improvement in attention and working memory performance (11); a third add-on trial with PREG (50 mg/d) demonstrated a significant reduction on the SANS negative dimension scores (blunted affect, avolition and anhedonia domains) and the Positive and Negative Syndrome Scale (PANSS) negative subscale scores (37) and amelioration of the visual attention deficit (38) in recent-onset schizophrenia or schizoaffective disorder receiving PREG compared to the placebo group. LT augmentation (400 mg/day) for 8 weeks was associated with significant reduction of anxiety, positive and general psychopathology scores, among patients with chronic schizophrenia or schizoaffective disorders (12). Both PREG and LT were found to be safe and well-tolerated in all trials.

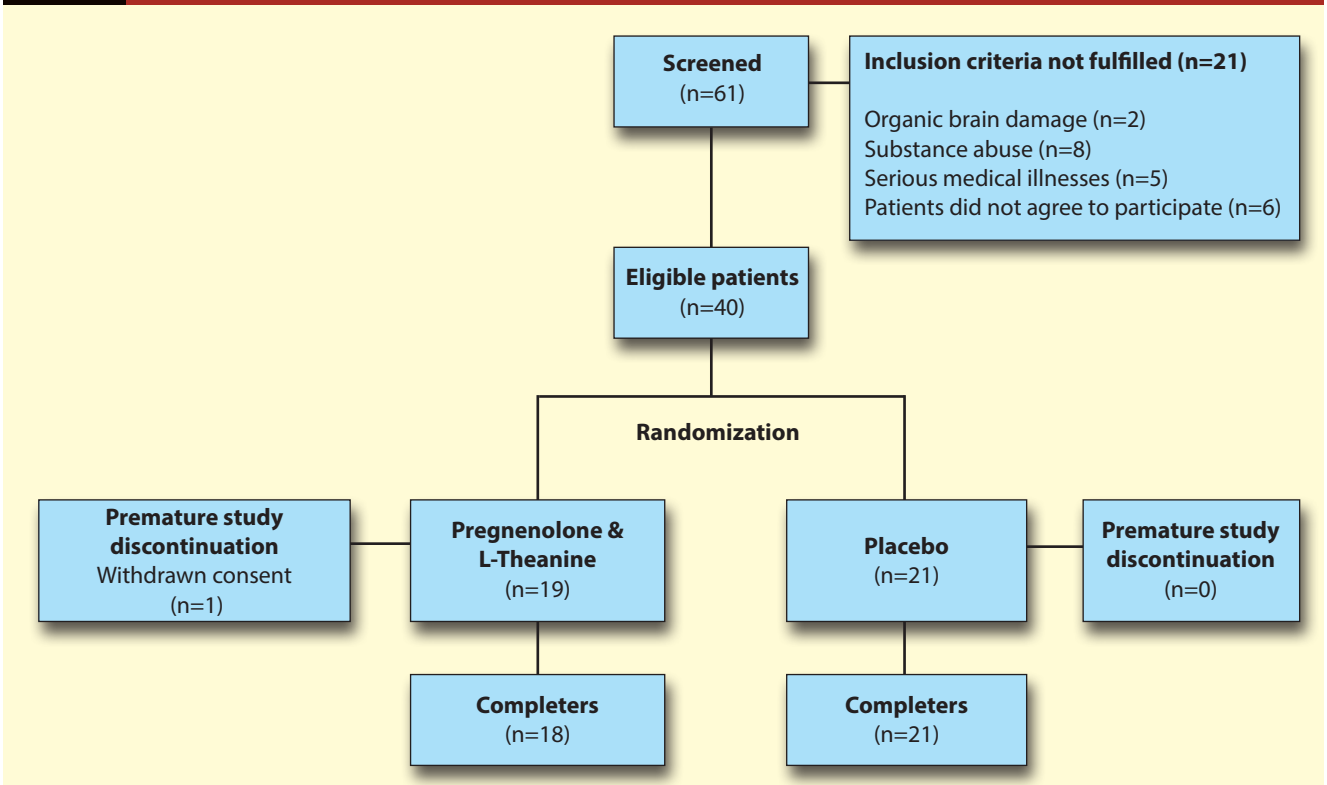
Given a strong rationale from preclinical studies and clinical experience with PREG and LT augmentation, we hypothesized that an add-on combination of these two agents (PREG and LT) might ameliorate both negative and anxiety symptoms in schizophrenia and schizoaffective disorder patients compared to placebo administration. The objective of the present trial was to evaluate the efficacy and safety of augmentation of antipsychotic treatment of patients with chronic schizophrenia and schizoaffective disorder with PREG and LT. Thus, this is the first trial to evaluate the combination of two adjunctive agents (PREG-LT) versus placebo in schizophrenia and schizoaffective disorder patients.

