Intranasal oxytocin treatment for social deficits and biomarkers of response in children with autism


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Autism spectrum disorder (ASD) is characterized by core social deficits. Prognosis is poor, in part, because existing medications target only associated ASD features. Emerging evidence suggests that the neuropeptide oxytocin (OXT) may be a blood-based biomarker of social functioning and a possible treatment for ASD. However, prior OXT treatment trials have produced equivocal results, perhaps because of variability in patients’ underlying neuropeptide biology, but this hypothesis has not been tested. Using a double-blind, randomized, placebo-controlled, parallel design, we tested the efficacy and tolerability of 4-wk intranasal OXT treatment (24 International Units, twice daily) in 32 children with ASD, aged 6–12 y. When pretreatment neuropeptide measures were included in the statistical model, OXT compared with placebo treatment significantly enhanced social abilities in children with ASD [as measured by the trial’s primary outcome measure, the Social Responsiveness Scale (SRS)]. Importantly, pretreatment blood OXT concentrations also predicted treatment response, such that individuals with the lowest pretreatment OXT concentrations showed the greatest social improvement. OXT was well tolerated, and its effects were specific to social functioning, with no observed decrease in repetitive behaviors or anxiety. Finally, as with many trials, some placebo-treated participants showed improvement on the SRS. This enhanced social functioning was mirrored by a posttreatment increase in their blood OXT concentrations, suggesting that increased endogenous OXT secretion may underlie this improvement. These findings indicate that OXT treatment enhances social abilities in children with ASD and that individuals with pretreatment OXT signaling deficits may stand to benefit the most from OXT treatment.

Significance

Autism spectrum disorder (ASD) is characterized by social deficits. Emerging evidence suggests that the neuropeptide oxytocin, which regulates mammalian social functioning, may be a promising treatment for ASD. However, prior oxytocin treatment trials in ASD patients have produced equivocal results, perhaps because of variability in patients’ underlying neuropeptide biology. Here we provide evidence that oxytocin treatment improves social abilities in children with ASD and that individuals with the lowest pretreatment blood oxytocin concentrations benefit the most from oxytocin administration. These findings reveal a personalized component to oxytocin treatment which may have important implications for accurately testing oxytocin’s therapeutic potential, both for ASD and for a broad range of developmental and psychiatric disorders in which patients exhibit social impairments.


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variability in responses to OXT (18, 19, 21), highlighting the need to identify specific factors that contribute to OXT efficacy.

Because ASD is an extremely heterogeneous disorder, one possible explanation for these ambiguous outcomes in OXT treatment trials is that individual differences in endogenous neuropeptide biology may influence the response to OXT treatment. Specifically, individuals with ASD who have pronounced OXT signaling deficits may benefit the most from OXT treatment. Studies have shown that some (18, 25, 26) but not all (27–29) individuals with ASD have lower plasma OXT concentrations than controls. Plasma OXT concentrations also positively predict social functioning in children with ASD, such that children with the lowest plasma OXT concentrations show the greatest social deficits (27). However, no prior trial has tested whether individual differences in pretreatment neuropeptide biology contribute to how and to what extent individuals with ASD respond to OXT treatment, nor has any prior trial directly tested whether a statistical model’s explanatory power is improved by the inclusion of such biomarkers. The present study was designed to address these critical gaps in knowledge. Using a double-blind, randomized, placebo-controlled, parallel design, we investigated whether 4-wk intranasal OXT treatment improves social abilities in children with ASD (as measured by the trial’s primary outcome measure, the Social Responsiveness Scale (SRS) Total Raw Score) and whether pretreatment blood measures of neuropeptide biology (i.e., OXT concentrations and OXTR and V1AR gene expression) predict the response to OXT treatment. As secondary outcome measures, we also tested whether OXT ameliorates other core (i.e., repetitive behaviors) or associated (i.e., anxiety) symptoms of ASD and whether OXT is well tolerated in children with ASD, because limited safety and tolerability data are available in pediatric populations.

**Results**

**Participants.** Thirty-two children with ASD (27 male, 5 female), aged 6–12 y, completed this clinical trial (see the CONSORT diagram, Fig. 1). Participant demographic characteristics are presented in Table 1. Participants’ stable concomitant medications, which did not differ by treatment condition, are presented in Table S1.

