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Proof-of-Concept Trial with the Neurosteroid Pregnenolone Targeting Cognitive and Negative Symptoms in Schizophrenia

Christine E Marx^{*,1,2,3}, Richard SE Keefe¹, Robert W Buchanan⁴, Robert M Hamer⁵, Jason D Kilts^{1,2,3}, Daniel W Bradford^{1,2}, Jennifer L Strauss^{1,2,3}, Jennifer C Naylor^{1,2,3}, Victoria M Payne^{1,2,3}, Jeffrey A Lieberman⁶, Adam J Savitz⁷, Linda A Leimone^{2,3}, Lawrence Dunn^{1,2}, Patrizia Porcu⁵, A Leslie Morrow⁵, Lawrence J Shampine^{1,2,3}

¹Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC, USA

²Durham Veterans Affairs Medical Center, Durham, NC, USA

³Veterans Affairs Mid-Atlantic Mental Illness Research, Education, and Clinical Center (VISN6), Durham, NC, USA

⁴Maryland Psychiatric Research Center, University of Maryland, Baltimore, MD, USA

⁵Department of Psychiatry, University of North Carolina, Chapel Hill, NC, USA

⁶Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York, NY, USA

⁷Department of Psychiatry, Weill Medical College of Cornell University, New York NY, USA

Abstract

The neurosteroid pregnenolone and its sulfated derivative enhance learning and memory in rodents. Pregnenolone sulfate also positively modulates NMDA receptors and could thus ameliorate hypothesized NMDA receptor hypofunction in schizophrenia. Furthermore, clozapine increases pregnenolone in rodent hippocampus, possibly contributing to its superior efficacy. We

*Correspondence: Dr CE Marx, Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham VA Medical Center, 508 Fulton Street, MHSL 116A, Durham, NC 27705, USA, Tel: +919 286 0411 Ext. 7426, Fax: +919 286 6811, marx0001@mc.duke.edu.

DISCLOSURE OF COMPENSATION OVER THE LAST 3 YEARS/CONFLICT OF INTEREST

Dr Marx has no compensation to disclose over the last 3 years. Dr Marx is a co-applicant on a pending US patent application on the use of neurosteroids for the treatment of central nervous system disorders. She is an unpaid scientific advisor and board member of NeuroScience Pharmaceuticals. Dr Keefe has received compensation from the following sources: Astra-Zeneca, Cyberonics, Gabriel Pharmaceuticals, Organon Pharmaceuticals, Otsuka, Pfizer, Saegis, Abbott, Acadia, BiolineRx, Bristol-Myers Squibb, Cephalo, Cortex, Dainippon Sumitomo Pharmaceutical, Eli Lilly, Johnson & Johnson, Lundbeck, Memory Pharmaceuticals, Merck, Orexigen, Sanofi/Aventis, Shering-Plough, Wyeth, and Xenoport. Dr Keefe is a contributor to a pending US patent application on the use of neurosteroids for the treatment of central nervous system disorders. Dr Keefe receives royalties for two of the cognitive test batteries used in this study, the BACS and the MATRICS Consensus Cognitive Battery. Dr Buchanan has received compensation from the following sources: Astra-Zeneca, GlaxoSmithKline, Janssen, Merck, Natixis Bleichroeder, Organon, Ortho-McNeil, Pfizer, Sanofi-Aventis, Solvay Pharmaceuticals, and Wyeth. Dr Hamer has received compensation from the following sources: Acadia, Allergan, Alpharma, Astra-Zeneca, Cenerx, Corcept, EnabledMD, Epix, Johnson & Johnson, Pfizer, Schwartz, Solvay Pharmaceuticals, Sanofi-Aventis, Takeda, and Wyeth. Dr Kilts, Dr Bradford, Dr Strauss, Dr Naylor, Dr Payne, Ms Leimone, Dr Dunn, Dr Porcu, Dr Morrow, and Mr Shampine have no compensation to disclose over the last 3 years. Dr Lieberman has received compensation from the following sources: Astra-Zeneca, Eli Lilly, Forest Laboratories, GlaxoSmithKline, Pfizer, Wyeth, Solvay Pharmaceuticals, Bristol-Myers Squibb, Janssen, and Repligen. Dr Savitz has received compensation from the following sources: Astra-Zeneca, Sanofi-Aventis, Janssen, and Pfizer.

therefore investigated adjunctive pregnenolone for cognitive and negative symptoms in patients with schizophrenia or schizoaffective disorder receiving stable doses of second-generation antipsychotics in a pilot randomized, placebo-controlled, double-blind trial. Following a 2-week single-blind placebo lead-in, patients were randomized to pregnenolone (fixed escalating doses to 500 mg/day) or placebo, for 8 weeks. Primary end points were changes in BACS and MCCB composite and total SANS scores. Of 21 patients randomized, 18 completed at least 4 weeks of treatment ($n = 9/\text{group}$). Pregnenolone was well tolerated. Patients receiving pregnenolone demonstrated significantly greater improvements in SANS scores (mean change = 10.38) compared with patients receiving placebo (mean change = 2.33), $p = 0.048$. Mean composite changes in BACS and MCCB scores were not significantly different in patients randomized to pregnenolone compared with placebo. However, serum pregnenolone increases predicted BACS composite scores at 8 weeks in the pregnenolone group ($r_s = 0.81$, $p = 0.022$). Increases in allopregnanolone, a GABAergic pregnenolone metabolite, also predicted BACS composite scores ($r_s = 0.74$, $p = 0.046$). In addition, baseline pregnenolone ($r_s = -0.76$, $p = 0.037$), pregnenolone sulfate ($r_s = -0.83$, $p = 0.015$), and allopregnanolone levels ($r_s = -0.83$, $p = 0.015$) were inversely correlated with improvements in MCCB composite scores, further supporting a possible role for neurosteroids in cognition. Mean BACS and MCCB composite scores were correlated ($r_s = 0.74$, $p < 0.0001$). Pregnenolone may be a promising therapeutic agent for negative symptoms and merits further investigation for cognitive symptoms in schizophrenia.

Keywords

schizophrenia; negative symptoms; cognitive symptoms; neurosteroid; pregnenolone; allopregnanolone

INTRODUCTION

Cognitive and negative symptoms in schizophrenia are frequently severe and strongly correlated with decreased functional outcome and quality of life (Buchanan, 2006; Buchanan *et al*, 2005; Green, 2006; Green *et al*, 2000; Harvey *et al*, 2004; Kirkpatrick *et al*, 2006; Marder, 2006; Marder and Fenton, 2004), but effective pharmacological interventions remain limited for these symptom domains (Buchanan, 2007; Buchanan *et al*, 2005, 2007; Carter *et al*, 2008; Kirkpatrick *et al*, 2006). Although first- and second-generation antipsychotic agents have been reported to produce improvements in cognitive and negative symptoms, the emerging scientific consensus appears to be that these effects are generally modest in scope, and that significant disabling symptoms frequently persist despite positive symptom reduction (Buchanan *et al*, 2005; Keefe *et al*, 2007; Kirkpatrick *et al*, 2006). New treatment strategies are therefore necessary to address these important dimensions of schizophrenia symptomatology.

The neurosteroid pregnenolone may represent a promising and mechanistically novel agent for cognitive and negative symptoms in schizophrenia. Pregnenolone (Flood *et al*, 1992) and its sulfated derivative, pregnenolone sulfate (Akwa *et al*, 2001; Darnaudery *et al*, 2002; Flood *et al*, 1995; Ladurelle *et al*, 2000; Mayo *et al*, 1993; Meziane *et al*, 1996; Pallares *et al*, 1998; Vallee *et al*, 1997, 2001), enhance learning and memory in animal models at

concentrations that are physiologically relevant and known to be present in human brain (Marx *et al*, 2006c; Weill-Engerer *et al*, 2002). For example, acute administration of pregnenolone or pregnenolone sulfate to rodents, delivered either i.c.v., i.p., or by direct hippocampal injection, results in behavioral performance increases in the T-maze, the Morris water maze, and the Y-maze (Akwa *et al*, 2001; Flood *et al*, 1992, 1995; Vallee *et al*, 1997). Pregnenolone sulfate positively modulates glutamatergic *N*-methyl-D-aspartate (NMDA) receptors (Bowlby, 1993; Irwin *et al*, 1994; Wu *et al*, 1991), enhances long-term potentiation in rat hippocampus (Sliwinski *et al*, 2004), and prevents learning and memory deficits induced by NMDA receptor antagonists, including MK-801 and DAP-V (Akwa *et al*, 2001; Cheney *et al*, 1995; Mathis *et al*, 1994, 1996; Romeo *et al*, 1994). Treatment with pregnenolone, which is metabolized to pregnenolone sulfate in humans following acute administration (Morley *et al*, 1997; Roberts, 1995), may thus represent a logical strategy for the amelioration of hypothesized NMDA receptor hypofunction in schizophrenia (Coyle, 2006; Javitt, 2004, 2007; Millan, 2005; Rujescu *et al*, 2006). Pregnenolone sulfate also increases acetylcholine release in multiple rodent brain regions (Darnaudey *et al*, 1998, 2002; Mayo *et al*, 1993; Pallares *et al*, 1998; Vallee *et al*, 1997) and prevents the memory-impairing effects of the muscarinic cholinergic receptor antagonist scopolamine (Meziane *et al*, 1996; Vallee *et al*, 2001), actions consistent with cognitive enhancement. In addition, clozapine markedly increases pregnenolone levels in rodent hippocampus, representing a candidate mechanism for its superior efficacy (Marx *et al*, 2006a). Converging evidence thus suggests that pregnenolone may have potential utility for the treatment of cognitive and negative symptoms in schizophrenia.

