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BRIEF REPORT

Brief Report: An Open-Label Study of the Neurosteroid Pregnenolone in Adults with Autism Spectrum Disorder

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Abstract The objective of this study was to assess the tolerability and efficacy of pregnenolone in reducing irritability in adults with autism spectrum disorder (ASD). This was a pilot, open-label, 12-week trial that included twelve subjects with a mean age of 22.5 ± 5.8 years. Two participants dropped out of the study due to reasons unrelated to adverse effects. Pregnenolone yielded a statistically significant improvement in the primary measure, Aberrant Behavior Checklist (ABC)-Irritability [from 17.4 ± 7.4 at baseline to 11.2 ± 7.0 at 12 weeks ($p = 0.028$)]. Secondary measures were not statistically significant with the exception of ABC-lethargy ($p = 0.046$) and total Short Sensory Profile score ($p = 0.009$). No significant vital sign changes occurred during this study. Pregnenolone was not associated with any severe side effects. Single episodes of tiredness, diarrhea and depressive affect that could be related to pregnenolone were reported. Overall, pregnenolone was modestly effective and well-tolerated in individuals with ASD.

Keywords Autism spectrum disorder · Pregnenolone · Neurosteroids · Irritability · Open-label trial

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Introduction

Autism spectrum disorder (ASD) is characterized by deficits in social communication and interactions, stereotypic behaviors, and restricted interests (APA 2013). In addition to these core symptoms, persons with ASD often suffer from irritability, which may manifest as tantrums, self-injury, and aggressive behaviors toward others. These symptoms often cause significant challenges to individuals with ASD and their families. Behavioral interventions are usually the first treatments for the associated behaviors, but when not effective and especially when the symptoms cause significant impairment in functioning, pharmacologic treatments for irritability may be considered. Currently, risperidone and aripiprazole are the only medications approved by the US Food and Drug Administration for the treatment of irritability in individuals with ASD. These atypical antipsychotic medications are effective in reducing irritability. However, adverse effects such as metabolic syndrome, extrapyramidal symptoms, and tardive dyskinesia can be devastating to the patients and their families. Therefore, effective medications with more tolerable side effect profiles will be more desirable.

One proposed model of ASD suggests that at least some forms of this disorder are a result of an altered ratio of excitation/inhibition (E/I) in key neural systems (Rubenstein and Merzenich 2003). Brain neurotransmission pathways based on gamma aminobutyric acid (GABA) are known to be inhibitory, while the pathways based on glutamate are known to be excitatory (Kandel et al. 2013). Mounting evidence from animal models and human studies support this hypothesis on E/I imbalance. A meta-analysis of transgenic mouse models of ASD revealed that the number of parvalbumin (PV)-positive GABAergic interneurons was reduced in the neocortex, suggesting that PV-

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circuit disruption may be relevant in the pathogenesis and pathophysiology of ASD (Gogolla et al. 2009). Further evidence of abnormalities of the GABAergic system in ASD was provided in postmortem and neuroimaging studies. Reduced expressions of specific subunits of $GABA_A$ as well as $GABA_B$ receptors were found in the postmortem brains of adults with ASD (Fatemi et al. 2010, 2014). Additionally, a recent preliminary positron emission tomography (PET) study showed lower densities of α1 and α5-containing $GABA_A$ receptors in the brains globally (but especially amygdala and nucleus accumbens) of high-functioning individuals with ASD when compared with healthy controls (Mendez et al. 2012). Collectively, modulation of the GABAergic pathways is predicted to change the E/I ratio and therefore may be considered as a strategy for treating irritability in individuals with ASD. In addition to the GABAergic pathways, emerging evidence has shown abnormalities of the glutamatergic pathways in ASD (Gai et al. 2012; O'Roak et al. 2011) as well. Given the alterations of the GABAergic and glutamatergic neurotransmission systems, we speculate that substances, which can modulate these systems may be relevant in the treatment of ASD.