Methods

Study Design

This was a single-center, double-blind, 8-week, randomized, placebo-controlled study conducted independent of commercial entities. The specific objective was to determine whether PREG-LT ameliorates severity of negative and anxiety symptoms among schizophrenia and schizoaffective disorder patients. Prior to starting the study, all subjects provided written informed consent after receiving a full explanation regarding the nature of the study, and its potential risks and benefits. The Institutional Review Boards of the Sha'ar Menashe Mental Health Center and the Israel Ministry of Health approved the study.

Figure 1 Patient Flow Through the Study



Participants

Participants were recruited from the services of Sha'ar Menashe Mental Health Center affiliated with the Rappaport Faculty of Medicine, Technion, Haifa (Israel), with a catchment area of approximately 800,000 residents. Recruitment was initiated in March 2011, ended in July 2013, and the last patient completed treatment in October 2013.

For inclusion in the study, patients were required to be between the ages of 18–65 years and meet criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)* (39) for a diagnosis of schizophrenia and schizoaffective disorder and suboptimal response to previous treatment, which was defined by the two criteria used in clinical trials (40): 1) persistent positive symptoms (hallucinations, delusions, or marked thought disorder) after at least 6 continuous weeks of antipsychotic treatment; and, 2) a poor level of functioning over the past 2 years, defined by the lack of competitive employment or enrollment in an academic or vocational program. We use the term "suboptimal response to treatment" to highlight that our criteria are different from "treatment resistance" (41).

In addition, patients were required to have: 1) a score of at least 3 on the Clinical Global Impression Scale (CGI-S) (42) at entrance to the study; 2) at least two weeks of ongoing treatment with current antipsychotic agents before the pre-treatment stabilization period; 3) stable symptoms throughout the 2-week pre-treatment stabilization period, with no more than a 20% change in the PANSS total score; 4) no change in anticholinergic, benzodiazepine, or mood stabilizer medications during the pre-treatment stabilization period, as well as no change in antipsychotics, anticholinergics, benzodiazepines, or mood stabilizers during the 8-week duration of the study; and, 5) ability to participate fully in the informed consent process, or have a legal guardian able to participate in the informed consent process.

Major exclusion criteria included: an unstable medical condition, any significant medical or neurological illnesses, pregnancy, and treatment with any steroid or hormonal supplement (e.g., estrogen). The absence of medical or neurologic illnesses was verified by means of a routine laboratory investigation that included blood cell count with differential, liver function tests, glucose and cholesterol levels, physical and neurologic examinations, reports of the patient's family physician, and medical records. It was forbidden to add any other psychoactive medication before entry or during the entire study period.

Study Procedure and Treatment

At the initial screening visit, a thorough clinical and psychiatric examination was performed on patients who met entry criteria. Severity of the disorder and symptoms

Table 1 Baseline Characteristics of the Population

Characteristics	Pregnenolone & L-Theanine (n=18)		Placebo (n=21)		Significance[§]	
	Mean	SD	Mean	SD	t (z)	P
Age (years)	32.2	7.6	33.0	6.7	0.4	0.72
Education (years)	9.0	3.0	9.1	3.5	0.1	0.97
Age of onset (years)	20.3	8.3	22.8	6.6	1.0	0.30
Body mass index (kg/m^2)	26.7	2.8	27.4	3.6	0.2	0.85
Number of admissions	6.7	7.4	7.1	7.6	0.2	0.84
Illness duration (years)	8.2	6.0	13.9	8.0	2.5	0.017
Negative symptoms (SANS)	58.5	9.6	59.8	9.1	0.4	0.65
Hamilton Scale for Anxiety (HAM-A)	9.7	3.6	9.8	3.5	0.1	0.93
General Functioning (GAF)	56.1	6.4	54.1	8.2	0.8	0.40
Illness severity (CGI-S)	4.4	0.7	4.3	0.5	0.6	0.56
PANSS Negative subscale	29.0	3.2	27.7	5.4	0.9	0.38
PANSS Positive subscale	24.4	4.5	22.4	6.8	1.1	0.29
PANSS General subscale	51.3	7.2	50.0	8.9	0.5	0.59
Daily dose (CPZ, mg/day)*	497	245	511	261	0.3	0.80
Side Effects						
Akathisia (BARS)	1.3	1.9	2.0	2.4	1.0	0.32
Dyskinesia (ESRS)	0.5	1.0	0.7	1.2	0.6	0.55
Parkinsonism (ESRS)	1.4	1.1	1.5	1.3	0.2	0.82
Dystonia (ESRS)	0.4	0.8	0.3	0.8	0.2	0.83
Gender (male/female)	16/2		19/2		χ^2=0.03, df=1, p=0.87	
Married	11.1% (2/18)		14.3% (3/21)		χ^2=1.8, df=1, p=0.75	
Schizophrenia, paranoid type	13	72.2%	16	76.2%	χ^2=0.08, df=1, p=0.78	
Schizophrenia (other types)[†] and schizoaffective disorder	5	27.8%	5	23.8%		
Antipsychotic Drugs FGAs/SGAs/COMB[‡]	8/7/3		7/8/9		χ^2=0.9, df=2, p=0.65	