Despite the availability of pregnenolone as a dietary supplement in the United States, very few clinical trials have investigated its use. Studies conducted in the 1940s and 1950s showed that pregnenolone 25–500 mg/day was safe and well tolerated in humans (Davison *et al*, 1950; Freeman *et al*, 1950a; Guest *et al*, 1950; Henderson *et al*, 1950; McGavack *et al*, 1951; Pincus and Hoagland, 1944, 1945a,b). A number of these earlier studies treated patients with inflammatory diseases, such as rheumatoid arthritis, and several reported improvements in symptoms and overall functioning following pregnenolone. According to these earlier reports, pregnenolone was very well tolerated with minimal side effects, and did not affect weight, heart rate, blood pressure (even in patients with hypertension), menstrual cycle, or glucose levels (either in diabetics or non-diabetics). In a more recent investigation, pregnenolone at low doses (15–30 mg/day) was generally well tolerated in healthy volunteers with no significant side effects compared with placebo (Meieran *et al*, 2004). Evidence to date thus suggests that pregnenolone has a favorable safety profile, although controlled trials remain very limited.

In this pilot study, we thus investigated pregnenolone as an adjunctive therapeutic strategy for cognitive and negative symptoms in patients with schizophrenia or schizoaffective disorder with an escalating fixed-dose approach, achieving a total dose of 500 mg/day (approaching the highest well tolerated dose reported in the existing literature) in the last 4 weeks of the study administered in divided doses (Freeman *et al*, 1950b; McGavack *et al*, 1951). Although human pharmacokinetic studies addressing acute administration are extremely few, a single oral dose of pregnenolone 175 mg approximately doubles serum levels over the course of 4–8 h (Roberts, 1995). As pregnenolone levels decrease by

approximately 60% with age (Morley *et al*, 1997; Roberts, 1995), and as we estimated that the mean study participant age would be greater than 40, we predicted that our dosing strategy of 500 mg/day of pregnenolone in the last 4 weeks of the study would likely produce pregnenolone levels that are close to those observed in young adulthood or up to twofold higher than typical young adult levels.

METHODS

This pilot investigation was a placebo-controlled, double-blind, parallel group, randomized trial of adjunctive pregnenolone for the treatment of cognitive and negative symptoms in patients with schizophrenia or schizoaffective disorder. Following a single-blind 2-week placebo lead-in phase (all patients), subjects were randomized to 8 weeks of treatment with adjunctive pregnenolone or placebo. Patients received a total of six study visits, which took place every 2 weeks. In addition, subjects received staggered telephone check-in calls to assess potential side effects every 2 weeks (ie, during alternate weeks when a study visit did not take place). The trial was conducted at a single site, the Durham Veterans Affairs Medical Center in Durham, North Carolina. The protocol was approved by the local institutional review board and conducted under FDA Investigational New Drug (IND) no. 71 768. The [ClinicalTrials.gov](https://clinicaltrials.gov) number for this study was NCT00560937.

Subjects

Subjects were outpatients between the ages of 18 and 65 years with schizophrenia or schizoaffective disorder diagnosed by DSM-IV/DSM-IV-TR criteria using the Structured Clinical Interview (First *et al*, 1996) and medical records. All subjects were between the ages of 18 and 65 years and provided informed consent. Duration of illness was more than 1 year for all enrolled patients. To be eligible for the trial, patients had to be taking a second-generation antipsychotic (aripiprazole, olanzapine, quetiapine, or risperidone) for at least 8 weeks, with no changes in antipsychotic dosing in the preceding 4 weeks. Patients receiving first-generation antipsychotics were excluded from this pilot investigation. Concomitant psychiatric medications were permissible (antidepressants, mood stabilizers, anticholinergics, and other), provided that patients were receiving stable doses of all of these medications in the 4 weeks preceding the trial and throughout the entire duration of the study. Any change in psychiatric medications at any point during the study rendered a patient ineligible to continue participation. Exclusionary were unstable medical or neurological illness, alcohol or other substance dependence within the last month (other than nicotine), use of oral contraceptives or other hormonal supplementation such as estrogen (although early studies suggested no effects on menstrual cycle, alterations in downstream pregnenolone metabolites, such as estradiol, could theoretically impact the efficacy of oral contraceptives or estrogen replacement), active expression of suicidal or homicidal ideation, pregnancy or breastfeeding, and known allergy to study medication.

During the screening visit (Visit 1), patients received a physical exam, vital signs, and electrocardiogram (EKG), as well as baseline laboratory measures consisting of serum electrolytes, glucose, creatinine, blood urea nitrogen (chemistry 7 panel), liver function tests, complete blood count (CBC), urinalysis, urine toxicology test, thyroid stimulating hormone

(TSH), and prolactin, and serum pregnancy test if female. These tests were also repeated at the completion of the study (Visit 6). In addition, patients received an interim EKG 4 weeks post-randomization, as well as a serum pregnancy test if female (Visit 4). Patients also received a chemistry 7 panel, CBC, and liver function tests at Visits 2, 3, 4, and 5 (ie, at all study visits). Psychiatric symptoms were assessed at baseline using the Scale for the Assessment of Negative Symptoms (Andreasen, 1983), the Positive and Negative Symptom Scale (PANSS) (Kay *et al*, 1987), the Calgary Depression Rating Scale (CDRS) (Addington *et al*, 1990), the Clinical Global Impression Improvement (CGI-I) and Severity (CGI-S) scales, the Barnes Akathisia Scale (BAS) (Barnes, 1989), the Simpson Angus Scale for Extrapyramidal Symptoms (SAS) (Simpson and Angus, 1970), the Quality of Life Scale (QOL) (Heinrichs *et al*, 1984), and the Hillside Adverse Events Form. Each side effect query was rated with the latter assessment instrument on a Likert scale with regard to both intensity and relationship to study drug. In addition, potential side effects were also assessed with this instrument during a scheduled phone check-in call every 2 weeks, which was staggered to study visits. Side effects were thus assessed at weekly intervals (either in person at a scheduled study visit or by phone check-in) using a structured rating scale. One research nurse with 5 years of experience in the conduction of schizophrenia clinical trials completed both psychiatric and cognitive assessments throughout the study, and was trained to one point of divergence on each PANSS item.

Cognitive symptoms were assessed by the Brief Assessment of Cognition in Schizophrenia (BACS) (Keefe *et al*, 2004, 2008) and the MATRICS Consensus Cognitive Battery (MCCB), (Nuechterlein and Green, 2006; Nuechterlein *et al*, 2008). To the best of our knowledge, no clinical trial to date has reported both of these cognitive assessments within the same study. The MCCB became available shortly before enrollment for this study. Given the advancement that the MCCB assessment tool represents in incorporating the latest expert opinion in this area, inclusion of the MCCB battery was judged imperative for this pilot investigation and provided an opportunity to obtain initial data with both cognitive instruments. The BACS and MCCB batteries were administered 2 weeks following the screening visit (following the completion of the 2-week placebo lead-in phase at Visit 2), and at two 4-week intervals thereafter (at mid point following randomization at Visit 4 and at the final visit (Visit 6). The BACS cognitive battery uses the following assessments in the respective targeted domains (Keefe *et al*, 2004, 2008): list learning (verbal memory), digit sequencing task (working memory), token motor task (motor speed), verbal fluency (processing speed), symbol coding (attention and processing speed), and the Tower of London test (executive functions/reasoning and problem solving). The MCCB uses the following assessments: Trail Making Test, Part A and the symbol coding subtest of the BACS (both assess speed of processing), the Hopkins Verbal Learning Test-Revised, immediate recall (three learning trials only) (verbal learning), the Wechsler Memory Scale, 3rd ed., spatial span subtest (working memory, nonverbal), the Letter-Number Span test (working memory, verbal), the Neuropsychological Assessment Battery, mazes subtest (reasoning and problem solving), the Brief Visuospatial Memory Test-Revised (visual learning), the Category Fluency Test (animal naming) (speed of processing), the Mayer-Salovey-Caruso Emotional Intelligence Test, managing emotions branch (social cognition), and the Continuous Performance Test, Identical Pairs version (attention/ vigilance). Of note,

the symbol coding subtest of the BACS was administered only once per visit at Visits 2, 4, and 6, although it is a component of both the BACS and MCCB cognitive batteries, to avoid potentially confounding practice effects.

Study drug (pregnenolone 50 mg (PREG)) and matching placebo identical in appearance was obtained from Douglas Laboratories (Pittsburgh, PA, USA), which provided certificates of analysis for pregnenolone 50 mg tablets and matching placebo. Study drug was dispensed every 2 weeks at each study visit at fixed escalating doses as follows:

Visit 1: Two-week single-blind placebo lead-in phase, one placebo tablet twice each day (all pts),

Visit 2: Randomization to PREG 50 mg twice each day (100 mg/day total) or placebo for 2 weeks,

Visit 3: PREG 150 mg twice each day (300 mg/day total) or placebo for 2 weeks,

Visit 4: PREG 250 mg twice each day (500 mg/day total) or placebo for 2 weeks,

Visit 5: PREG 250 mg twice each day (500 mg/day total) or placebo continued for 2 weeks.

Patients thus received PREG 500 mg/day in divided doses during the last 4 weeks of the study. Patients also received the Hillside Adverse Events Form at Visits 2–6, and during staggered telephone check-in calls every 2 weeks, beginning 1 week into the single-blind placebo lead-in phase.

