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Oxytocin treatment in children with Prader–Willi syndrome: A double-blind, placebo-controlled, crossover study

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Abstract

Prader–Willi syndrome (PWS) is a rare, complex multisystem genetic disorder which includes hypothalamic dysfunction, hyperphagia, cognitive and behavioral problems, increased anxiety, and compulsive behaviors. Individuals with PWS have a deficit of oxytocin producing neurons in the paraventricular nucleus of the hypothalamus. Oxytocin plays a role in regulation of feeding behaviors, social interactions, and emotional reactivity, which are all issues that significantly affect the quality of life for individuals with this syndrome. We performed a double-blind, placebo-controlled, crossover study in 24 children with PWS at three academic institutions using 5 days of intranasal oxytocin (IN-OT) or 5 days of intranasal placebo spray, followed by a 4 week washout period, and then patients returned for 5 days of treatment with the alternate source. Questionnaires, including the Aberrant Behavior Checklist, Social Responsiveness Scale, Repetitive Behavior Scale − Revised, and the Hyperphagia Questionnaire, as well as Clinical Global Impression scales were administered. Blood testing for sodium, potassium, and glucose levels on days 2, 4, and 6, and a 24 hr diet recall. All scales factor improvement from Day 3 to Day 6 favored oxytocin over placebo. No single factor showed a statistically significant difference ($P < 0.05$) between groups at Day 6. The drug effect appeared to be diminished at Day 14. There was no evidence of a difference between oxytocin and placebo in safety lab parameters, 60 min post dose vital signs, weight, or diet parameters. The results from this study suggest that low dose intranasal oxytocin is

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

safe for individuals with PWS and may result in reduction in appetite drive, and improvements in socialization, anxiety, and repetitive behaviors. Further, long-term studies with a larger population of participants are necessary to confirm these findings. The results of this study are encouraging that oxytocin may be a safe and effective treatment for many of the issues that negatively impact individuals with PWS.

Keywords

hyperphagia; oxytocin; Prader–Willi syndrome

1 | INTRODUCTION

Prader–Willi syndrome (PWS) is a rare, complex multisystem genetic disorder arising from the lack of expression of paternally inherited imprinted genes in the 15q11-q13 chromosomal region. The syndrome includes severe neonatal hypotonia with decreased appetite and impaired suck necessitating assisted feeding (nutritional phase 1a), followed by a period of improved appetite (nutritional phase 1b) (Miller et al., 2011). Weight gain without a change in appetite begins around age 2 years (nutritional phase 2a) (Goldstone, Holland, Butler, & Whittington, 2012; Miller et al., 2011). Then an increased interest and awareness of food, with increased anxiety and behavioral problems typically begins around age 4–5 years (nutritional phase 2b). This is followed by an insatiable appetite with worsening behavioral manifestations beginning around age 8 years (nutritional phase 3) (Goldstone et al., 2012; Miller et al., 2011). These appetite issues are combined with other endocrine problems probably due to hypothalamic dysfunction. The pathophysiological mechanism of the occurrence of the various nutritional phases of PWS is unknown.

Swaab, Purba, & Hofman, (1995) reported a deficit in the oxytocin (OT)-producing neurons of the paraventricular nucleus (PVN) in the brain individuals with PWS and Bittel and colleagues reported decreased oxytocin receptor (OTR) gene function in PWS (Bittel, Kibiryeva, Sell, Strong, & Butler, 2007). In addition with its well-known anorexigenic effect, OT is involved in establishing and maintaining social standards. In a PWS knock-out mouse model for the *Magel2* gene, there is a significant reduction in OT, and the mice with this mutation that live into adulthood have reduced interest in new partners, reduced novel object exploration, and alter special learning deficits (Dombret et al., 2012). In these mice, a single OT injection at 5 hr of life prevented early death observed in 50% of newborn mice by recovering a normal suck (Schaller et al., 1995), while daily oxytocin administration from birth to day 7 of life prevented abnormalities in social behavior and special learning deficits (Meziane et al., 2015). These findings suggest that cognitive and behavioral phenotypes seen in PWS can be rescued postnatally.