*CPZ=chlorpromazine equivalent, mg/day. [†]Schizophrenia, other types *(DSM-IV)*: 295.1 (n=2), 295.6 (n=4), 295.9 (n=2), and schizoaffective disorder (295.7; PREG-LT: n=1, placebo group: n=1). [‡]FGAs=first-generation antipsychotics (chlorpromazine, haloperidol, haloperidol decanoate, perphenazine, zuclopenthixol, zuclopenthixol decanoate, fluphenazine decanoate); SGAs=second-generation antipsychotics (risperidone, olanzapine, quetiapine, ziprasidone, clozapine); COMB=both types of antipsychotic medications (combined therapy or antipsychotic polypharmacy). [§]Continuous variables were compared using the two-tailed t-test, or the Wilcoxon signed-rank test (z) for assessing the difference in medians. Differences in frequency of categorical variables were examined with the chi-square test (χ^2).

were evaluated with the CGI-S and the PANSS. Patients were required to continue to take their regular medications. Patients who were clinically stable for the two weeks of the *lead-in phase* (with no more than a 20% change in PANSS total score) were randomized into 2 groups: patients who received PREG-LT and patients who received a placebo, each for 8 weeks in a double-blind manner. The randomization procedure was performed using Random Allocation Software (Version 1.0, May 2004; available at: http://mahmood-saghaei.tripod.com/Softwares/randalloc.html).

We used 50 mg/day of PREG and 400 mg/day of L-theanine (Suntheanine™), taking into account findings from our previous studies (12, 37) (the supplier: BioSynergy Health Alternatives; http://www.biosynergy.com/). PREG, LT and placebo were packaged in identical appearing capsules, and prescribed only as study medication. Study medications were prescribed to be taken on a twice-per-day schedule and dispensed on a weekly basis. Patients were given two extra days of medication in case of a missed appointment. Compliance was monitored through weekly capsule and bottle counts, and interviews. The next week's supply of medication was not dispensed until all rating assessments were completed. During the course of the 8-week study, patients who took at least 75% of prescribed medication were considered compliant and entered into the efficacy analysis. Follow-up visits for psychiatric and safety assessments were conducted at baseline and weeks 2, 4, 6, and 8.

Outcome Measures

All outcome measures were performed by psychiatrists who were blind to the patients' medication. The primary rating tools were the SANS and the Hamilton Scale for Anxiety (HAM-A). The SANS assesses five symptom complexes to obtain clinical ratings of negative symptoms in patients with

schizophrenia. They are: affective blunting; alogia (impoverished thinking); avolition/apathy; anhedonia/asociality; and disturbance of attention. Assessments are conducted on a six-point scale (0=not at all to 5=severe) (43).

The HAM-A consists of 14 items, each defined by a series of symptoms, and measures both psychic anxiety (mental agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety) (44).

Secondary outcome measures included the PANSS, the Global Assessment of Functioning (GAF), and the Extrapyramidal Symptom Rating Scale (ESRS). Of the 30 items included in the PANSS, 7 constitute a Positive Scale, 7 a Negative Scale, and the remaining 16 a General Psychopathology Scale. The scores for these scales are arrived at by summation of ratings across component items. Therefore, the potential ranges are 7 to 49 for the Positive and Negative Scales, and 16 to 112 for the General Psychopathology Scale (45).

The GAF measures symptoms, impairment and functioning in clinical and research settings. Clinicians rate clients on a 1 to 100 scale in terms of their psychological, social, and occupational functioning (American Psychiatric Association, 2000). The scale includes 10 sets of anchor descriptions spaced at 10-point intervals. Anchors allow clinicians to consider both symptom severity and social/occupational functioning in their ratings.

The ESRS assesses four types of drug-induced movement disorders: Parkinsonism, akathisia, dystonia, and tardive dyskinesia (46). The CGI-S was used for the lead-in phase and baseline assessments.