Neurosteroid Analyses

Pregnenolone and allopregnanolone levels in serum were determined by a highly sensitive and specific gas chromatography/mass spectrometry (GC/MS) method in the negative ion chemical ionization mode, as described earlier (Marx *et al*, 2006d, e). One ml of serum was extracted three times in ethyl acetate before high performance liquid chromatography (HPLC) purification using tetrahydrofuran, ethanol, and hexane in the mobile phase. All samples were injected in duplicate. The mean coefficients of variation for pregnenolone and allopregnanolone were 1.9 and 4.9%, respectively. The limit of detection with this method was 2 pg for both pregnenolone and allopregnanolone. Pregnenolone sulfate levels in serum were determined by radioimmunoassay as described earlier (Porcu *et al*, 2008), with modifications. Antiserum for pregnenolone sulfate (MP Biomedicals, Orangeburg, NY, USA) was diluted 1:300 to allow quantification of high pregnenolone sulfate levels observed in this study following treatment with adjunctive pregnenolone. The sensitivity of the assay was 50 pg; the mean intra-assay coefficient of variation was 13.1%. All other serum steroid levels were determined using the commercially available radioimmunoassay kits according to the manufacturer's directions (progesterone, cortisol, testosterone, free testosterone, and DHEAS: Diagnostic System Laboratories, Webster, TX, USA). DHEA serum levels were determined using a kit from ICN Pharmaceuticals (Costa Mesa, CA, USA).

Statistical Analysis

Primary end points in this pilot investigation were changes from baseline at Visit 2 following a 2-week placebo lead-in period (all patients) to final assessment at Visit 6 in cognitive symptoms (as assessed by BACS and MCCB composite scores) and negative symptoms (as assessed by SANS total scores). If the final Visit 6 assessment was missing for a subject (8 weeks of treatment with pregnenolone or placebo post-randomization), Visit 4 was carried forward for BACS, MCCB, SANS, and other psychiatric assessments (4 weeks of treatment with pregnenolone or placebo post-randomization). Student's *t*-tests were conducted on the change scores between treatment groups in this proof-of-concept study (two-tailed- α $p=0.05$ for each statistical test). Spearman's correlation coefficients were determined to assess the relationship between increases in serum pregnenolone levels and cognitive and psychiatric improvements, as well as the relationship between baseline neurosteroid levels and changes in these outcome measures following treatment with pregnenolone. Paired *t*-tests were used to assess pregnenolone and pregnenolone metabolite level changes in serum post-treatment.

RESULTS

Subject Characterization

Of 28 subjects who received a screening visit, 21 met entry criteria and were randomized at Visit 2 to 8 weeks of treatment with adjunctive pregnenolone or placebo, following a 2-week single-blind placebo lead-in phase for all patients. The number of subjects who completed 4 or more weeks of treatment with pregnenolone or placebo post-randomization was 18 (86%), with 17 patients completing the entire 8-week study post-randomization (81%). One patient randomized to the placebo group completed only 4 weeks of the study secondary to requiring an increase in antipsychotic dose, thus excluding this subject from continued participation (Visit 4 assessments were thus carried forward for the statistical analyses). Baseline demographic and clinical characteristics are presented in Table 1. The mean age of subjects was 49.43 years (± 12.19 SD) for the placebo group and 52.68 years (± 6.31 SD) for the pregnenolone group. The study sample contained only one female subject, reflecting the approximate demographic of the population with psychotic disorders treated at the Durham Veterans Affairs Medical Center.

Of 18 randomized subjects completing at least 4 weeks of the study, 3 were treated with aripiprazole, 8 with olanzapine, 3 with quetiapine, and 4 with risperidone (Table 1). Three of the 21 patients who were randomized did not complete at least 4 weeks of treatment with pregnenolone or placebo for the following reasons: (1) one patient began truck-driving school shortly after randomization at Visit 2 and his new schedule and location could not accommodate study visits; (2) one patient arrived intoxicated at Visit 4 following heavy alcohol and cocaine use that morning, and had taken his oral hypoglycemic without breakfast. He subsequently sustained an episode of hypoglycemia requiring medical attention that was believed to be unrelated to study drug, per internal medicine physician evaluation on the day of the event; and (3) one patient received an increase in antipsychotic prescribed by the subject's regular psychiatrist between Visit 2 and Visit 3, hence making the patient ineligible to continue in the study.

Missing data were addressed in the following manner. For cognitive assessments, any missing subscale was imputed as directed by BACS and MCCB guidelines, and these imputed values are included in the cognitive subscale tables for the BACS and MCCB described below. There were relatively few missing cognitive data for either the BACS or MCCB instruments, and testing sessions were generally very well tolerated. For the BACS, 1% of the total number of administered subscales was missing and required imputation (one symbol coding test and two token motor tests). For the MCCB, 1.5% of the total number of administered subscales was missing (two continuous performance tests (CPT) and five social cognition tests (MSCEIT)). One patient received BACS and MCCB cognitive batteries and CGI assessments at baseline, but could not complete several other rating scales (PANSS, SANS, Calgary Depression Rating Scale) secondary to a scheduling conflict. As sleep deprivation for 20–25 h significantly impacts cognitive testing performance in a manner comparable with a blood alcohol level of 0.10% (Dawson and Reid, 1997; Lamond and Dawson, 1999), cognitive data for one patient who reported sleep deprivation for the preceding 24 h at the final study visit could not be used; this patient's interim cognitive assessments from Visit 4 were thus carried forward for the statistical analyses. Other missing assessments were addressed through case-wise deletion (3 WRAT and 1 Calgary Depression Rating Scale), as were three outlying steroid levels greater than 2.4 SDs above the mean that were omitted from analyses as per statistical consultation. Of the serum estradiol and free testosterone levels determined at baseline and at the final study visit in both the pregnenolone and placebo groups ($n = 35$; one randomized patient did not reach the final study visit as described above), six estradiol and two free testosterone levels were below the limits of detection of the RIA kits used in this investigation (8 and 0.15 pg/ml, respectively), and are thus not included in Table 5.

Pregnenolone Significantly Reduces SANS Scores Compared with Placebo

Baseline SANS scores were similar in both the pregnenolone and placebo groups (50.75 ± 12.21 and 47.56 ± 12.09 SD, respectively). Patients randomized to pregnenolone completing at least 4 weeks of treatment post-randomization demonstrated significantly improved SANS scores (mean change 10.38 ± 10.18 SD) compared with patients receiving placebo (mean change 2.33 ± 4.42 SD), unpaired Student's *t*-test of SANS change from baseline $t = 2.16$, *df* 15, $p = 0.048$, Table 2. Pregnenolone thus outperformed placebo by approximately 8 points on the SANS assessment, a difference that may be clinically relevant. SANS subscales are also provided in Table 2, although this information should be interpreted with caution given the small number of subjects in this study. This table does, however, provide data for future hypothesis testing with regard to domains of the SANS that may be responsive to a pregnenolone intervention. In the subscale analyses, uncorrected for multiple comparisons in this exploratory investigation, patients randomized to pregnenolone demonstrated significantly greater improvements in the 'affect' subscale of the SANS ($t = 2.33$, *df* 15, $p = 0.035$). There was also a trend for improvements in the 'alogia' SANS subscale ($t = 1.83$, *df* = 15, $p = 0.087$) in the pregnenolone group.

BACS and MCCB Assessments

The mean composite changes in BACS and MCCB scores at 8 weeks compared with baseline were not significantly different in patients randomized to pregnenolone compared

with the placebo group. The mean improvements in BACS composite scores in the pregnenolone group (z -score change 0.60 ± 0.78 SD) were nonsignificantly greater than those in the placebo group (z -score change 0.22 ± 0.47 SD) by a z -score of approximately 0.4 and approached a medium effect size in this small pilot study ($p = 0.22$, $t = 1.27$, $df = 16$), Table 3. Individual BACS subscales are also presented in Table 3 for descriptive purposes to inform future hypothesis testing. BACS subscales that may demonstrate improvements in the pregnenolone group compared with the placebo group are verbal memory and the Tower of London tests (both with z -score improvements 0.61 greater than placebo), followed by the token motor and digit sequencing tasks in which the pregnenolone group demonstrated z -score improvements of 0.42 and 0.25, respectively, greater than placebo (Table 3). Mean improvements in MCCB composite scores were the same post-treatment in the pregnenolone (mean t -score change 7.00 ± 8.87 SD) and placebo groups (mean t -score change 7.00 ± 4.95 SD), a finding that appears driven by improvements in the MSCEIT subscale in the placebo group (Table 4). MCCB subscales that may demonstrate improvement include attention/vigilance (t -score improvement 6.89 greater than placebo), working memory (t -score improvement 4.00 greater than placebo), and verbal learning (t -score improvement 2.67 greater than placebo). Composite MCCB scores and BACS composite scores administered at baseline and post-treatment were positively correlated (Spearman's correlation coefficient 0.74, $p < 0.0001$, $n = 36$).

In the group randomized to pregnenolone, increases in serum levels of this neurosteroid predicted improvements in BACS composite scores (Spearman's $r_s = 0.81$, $n = 8$, $p = 0.022$), suggesting that peripheral pregnenolone levels may potentially have biomarker utility for the assessment of clinical response (Figure 1a). Serum pregnenolone levels tended to nonsignificantly predict improvements in MCCB composite scores (Spearman's $r_s = 0.62$, $n = 8$, $p = 0.115$), data not shown. Spearman's correlation coefficients were determined (rather than Pearson correlation coefficients) to reduce the influence of outliers. Serum increases in the GABAergic neurosteroid allopregnanolone also predicted improvements in BACS composite scores, Figure 1b (Spearman's $r_s = 0.74$, $n = 8$, $p = 0.046$). Serum increases in pregnenolone sulfate levels did not predict improvements in the BACS or MCCB composite scores ($p > 0.05$ for both assessments). However, baseline pregnenolone sulfate levels were inversely correlated with improvements in MCCB composite scores (Spearman's $r_s = -0.83$, $n = 8$, $p = 0.015$), suggesting that patients with lower pregnenolone sulfate levels at baseline experienced greater cognitive improvements as assessed by the MCCB following treatment with adjunctive pregnenolone (Figure 1c). Similarly, baseline pregnenolone (Spearman's $r_s = -0.76$, $n = 8$, $p = 0.037$) and allopregnanolone (Spearman's $r_s = -0.83$, $n = 8$, $p = 0.015$) levels were also inversely associated with improvements in MCCB composite scores. There was a trend for baseline pregnenolone levels to be inversely associated with improvements in BACS composite scores ($r = -0.71$, $n = 8$, $p = 0.058$).