Neurosteroids are steroids synthesized within the central nervous system as well as in other steroidogenic organs/tissues. In contrary with the classical actions through intracellular receptors, extensive evidence has established that neurosteroids can exert rapid, potent actions at the cell membrane via allosteric interactions with the $GABA_A$ receptor. Pregnenolone is a naturally occurring neurosteroid directly metabolized from cholesterol and the precursor of virtually all neurosteroids. This hormone has been used as a dietary supplement. When orally administered in humans, pregnenolone is converted to multiple metabolites. Among them, the most abundant metabolites are allopregnanolone and pregnenolone sulfate (Sripada et al. 2013). In contrast, cortisol level was shown to remain the same after administration of pregnenolone (Marx et al. 2009). Allopregnanolone has been shown to regulate $GABA_A$ receptors through two discrete transmembrane sites (Hosie et al. 2006) and positively modulate $GABA_A$ receptors (Majewska et al. 1986). The sulfated form of pregnenolone was recently found to stimulate the trafficking of N-methyl-D-aspartate (NMDA) receptors to neuronal surface (Kostakis et al. 2013). The pharmacologic actions of allopregnanolone and pregnenolone sulfate may potentially be relevant mechanisms of action in relieving symptoms in ASD.

Oral administration of pregnenolone was recently shown to be associated with enhanced activation of neurocircuits controlling emotion regulation (Sripada et al. 2013). Compared with placebo, allopregnanolone (as measured after taking pregnenolone) was associated in healthy adults with increased activity in the dorsal medial prefrontal cortex and enhanced connectivity between the amygdala and dorsal medial prefrontal cortex, an effect that was associated with reduced self-reported anxiety. Pregnenolone was shown to be superior over placebo treatments in at least three randomized, placebo-controlled, double-blind trials of psychiatric disorders (Marx et al. 2009; Osuji et al. 2010; Ritsner et al. 2010). In patients with co-morbid major depressive and substance disorders, pregnenolone reduced the Hamilton rating scale for depression (HRSD) scores in a post hoc analysis of completers (Osuji et al. 2010). Pregnenolone was also shown to improve negative symptoms in patients with schizophrenia (Marx et al. 2009). Finally, a low dose of pregnenolone given adjunctively to patients with schizophrenia and schizoaffective disorder ameliorated positive symptoms and resulted in improvement in attention and working memory (Ritsner et al. 2010). Pregnenolone was also found to be well tolerated by participants in all reported studies to date.

In light of the above-discussed evidence, we completed an open-label, 12-week trial of pregnenolone, a dietary supplement, in adults with ASD with the objectives of examining its effect on irritability and to assess its safety and tolerability in this population.

Methods

This was a 12-week, open-label study of pregnenolone in twelve adults with ASD conducted in the authors' home institution between November 2011 and September 2013. Informed consent was signed by the legal guardians and assent was obtained from participants when possible. Subjects were then screened and inclusion and exclusion criteria were assessed. No changes in eligibility criteria were applied throughout the study. This investigation was approved by the Institutional Review Board at the authors' home institution. An investigational new drug application (#109191) was filed with the Food and Drug Administration. The full trial protocol is available upon request.

Participants

Fifteen individuals signed consent forms for this study, but only 12 met inclusion/exclusion criteria and were included in this prospective study. Subjects with and without intellectual disability were enrolled. Inclusion criteria consisted of the following: (a) outpatients 18–45 years of age; (b) males and females who are physically healthy; (c) diagnosis of autistic disorder based on expert clinical evaluation and DSM-IV-TR criteria, and confirmed using the Autism Diagnostic Interview-Revised (Lord et al. 1994), and the Autism Diagnostic Observation Schedule