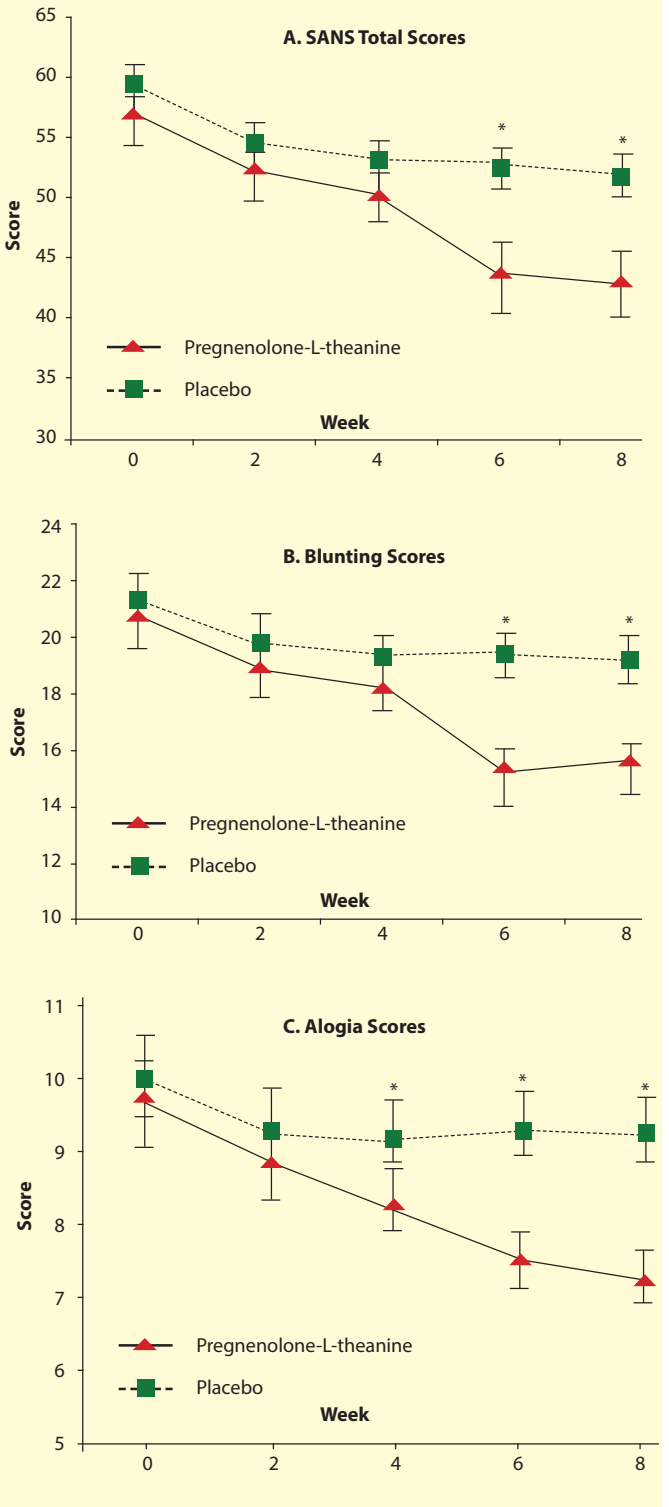
Safety evaluations included solicited adverse event reporting, tests of neurological status, routine laboratory tests, electrocardiogram monitoring, and recording of vital signs and body weight. These evaluations were repeated every 2 weeks for the duration of the study in addition to the regular above described clinical ratings. Patient ratings were performed by all three of the authors who were trained before the study to produce acceptable levels of inter-rater reliability, estimated by intraclass correlation coefficient (ICC), for the primary diagnosis, CGI-S, SANS, HAM-A, PANSS, GAF, and ESRS (ICC=0.92, 0.83, 0.89, 0.79, 0.81, and 0.80, respectively). Throughout the study, the same rater conducted most ratings for each patient.

Statistical Analysis

General linear ANOVA model with fixed, repeated-measures factors for time and treatment was used. Main effects for time and treatment, as well as their interaction, were included in the model along with a fixed main effect for treatment order. The Shapiro-Wilk test was used to determine the normality of the data.

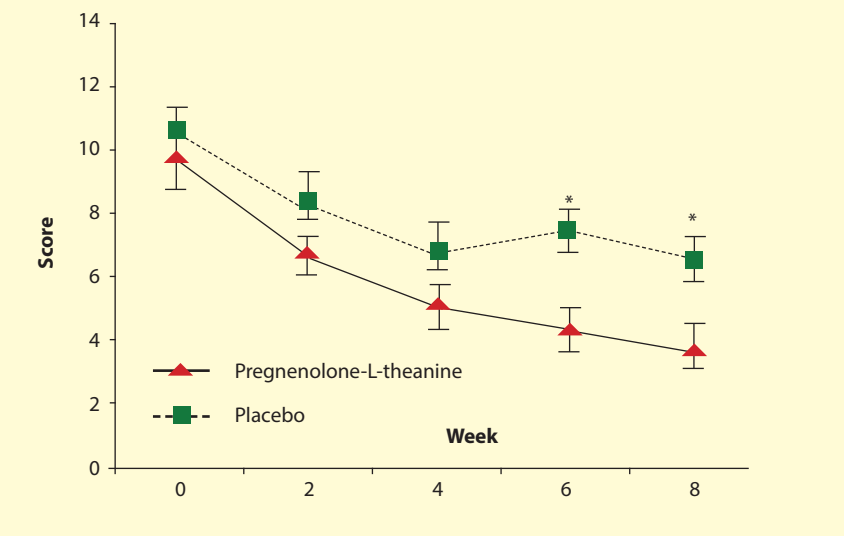
Two-step statistical analysis was performed. First, effects of PREG-LT administration on the primary and sec-

Figure 2 Severity of Negative Symptoms (SANS Scores) Among Patients with Combined Pregnenolone and L-Theanine Augmentation Compared to Placebo over Time



A. SANS total scores; B. Blunting scores; C. Alogia scores. SANS=Scale for the Assessment of Negative Symptoms. Mean raw scores and SE are shown. Significance: blunted affect (from 4th week; $F_{1,179}$=7.7, p=0.006), alogia (from 2nd week; $F_{1,179}$= 6.9, p=0.009), anhedonia (from 4th week; $F_{1,179}$= 6.4, p=0.012) scores. *Indicated significant between groups differences.