**Evaluation of Biomarker Inclusion in the Statistical Model.** To test our a priori hypothesis that endogenous pretreatment neuropeptide measures would enhance our ability to detect OXT treatment effects, we first directly compared the $R^2$ of two statistical models for our primary outcome measure (i.e., the SRS Total Raw Score). Failure to include the biomarker measures in our model resulted in an $R^2$ of only 51%, with OXT-treated individuals showing only marginal (and nonsignificant) improvement in social abilities compared with placebo-treated individuals ($F_{1,27} = 3.716; P = 0.0645$). However, when the biomarker measures were included in the model (see Results below), the $R^2$ increased significantly, from 51 to 73% ($F_{6,21} = 3.131; P = 0.0237$), corresponding to an improvement of 33.89 points in the small-sample-size–corrected version of the Akaike information criterion (AICc). Thus, the inclusion of patients’ endogenous pretreatment neuropeptide measures increased the explained variation in SRS improvement significantly, by 43%. Consequently, the biomarker measures were included in all subsequent analyses detailed below.

**Effects of OXT Treatment on Social Abilities.** OXT-treated individuals showed greater improvement in social abilities (as measured by the SRS Total Raw Score) than placebo-treated individuals following completion of the 4-wk trial ($F_{1,21} = 5.6083; P = 0.0275$) (Fig. 2A). As illustrated in Fig. 2B, no OXT-treated
individual’s social abilities worsened, but 6/16 (37.5%) of placebo-treated individuals’ did so. Pretreatment OXT concentration also negatively predicted the magnitude of the improvement in the SRS Total Raw Score ($F_{1,21} = 11.4577; P = 0.0028$) (Fig. 2B), such that individuals with the lowest pretreatment OXT concentrations improved the most following 4-wk treatment (Fig. 2B). No effect of pretreatment neuropeptide receptor gene expression on improvement in social abilities (or any other measures) was discerned, but inclusion of this measure in the model did increase explanatory power to the analyses.

Unexpectedly, pretreatment blood OXT concentration did not differ in the prediction of treatment outcomes between OXT- and placebo-treated individuals ($F_{1,19} = 0.5980; P = 0.4488$). In other words, the pretreatment blood OXT concentration predicted treatment efficacy in both groups. To ensure that this finding was not an artifact of insuficient power to detect an interaction, we performed a follow-up analysis. We hypothesized that the observed social improvement in some placebo-treated participants might coincide with increased endogenous OXT secretion. If so, the change in pretreatment to posttreatment endogenous plasma OXT concentration should predict the magnitude of the placebo response, but no such relationship should exist in OXT-treated participants (whose measured OXT concentrations would comprise both endogenous and also variable quantities of exogenous OXT). Accordingly, we tested whether the posttreatment increase in plasma OXT concentration predicted greater improvement in the SRS Total Raw Score. As predicted, we observed a treatment condition × OXT concentration interaction ($F_{1,18} = 6.0333; P = 0.0244$) (Fig. 2C), whereby larger increases in posttreatment plasma OXT concentrations predicted larger SRS improvement in placebo-treated ($F_{1,18} = 16.5280; P = 0.0007$) but not in OXT-treated ($F_{1,18} = 0.3067; P = 0.5865$), individuals.

Effects of OXT Treatment on Other Core and Associated Symptom Measures. OXT’s effects were specific to social functioning, because OXT compared with placebo treatment did not significantly reduce repetitive behaviors [as measured by the Repetitive Behaviors Scale-Revised (RBS-R) Total Score ($F_{1,20} = 0.0010; P = 0.9757$) (Fig. S1A) or any of the RBS-R subscales], nor did pretreatment plasma OXT concentrations predict posttreatment RBS-R Total Score either as an interaction with treatment condition ($F_{1,20} = 0.0168; P = 0.8983$) or as a main effect ($F_{1,20} = 0.0359; P = 0.8517$). Additionally, no relationships were discerned between pretreatment plasma OXT concentrations and any RBS-R subscales. Similarly, OXT compared with placebo treatment did not reduce anxiety symptoms (as measured by the Spence Children’s Anxiety Scale Total Score, hereafter “Spence”) ($F_{1,18} = 0.9626; P = 0.3395$) (Fig S1B), nor did pretreatment plasma OXT concentrations predict posttreatment Spence Total Score either as an interaction with treatment condition ($F_{1,18} = 1.5400; P = 0.2325$), or as a main effect ($F_{1,18} = 0.4176; P = 0.5263$).

### Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$N$</th>
<th>Female</th>
<th>Male</th>
<th>Caucasian</th>
<th>Asian</th>
<th>Other</th>
<th>Age, y</th>
<th>Full-scale IQ</th>
<th>Pretreatment SRS Total Raw Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytocin</td>
<td>14</td>
<td>1</td>
<td>13</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>9.35 ± 2.34</td>
<td>65.21 ± 28.91</td>
<td>106.61 ± 30.65</td>
</tr>
<tr>
<td>Placebo</td>
<td>18</td>
<td>4</td>
<td>14</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>8.13 ± 1.87</td>
<td>67.39 ± 26.43</td>
<td>106.33 ± 25.00</td>
</tr>
</tbody>
</table>

$\chi^2$ was used to test whether the distribution of individuals randomized to the treatment conditions differed by sex and by ethnicity; no significant effects were found. For age, IQ, and pretreatment SRS Total Raw Score, differences between groups were tested with a simple one-way general linear model; no significant effects were discerned. The values are reported as mean ± SD.

![Fig. 2](image-url)  

**Fig. 2.** OXT treatment efficacy and biomarkers of social improvement. (A) OXT compared with placebo treatment enhances social abilities in children with ASD as measured by improvement in the trial’s primary outcome measure, the SRS. Data are presented as least squares means ± SE. (B) The pretreatment blood OXT concentration robustly predicts treatment response, such that individuals with the lowest pretreatment OXT concentrations show the greatest SRS Total Raw Score improvement. (C) Study placebo responders are identifiable by a robust posttreatment increase in blood OXT concentrations that accompany their SRS Total Raw Score improvement. Data are corrected for the blocking factors in the analysis. Placebo-treated children are depicted in blue; OXT-treated children are depicted in orange.
Effects of OXT Treatment on Safety and Tolerability Measures. OXT treatment in children with ASD was well tolerated with minimal side effects. There were no significant differences in the adverse event rates reported in the OXT-treated and the placebo-treated groups as assessed by parent ratings on the Dosage Record Treatment Emergent Symptom Scale (DOTES) (Table S2). Two individuals randomized to OXT treatment did not complete 4-wk assessments. One participant refused to take the nasal spray at the time of the first dose, and one participant was provided with a supply of OXT but did not return to the clinic following 4-wk treatment. (Attempts to bring the latter participant back to the clinic were unsuccessful.) No significant changes from baseline in height or weight or in the majority of vital signs measurements were observed after 4-wk treatment (Table S3). Systolic standing blood pressure did show an increase in OXT-treated but not in placebo-treated participants ($P = 0.0218$). However, this effect was not significant once multiple comparisons were considered (i.e., six blood pressure measurements were performed, so the adjusted significance level was set at $P < 0.0083$). Change in systolic blood pressure (standing–sitting) did show a significant treatment condition × time point interaction ($P = 0.0004$) that remained significant once multiple comparisons were considered. This finding was unrelated to pretreatment blood pressure measurements. Placebo responses for plasma OXT concentrations showed the greatest social improvement, but other trials have not (21, 22). However, participants who did not complete treatment for ASD have been shown to have increased social abilities in children with ASD when accounting for pretreatment blood OXT concentrations predicted OXT treatment response. Despite preclinical evidence indicating that OXT may be a promising therapeutic for human social impairments, prior clinical trials found no differences in social abilities between treatment groups (19, 20). Our findings for ASD may be attributable, at least in part, to variability in pretreatment social abilities, which might be attributable, at least in part, to variability in participants' social abilities.