Central release of oxytocin and its closely related peptide, arginine vasopressin (AVP), are involved in aspects of social cognition and function including social recognition, social memory, affiliative behaviors, mother-infant and male-female pair-bond formation, separation distress, and other aspects of social attachment (Bachner-Melman and Ebstein, 2014). Oxytocin is also involved in regulating stress response and studied extensively

because of its unique role in influencing social and repetitive behaviors (Yee et al., 2016). There is potential to generate animal models with behavioral deficits that may relate to autism. In animal models, oxytocin has been shown to play a critical role in social processing, recognition, and bonding, and also to influence stereotyped behaviors such as exaggerated grooming (Yee et al., 2016). In mammals, OTRs are expressed at higher levels in early development. OT knockout mice have been shown to maintain olfaction and cognitive performance, but suffer deficits in social recognition that were recovered by intraventricular OT but not by AVP administration (Sala et al., 2011). Compared to wild-type, OTR knockout mice emit fewer ultrasonic vocalizations in response to social isolation, experience deficits in social discrimination, and demonstrate more aggressive behavior.

The oxytocin system appears to be dysfunctional in individuals with PWS. In adults with PWS, the number of oxytocin-expressing neurons in the hypothalamus was significantly decreased and plasma levels of oxytocin were relatively low in relation to their obesity (Bittel et al., 2007; Swaab et al., 1995). However, 23 children with PWS between 5 and 11 years of age were noted to have high plasma levels of oxytocin (Johnson, Manzardo, Miller, Driscoll, & Butler, 2016). As individuals with PWS have symptoms consistent with oxytocin deficiency, including poor suck and swallow as infants, hyperphagia in childhood/adulthood, anxiety, and repetitive behaviors, it is hypothesized that these high serum levels of oxytocin could be due to disruption of the oxytocin receptor responsivity or overproduction of non-biologically active form of oxytocin (Johnson et al., 2016). If this were the case, then supplementation with the active formulation of oxytocin could be beneficial, even in the face of elevated serum levels of oxytocin.

Several studies in humans have investigated the effects of intranasal oxytocin (IN-OT). IN-OT is absorbed through the highly permeable nasal mucosa, and shown to pass the blood-brain-barrier, with ease to self-administer. However, the published studies on the effects of IN-OT in PWS are contradictory. In a double blind placebo study of adults with PWS, a single dose of IN-OT significantly decreased depressive mood tendencies and tantrums while increasing trust in others, with supportive data to decrease appetite with higher satiety (Tauber et al., 2011). These results were recorded for 2 days following a single IN-OT dose administration. However, another study of 30 adolescents and adults with PWS with twice daily dosing of IN-OT found no improvements in behaviour, hyperphagia, or compulsivity, but an increase in temper outbursts with higher doses of oxytocin (Einfeld et al., 2014).

The need for treatment of hyperphagia and behavioral issues in children with PWS, along with the *Magel2* knock-out mice data, and the general safety of IN-OT administration in humans, prompted us to evaluate the tolerance and effects of IN-OT in children with PWS ages 5–11 years who were in nutritional phases 2b or 3 (increased appetite and interest in food).

2 | MATERIALS AND METHODS

This study was a two period two treatment crossover study with 24 patients randomized equally to receive either IN-OT followed by placebo or placebo followed by IN-OT. A minimum 4 week washout period was required between periods.

Initial studies of an intervention in a new population, especially young children, are always small in sample size. The goal in these studies is not to demonstrate efficacy, but rather to ensure there are no dangerous safety issues and also to potentially test out various instruments and procedures for larger studies (Lancaster, 2015).

The sample size of this study was based on a prior specified 0.33 difference in an adverse event incidence difference between IN-OT and placebo. It was not anticipated that efficacy measures would show statistical significance, therefore, the emphasis for efficacy was on the observed 95% confidence intervals between IN-OT and placebo administration (Lancaster, Dodd, & Williamson, 2004).