Figure 3 Severity of Anxiety (HAM-A Scores) Among Patients with Combined Pregnenolone and L-Theanine Augmentation Compared to Placebo over Time



HAM-A=Hamilton Scale for Anxiety. Mean raw scores and SE are shown (PREG with L-theanine compared with placebo: $F_{1,179}$=7.2, p=0.008). *Indicated significant between groups differences.

ondary outcome measures were determined by analysis of the three-way ANOVA: 1) "treatment condition" using scale ratings for the two treatment arms (PREG-LT versus placebo); 2) "time" factor using data obtained at five time points (at baseline, weeks 2, 4, 6, and 8); and, 3) *DSM-IV* diagnosis (295.3 versus other subtypes). The second step was based on the idea of controlling for factors (covariates) and how the inclusion of additional factors can increase the statistical power (sensitivity) of our design. A possible effect of the covariates such as treatment with antipsychotics (FGAs, SGAs, and COMB; chlorpromazine equivalent, body mass index), concomitant drugs ("yes" or "no;" mood stabilizers, benzodiazepines, and anti-Parkinson agents), side effects (ESRS), and the duration of the illness (years) were included in the ANOVA model for assessment of their effects on outcome variables.

Post hoc analysis was carried out in cases of significant outcomes, using the Tukey-Kramer multiple-comparison test using range distribution. This test was applied to examine all pairs of treatment means; the error rate is experiment-wise.

The simple definition of effect size (ES) is the magnitude of an effect. The ES is a way of quantifying the effectiveness of a particular intervention relative to some comparison. An effect size (Cohen's d) was calculated for changes in raw scores of outcome variables between baseline and endpoint (week 8) for between-group comparisons by the estimated pooled standard deviation (SD). A small effect size was defined as d≥0.2, a moderate effect size as d≥0.5, and a large effect size as d≥0.8 (47). The Bonferroni correction was also applied. Chlorpromazine equivalent (CPZ) doses were calculated based on published data (48, 49).

The data were expressed as the mean ± SD (standard deviation) or ± SE (standard error). Continuous variables were compared using the two-tailed t-test, or the Wilcoxon signed-rank test (z) for assessing the difference in medians. Differences in the frequency of categorical variables were examined with the chi-square test (χ^2). For all analyses, the level of statistical significance was defined as an alpha less than 0.05, 2-tailed. The NCSS-2000 PC program (50) was used for all analyses.

Results

Sample Composition

Figure 1 presents a flow diagram of the study population. Of 61 screened subjects with ongoing residual symptoms, 21 patients did not enter the study. Forty enrolled patients were randomly assigned to receive 50 mg/day of PREG and 400 mg/day L-theanine (n=19), and placebo (n=21). Of the 40 patients randomized to this trial, one patient dropped out (PREG-LT group) between 2 and 4 weeks due to withdrawal of consent. The missing data of one subject who failed to complete at least 4 weeks of double-blind treatment (selected a priori) were excluded. Thus, data from 39 patients who completed the lead-in phase and all 8 weeks of the study were included in the analysis.

Table 2 Changes in Outcome Measures from Baseline and Endpoint Assessments, and Between Two Treatment Groups

Variables	Pregnenolone & L-Theanine (n=18)				Placebo (n=21)				Significance (ANOVA)†			
	Changes between baseline and endpoint		95% CI* for mean differences		Changes between baseline and endpoint		95% CI* for mean differences		Treatment condition (df=1,179)†		Time (df=4,179)‡	
	Mean	SD	Lower	Upper	Mean	SD	Lower	Upper	F	p	F	p
Negative symptoms (SANS), total score	-16.0	14.0	-23.0	-9.1	-9.8	10.5	-14.6	-5.0	7.6	<0.01	4.3	<0.001
Blunted affect	-6.0	5.1	-8.6	-3.5	-3.2	4.6	-5.3	-1.1	7.7	<0.01	3.2	<0.01
Alogia	-3.0	3.5	-4.8	-1.2	-1.3	2.4	-2.4	-0.2	6.9	<0.01	2.9	<0.05
Anhedonia	-2.2	3.6	-4.0	-0.4	-2.1	2.5	-3.3	-1.0	6.4	0.01	2.6	<0.05
Avolition	-3.0	2.2	-4.1	-1.9	-1.9	2.5	-3.0	-0.8	3.8	>0.05	4.8	<0.001
Attention	-1.7	1.9	-2.7	-0.8	-1.2	1.2	-1.7	-0.6	0.4	>0.05	5.1	<0.001
Anxiety (HAM-A)	-6.0	3.2	-7.6	-4.5	-4.3	4.2	-6.1	-2.4	7.2	<0.01	6.8	<0.001
General Functioning (GAF)	6.8	5.6	3.5	10.0	5.5	6.3	2.2	8.8	7.1	<0.01	4.2	<0.01

*CI=confidence interval 95%. †Pregnenolone and L-theanine versus placebo. ‡Week 0, 2, 4, 6, and 8. SANS=Scale for the Assessment of Negative Symptoms. HAM-A=Hamilton Scale for Anxiety. GAF=Global Assessment of Functioning.