Psychiatric Assessments

Patients randomized to pregnenolone demonstrate significantly improved final CGI-I scores (2.11 ± 0.33 SD), compared with the group randomized to placebo (2.89 ± 0.78 SD), unpaired Student's t -test $p = 0.015$, $t = 2.75$, $df = 16$ (CGI-I of 2 = much improved; CGI-I of 3 = minimally improved), Table 5. The change was not reflected in CGI-S scores, however,

with both pregnenolone and placebo showing persistent moderate symptoms (4.00 ± 0.50 and 4.00 ± 0.00 at baseline, respectively; 3.89 ± 0.33 and 4.00 ± 0.00 post-treatment, respectively), Table 5. PANSS scores were decreased by 4.43 ± 3.59 more points in the pregnenolone group compared with the placebo group, but this finding was not significant ($p = 0.24$, $t = 1.23$, $df = 15$), Table 5. There was a trend for pregnenolone administration to decrease the positive symptom subscale of the PANSS ($p = 0.069$, $t = 1.96$, $df = 15$), but not the negative symptom or general subscales (Table 5). Negative symptom subscale scores of the PANSS were significantly correlated with total SANS scores in this pilot investigation (Spearman's $r = 0.58$, $p = 0.0004$, $n = 33$, xy pairs consisting of baseline and week 8 scores for all patients for whom these data are available). Other psychiatric assessments did not show significant differences following treatment with pregnenolone compared with placebo (see Table 5 for summary of clinical rating scales).

Pregnenolone Administration Results in Increases in Serum Pregnenolone and Specific Downstream Metabolite Levels, Including the GABAergic Neurosteroid Allopregnanolone

Paired t -tests were used to determine if pregnenolone administration alters the serum concentrations of a number of downstream metabolites, including the GABAergic neurosteroid allopregnanolone and the NMDA receptor modulator pregnenolone sulfate. Treatment with pregnenolone resulted in fourfold elevations in serum levels of pregnenolone (paired t -test $p = 0.017$, $t = 3.11$, $df = 7$), tripled serum pregnenolone sulfate levels (paired t -test $p < 0.0001$, $t = 10.44$, $df = 8$), and increased the GABAergic neurosteroid allopregnanolone fivefold (paired t -test $p = 0.009$, $t = 3.59$, $df = 7$) (Table 6). Pregnenolone administration also increased serum progesterone over fourfold and DHEAS levels by approximately 16% (Table 6). Treatment with pregnenolone did not increase serum testosterone, free testosterone, cortisol, DHEA, estradiol, or androstenedione levels (Table 6).

Side Effect Profiles in Patients Randomized to Pregnenolone and Placebo

There were no significant changes in most laboratory parameters, including glucose, triglycerides, HDL, TSH, and prolactin post-treatment compared with baseline in the pregnenolone group (Table 7). Cholesterol levels were significantly decreased following treatment with pregnenolone compared with baseline (paired t -test $t = 3.53$, $df = 8$, $p = 0.008$); however, not all blood draws were conducted in a fasting state. Serum LDL levels were also significantly reduced in the pregnenolone group post-treatment (paired t -test $t = 5.16$, $df = 6$, $p = 0.002$). There was no change in EKG QTc interval following treatment with pregnenolone (Table 7). Pregnenolone was very well tolerated. The only side effects present in the pregnenolone group to a greater degree than the placebo group were two reports of mild restlessness, one report of mild muscle pain/stiffness, and one report of mild cold extremities (Table 8).

DISCUSSION

The main finding of this pilot randomized placebo-controlled trial is that adjunctive pregnenolone significantly reduces negative symptoms in patients with schizophrenia or schizoaffective disorder as assessed by the SANS. In addition to the SANS, the two other

primary end points for this study were the BACS and MCCB cognitive assessment batteries. The mean composite changes in BACS and MCCB scores post-treatment compared with baseline were not significantly different in patients randomized to pregnenolone compared with the placebo group. However, increases in serum pregnenolone and allopregnanolone levels predicted BACS composite scores post-treatment in the pregnenolone group. In addition, patients randomized to pregnenolone who demonstrated lower pregnenolone sulfate, pregnenolone, and allopregnanolone levels at baseline showed greater improvements in MCCB composite scores. These data suggest that adjunctive pregnenolone may have utility for negative symptoms and merits further investigation for cognitive symptoms in schizophrenia. Patients receiving adjunctive pregnenolone also demonstrated significantly improved CGI-I scores, although CGI-S scores remained unchanged. These findings, related results, and potential pregnenolone mechanisms of action are discussed below.

Adjunctive Pregnenolone Significantly Reduces Negative Symptoms

Treatment with adjunctive pregnenolone significantly reduced negative symptoms as assessed by SANS scores in patients with schizophrenia or schizoaffective disorder compared with placebo. Although these results should be interpreted with caution given small sample sizes, a significant effect on negative symptoms following pregnenolone treatment is encouraging and merits further investigation. In our exploratory analyses, individual subscales of the SANS were also investigated. Results suggest that the greatest improvements in negative symptoms following adjunctive pregnenolone occurred in the ‘affective flattening or blunting’ domain, with the next most pronounced improvements occurring in the ‘alogia’ and ‘anhedonia-asociality’ subscales. The change in ‘affective flattening or blunting’ was significantly greater in the pregnenolone group, uncorrected for multiple comparisons in this pilot study. As greater negative symptom improvement in the pregnenolone group appeared to occur among SANS subscales containing items that may overlap with depressive symptomatology (‘affective flattening or blunting’ and ‘anhedonia-asociality’), it is perhaps worth noting that patients with depressive symptoms demonstrate reductions in cerebrospinal fluid (CSF) pregnenolone levels (George *et al*, 1994). It is therefore possible that supplementation with pregnenolone may be particularly efficacious for negative symptoms with potential affective dimensions. Replication of these findings in a larger cohort of patients will thus be necessary to confirm potential pregnenolone effects on specific SANS subscale scores as well as total SANS scores, and to investigate the mechanism(s) of action contributing to the possible mitigation of negative symptoms by this neurosteroid.

A number of pregnenolone characteristics may play roles in the production of the aforementioned potential therapeutic effects. For example, pregnenolone enhances myelination (Koenig *et al*, 1995), increases neuritic outgrowth (Fontaine-Lenoir *et al*, 2006), and impacts microtubule polymerization and stability (Fontaine-Lenoir *et al*, 2006; Hsu *et al*, 2006; Murakami *et al*, 2000), actions that may be relevant to the pathophysiology of schizophrenia (Benitez-King *et al*, 2004; Glantz and Lewis, 2000; Hakak *et al*, 2001; Tkachev *et al*, 2007). Furthermore, we have determined earlier that clozapine markedly elevates pregnenolone levels in rat hippocampus and serum to a greater degree than other second-generation antipsychotics at doses producing comparable striatal D₂ receptor

occupancies, representing a candidate mechanism for its superior efficacy (Marx *et al*, 2006a). Recent evidence that clozapine enhances the binding of the translocator protein involved in the rate-limiting step leading to pregnenolone formation from cholesterol (formerly referred to as the peripheral-type benzodiazepine receptor, (Lacapere and Papadopoulos, 2003)) supports a role for this neurosteroid in clozapine mechanisms of action (Danovich *et al*, 2008). In addition, pregnenolone is present in human brain tissue at physiologically relevant concentrations in the nanomolar range that are greater than 10-fold higher than those frequently observed in serum or plasma (Marx *et al*, 2006c) and known to positively impact learning and memory (Flood *et al*, 1992). Pregnenolone is elevated in posterior cingulate and parietal cortex in patients with schizophrenia compared with control subjects, a finding that may represent compensatory changes and/or drug effects (Marx *et al*, 2006c), given earlier evidence that pharmacological agents such as clozapine and olanzapine elevate neurosteroids (Barbaccia *et al*, 2001; Marx *et al*, 2003, 2006a, b). As neurosteroid induction may contribute to the therapeutic efficacy of certain antipsychotics, it is therefore logical to target pregnenolone as an adjunctive strategy in patients with schizophrenia.

On the basis of a strong rationale for possible NMDA receptor hypofunction in schizophrenia (Coyle, 2006; Javitt, 2004, 2007; Millan, 2005; Rujescu *et al*, 2006), a number of earlier investigations using compounds with modulatory activities at NMDA receptors such as D-serine (Heresco-Levy *et al*, 2005; Tsai *et al*, 1998, 1999), glycine (Buchanan *et al*, 2007; Heresco-Levy *et al*, 1996, 1999, 2004; Javitt *et al*, 2001), and D-cycloserine (Buchanan *et al*, 2007; Cascella *et al*, 1994; Duncan *et al*, 2004; Goff *et al*, 1995, 1996, 1999a, b; Rosse *et al*, 1996; van Berckel *et al*, 1996) have been undertaken, with somewhat mixed findings. Because our data demonstrate that adjunctive pregnenolone administration significantly elevates serum levels of pregnenolone sulfate, a positive NMDA receptor modulator, it is possible that metabolism to this neurosteroid may contribute to its therapeutic efficacy for negative symptoms through this mechanism. It is unknown, however, if pregnenolone-induced increases in serum pregnenolone sulfate also lead to elevated pregnenolone sulfate levels in human brain. A rodent investigation suggests this may be the case (Wang *et al*, 1997), but the accurate determination of pregnenolone sulfate levels in brain tissue has also been the subject of several recent inquiries addressing possible confounding methodological challenges in the quantification of this neurosteroid (Ebner *et al*, 2006; Higashi *et al*, 2003a, b; Liere *et al*, 2004; Liu *et al*, 2003; Schumacher *et al*, 2008). It is therefore possible that other mechanisms contribute to potential pregnenolone effects on negative symptoms that are unrelated to the NMDA receptor modulatory actions of its sulfated metabolite.