(Lord et al. 2000); (d) clinical global impression –severity (CGI-S) greater than or equal to 4; (e) care provider who could reliably bring subject to clinic visits, could provide trustworthy ratings, and interacted with subject on a regular basis; (f) ability of subject to swallow the compound; (g) stable concomitant medications for at least 2 weeks (4 weeks if patient took fluoxetine); and (h) no planned changes in psychosocial interventions during the open-label pregnenolone trial. Exclusion Criteria included the following: (a) DSM-IV-TR diagnosis of schizophrenia, schizoaffective disorder, or psychotic disorder NOS; (b) prior adequate trial of pregnenolone; (c) active medical problems such as unstable seizures, or significant physical illness (e.g., serious liver or renal pathology); (d) pregnancy or sexually active females (as determined by a urinary pregnancy test in the beginning of the study); and (e) subjects taking oil or fat-based nutritional supplements would be excluded from the study except they had been off these compounds for at least 4 weeks.

Pharmacological Intervention

Pregnenolone capsules were obtained from Pure Encapsulations (Sudbury, MA, USA). The capsules combined a 99 % purity pregnenolone powder with hypo-allergenic plant fiber (cellulose) in a vegetarian capsule (derived from cellulose and water). Pregnenolone was initiated at a dose of 50 mg twice daily for the first 2 weeks and was increased by 50 mg twice daily every 2 weeks until reaching the maximal dosage of 250 mg twice daily from weeks 9 to 12. If subjects could not tolerate a specific dose, s/he would be maintained at the highest tolerated dose. At the end of this initial phase, pregnenolone was decreased by 50 mg twice a day every 3 days until it was discontinued.

Assessments

Primary outcome measures included the ABC-I. Secondary measures included the other subscales of ABC, Short Sensory Profile (SSP), Social Responsiveness Scale (SRS), and Vineland Adaptive Behavior Scale (VABS). The ABC, SSP, SRS, VABS were administered at baseline, at the end of the 12-week treatment. Participants were also invited back for a follow-up visit 4 weeks after the end of the trial to monitor the effect of tapering and discontinuation of the medication. On an exploratory basis, ABC was also monitored at 2, 4, 6, 8 and 10 weeks. Vital signs and Dosage Record and Treatment Emergent Symptom Scale (DOTES) were monitored for adverse events in each visit. In addition, EKG and laboratory tests (complete metabolic panel, complete blood count with differential, cholesterol panel, and routine urinalysis) were performed at baseline and at the end of treatment phase at Week 12. Urine toxicology screen and pregnancy test (for female subjects) were performed at screening phase.

Briefly, the ABC is a standardized scale, comprising 58 items, for assessing problem behavior in subjects with intellectual disability and developmental disabilities (Aman et al. 1985). The checklist was empirically derived from ratings on approximately 1,000 subjects, and the items resolve into five subscales: irritability, lethargy/social withdrawal, stereotypic behavior, hyperactivity, and inappropriate speech. High ABC scores indicate more severe behavioral symptoms. The SSP is a 38-item parent report questionnaire that evaluates sensory abnormalities and compares to available normative data (Tomchek and Dunn 2007). The items in SSP are written such that low scores reflect undesirable and abnormal behaviors. The SRS is a 65-item parent report questionnaire designed to measure the severity of autism spectrum symptoms as they occur in natural social settings (Constantino et al. 2003). High scores in SRS indicate more severe behavioral symptoms. VABS is a 433-item parent report questionnaire designed to assess personal and social skills needed for everyday living (Sparrow et al. 2005). Low scores in VABS suggest lower adaptive abilities.

Statistical Analyses

Data analysis was conducted with SPSS 19 (SPSS Inc., Chicago, IL, USA). Paired, 2-tailed student t-tests were performed to compare primary and secondary outcome measures between baseline and at 12 weeks using last observation carried forward approach. Differences were considered significant with $p < 0.05$. Effect sizes (Cohen's d) for statistically significant findings were calculated by the following equation:

$$\text{Cohen's d} = (\text{Measure}_{\text{end}} - \text{Measure}_{\text{baseline}}) / \text{Measure}_{\text{stdev,pool}}$$

where $\text{Measure}_{\text{end}}$ is the mean of the outcome measure at the end of treatment; $\text{Measure}_{\text{baseline}}$ is the mean of the outcome measure at baseline; and $\text{Measure}_{\text{stdev,pool}}$ is the pooled standard deviation.