The baseline characteristics of the 39 subjects are listed in Table 1. As can be seen, among the participants there were no notable imbalances between the two treatment groups in age, gender, marital status, age at onset of illness or number of hospital admissions. Illness duration was significantly longer among the placebo group compared to PREG-LT group participants (p=0.017). Thirty-seven participants were diagnosed with schizophrenia and 2 participants with schizoaffective disorder (295.7; PREG-LT: n=1, placebo group: n=1). All participants had received an antipsychotic medication for at least three months. There were no between-group differences regarding the distribution of medication type or antipsychotic medications classified as first-generation antipsychotic (FGA), second-generation antipsychotic (SGA), and combination (COMB) ($\chi 2$=0.9, df=2, p=0.65).

There were no significant between-group differences between patients who were randomized to receive PREG-LT or placebo on the baseline mean scores for the CGI-S, PANSS and SANS subscales, HAM-A, GAF, or ESRS scales. Comparisons between PREG-LT and placebo groups for the 2-week lead-in phase and at baseline assessments of PANSS, SANS, and GAF scale scores did not reach significant levels of difference between the two treatment arms (all p's>0.05, data not shown).

Effectiveness

Negative Symptoms

As shown in Table 2, patients randomized to PREG-LT demonstrated significant reduction (from 4th week) in SANS total scores (-16.0±14.0) compared with patients who received placebo (-9.81±10.5; $F_{1,179}$=7.6, p=0.006) with moderate effect sizes (Cohen's d=0.50, 95% CI from -1.14 to 0.14). PREG-LT group patients significantly improved on three SANS domain scores compared to the placebo group: blunted affect (from 4th week; $F_{1,179}$=7.7, p=0.006; d=-0.57, 95% CI from -1.21 to 0.07), alogia (from 2nd week; $F_{1,179}$=6.9, p=0.009; d=-0.56, 95% CI from -1.21 to 0.08), anhedonia (from 4th week; $F_{1,179}$=6.4, p=0.012), but not on avolition ($F_{1,179}$=3.8, p=0.052) and inattention ($F_{1,179}$=0.4, p=0.55) scores (see Figure 2).

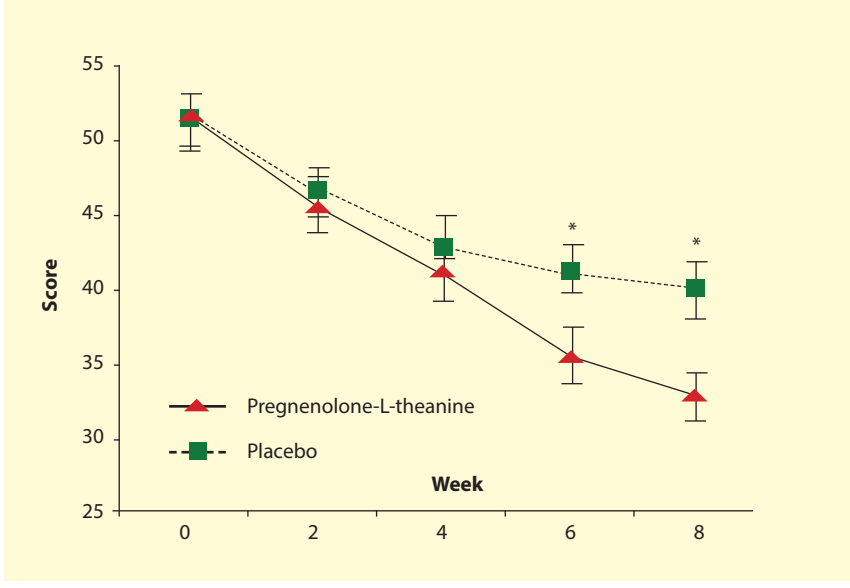
Anxiety Symptoms

In addition, in the PREG-LT augmentation group there was a significant amelioration of total HAM-A scores (onset of improvement on the 4th week) compared with placebo ($F_{1,179}$=7.2, p=0.008), with a small effect size (d=-0.44, 95% CI from -1.08 to 0.20) (see Figure 3). PREG-LT augmentation also improved severity of anxiety (HAM-A) scores compared with placebo ($F_{1,179}$=7.2, p=0.008). In the HAM-A item analysis, patients randomized to PREG-LT demonstrated significantly greater improvements compared to the placebo group in anxious mood ($F_{1,195}$=5.2, p=0.024), tension ($F_{1,195}$=16.1, p<0.0001), and cardiovascular symptoms ($F_{1,195}$=6.7, p=0.010) scores.