Adjunctive Pregnenolone and Cognition in Schizophrenia

Serum increases in pregnenolone in the group randomized to this neurosteroid are significantly correlated with cognitive improvement following this intervention as determined by the BACS assessment battery, a finding that may be encouraging with regard to the therapeutic potential of pregnenolone for cognitive symptoms in schizophrenia. This correlation may also highlight the biomarker potential of serum pregnenolone levels in predicting therapeutic response. Along these lines, lower baseline pregnenolone sulfate, pregnenolone, and allopregnanolone levels are also associated with greater improvements in

MCCB composite scores. It is therefore possible that the determination of peripheral neurosteroid levels may have predictive utility for the identification of schizophrenia patients who would potentially benefit from a pregnenolone intervention and for the prediction of clinical efficacy.

This is the first report to the best of our knowledge to compare the BACS and MCCB cognitive assessment batteries within the same clinical trial. In this study, the composite scores of the BACS and MCCB assessments are positively correlated (Spearman's $r_s = 0.74$, $n = 36$, $p < 0.0001$). Treatment with adjunctive pregnenolone non-significantly improved composite BACS scores compared with placebo by a z -score of approximately 0.4, approaching a medium effect size if replicated in future studies adequately powered to detect this difference. There was no difference in mean composite MCCB t -score changes in the pregnenolone group compared with the placebo group post-treatment, a finding that appears to be driven by improved performances on the MSCEIT (social cognition domain test) by the placebo group. It is not clear why the placebo group outperformed the pregnenolone group on this particular MCCB measure, and this finding may have been impacted by a relatively larger number of missing MSCEIT assessments compared with other MCCB subscales. Larger randomized controlled trials will clearly be required to confirm initial findings regarding the potential for cognitive enhancement following treatment with pregnenolone.

Adjunctive Pregnenolone and Other Psychiatric Outcome Measures

Patients randomized to pregnenolone demonstrate significantly greater improvements in CGI-I scores, demonstrating a mean post-treatment CGI-I score of 2.11 (2 = much improved) compared with mean CGI-I scores of 2.89 in patients receiving placebo (3 = minimally improved). A significant finding in this overall improvement measure ($p < 0.015$) is potentially encouraging in a pilot investigation of this size, but this result will require replication in a larger study, particularly as changes in the CGI-S measure were similar in both groups. Another measure of general patient functioning, the Heinrich Carpenter Quality of Life Scale, was not significantly altered post-treatment in this initial proof-of-concept trial, but an improvement of 5.27 points in the pregnenolone group compared with the placebo group was in the predicted direction (with possibly the greatest improvement in the 'interpersonal and social work' subscale). Together these findings suggest a potential signal for overall improvement following pregnenolone administration, but adequately powered controlled trials will be required to test this possibility.

With regard to PANSS total scores, patients randomized to pregnenolone demonstrated slightly greater improvements in this outcome measure compared with placebo (4.43 points), but the finding was not statistically significant. The negative symptom subscale of the PANSS was nonsignificantly improved to a modest degree by 1.53 points in the pregnenolone group compared with the placebo group. This result is not necessarily surprising, however, as the SANS demonstrates greater sensitivity for the assessment of this symptom domain compared with the PANSS negative symptom subscale, and hence the absence of a statistically significant effect may reflect limited power in this initial study of nine subjects per group. Nonetheless, the negative symptom subscale of the PANSS was

significantly correlated with the SANS. Finally, there was a trend for treatment with pregnenolone to reduce the positive symptom subscale of the PANSS ($p < 0.07$), a finding that merits further investigation.

Pregnenolone Levels in Serum and Pregnenolone Metabolism Profiles: Candidate Biomarkers and Potential Relevance to Mechanisms of Action

Adjunctive pregnenolone elevates serum pregnenolone levels—Pregnenolone administration in this study results in approximately fourfold elevations in serum pregnenolone to physiologically relevant nanomolar levels. Preclinical data support the possibility that elevations of pregnenolone levels to this extent could have significant beneficial effects. For example, pregnenolone enhances learning and memory in rodent models at even lower concentrations than the nanomolar serum concentrations achieved in the current investigation (Flood *et al*, 1992). As pregnenolone is lipophilic and readily crosses the blood–brain barrier, it is likely that brain concentrations of pregnenolone in humans are also elevated following pregnenolone administration, as suggested by animal studies (Wang *et al*, 1997). Furthermore, we have demonstrated earlier that serum pregnenolone levels are closely correlated with hippocampal pregnenolone levels in rats (Marx *et al*, 2006a), and that CSF pregnenolone levels in humans are correlated with temporal cortex pregnenolone levels within the same subject cohort (Naylor *et al*, 2008). Serum pregnenolone (and possibly other neurosteroids) may thus serve as a proxy or surrogate marker for brain pregnenolone levels, potentially providing data that may be relevant to the prediction of clinical response.

Pregnenolone metabolism to other neurosteroids

Metabolism to pregnenolone sulfate—Our data demonstrate that pregnenolone administration triples serum levels of its sulfated derivative, pregnenolone sulfate. Pregnenolone sulfate levels attained in serum following pregnenolone administration are very consistent with doses required to achieve its positive effects on learning and memory in rodent models (Akwa *et al*, 2001; Flood *et al*, 1992, 1995; Mathis *et al*, 1996; Meziane *et al*, 1996). Furthermore, recent evidence suggests that pregnenolone sulfate may positively modulate NMDA receptors at concentrations in the nanomolar range at binding sites distinct from a number of known NMDA receptor targets (Johansson *et al*, 2008). Pregnenolone sulfate in the nanomolar range also enhances long-term potentiation (Sliwinski *et al*, 2004) and influences GABA release (Mtchedlishvili and Kapur, 2003) in rodents. In addition to these actions, pregnenolone sulfate may increase neurogenesis in rodent hippocampus (Mayo *et al*, 2005). In this study, patients with lower serum pregnenolone sulfate levels at baseline demonstrated greater cognitive improvements as assessed by the MCCB. It is therefore possible that a subset of patients with schizophrenia may demonstrate relative pregnenolone sulfate deficits (as well as lower baseline pregnenolone and allopregnanolone levels) that are potentially restored to optimal levels with pregnenolone administration. Pregnenolone metabolism to pregnenolone sulfate could thus result in a number of additional mechanistic contributions for this neurosteroid and its effects on cognitive and negative symptoms, including the amelioration of hypothesized NMDA receptor hypofunction.

Metabolism to the GABAergic neurosteroid allopregnanolone—Pregnenolone administration results in fivefold elevations in serum allopregnanolone levels in this pilot investigation, and it is possible that pregnenolone metabolism to this downstream GABAergic neurosteroid metabolite may play a role in its therapeutic efficacy. Supporting this possibility, increases in allopregnanolone predicted cognitive improvement as assessed by BACS composite scores. Given compelling evidence for a GABAergic deficit in patients with schizophrenia (Benes and Berretta, 2001; Benes *et al*, 2007; Guidotti *et al*, 2005; Lewis *et al*, 2003, 2004, 2005) and the fact that allopregnanolone potentiates GABA_A receptor responses to a greater degree than benzodiazepines or barbiturates (Majewska *et al*, 1986; Morrow *et al*, 1987, 1990), it is possible that pregnenolone metabolism to the GABAergic neurosteroid allopregnanolone may contribute to its mechanism(s) of action.

Allopregnanolone elevations resulting from treatment with pregnenolone may also play a role in other physiological processes related to schizophrenia, as this GABAergic neurosteroid enhances neurogenesis (Wang *et al*, 2005) and shows multiple pronounced neuroprotective properties (Djebaili *et al*, 2005; Griffin *et al*, 2004; Lockhart *et al*, 2002; Mellon *et al*, 2008; Sayeed *et al*, 2006; Xilouri and Papazafiri, 2006). In addition, it enhances myelination (Ghoumari *et al*, 2003) and possesses anticonvulsant actions (Kokate *et al*, 1994, 1996). Furthermore, allopregnanolone shows multiple anti-inflammatory effects (He *et al*, 2004; VanLandingham *et al*, 2007) and decreases apoptosis (Charalampopoulos *et al*, 2004, 2006; Xilouri and Papazafiri, 2006), actions that may be relevant to schizophrenia (Dickerson *et al*, 2007; Glantz *et al*, 2006; Jarskog *et al*, 2005; Knight *et al*, 2007; Lencz *et al*, 2007). Finally, allopregnanolone levels are decreased in postmortem brain tissue from patients with Alzheimer's disease (Marx *et al*, 2006e), and hence the restoration of allopregnanolone levels by precursor loading with pregnenolone may be a logical approach for a number of disorders in which the disruption of cognition is a salient characteristic.