The study was performed under the auspices of the NIH-funded Rare Disease Center Network at three of the sites designated for study of PWS: University of Florida, Kansas University Medical Center, and University of California, Irvine. Determination of nutritional phase was done by the PI at each site based on the nutritional phases described by this consortium in 2011 (Miller et al., 2011). Validated questionnaires were used in the study to evaluate the effects of IN-OT on behavior, socialization, and anxiety, including: Aberrant Behavior Checklist (ABC); Repetitive Behavior Scale-Revised (RBS-R) (Lam and Aman, 2007); and Social Responsiveness Scale (SRS) (Constantino et al., 2003). Additionally, the Hyperphagia Questionnaire (HQ) (Dykens, Maxwell, Pantino, Kossler, & Roof, 2007) was administered to determine the effects on appetite in PWS. All questionnaires were modified to reflect that the respondent was answering the questions for the past 24 hr only, as they were used repeatedly during the course of the study. A standardized Clinical Global Impression (CGI) scale was given daily in person by the study coordinator during the study, as well as by phone on Day 14. Assessment of the Clinical Global Improvement (CGI) was based on the results from Day 7 (immediately after completing treatment with IN-OT or placebo) and Day 14 (after they had been off treatment for 1 week).

A 24 hr dietary recall was obtained from parents on Days 2, 4, and 6 during the study and was analyzed by a registered dietitian using the Nutrition Data System for Research (NDSR) software versions 2013–2014. NDSR is a program which was developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN. Final calculations were completed using NDSR version 2014. The NDSR time-related database updates analytic data while maintaining nutrient profiles true to the version used for data collection. NDSR provides a complete nutrient profile for all foods in the database. If an analytic value is not available for a nutrient in a food, NCC calculates the value based on the nutrient content of other nutrients in the same food or on a product ingredient list, or estimates the value based on the nutrient content of similar foods. A missing value is allowed only if 1) the value is believed to be negligible; 2) the food is usually eaten in very small amounts; 3) it is unknown if the nutrient exists in the food at all; or 4) there is no way to estimate the value because the food is unlike any other. The NDSR software utilizes the multiple-pass system of the interview methodology. The primary caregiver for the child typically provided the report, but review of portion sizes and food types was done with the parents to ascertain, to the extent possible, that the information we received was accurate and complete.

Participants came in within 7 days prior to participating in the study for screening blood work and determination of nutritional phase. They were seen in the outpatient Clinical Research Center at each institution for all of the evaluations. All measurements were done at the same time each morning by the same nurse on the same equipment and all questionnaires were administered to the parents in person by the study coordinator for the site. On Day 1 of the study they came in for administration of questionnaires and physical examination. Participants were given either 16 IU of IN-OT (8 IU per nostril) or 16 IU of placebo from Days 2–6 of the study. Vital signs were monitored every 15 min for 1 hr following administration of the medication/placebo, daily weights were obtained, nasal, and oropharynx examination was done by the site PI, and review of adverse events was performed daily. Questionnaires were administered on Days 1, 4, 6 while the participants were being treated, as well as on Day 14 (by phone by the site PI). Blood work for various hormone analyses, as well as sodium, potassium, and glucose levels was monitored on Days 2, 4, and 6 of each visit. Parents provided a 24 hr diet history for the children on Days 2, 4, and 6.

Scale factors were analyzed using a mixed model repeated measures analysis with improvement from Day 1 to Day 6 as the dependent variable and treatment and period as the dependent factors. A restricted maximum likelihood procedure was used to estimate the parameters in the model. Ninety-five percent confidence intervals were constructed comparing improvement on IN-OT versus placebo using the inversion of the Wald test approach. All analyses were conducted with the use of SAS PROC MIXED Version 9.2.

Non-fasting blood was collected from each study participant into 6.0 ml K_2EDTA BD Vacutainer Plus Blood Collection tubes containing either 100 μl of AEBSF (120 mg/ml in water) for the assessment of acylated and un-acylated ghrelin isoforms, or 100 μl of aprotinin (11,063 KIU/ml) for the assessment of cortisol, insulin, leptin, orexin-A, and oxytocin. The samples were then centrifuged within 30 min of collection at 3,000*g* for 15 min at +4°C. Plasma samples were aliquoted into 1 ml cryo-tubes and stored at −80°C until use.