PANSS Subscales

In the subscale of negative symptoms, patients who received PREG-LT demonstrated significantly greater improvement compared to those in the placebo group ($F_{1,179}$=4.2, p=0.043) and general psychopathology ($F_{1,179}$=3.9, p=0.048) ratings (see Figure 4). However, between-group differences on PANSS-P subscale did not reach a significant level ($F_{1,179}$=2.5, p=0.055).

Figure 4 Severity General Psychopathology (PANSS Scores) Among Patients with Combined Pregnenolone and L- Theanine Augmentation Compared to Placebo over Time



PANSS=Positive and Negative Syndrome Scale. Mean raw scores and SE are shown. *Indicated significant between groups differences.

General Functioning

PREG-LT patients also demonstrated significant improvement on the GAF scale compared to the placebo group ($F_{1,179}$=7.1, p=0.008) with a small effect size (d=0.21, 95% CI from -0.42 to 0.84).

The Bonferroni correction for four scales (SANS, HAM-A, PANSS and GAF) was applied (p=0.05/4=0.0125). After the Bonferroni correction, improvement in total SANS, HAM-A, and GAF scales remained significant (p<0.05), but not in PANSS subscale scores.

As Table 2 shows, both PREG-LT and placebo augmentation resulted in statistically significant decreases from baseline to endpoint of the study on the SANS, HAM-A, and GAF measures ("time"). However, no significant "treatment conditions" by "time" interactions for these scales were indicated (all p values >0.05).

Antipsychotic Agents

There was no difference between both treatment groups regarding the distribution of medication type (χ^2=0.9, df=2, p=0.65) and chlorpromazine equivalents (CPZ; p>0.05; see Table 1). When outcome variables were adjusted for type (FGAs, SGAs, COMB), CPZ equivalents of antipsychotic agents, and body mass index, patients receiving PREG-LT demonstrated significant improvement compared to the placebo group with respect to SANS, PANSS and HAM-A (ANOVA; p<0.01), whereas between-group differences on GAF ratings lost their significance (p>0.0125).

Effect of Other Covariates

No significant main effect of the *DSM-IV* diagnosis, duration of illness (years), concomitant treatment with mood stabilizers, benzodiazepines, antidepressants and anti-Parkinson agents, and interactions (arms by visits) on SANS, HAM-A, PANSS and GAF ratings was observed (all p values >0.05).

Side Effects, Tolerability and Safety

No differences between the two treatment arms were noted on ESRS scores: Parkinsonism ($F_{1,185}$=3.7, p=0.056), akathisia ($F_{1,185}$=0.8, p=0.35), dystonia ($F_{1,185}$=1.0, p=0.32), and tardive dyskinesia ($F_{1,185}$=0.1, p=0.82). Both PREG-LT and placebo augmentation resulted in unchanged ESRS scores from baseline to end point of the study without significant interaction: treatment arms × time (all p's>0.05). No treatment-related adverse events occurred in either group. There were no clinically significant changes in vital signs, electrocardiograms, or clinical laboratory variables associated with treatment. Thus, the administration of PREG-LT was well-tolerated.

Discussion

To the best of our knowledge, the present study is the first that has evaluated a combination of PREG-LT, two augmentive agents, in a randomized, double-blind, placebo-controlled add-on clinical trial in schizophrenia. The obtained findings suggest that:

Table 3 Comparison of Published and Present Clinical Trials with Pregnenolone and L-Theanine for Treatment of Patients with Schizophrenia and Schizoaffective Disorder

	Pregnenolone (PREG)			L-Theanine	Pregnenolone plus L-Theanine
Characteristics	Marx et al. (2009)	Ritsner et al. (2010)	Ritsner et al. (2013); Kreinin et al. (2013)	Ritsner et al. (2010)	Kardashev, Ratner, Ritsner (Present study)
Participants (treatment/placebo)	9/9	16/10/16	25/27	25/27	18/21
Antipsychotic drugs: FGAs/SGAs/COMB*	SGAs	FGAs, SGAs, COMB	FGAs, SGAs, COMB	FGAs, SGAs, COMB	FGAs, SGAs, COMB
Illness duration (mean±SD, yr.)	>1	15.1±8.0	2.7±1.5	12.3±8.6	11.2±7.6
Daily dose (mg/day)					
PREG	500	30 or 200	50	-	50
L-theanine	-	-	-	400	400
Length of trial (weeks)	8	8	8	8	8
Negative symptoms					
SANS, total	0.048	-	0.003	-	0.006
PANSS negative	n.s.	n.s.	0.0017	n.s.	0.043
Positive symptoms (PANSS)	n.s.	0.010	n.s.	0.009	n.s.
General psychopathology (PANSS)	n.s.	n.s.	n.s.	<0.001	0.048
Anxiety (HAM-A, total)	-	-	-	0.015	0.008
General functioning (GAF)	-	n.s.	n.s.	n.s.	0.008
Side effects (ESRS etc.)	-	0.049	n.s.	n.s.	n.s.
Cognitive deficit	n.s.	Attention Memory	Attention	n.s.	-

*FGAs=first-generation antipsychotics; SGAs=second-generation antipsychotics; COMB=both types of antipsychotic medications. SANS=Scale for the Assessment of Negative Symptoms. PANSS=Positive and Negative Syndrome Scale. HAM-A=Hamilton Scale for Anxiety. GAF=Global Assessment of Functioning. ESRS=Extrapyramidal Symptom Rating Scale.