Metabolism to progesterone intermediary—Abundant evidence demonstrates that progesterone plays an important role in a number of brain functions in addition to its well-characterized non-central nervous system effects. Progesterone is present in both male and female human brain in nanomolar concentrations (Lacroix *et al*, 1987; Lanthier and Patwardhan, 1986; Weill-Engerer *et al*, 2002). Progesterone administration results in pronounced neuroprotective effects in rodent models of traumatic brain injury (Djebaili *et al*, 2004, 2005; He *et al*, 2004; Jones *et al*, 2005; Robertson *et al*, 2006; Roof *et al*, 1997), and also positively impacts myelination processes (Azcoitia *et al*, 2003; Ghoumari *et al*, 2003; Ibanez *et al*, 2003; Koenig *et al*, 1995; Schumacher *et al*, 2000) and dendritic outgrowth during development (Sakamoto *et al*, 2001). Extending these rodent investigations, a recent randomized controlled clinical trial reported that intravenous progesterone for 3 days reduced 30-day mortality rates by over 50% in acute moderate-to-severe traumatic brain injury (Wright *et al*, 2007), strongly supporting a neuroprotective role for this neurosteroid. This pivotal study has been described as the first successful clinical trial of a pharmacological agent for traumatic brain injury in 40 years, with no adverse events attributable to the intervention (Stein, 2008; Wright *et al*, 2007). Furthermore, these promising clinical findings have recently been replicated, and also extended to demonstrate

enduring therapeutic progesterone effects 6 months following traumatic brain injury (Xiao *et al*, 2008).

In this study, pregnenolone administration results in elevated progesterone levels, a finding that is not surprising as progesterone is an allopregnanolone precursor and a pregnenolone metabolite, hence representing an intermediary neurosteroid in the biosynthetic pathway. The mean serum progesterone elevations to 2.17 ng/ml following adjunctive pregnenolone in this pilot study represent levels that are somewhat higher than typical male serum progesterone ranges defined by the commercially available radioimmunoassay kit used in this investigation (serum range for males 0.10–1.17 ng/ml), but less than or comparable with those achieved in human male brain (Lacroix *et al*, 1987; Lanthier and Patwardhan, 1986; Weill-Engerer *et al*, 2002). It is also possible that induction of this neurosteroid intermediary may be relevant to pregnenolone mechanisms of action primarily because it is readily metabolized to the neuroprotective GABAergic neurosteroid allopregnanolone, a possibility supported by animal models of stroke (Sayeed *et al*, 2006) and seizures (Kokate *et al*, 1999).

Possible metabolism to DHEAS—DHEAS levels are significantly increased following pregnenolone administration, but the magnitude of this effect is relatively modest (approximately 16%). Given less pronounced increases in serum DHEAS levels following treatment with pregnenolone as well as the small sample size of the current pilot study, this result will require replication in a larger cohort of subjects. Nonetheless, an earlier investigation determined that DHEA administration significantly attenuated negative symptoms in patients with schizophrenia and resulted in concomitant elevations of its sulfated derivative, DHEAS (Strous *et al*, 2003). It is therefore possible that pregnenolone metabolism to DHEAS may be relevant to its therapeutic mechanism(s) of action.

Absence of conversion to other steroids—Pregnenolone administration for 8 weeks to patients with schizophrenia or schizoaffective disorder did not result in downstream elevations of cortisol, testosterone, free testosterone, DHEA, androstenedione, or estradiol (Table 6). As pregnenolone is a potential precursor to a number of steroids, pregnenolone could theoretically be metabolized to all of these molecules. Our data suggest, however, that pregnenolone metabolism is weighted toward biosynthetic pathways resulting in pregnenolone sulfate and allopregnanolone formation, rather than toward glucocorticoid or sex steroid synthesis involving testosterone or estradiol.

Tolerability and Safety

Pregnenolone was very well tolerated at the doses used in this pilot randomized controlled trial. Side effects reported at greater frequency than placebo included two instances of mild restlessness, one instance of mild cold extremities, and one instance of mild muscle pain/stiffness. No patients receiving pregnenolone experienced a serious adverse event related to study medication. Patients randomized to pregnenolone did not demonstrate significant weight gain, blood pressure, or pulse rate changes during the course of this study. Glucose levels were unchanged, as were serum electrolyte panels, TSH, prolactin levels, and liver function tests. Cholesterol levels were significantly decreased following 8 weeks of treatment with pregnenolone, but this result should be interpreted with caution, as not all

blood draws were conducted in a fasting state. Interestingly, serum LDL levels were significantly decreased following adjunctive pregnenolone, a finding that may merit further investigation. As anticipated, pregnenolone serum levels at the completion of the study achieved or slightly exceeded pregnenolone levels observed in young adulthood (Morley *et al*, 1997).

Limitations

One of the main limitations of this pilot clinical trial is small sample size, and these initial results will clearly require replication in a larger cohort. In addition, only patients receiving second-generation antipsychotics were enrolled in this investigation, and hence it is unclear if these results are potentially generalizable to patients receiving first-generation agents. Only one female patient was enrolled in this study, constituting another limitation with regard to generalizability. The presence of other psychiatric medications in addition to second-generation antipsychotics was not exclusionary, possibly representing a confounding element, although no dose changes were permitted 4 weeks before the study and throughout the study duration. The optimal dosing for pregnenolone is not clear and pharmacokinetic data are currently very limited. Our dosing strategy in this study was very well tolerated and resulted in a significant reduction in negative symptoms and a possible signal for cognitive symptoms, but it is not known at present if other dosing approaches could potentially be more efficacious. These issues will need to be addressed in larger controlled studies. Using the current preliminary data to estimate statistical power requirements, a future investigation to begin to test hypotheses generated in this proof-of-concept study would optimally include at least 88 subjects (44 per group) to detect significant improvements in cognitive assessment composite scores following treatment with pregnenolone. A co-primary functional outcome measure would also be advantageous. Fewer patients would be required to test the hypothesis that pregnenolone significantly attenuates negative symptoms in schizophrenia. Other areas that will need to be addressed in future studies include potential sources of variability in neurosteroid levels, which may be impacted by factors such as age, smoking status, and concurrent medication use.

Summary

The results of this pilot randomized controlled trial investigating adjunctive pregnenolone as a treatment strategy for negative and cognitive symptoms in schizophrenia are promising and merit further study in a larger cohort of patients to attempt to replicate these initial findings. Pregnenolone was very well tolerated in this study and showed no untoward effects on weight, blood pressure, pulse, glucose, cholesterol, prolactin, or other monitored laboratory parameters. Candidate mechanisms for pregnenolone efficacy are diverse, and a number of theoretically coherent possibilities are supported by the existing preclinical and clinical literature. If these initial pilot findings are confirmed in larger randomized controlled trials, pregnenolone may represent a novel therapeutic advance for the treatment of cognitive and negative symptoms in schizophrenia.

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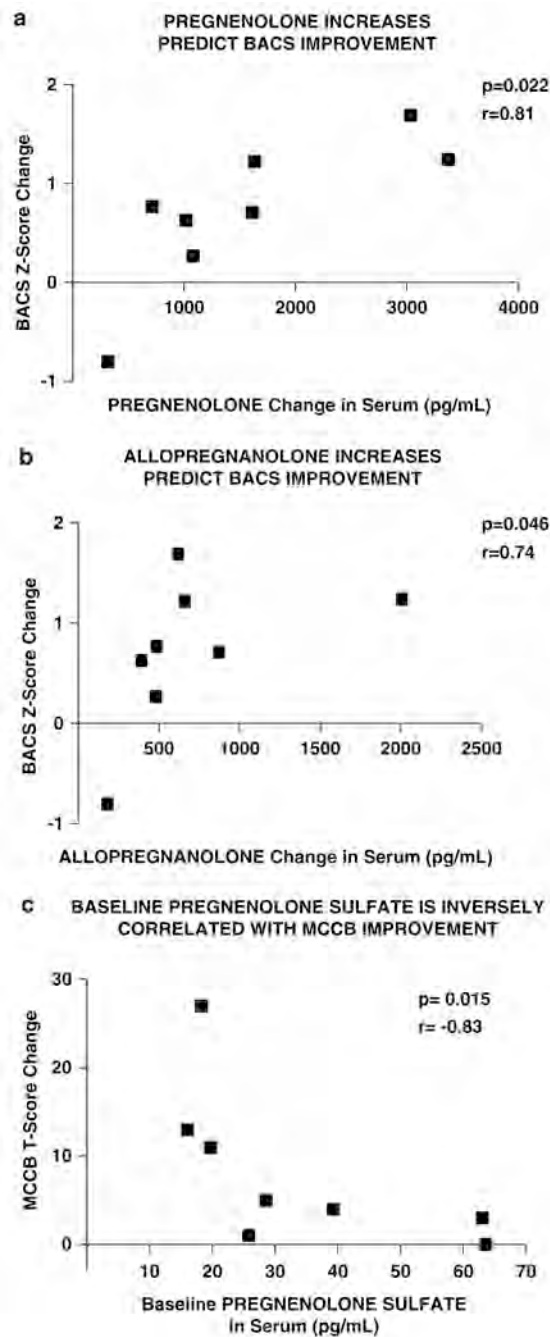


Figure 1.

(a) Increases in serum pregnenolone following treatment with this neurosteroid are correlated with improvements in cognitive performance, as assessed by composite BACS z-scores. (b) Increases in serum allopregnanolone following treatment with pregnenolone are correlated with cognitive improvement, as assessed by composite BACS z-scores. (c) Baseline pregnenolone sulfate levels are inversely associated with cognitive improvement, as assessed by composite MCCB *t*-scores.