Cortisol was assayed using an ELISA kit purchased from Abcam, MA. Insulin and leptin were assayed using ELISA kits obtained from EMD Millipore, MA. Concentrations of orexin-A and oxytocin were measured using fluorescent ELISA kits obtained from Phoenix Pharmaceuticals, Inc., CA. Plasma samples were diluted 1:2 in assay buffer for both orexin-A and oxytocin ELISAs. Acylated ghrelin and unacylated ghrelin were measured using ELISA kits purchased from Cayman, MI. Plasma samples were diluted 1:2 and 1:5 in assay buffer for acylated ghrelin and unacylated ghrelin, respectively. On all assays, samples were assayed in duplicates and procedures performed as per instructions of the manufacturer. The ELISA data were normalized for inter-assay variability to three internal controls that were included with every assay performed.

3 | RESULTS

Twenty four individuals with PWS, ages 5–11 years, participated in this study. Recruitment was rapid with all subjects enrolled within a 6 month time frame. There were no dropouts or

withdrawals. There were nine females/15 males. Appropriate genetic testing was used to classify individuals with PWS into the appropriate molecular class—paternal deletion, maternal uniparental disomy (UPD), or imprinting defect (ID) (Buiting et al., 2014; Driscoll et al., 2016). There were 18 participants with deletion, 5 with UPD, and 1 with ID (Table 1).

There was no evidence of a difference between oxytocin and placebo in safety lab parameters measured in 60 min post dose vital signs, or in weight. Pre- and post-dose blood pressure, heart rate, temperature, and respiratory rate were not significantly different, nor were they different in those treated with oxytocin versus placebo. The weight of the participants remained stable over the time of evaluation and did not differ between treatment groups ($P = 0.396$). Sodium levels, blood glucose levels, and insulin levels were all within normal range prior to treatment, and were not significantly different on follow-up measurement on Days 2, 4, and 6. IN-OT had an excellent safety profile in this study with few adverse events reported (Table 2). The most frequent adverse event with oxytocin versus placebo was nasal irritation ($P = 0.125$). The only other adverse event noted was irritability ($n = 2$) and increased irritability ($n = 2$) on IN-OT, each only occurring 1 day during the study and self-resolved.

Baseline scores and ranges on the questionnaires did not differ between participants at either week of the study. Figure 1 shows a Forest plot of 95% confidence interval for each scale factor. The 17 endpoints were all a priori defined. While there is heterogeneity in variability in the different endpoints, as indicated by the different lengths of the confidence intervals, all are centered in favor of IN-OT at Day 6. All confidence intervals overlap with zero. The same 17 endpoints at Day 14 are shown in Figure 2. Generally, the confidence intervals appear to be shifted toward zero with two endpoints, hyperphagia behaviors, and the social responsiveness total score now favoring placebo. Shifts toward zero are consistent with the diminishment of study drug effect, as would be expected since they were off of the study drug for one week at the time of evaluation.

The most clinically relevant effect noted by parents with IN-OT compared to placebo was a reduction in anxiety for the participants in nutritional phases 2b and 3. This manifested as decreased self-injurious behavior (e.g., skin picking, nail biting), as noted on the RBS-R. Additionally, there were positive trends on all of the measures assessed by the RBS-R with IN-OT over placebo, including: decreased compulsive behaviors, decreased ritualistic behavior/insistence on sameness, decreased stereotypic behavior, and decreased restricted interests. Note that the RBS-R result in Figure 1, which have the left endpoint closest to zero, indicating the most significant change from baseline with IN-OT over placebo.

Evaluation of the ABC also revealed improvements with IN-OT over placebo as decreased lethargy and decreased inappropriate speech (repetitive questioning). Additionally, there were improvements in every subscale measured by the ABC with IN-OT versus placebo, including decreased irritability, decreased stereotypical behaviors, and decreased hyperactivity/non-compliant behaviors.