1) PREG-LT augmentation was superior to placebo in the treatment of persistent negative symptoms (blunted affect, alogia, and anhedonia), anxiety (anxious mood, tension, and cardiovascular symptoms) and general functioning in schizophrenia patients. The beneficial effect of PREG-LT was noticed from the 4th week of treatment. The effect sizes (d) for symptom changes were moderate;
2) beneficial effects of PREG-LT augmentation were unrelated to the schizophrenia subtype, the duration of the illness, concomitant treatment, or type and CPZ equivalents of antipsychotic agents; and,
3) PREG-LT administration was found to be a safe and well-tolerated medication; 50 mg/day of PREG and 400 mg/day of LT did not produce side effects. PREG-LT did not induce amelioration of antipsychotic-related side effects.

The present study with PREG-LT confirms an ameliorative effect of PREG on negative symptoms (13, 37) and a beneficial effect of LT on anxiety and general psychopathology (12), but not on positive symptoms or extrapyramidal side effects (11) (see Table 3). However, this assertion in the present design might not be sustained because there is no just PREG arm or no just LT arm; therefore, we cannot say which particular substance contributed to the improvement. Furthermore, PREG-LT augmentation indicated significant improvement in general functioning (GAF), which was reported in previous trials. Thus, augmentation with PREG-LT not only replicated the beneficial effect of PREG on negative symptoms and LT on anxiety, but the combined treatment demonstrated a possible interplay effect, such as improvement in general functioning of patients. Further replication of this finding is warranted.

While the precise factors and specific mechanisms underlying improvement in negative symptoms following adjunctive therapy with PREG and LT are not yet understood, they may be related to brain protective and neurotrophic mechanisms. Indeed, PREG shows multiple pronounced neuroprotective properties: it regulates the growth of neurons and cerebral brain-derived neurotrophic factor (BDNF) levels, enhances myelination and synaptogenesis in the brain, and demonstrates other neuroprotective properties (16, 17). Likewise, LT protects brain cells against excitotoxicity by calming the nerve networks in the brain (51, 52). Furthermore, LT has antagonistic effects on NMDA receptors (53), and it increases BDNF levels (54). Decreased serum BDNF levels were found to be associated with reduction of dysphoric mood and anxiety symptom scores during LT augmentation, but not with placebo (55).

The next possible target for a combined beneficial effect of PREG-LT augmentation might be modulation of the main neurotransmitter signaling systems. The modulatory effect of PREG and its metabolites (PREGS, DHEA, DHEAS) on $GABA_A$, NMDA, sigma-1, cholinergic, and dopamine receptors (19, 20) may lead to important changes for neuronal excitability and possibly account for PREG's influence on clinical improvement. On the other hand, LT increases CNS levels of DA, 5-HT, GABA, as well as inhibits NMDA receptors (32-35), which might explain the anti-anxiety properties, mood-enhancing and relaxation effects of LT administration (26, 27).

Combinations of neuroprotective agents and/or antioxidants are used in animal models and human studies as well. The results of these trials are very encouraging (56-58). We can speculate that concomitant administration of PREG and LT has a more protective effect and possible synergistic modulatory effect on brain neurotransmitter signaling systems than either drug alone. Further testing of this hypothesis is warranted.

Limitations of this study include the relatively small sample size of patients and the relatively short duration of the study. The effects of PREG and LT were not investigated separately in this trial; however, they have previously been tested separately by the same design. Since we have some concerns about clinical versus statistical significance, larger studies should be conducted to establish the potential utility of the treatment. An improvement in our placebo group was not a specific limitation. Indeed, improvements in placebo groups of antipsychotic and antidepressant trials account for a major part of the expected drug effects (59). For example, the placebo effect accounted for 68% of the effect in the drug groups in the study by Rief et al. (60). Furthermore, placebo group improvement in pharmacotherapy trials has been increasing over time across several pharmacological treatment areas (61).

Thus, these initial results clearly require replication in a larger cohort. However, we assume that PREG-LT augmentation of antipsychotic therapy can ameliorate negative and anxiety symptoms in schizophrenia. Therefore, these positive findings support continuing the study of PREG and LT augmentation of antipsychotic medication in patients suffering from chronic schizophrenia with negative and anxiety symptoms.

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