Table 1

Baseline Demographic and Clinical Characteristics

	Placebo (n = 9)		Pregnenolone (n = 9)	
Male/female	9/0		8/1	
Age (years)	49.43 ± 12.19		52.68 ± 6.31	
Schizophrenia	5		6	
Schizoaffective disorder	4		3	
Caucasian	3		3	
African American	5		6	
Other	1		0	
WRAT	100.1 ± 15.33		101.1 ± 10.94	
	Placebo		Pregnenolone	
Antipsychotic (mg/day)	Number of subjects	Mean dose ± SD	Number of subjects	Mean dose ± SD
Aripiprazole	1	15 ± 0	2	30 ± 21
Olanzapine	5	18 ± 2.7	3	13.3 ± 7.6
Quetiapine	1	300 ± 0	2	400 ± 283
Risperidone	2	5 ± 1.4	2	6 ± 0

Table 2
Scale for the Assessment of Negative Symptoms (SANS) Subscales and SANS Total Score

	Week 0		Week 4		Week 8		Change from week 0 to week 8		Statistics			
	Placebo (n=9) (mean ± SD)	PREG (n=8) (mean ± SD)	Placebo (n=9) (mean ± SD)	PREG (n=8) (mean ± SD)	Placebo (n=9) (mean ± SD)	PREG (n=8) (mean ± SD)	Placebo (mean ± SD)	PREG (mean±SD)	Difference	t	df	P
<i>SANS subscales</i>												
Affect	12.78 ± 3.96	16.13 ± 4.97	13.44 ± 4.45	14.25 ± 3.15	13.00 ± 4.36	11.88 ± 0.35	0.22 ± 1.04	-4.25 ± 1.68	-4.47 ± 1.92	2.33	15	0.035*
Alogia	8.78 ± 3.80	8.75 ± 3.81	8.44 ± 3.71	7.75 ± 3.24	8.44 ± 3.71	6.88 ± 2.10	-0.33 ± 0.33	-1.88 ± 0.81	-1.54 ± 0.84	1.83	15	0.087#
Avolition/ apathy	8.44 ± 2.40	8.25 ± 1.39	8.56 ± 2.46	8.13 ± 0.99	8.11 ± 2.71	8.00 ± 0.53	-0.33 ± 0.44	-0.25 ± 0.31	0.08 ± 0.55	0.15	15	0.88
Anhedonia/ sociality	11.56 ± 5.62	12.00 ± 4.54	11.11 ± 5.49	10.38 ± 4.07	10.78 ± 5.09	9.63 ± 3.50	-0.78 ± 0.46	-2.38 ± 1.30	-1.60 ± 1.31	1.22	15	0.24
Attention	6.00 ± 1.00	5.63 ± 1.06	5.78 ± 1.20	5.25 ± 1.04	4.89 ± 1.45	4.00 ± 0	-1.11 ± 0.35	-1.63 ± 0.38	-0.51 ± 0.51	1.00	15	0.33
SANS total	47.56 ± 12.09	50.75 ± 12.21	47.33 ± 11.68	45.75 ± 8.84	45.22 ± 11.96	40.38 ± 3.50	-2.33 ± 4.42	-10.38 ± 10.18	-8.04 ± 3.73	2.16	15	0.048*

Unpaired Student's *t*-tests conducted on change scores at week 8 compared with week 0.

* $p < 0.05$

$p < 0.10$.

Table 3
Brief Assessment of Cognition in Schizophrenia (BACS) Subscales and BACS Composite Score

	Week 0 raw scores			Week 4 raw scores			Week 8 raw scores			Change in Z-scores from week 0 to week 8		
	Placebo (mean ± SD)	PREG (mean ± SD)		Placebo (mean ± SD)	REG (mean ± SD)		Placebo (mean ± SD)	PREG (mean ± SD)		Placebo (n=9) (mean ± SD)	PREG (n=9) (mean ± SD)	Difference
<i>BACS subscales</i>												
Verbal memory	35.33 ± 1233	36.78 ± 7.82		37.89 ± 10.51	39.00 ± 7.12		37.22 ± 11.29	43.78 ± 9.80		0.22 ± 0.71	0.83 ± 0.88	0.61 ± 0.38
Digit sequencing	18.44 ± 3.17	15.78 ± 3.99		19.56 ± 3.17	17.22 ± 3.35		19.78 ± 4.79	18.44 ± 3.91		0.25 ± 0.61	0.50 ± 0.63	0.25 ± 0.29
Token motor	53.67 ± 16.99	46.44 ± 17.54		53.11 ± 14.18	55.56 ± 18.02		53.33 ± 11.31	52.22 ± 14.12		-0.02 ± 1.04	0.40 ± 1.67	0.42 ± 0.66
Verbal fluency	44.00 ± 12.43	45.78 ± 12.56		42.78 ± 9.50	45.11 ± 9.97		44.11 ± 11.61	45.22 ± 7.87		0.01 ± 0.60	-0.05 ± 0.75	-0.06 ± 0.32
Symbol coding	36.78 ± 9.32	36.89 ± 13.39		39.44 ± 9.61	44.78 ± 11.97		41.22 ± 10.01	38.67 ± 13.61		0.27 ± 0.55	0.11 ± 0.46	-0.16 ± 0.24
Tower of London	14.78 ± 7.89	13.22 ± 6.26		15.11 ± 6.68	15.22 ± 6.06		15.56 ± 6.15	16.22 ± 4.94		0.21 ± 1.62	0.82 ± 1.79	0.61 ± 0.80
<i>BACS Z-scores</i>												
	Placebo (mean ± SD)	PREG (mean ± SD)		Placebo (mean ± SD)	PREG (mean ± SD)		Placebo (mean ± SD)	PREG (mean ± SD)		Placebo (n=9) (mean ± SD)	PREG (n=9) (mean ± SD)	Difference
BACS composite	-1.28 ± 0.96	-1.54 ± 0.96		-1.14 ± 0.80	-1.04 ± 0.75		-1.06 ± 0.88	-0.93 ± 0.87		0.22 ± 0.47	0.60 ± 0.78	0.38 ± 0.30

Table 4
 Measurement and Treatment Research to Improve Cognition in Schizophrenia (MCCB) Subscales and Composite Score

	Week 0 raw scores			Week 4 raw scores			Week 8 Raw scores			Change in domain T-scores from week 0 to week 8		
	Placebo (n=9) (mean ± SD)	PREG (n=9) (mean ± SD)		Placebo (n=9) (mean ± SD)	PREG (n=9) (mean ± SD)		Placebo (n=9) (mean ± SD)	PREG (n=9) (mean ± SD)		Placebo (n=9) (mean ± SD)	PREG (n=9) (mean ± SD)	Difference
<i>MCCB subscales</i>												
Trail Making Test, Part A	56.11 ± 30.29	42.11 ± 8.37		42.33 ± 11.62	38.33 ± 7.76		44.11 ± 10.52	34.33 ± 7.12		4.67 ± 7.50	544 ± 8.69	078 ± 3.83
BACS, symbol coding subtest	36.78 ± 9.32	36.89 ± 13.39		39.44 ± 9.61	44.78 ± 11.97		41.22 ± 10.01	38.67 ± 13.61				
Category fluency test, animal naming	18.00 ± 5.63	18.33 ± 5.17		16.44 ± 4.64	18.44 ± 3.78		18.67 ± 4.21	20.44 ± 4.10				
Speed of processing												
Hopkins Verbal Learning Test	21.00 ± 6.42	18.56 ± 6.39		20.33 ± 6.14	20.89 ± 5.33		21.11 ± 7.49	21.00 ± 5.24		0.78 ± 5.07	344 ± 2.92	2.67 ± 1.95
Verbal learning												
Wechsler Memory Scale, 3rd ed., spatial span subtest	12.00 ± 3.67	13.11 ± 2.93		12.11 ± 3.41	14.33 ± 3.35		12.78 ± 2.54	14.22 ± 3.15		1.11 ± 8.15	5.11 ± 6.83	4.00 ± 3.54
Working memory												
Letter-Number Span test	13.22 ± 2.91	11.44 ± 3.13		13.33 ± 3.91	12.67 ± 3.08		13.11 ± 4.11	13.11 ± 3.86				
Reasoning and problem solving												
Neuropsychological Assessment	11.11 ± 3.92	13.56 ± 6.42		13.33 ± 6.40	15.67 ± 6.24		15.00 ± 6.87	17.33 ± 6.30		6.22 ± 9.01	6.22 ± 10.80	0.00 ± 4.69
Battery, mazes subtest												
Brief Visuospatial Memory Test— Rev.	16.33 ± 8.15	20.22 ± 8.63		18.78 ± 9.04	21.33 ± 10.07		20.78 ± 10.24	22.33 ± 9.30		7.56 ± 13.44	3.78 ± 7.87	-3.78 ± 5.19
Visual learning												
Mayer-Salovey-Caruso Emotional Intelligence Test, managing emotions branch	86.87 ± 13.30	86.89 ± 14.84		90.85 ± 16.12	90.60 ± 13.55		93.92 ± 12.43	86.10 ± 16.81		8.33 ± 6.60	-0.89 ± 15.57	-9.22 ± 5.64
Social cognition												
Continuous Performance	1.96 ± 0.97	1.42 ± 0.93		2.10 ± 1.11	1.37 ± 1.24		2.01 ± 1.19	1.91 ± 1.05		0.67 ± 5.45	7.56 ± 19.45	6.89 ± 6.73
Attention/vigilance												