IN-OT also had positive effects on hyperphagia in individuals with PWS who were in nutritional phases 2b and 3. By Day 6 of oxytocin treatment there was a 2.5 point decrease

in the HQ total score, as compared to a 1.6 point decrease in those on placebo. Food-related behaviors and appetite drive showed a trend of decreasing on IN-OT as compared to placebo. From the NDSR data analysis, there was a trend toward less consumption of carbohydrates while on IN-OT, with an average reduction of 12 g per day from Day 1 to Day 6, compared to an increase of 18 g per day of carbohydrate intake from Day 1 to Day 6 while on placebo.

On Day 7, the CGI, as rated by the parents, had improved for IN-OT compared to placebo. The CGI assessed parental impression of global improvement in symptoms, severity of symptoms, and efficacy of treatment. Many parents specifically noted an improvement in skin-picking and other self-injurious behaviors when the children were on IN-OT versus placebo. Interestingly, although the treatment effect had diminished by Day 14 after IN-OT, the parents continued to report significant improvement in symptoms compared to baseline. This finding suggests that, as was reported by Tauber et al. (2011) that some effects of IN-OT may last longer in individuals with PWS compared to the general population.

4 | DISCUSSION

This is the first reported trial of IN-OT in young children with PWS. IN-OT was found to be safe for these children over a 5 days study period, with no evidence of changes in vital signs, laboratory testing, or weight. No serious adverse events were associated with IN-OT.

At Day 6, we found that the change from baseline for all 17 behavioral, socialization, anxiety, and appetite endpoints was numerically superior while on IN-OT as compared to placebo. The most clinically significant improvement noted by parents was a decrease in overall anxiety when children were on IN-OT. Similar to what was seen in the Tauber study in adults with PWS (Tauber et al., 2011), we noted improvement in self-injurious behaviour with IN-OT compared to placebo, which could be a manifestation of decreased anxiety.

It is natural to ask whether the changes we saw from baseline with IN-OT compared to placebo could be due to chance. This is difficult to answer precisely because the 17 endpoints are not independent—many measure similar underlying behavior, therefore there is certainly some correlation between endpoints. If we disregard the magnitude of the superiority of oxytocin over placebo, and if the two groups are equivalent on all endpoints, then this is sign test situation with 17 potentially correlated endpoints. When six independent endpoints all indicate superiority or one group over another group, then the sign test is statistically significant at 0.05 Level. Therefore, for the results of this trial to not be considered statistically significant, one would have to believe that the 17 endpoints in this study have less information than six independent endpoints. Whether this is true can be debated however clearly, the results of this trial show a trend toward significance and therefore provide support for a larger study (McGrath, 2013).

Individuals with PWS have dysfunctional behavioural patterns which inhibit normal socialization. They have increased anxiety, stereotypic behaviours, and temper tantrums, which can cause them to have poor relationships with their peers (Tauber et al., 2011). They also can have difficulties understanding social situations and interpreting social cues (Bull,

Oliver, Callaghan, & Woodcock, 2015; Rice and Einfeld, 2015). Oxytocin nasal spray can enhance emotional recognition processing and also may decrease stereotypic, repetitive behaviours. However, to date there are two published studies evaluating the effects of IN-OT in individuals with PWS—one which showed positive results with a single IN-OT administration (Tauber et al., 2011), and one which showed no benefits in terms of food-related or repetitive behaviours and increased temper outbursts with an 8 week course of IN-OT (Einfeld et al., 2014).

Given the positive trend on all variables in our study with IN-OT administration, it is possible that the previously published negative results of IN-OT in adults and adolescents with PWS were simply due to an overdose of the hormone (Einfeld et al., 2014). In animal models there is considerable evidence that oxytocin can bind to and affect receptors for the closely related peptide, arginine vasopressin (AVP); particularly the V1A receptor subtype (Koenig, Klin, & Schultz, 2004). While OT exposure is associated with positive sociality and reduced anxiety, AVP is anxiogenic (Francis et al., 2014; Insel, 2010; Manning et al., 2012). At higher levels. OT and AVP are partial agonists for their homologous receptors (Wigger et al., 2004). Thus, the high doses of IN-OT used in the Einfeld study could have saturated the OT receptors and bound to the AVP receptors, thus resulting in increased emotional responses and anxiety, which manifested as increased temper tantrums. As individuals with PWS may have reduced oxytocin (Bittel et al., 2007) even a minimal overdose of IN-OT could presumably result in increased binding of the oxytocin to the AVP receptors, which could create negative/unwanted behaviors.