Additional exploratory analyses were performed on the primary outcome measure (ABC-I) and included repeated measures ANOVA in analyzing longitudinal data in the treatment phase and a paired, 2-tailed student t test comparing week 12 and the follow-up visit after pregnenolone was discontinued Table 1.

Results

Ten men and two women with ASD (mean age 22.5 ± 5.8 years; range 18.1–35.5 years; 9 Caucasians and

Table 1 Clinical measures changes with pregnenolone treatment

Clinical measures	Baseline		Week 12[a]		Paired t test		Effect size
	Mean	SD	Mean	SD	t	*p*	Cohen's d
Aberrant behavioral checklist							
ABC-Irritability	17.4	7.4	11.2	7.0	2.5	0.028*	−0.86
ABC-lethargy/social withdrawal	18.1	8.0	12.8	8.7	2.3	0.046*	−0.63
ABC-stereotypy	9.8	5.5	8.7	6.5	0.7	0.522	−0.18
ABC-hyperactivity	20.5	16.1	16.1	8.9	1.8	0.098	−0.34
ABC-inappropriate speech	5.8	4.3	4.8	4.4	1.0	0.356	−0.23
Short sensory profile—total score	137.7	21.5	147.6	15.3	−3.2	0.009*	0.53
Social responsiveness scale—total score	84.9	8.1	84.5	9.2	0.2	0.848	−0.05
Vineland[b]—adaptive behavior composite score	37.3	13.1	42.9	16.5	−1.3	0.224	0.38

[a] Week 12: end of the active treatment phase

[b] Vineland: Vineland adaptive behavior scales

* $p < 0.05$

3 Asians) met the study criteria for inclusion in this open-label study. One participant failed inclusion criteria due to incidental findings on the baseline EKG (right bundle branch block and possible right ventricular hypertrophy). All participants were taking psychotropic medications with the exception of one subject. Concomitant medications were maintained at the same dosage throughout the study and 8 participants were on second-generation anti-psychotics, 3 on selective serotonin reuptake inhibitors, 4 on mood stabilizers (including lithium, valproic acid, carbamazepine), 1 on buspirone, 1 on trazodone, 1 on diazepam, 1 on zolpidem, 2 on melatonin, and 1 on benadryl.

Treatment Response

Pregnenolone yielded a statistically significant improvement in the primary measure, ABC-I at 12 weeks [from 17.4 ± 7.4 at baseline to 11.2 ± 7.0 at 12 weeks ($p = 0.028$, $df = 11$, $t = 2.5$); $d = -0.86$]. Secondary measures were not statistically significant with the exception of ABC-lethargy/social withdrawal (ABC-L/SW; $p = 0.046$, $df = 11$, $t = 2.3$; $d = -0.64$) and total SSP score ($p = 0.009$, $df = 10$, $t = 3.2$; $d = 0.53$). Exploratory repeated measures analysis of the longitudinal ABC-I data revealed a trend toward statistical significance (Greenhouse-Geisser: $F = 2.17$; $p = 0.089$). No difference in the primary outcome measure (ABC-I) was observed between week 12 follow-up visit and 4 weeks after the end of the treatment phase (week 12: 11.9 ± 6.8; week 16: 12.6 ± 7.7; $t = -0.606$, $df = 10$, $p = 0.558$).

Safety Measures and Adverse Effects

During the 12-week treatment period, two participants dropped out of the study. The parents of one of the participants decided to withdraw from the study after 4 weeks (day 29) since patient was getting frustrated from taking too many pills twice a day. Another participant exhibited worsening of baseline behaviors during week 8, which led mother to withdraw him from the study at day 58. The decrease and discontinuation of pregnenolone did not lead to the amelioration of behaviors, which led to the adjustment of the baseline medications that he was taking.