Discussion

Here we studied whether intranasal OXT treatment improved social abilities in children with ASD when accounting for pretreatment variation in neuropeptide biology and tested whether pretreatment blood OXT concentrations predicted OXT treatment response. Despite preclinical evidence indicating that OXT may be a promising therapeutic for human social impairments, prior clinical trials found no differences in social abilities between treatment groups (19, 20). Our findings for ASD may be attributable, at least in part, to variability in pretreatment social abilities, which might be attributable, at least in part, to variability in participants' social abilities. Using a double-blind, randomized, placebo-controlled, parallel design, we found that 4-wk OXT compared with placebo treatment significantly enhanced social abilities in children with ASD. We also found that pretreatment blood OXT concentrations predicted treatment response, such that individuals with the lowest pretreatment OXT concentrations showed the greatest social improvement. Importantly, we also confirmed that failure to include participants' endogenous pretreatment neuropeptide measures in our statistical model would have resulted in a negative trial, whereas inclusion of these biomarker measures improved the explanatory power of our model by 43%. Results from the present study indicate that OXT administration enhances social abilities in children with ASD and that individuals with pretreatment OXT signaling deficits may stand to benefit most from OXT administration. Although confirmatory evidence from larger-scale, biomarker-stratified OXT treatment trials is needed, our findings suggest that OXT treatment has the potential to reduce suffering in ASD patients by enhancing quality of life through improved social abilities.

Materials and Methods

Study Regulatory Approval. This study was conducted in the Autism and Developmental Disabilities Clinic (ADDCC) in the Division of Child and Adolescent Psychiatry at Stanford University. Recruitment began in June 2012 and ended in April 2016. Before initiating this trial, an Investigational New Drug application (no. 114664) was filed with the Federal Drug Administration, and this study was approved by the Internal Review Board of the Stanford University School of Medicine. This trial was also registered on ClinicalTrials.gov (NCT01624194). Parents and/or legal guardians of the study's participants provided informed written consent before the initiation of any experimental procedures. If the child was deemed intellectually capable of understanding the study, written assent was also obtained from the child. Finally, this study was overseen by an independent Data Safety Monitoring Board comprised of clinicians with expertise in clinical trials, ASD, and/or pediatric medical care. The trial protocol is available upon request.

Participant Recruitment and Eligibility Criteria. Children with a diagnostic history of ASD were recruited to participate in this study. Participants were

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recruited through (i) the Autism and Developmental Disorders Research Registry at Stanford University, (ii) flyers posted in the ADDC or in the surrounding community (e.g., pediatrics offices, (iii) www.signup4AD.org, a website targeted to parents of children with autism, and (iv) special events (e.g., the Bay Area Autism Speaks Walk). Participants were telephone-screened for initial study eligibility and then underwent a medical assessment (including ECG, heart rate, and blood pressure measurement) as well as a comprehensive psychiatric evaluation. The psychiatric evaluation included determination of the accuracy of the child’s previous ASD diagnosis based on Diagnostic and Statistical Manual of Mental Disorders IV-TR Text Revision (DSM-IV-TR) (34) or DSM-5 (1) criteria, which was confirmed with research diagnostic methods [i.e., the Autism Diagnostic Instrument-Revised (35) and the Autism Diagnostic Observation Schedule using the revised algorithms (36)] conducted by research staff trained by a research-eligible clinician.

In addition to meeting diagnostic criteria for ASD, other study inclusion criteria included (i) medically healthy outpatients between 6 and 12.92 y of age with (ii) an IQ >40 (as determined by the Stanford-Binet Intelligence Scales, fifth edition (37)), (iii) a Clinical Global Impression severity rating of ≥4 (38), (iv) a care provider who could reliably bring the participant to clinic visits, provide trustworthy ratings, and interact with the participant on a regular basis, (v) stable medications for at least 4 wk, (vi) no planned changes in psychosocial interventions during the trial, and (vii) willingness to provide informed consent.

Study exclusion criteria included (i) prior or current use of OXT; (ii) DSM-IV-TR or DSM-5 diagnosis of schizophrenia, schizoaffective disorder, or psychotic disorder; (iii) regular nasal obstruction or nosebleeds; (iv) active medical problems: unstable seizures or significant physical illness (e.g., serious liver, renal, or cardiac pathology); (v) sensitivity to preservatives (e.g., chlorobutanol hemiolydate); (vi) evidence of a genetic mutation known to cause autism spectrum disorder; (vii) significant hearing or vision impairments; (viii) habitual consumption of large volumes of water; (ix) pregnancy, breastfeeding, or child birth within the last 6 mo; or (x) sexually active females not using a reliable method of contraception.