However, despite positive effects of IN-OT in short-term studies such as this one, there is concern that longer-term administration of oxytocin could result in lack of positive effects. In mice, long term administration of oxytocin results in reduced oxytocin receptors throughout the brain (Huang et al., 2014). Long-term studies of oxytocin in individuals with autism have had discrepant results, with some reporting no improvement in symptoms with long-term administration of oxytocin, while others demonstrate positive responses to long-term administration of IN-OT (Okamoto, Ishitobi, Wada, & Kosaka, 2016). It is plausible that the longer-term administration of IN-OT in the Einfeld study could have downregulated to oxytocin receptors. It is known that exposure to oxytocin leads to desensitization of the oxytocin receptor in order to protect cells from overstimulation (Conti, Sertic, Reversi, & Chini, 2009). This desensitization can occur very rapidly, within seconds or minutes, and is observed in the majority of G protein-coupled receptors, including the oxytocin receptor (Passoni, Leonzino, Gigliucci, Chini, & Busnelli, 2016). However, studies have shown that these receptors are recycled back to the cell surface once oxytocin levels have decreased (Passoni et al., 2016). Therefore, it is possible that longer-term, daily dosing could cause the oxytocin receptor to be internalized into the cells and not be able to be recycled to the cell surface. Therefore, it is critical that both longer-term and dose-finding studies be explored in this vulnerable population before clinicians prescribe this medication. An additional concern about longer-term use of oxytocin, is that the oxytocin receptor expression is susceptible to epigenetic changes, as it is modulated by DNA methylation of its promoter (Harony-Nicolas et al., 2014). Therefore, there is concern that chronic administration of exogenous oxytocin may result in transcriptional silencing of the oxytocin receptor through epigenetic mechanisms.

While we did not find any significant correlations between anorexogenic (orexin-A, leptin, oxytocin) or orexogenic (ghrelin) hormones with IN-OT in this short-term study, the lack of differences could be due to the short time frame in the study or to the methods of measurement used. Future studies of IN-OT in PWS will need to include larger sample sizes, longer duration of treatment with dose-optimization protocols, and determination of the bioavailability of the intranasal oxytocin used in this study. Evaluations by teachers, therapists, or physicians, in addition to just parental questionnaires would also be beneficial in future studies. However, the positive results in this short-term study of children with PWS across all of the domains measured, are promising that IN-OT may offer some relief of core PWS symptoms for individuals with this rare genetic obesity-related disorder. However, longer, dose-finding studies are necessary.