Table 2 Vital signs at baseline and end of pregnenolone treatment at week 12

Clinical measures	Baseline N = 12		End of treatment N = 12		Paired t test	
	Mean	SD	Mean	SD	t	*p*
Systolic blood pressure (sitting)	119	15	121	11	−0.663	0.521
Diastolic blood pressure (sitting)	74	9	76	6	−0.672	0.515
Pulse (sitting)	85	13	83	14	0.5	0.627
Systolic blood pressure (standing)	117	14	120	11	−0.734	0.478
Diastolic blood pressure (standing)	75	7	77	6	−1.081	0.303
Pulse (standing)	92	11	89	17	0.887	0.394
Weight (pounds)	179	74	180	76	−0.223	0.828

Pregnenolone was not associated with any severe adverse effects. Single episodes of tiredness (n = 1), diarrhea (n = 1), and depressive affect (n = 1) that could possibly be related to pregnenolone were reported. A few other adverse events with remote chance to be related to the medication were reported: increased excitement/agitation (n = 3), sleep problems (n = 1), drowsiness (n = 1), anorexia/decreased appetite (n = 2), increased motor activity (n = 1), sweating (n = 1), constipation (n = 1), diarrhea (n = 1), tremor (n = 1), and depressive affect (n = 1). No significant vital sign or EKG changes occurred in any study participants (Table 2). No abnormal laboratory tests were caused by pregnenolone.

Discussion

This hypothesis-generating study represents the first attempt to provide preliminary support for the use of pregnenolone, a neurosteroid and an oral supplement, in the treatment irritability in individuals with ASD. Irritability is a non-specific mood state, which is postulated to be controlled by neural processes responsible for "top down inhibition" and "bottoms-up drive" (Siever 2008). Generally, the prefrontal cortex (PFC) and anterior cingulate cortex (ACC) are thought to be the neural substrate for "top down inhibition", whereas the amygdala and insula are often associated with "bottoms-up drive". When these regions are dysfunctional, it is suggested that affected individuals will exhibit irritability more severely and readily. In typically developing individuals, cognitive control was shown to be associated with optimal functional connectivity between dorsolateral PFC and parietal cortex; in contrast, in ASD, cognitive control was shown to be controlled by the ventrolateral PFC and ACC (Solomon et al. 2013). Compared to typically developing controls, participants with ASD were shown to have reduced functional connectivity between the insula and specific brain regions involved in emotional processing (e.g. amygdala) and sensory processing (e.g. somatosensory cortex) (Ebisch et al. 2011). Furthermore, emerging evidence has shown that alterations of the GABAergic system in ASD were also present in some of the same areas responsible for "top down inhibition" and "bottoms-up drive". For example, decreased $GABA_A$ receptors have been found in the ACC of postmortem brains of persons with ASD (Oblak et al. 2009). As mentioned, a recent pilot PET study showed lower densities of $\alpha 1$ and $\alpha 5$-containing $GABA_A$ receptors in the amygdala of high-functioning individuals with ASD when compared with healthy controls (Mendez et al. 2012). Given the above evidence, pregnenolone might exert its pharmacologic effects through its GABAergic metabolite allopregnanolone. Interestingly, a synthetic analog of allopregnanolone, ganaxolone, is currently being tested for treating children with fragile X syndrome, a syndromic form of ASD.

The effect size for pregnenolone at 12 weeks on ABC-I in adults with ASD in the current study (−0.86) is large, but is generally lower than the effect sizes for atypical antipsychotics in early open-label studies in ASD. For example, in an 8-week open-label study of paliperidone in 25 adolescents and young adults with ASD, the effect size for the medication on ABC-I was −2.2 (Stigler et al. 2012). The outcomes for open-label trials of olanzapine in children and adolescents with ASD were mixed [d = −2.1 (Fido and Al-Saad 2008) and −0.4 (Kemner et al. 2002) on ABC-I]. In one of risperidone's earliest investigations in the treatment of disruptive behaviors in children with ASD, 12-week treatment resulted in effect sizes of −1.0 and −0.8 in the items "Irritable" and "Aggression" in the Clinician-Rated Visual Analog Scales (McDougle et al. 1997). The effect size for aripiprazole at 14 weeks on ABC-I in children and adolescents with ASD was very large [−2.8 (Stigler et al. 2009)]. Overall, when comparing open-label studies, pregnenolone appears to be less potent than atypical antipsychotics in the treatment of irritability and associated behaviors in ASD.