Table 5

Psychiatric Rating Scales by Treatment Group

	Week 0		Week 4		Week 8		Change from Week 0 to Week 8		Statistics			
	Placebo (mean ± SD)	PREG (mean ± SD)	Placebo (mean ± SD)	PREG (mean ± SD)	Placebo (mean ± SD)	PREG (mean ± SD)	Placebo (n) (mean ± SD)	PREG (n) (mean ± SD)	Difference	t	df	p-value
PANSS total	72.89 ± 6.58	72.50 ± 14.65	70.44 ± 6.31	67.88 ± 10.52	67.44 ± 6.02	62.63 ± 6.23	-5.44 ± 5.27 (9)	-9.88 ± 9.23 (8)	-4.43 ± 3.59	1.23	15	0.24
<i>PANSS subscores</i>												
Positive symptoms	13.89 ± 3.02	13.63 ± 4.87	13.22 ± 2.05	12.78 ± 3.83	12.89 ± 3.82	11.89 ± 4.01	-1.00 ± 1.58 (9)	-2.63 ± 1.85 (8)	-1.63 ± 0.83	1.96	15	0.069#
Negative symptoms	24.11 ± 3.72	23.38 ± 3.93	23.67 ± 3.04	23.22 ± 4.47	22.89 ± 2.42	20.56 ± 2.01	-1.22 ± 2.68 (9)	-2.75 ± 3.01 (8)	-1.53 ± 1.38	1.11	15	0.29
General	34.89 ± 3.22	35.50 ± 6.70	33.56 ± 2.96	34.33 ± 4.80	31.67 ± 2.78	31.22 ± 2.82	-3.22 ± 3.77 (9)	-4.50 ± 4.90 (8)	-1.28 ± 2.11	0.61	15	0.55
CGI-I	—	—	—	—	2.89 ± 0.78	2.11 ± 0.33	—	—	—	2.75	16	0.014*
CGI-S	4.00 ± 0.00	4.00 ± 0.50	—	—	4.00 ± 0.00	3.89 ± 0.33	0.00 ± 0.00 (9)	-0.11 ± 0.33 (9)	NA	—	—	—
Calgary Depression Scale	2.88 ± 4.05	2.43 ± 3.41	—	—	0.88 ± 1.25	0.86 ± 1.86	-2.00 ± 3.66 (8)	-1.57 ± 2.15 (7)	0.43 ± 1.58	0.27	13	0.79
Simpson-Angus Scale	0.56 ± 0.73	1.00 ± 1.69	0.89 ± 1.17	1.25 ± 1.67	0.67 ± 0.87	0.88 ± 1.36	0.11 ± 0.60 (9)	-0.13 ± 0.83 (8)	-0.24 ± 0.35 NA	0.68	15	0.51
Barnes Akathisia Scale	0.00 ± 0.00	0.38 ± 1.06	0.33 ± 1.00	0.38 ± 1.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 (9)	-0.38 ± 1.06 (8)	NA	—	—	—
AIMS	1.67 ± 2.06	1.63 ± 2.13	1.00 ± 1.66	0.88 ± 1.25	1.22 ± 1.79	0.63 ± 0.92	-0.44 ± 1.88 (9)	-1.00 ± 1.85 (8)	-0.56 ± 0.91	0.61	15	0.55
Heinrich Carpenter Quality of Life Scale	38.29 ± 14.92	47.48 ± 14.76	—	—	40.95 ± 15.25	55.41 ± 17.85	2.66 ± 7.23 (8)	7.93 ± 12.40 (8)	5.27 ± 5.07	1.04	14	0.32
<i>Heinrich Carpenter Subscores</i>												
Interpersonal and social work	11.91 ± 6.90	17.86 ± 9.11	—	—	13.70 ± 6.37	23.29 ± 8.04	1.79 ± 4.14 (8)	5.43 ± 8.09 (8)	3.64 ± 3.21	1.13	14	0.28
Occupational role function	1.63 ± 4.60	1.88 ± 5.30	—	—	1.75 ± 4.95	2.75 ± 6.32	0.13 ± 0.35 (8)	0.88 ± 1.64 (8)	0.75 ± 0.59	1.26	14	0.23
Other residual symptoms	24.75 ± 6.76	27.75 ± 5.90	—	—	25.50 ± 6.30	29.38 ± 6.65	0.75 ± 3.01 (8)	1.63 ± 5.32 (8)	0.88 ± 2.16	0.41	14	0.69

NA, no calculation secondary to no change in CGI-S and Barnes Akathisia Scale scores in the placebo group.

Unpaired Student's *t*-tests conducted on change scores at week 8 compared with week 0 (except for CGI-I, unpaired Student's *t*-test conducted on week 8 assessments).

$p < 0.10$

 $p > 0.05$
*

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Table 6

Additional Steroid Levels at Baseline and Post-Treatment with Pregnenolone

	Placebo (n=8, unless specified)				Pregnenolone (n=9, unless specified)					
	Week 0 (mean ±SD)	Week 8 (mean ±SD)	t	df	p-Value	Week 0 (mean ±SD)	Week 8 (mean ±SD)	t	df	p-Value
Pregnenolone (pg/ml)	598.1 ±329.6	514.01± 122.5	1.05	7	0.33	499.9 ±313.6 (n=8)	2096.0±9994 (n=8)	4.16	7	0.0042 *
Pregnenolone sulfate (ng/ml)	52.2±21.7	41.8±24.3	1.98	7	0.09#	33.4± 18.3	94.4± 14.3	10.44	8	<0.0001 *
Allopregnanolone (pg/ml)	105.6±41.0	99.8±51.4	0.43	7	0.68	110.7±78.9 (n=8)	823.1 ±528.9 (n=8)	3.59	7	0.0088 *
Cortisol (µg per 100 ml)	8.88±5.13	8.84±4.54	0.04	7	0.97	8.08 ±2.80	7.96±3.85	0.13	8	0.90
Total testosterone (ng per 100 ml)	399.31 ±203.21	446.76± 140.36	1.03	7	0.34	302.83± 158.53	303.08± 156.05	0.01	8	0.99
Free testosterone (pg/ml)	7.96±2.45	9.34±2.95	1.49	7	0.18	7.96± 1.07 (n=8)	7.31 ±2.04 (n=8)	0.79	7	0.46
DHEA (ng/ml)	7.09 ±3.11	8.22±4.19	2.01	7	0.09#	7.85±5.94	6.33±3.26	1.48	8	0.18
DHEAS (µg per 100ml)	151.58± 108.99	160.96± 114.38	0.75	7	0.48	124.72± 86.52	144.87±97.30	3.61	8	0.007 *
Progesterone (ng/ml)	0.54±0.24	0.53±0.23	0.39	7	0.71	0.38±0.15 (n=8)	2.17±064 (n=8)	7.47	7	0.0001 *
Estradiol (pg/ml)	24.16±842 (n=6)	25.66 ± 10.18 (n=6)	0.48	5	0.65	24.65±6.21 (n=7)	25.42±4.73 (n=7)	0.32	6	0.76
Androstenedione (ng/ml)	3.71 ±2.28	3.32± 1.45	0.93	7	0.38	2.93± 1.30	3.01 ± 1.66	0.22	8	0.83

Paired *t*-tests conducted on week 0 and week 8 steroid levels in patients receiving pregnenolone or placebo.

* $p < 0.05$

$p < 0.10$

Table 7

Serum Analytes and EKG QTc Interval at Screening and Final Visit in Patients Randomized to Pregnenolone

	Pregnenolone (n= 9, unless specified)		<i>t</i>	df	<i>p</i> -value
	Screening (mean ± SD)	Week 8 (mean ± SD)			
Glucose (mg per 100 ml)	117.9 ±36.2	119.0± 33.5	0.15	8	0.88
Cholesterol (mg per 100 ml)	185.8 ±43.3	166.6 ±36.2	3.53	8	0.008*
HDL (mg per 100ml)	39.3 ± 16.5 (n= 8)	37.0 ± 17.4 (n= 8)	1.18	7	0.28
LDL (mg per 100 ml)	119.1 ±26.0 (n= 7)	90.9 ±29.4 (n= 7)	5.16	6	0.002*
Triglycerides (mg per 100 ml)	194.3 ±87.8	237.6 ± 119.5	1.79	8	0.11
TSH (μU/ml)	145 ± 0.64	1.26 ± 0.72	0.84	8	0.42
Prolactin (ng/ml)	7.69 ±5.91	7.85 ± 6.47	0.34	8	0.74
EKG QTc interval (ms)	414.8 ± 19.2	406.8 ± 20.9	1.19	8	0.27

Paired *t*-tests conducted on serum analytes and EKG QTc interval at screening and week 8 in patients receiving pregnenolone.*
p < 0.05.

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Table 8

Side Effect Profiles in Patient Receiving Placebo and Pregnenolone

SYMPTOM	Placebo (<i>n</i> = 9 patients)	Pregnenolone (<i>n</i> = 9 patients)
	<i>n</i> (%) ⁱ	<i>n</i> (%)
Disorientation (date, address, mayor, MD name)	2 (22%)—mild (both patients)	2 (22%)—mild (both patients)
Decreased interest in sex	2 (22%)—mild (both patients)	1(11%)—mild
Impaired sexual performance	2 (22%)—mild (both patients)	0
Hypertension	1(11%)— mild < 145/90)	1(11%)—mild (< 145/90)
Excitation/agitation	1(11%)—mild	0
Dry mouth	1(11%)—mild	1(11%)
Malaise	1(11%)—mild	0
Blurred vision	1(11%)—mild	0
Restlessness	0	2 (22%)—mild (both patients)
Muscle pain/stiffness	0	1(11%)—mild
Cold extremities	0	1(11%)—mild
Tremor	0	0
Headache	0	0
Insomnia	0	0
Drowsiness	0	0
Rigidity	0	0
Akathisia	0	0
Diarrhea	0	0
Nasal congestion	0	0
Sweating	0	0
Joint pain/stiffness	0	0
Peripheral edema	0	0
All other side effects	0	0