Pharmacological Intervention. Synctionin nasal spray (Novartis) was purchased from a Swiss pharmacy (Dr. Noyer Apotheke). The placebo solution was prepared with the same pharmacy and used for the active solution except the OXT compound. A pharmacist next transferred 35 mL of Synctionin [40 International Units (IU)/mL] or placebo into a disposable nasal applicator to ensure that the drug and placebo applicators were visually indistinguishable to the research team. These applicators were coded and given to the Stanford Health Care’s Investigational Drug Service (IDS) for refrigerated storage (2–8 °C) and subsequent dispensing.

After the screening phase, pretreatment baseline measures were obtained from participants and parents to meet inclusion and exclusion criteria. These measures included assessments to determine participants’ symptom severity and phenotype, evaluations for safety/tolerability monitoring, and blood sample collection for later biomarker quantification. One to four weeks later, participation was randomly assigned (1:1) to a treatment condition (i.e., OXT or placebo), stratified by gender. Randomization was performed by an IDS pharmacist using randomization.com. Each participant was given a participant identification number by the research staff to allow the research team to remain blinded throughout the trial’s duration.

Parents were trained in the clinic by research staff to administer the nasal spray to their child. The first dose, three puffs per nostril (4 IU per puff), for a total dose of 24 IU OXT or placebo was administered in the ADDC. Vital signs were measured before and 30 min after initial single-dose nasal spray administration to monitor for acute, unanticipated reactions to the drug. Participants’ parents then were provided with a 4-wk drug supply and were responsible for their child’s continued twice daily dosing (24 IU per dose, 48 IU/d) at home. Participants were instructed to keep the drug refrigerated with only brief room temperature excursions (i.e., for dosing). On completion of the 4-wk treatment period, participants returned with their parent to the clinic, and behavioral data, safety/tolerability data, and blood samples were again collected.

Blood Sample Collection and Processing Procedures. Twenty milliliters of whole blood was drawn from the child’s antecubital region by a pediatric phlebotomist using standard protocols at Lucile Packard Children’s Hospital outpatient laboratory. Whole blood was collected into chilled EDTA-treated vacutainer tubes and was placed on wet ice immediately. Samples were centrifuged promptly (1,600 × g at 4 °C for 15 min), and the plasma fraction was aliquoted into polypropylene tubes and flash-frozen on dry ice. Whole blood was also collected into PAXgene RNA tubes (Qiagen) and processed per the manufacturer’s instructions. All samples were stored at −80 °C until quantification.

Plasma OXT Quantification. Plasma OXT concentrations were quantified using a commercially available enzyme immunoassay kit (Enzo Life Sciences, Inc.). The kit is highly specific and sensitive to human OXT and does not detect related peptides (i.e., cross-reactivity with vasopressin is 0.6%). A technician blinded to treatment condition performed sample preparation and OXT quantification following established procedures (27). Briefly, plasma samples (1,000 μL per participant) were extracted using Strata-X columns (Phenomenex Inc.) and evaporated using compressed nitrogen. Each evaporated sample was reconstituted in 250 μL of assay buffer before OXT quantification to provide a sufficient sample volume to run each participant’s sample in duplicate wells (100 μL per well). This practice ensured that the plated samples contained sufficiently high OXT quantities to be read above the limit of detection (11.7 pg/mL). Samples were assayed with a tunable microplate reader ( Molecular Devices) for the 96-well format per the manufacturer’s instructions.

Quantification of OXTR and V1A Gene Expression Levels. Total RNA was isolated and purified using a PAXgene blood RNA kit from blood stabilized in PAXgene RNA tubes (Qiagen). The first-strand cDNA synthesis reaction was carried out with the Quantitect reverse transcription kit (Qiagen) with a starting RNA quantity of 1 μg in a 20-μL final volume. The primer sequence information for OXTR and V1AR genes was obtained from published studies and was designed as follows: OXTR forward 5′-GTCGAAACCTTCAAGGAATT-3′ and reverse 5′-AGCTGACCTTA-3′ (29); V1AR forward 5′-TGATGTGGTTTGTTCATGCTTA-3′ and reverse 5′-TATGATGTGGTTTGTTCATGCTTA-3′ (40). Two housekeeping genes, hypoxanthine phosphoribosyltransferase 1 (HPRTT); forward 5′-GGACAGGACGTTAACGTTGC-3′ and reverse 5′-ATAGCCCCCTTTT-GAGCACA-3′ (40); and ubiquitin C (UBC; forward 5′-GGCTGTCCTAAAGAC-TCCGGC-3′ and reverse 5′-GTCACCAAGCTCCTGCTTA-3′ (40)) were selected for normalization using geNorm. qPCR was performed on the StepOnePlus Real-Time PCR System (Life Technologies) with SYBR Green (Thermo Fisher Scientific). CDNA was PCR amplified in triplicate, and cycle threshold (Ct) values from each sample were obtained using StepOnePlus software. Analyses were conducted using the comparative Ct method (2−ΔΔCt) (41).