Pregnenolone's main active metabolite, allopregnanolone, is a positive modulator of the $GABA_A$ receptor. As mentioned earlier, individuals with ASD were shown to have lower $GABA_A$ receptor density in the amygdala (Mendez et al. 2012). As social processes are at least partially moderated by the amygdala, increase in allopregnanolone levels in the brain after administration of pregnenolone can potentially increase the GABAergic tone in the amygdala. Interestingly, this hypothesis is consistent with the improvement in ABC-L/SW score at the end of pregnenolone treatment. However, the improvement in ABC-L/SW score was inconsistent with the unchanged SRS total score. This discrepancy is possibly related to the fact that the two rating scales assess different social domains, or simply, changes observed on the ABC-withdrawal are false positive. Hence this observation should be interpreted with caution.

Pregnenolone was overall well tolerated, with few side effects and no severe adverse effects reported in this study. Additionally, due to pregnenolone sulfate's known role in activating TRPM3 (Naylor et al. 2010), a subtype of transient receptor potential (TRP) channels expressed in the brain and kidneys, we monitored cardiovascular effects carefully by measuring vital signs in all subjects and EKGs were obtained at baseline and at the end of the trial. During this 12-week study, we did not find any significant changes in blood pressure, pulse, and body weight and no EKG changes were observed. These observations suggest that pregnenolone is relatively safe in adults with ASD. However, further studies are needed before final conclusions can be made.

Findings from this study should be cautiously interpreted in light of several limitations. First, this is an open-label trial with a very small sample size. Placebo response is commonly observed in open-label trials. Second, although pregnenolone is known to metabolize mainly to allopregnanolone and pregnenolone sulfate [and not other downstream metabolites such as cortisol; (Marx et al. 2009)], no measures of plasma or salivary concentrations of these metabolites were completed in this study. Third, although the ABC-I at 12 weeks was significantly lower than baseline value, our exploratory repeated measures ANOVA analyses did not reach statistical significance. The discrepancy is likely due to the small sample size. Fourth,

inclusion criteria did not include a cut-off for ABC-I. Finally, no changes in ABC-I were observed after pregnenolone was discontinued. This might suggest that improvement observed in the study might be unrelated to the study medication. It might also be related to a long-lasting effect of pregnenolone and a longer follow-up period might be necessary.

In conclusion, in this pilot study, pregnenolone, an oral supplement, was modestly effective and was overall safe and well tolerated in individuals with ASD. More importantly, this investigation has generated potentially important hypotheses for the treatment of ASD. It supports further studies in exploring pregnenolone's effects on reducing irritability, improving social functioning (a core symptom of ASD), and attenuating sensory abnormalities. As the currently FDA-approved medications for the treatment of irritability in ASD have significant serious adverse side effects, pregnenolone may be a potential alternative due to its milder side effect profile. Furthermore, social and sensory deficits are among the core features of ASD and no effective treatment is currently available. Therefore, randomized large trials of pregnenolone and its associated neurosteroids targeting irritability, abnormal social interactions, and/or sensory aberrations may potentially lead to novel treatments for these symptoms in ASD.

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Conflict of interest Over the last 3 years, Dr. A.Y. Hardan has received research support and honorarium for consulting from the following companies: Bristol-Myers Squibb, Roche, Forest, and IntegraGen. Drs. L. K. Fung, J. Phillips, F. Haddad and Ms. R. A. Libove did not report any conflict.

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