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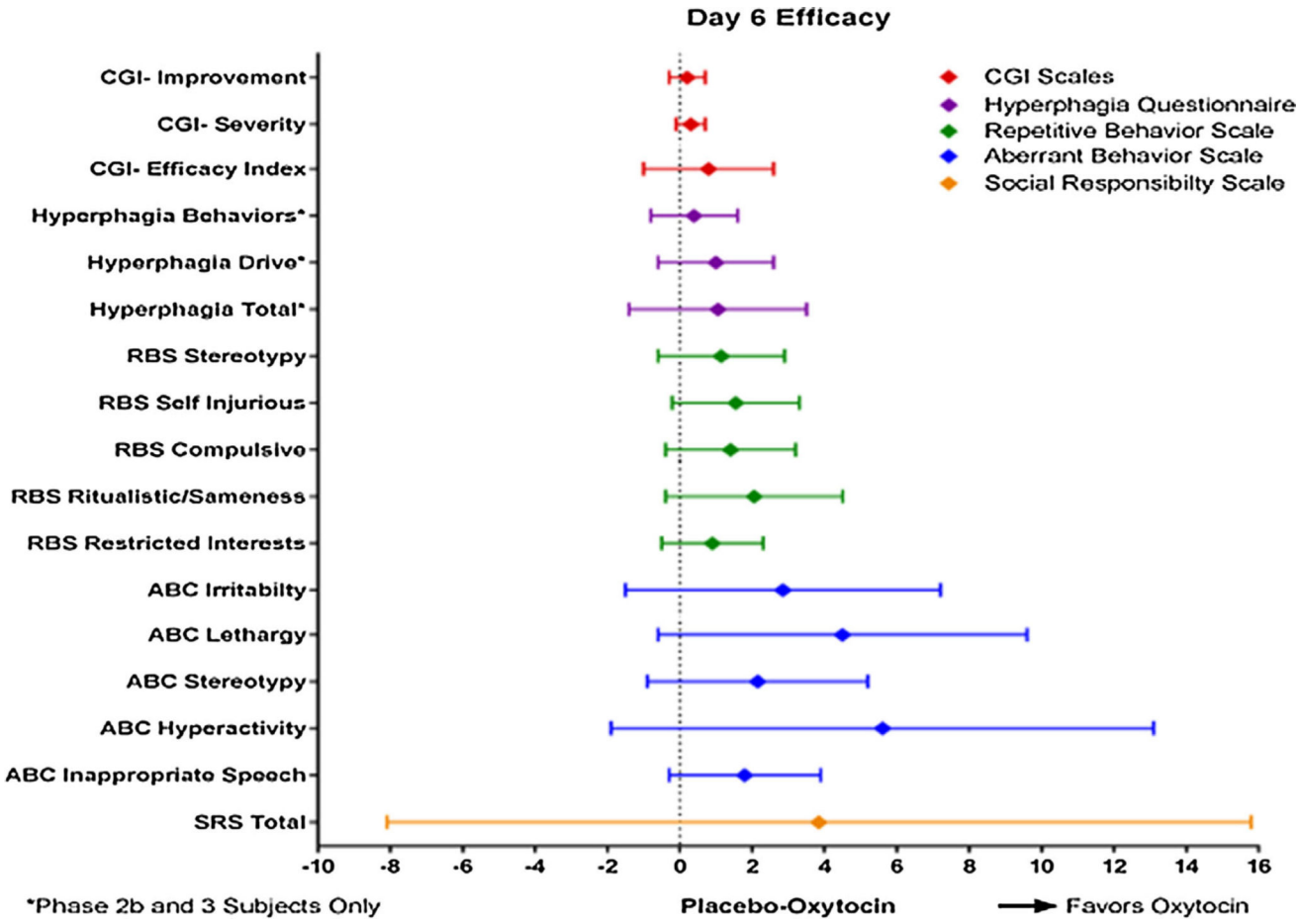

FIGURE 1.
Efficacy of IN-OT at Day 6 [Color figure can be viewed at wileyonlinelibrary.com]