Outcome Measures. The trial’s primary outcome measure was improvement in social abilities as measured by the SRS. The SRS is a norm-referenced measure that assesses a child’s social functioning and the impact of social and nonsocial problems on the child’s daily living (42). The SRS is a sensitive measure (i.e., it strongly correlates with DSM criterion scores) with high reliability. The trial’s secondary outcome measures included treatment efficacy in nonsocial symptom domains and OXT safety/tolerability. Treatment effect generalizability was evaluated using (i) the RBS-R (44), which measures a comprehensive list of repetitive and stereotyped behaviors, and (ii) The Spence Children’s Anxiety Scale (45), which assesses the trait anxiety symptoms broadly in line with DSM dimensions of anxiety disorder. Anxiety was selected as an outcome measure because of its significant comorbidity with ASD (46), its negative relationship with endogenous OXT concentrations in humans (47), and OXT’s anxiolytic effects in mice (48). Drug safety and tolerability were evaluated using (i) the DOTES, a rating scale that assesses the presence, frequency, and severity of side effects (38), and (ii) safety/clinical biosignals (i.e., blood pressure, heart rate, temperature) and in height and weight.

Statistical Analyses. Data were managed using REDCap (49) and analyzed using Least-Squares General Linear Models (LS-GLM) in JMP Pro 13 (SAS Institute Inc.). To identify a robust model for our primary outcome measure, initial analyses included IQ, age, and ethnicity as blocking factors. Gender was also included as a blocking factor to enable the use of the SRS Total Raw Score rather than the gender-normed T-Score, which has lower resolution. The pretreatment SRS Total Raw Score was included as a blocking factor to account for the range of possible social ability improvement and thus to reduce possible floor or ceiling effects. Finally, we included treatment condition (i.e., OXT or placebo) as a main effect to test our primary outcome measure and pretreatment blood OXT concentration and neuropeptide receptor gene expression level (expressed as a V1AR:OXTR ratio to account for within-individual differences in baseline expression) as biomarkers hypothesized to affect treatment efficacy. We also tested for treatment condition × biomarker interactions, because a predictive biomarker generally should predict treatment outcome only in drug-treated individuals.

Initial analyses showed that age and ethnicity introduced collinearity and did not improve the R2 of the model; therefore these factors were removed from our final best practice for linear models (50). Similarly, nonsignificant bio- marker interactions were removed to avoid confounds of marginality for the main effects (but see Table S4 for tested interactions). The final model contained IQ, gender, and pretreatment SRS Total Raw Score as blocking.
variables and treatment condition, pretreatment blood OXT concentration, and VIAR-OXTR ratio as hypothesis-driven main effects (Table S5). To assess the impact of the biomarkers, we also ran the same model with the total score for the SRS, RBS-R, and Spence. However, because psychometric validity for the RBS-R Total Score is not well established, we also performed the same analyses on each RBS-R subscale but corrected our critical P value to 0.0083 to protect against multiple comparisons and to achieve the same family-level significance as the total score. Similarly, to minimize the risk of false discovery, blocking factors were not tested for significance (50). The assumptions of LS-GLM (linearity, homogeneity of variance, and normality of error) were confirmed post hoc, and suitable transformations were applied as needed. Post hoc tests were performed as planned contrasts and were Bonferroni corrected for multiple comparisons. Least squares means ± SE are reported. Least squares means are model-derived means controlling for other factors in the analysis and correspond to the model coefficients represented by the F-ratio.

Adverse event data were analyzed with a repeated-measures restricted maximum likelihood mixed model using the treatment condition × time point interaction to test whether treatment affected the means, controlling for any pretreatment baseline differences. Suitable transformations were applied as needed. Significant results were examined and Tukey-corrected for multiple comparisons.

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