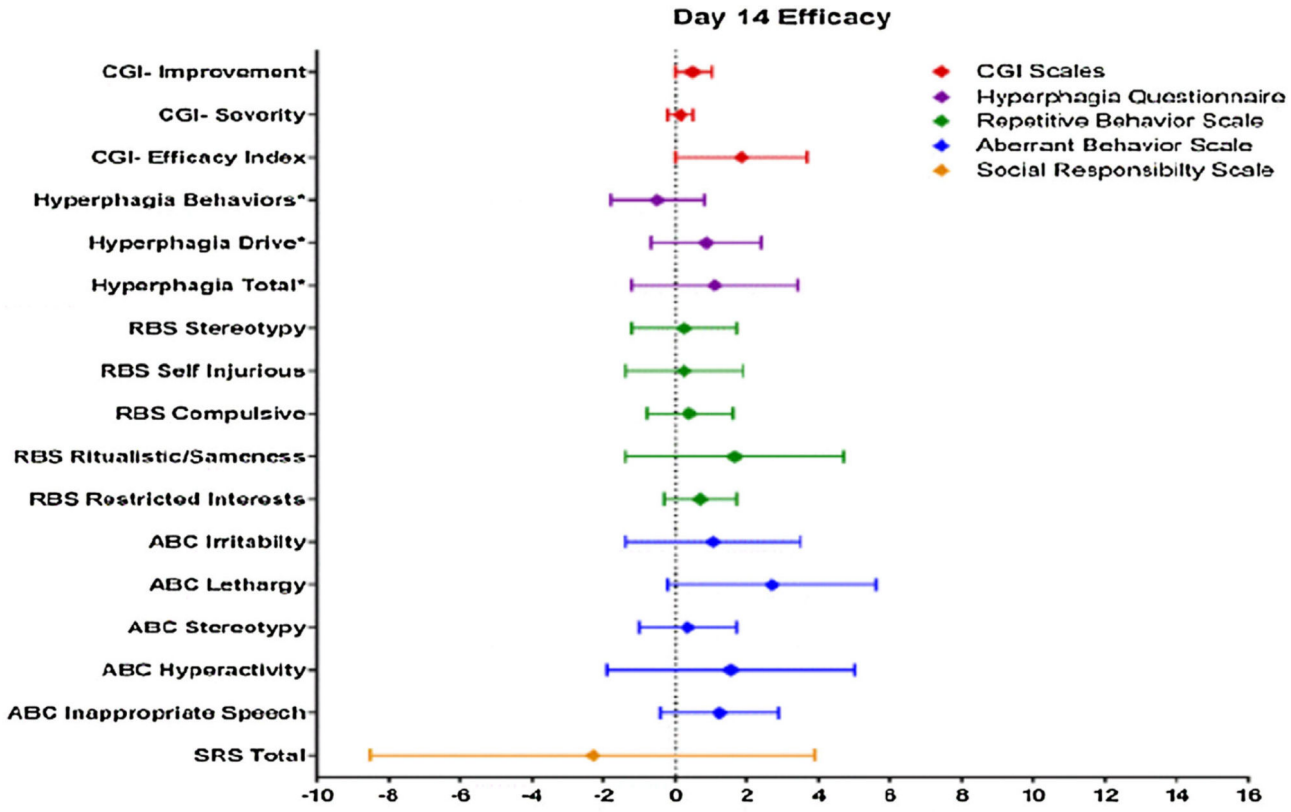

FIGURE 2.

Efficacy of IN-OT at Day 14 (off drug/placebo for 7 days) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1

Summary of baseline demographics

	Placebo-oxytocin, *N* = 12	**Oxytocin-placebo, *N* =12**	**Overall, *N* = 24**
Gender			
Male	4	8	9 (38%)
Female	5	7	15 (62%)
Ethnicity			
Hispanic	0	1	1 (4%)
Non-Hispanic	12	11	23 (96%)
Race			
Asian	0	1	1 (4%)
African–American	0	1	1 (4%)
Caucasian	12	10	22 (92%)
Nutritional phase			
2a	0	1	1 (4%)
2b	8	6	14 (58%)
3	4	5	9 (38%)
Age			
Mean	8.4	8.1	8.2
SD	2.1	1.4	1.7
Min	5.9	5.5	5.5
Max	11.8	10.2	11.8
PWS type			
Deletion	8	10	18
Uniparental disomy	4	1	5
Imprinting defect	0	1	1

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TABLE 2

Adverse events with IN-OT

		Attribution#									
Systems affected (CTCAE 4.0)		**Total**		**Serious-related**		**Serious-unrelated**		**Non-serious-related**		**Non-serious-unrelated**	
Category	**AE term**	**Number of subjects out of 24 eligible**	**Number of adverse events**	**Number of subjects**	**Number of adverse events**	**Number of subjects**	**Number of adverse events**	**Number of subjects**	**Number of adverse events**	**Number of subjects**	**Number of adverse events**
General disorders and administration site conditions	Fatigue	2	4	0	0	0	0	2	4	0	0
	General disorders and administration site conditions –nasal irritation McNemar Test *P*-value = 0.125	10	26	0	0	0	0	10	25	1	1
	Irritability	2	2	0	0	0	0	2	2	0	0
Skin and subcutaneous tissue disorders	Pain of skin	1	1	0	0	0	0	0	0	1	1
	Rash maculo-papular	1	2	0	0	0	0	1	2	0	0
			Not serious				**Serious**				**Total**
Total number of adverse events			35				0				35
Attribution#											
Probably not related			2				0				2
Possibly related			33				0				33
Outcome											
Recovered/resolved without sequelae			35				0				35