BRAIN
CLINICAL TRIAL

Effect of a novel nasal oxytocin spray with enhanced bioavailability on autism: a randomized trial

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Although intranasal oxytocin is expected to be a novel therapy for the core symptoms of autism spectrum disorder, which has currently no approved medication, the efficacy of repeated administrations was inconsistent, suggesting that the optimal dose for a single administration of oxytocin is not optimal for repeated administration.

The current double-blind, placebo-controlled, multicentre, crossover trial (ClinicalTrials.gov Identifier: NCT03466671) was aimed to test the effect of TTA-121, a new formulation of intranasal oxytocin spray with an enhanced bioavailability (3.6 times higher than Syntocinon® spray, as assessed by area under the concentration–time curve in rabbit brains), which enabled us to test a wide range of multiple doses, on autism spectrum disorder core symptoms and to determine the dose–response relationship. Four-week administrations of TTA-121, at low dose once per day (3 U/day), low dose twice per day (6 U/day), high dose once per day (10 U/day), or high dose twice per day (20 U/day), and 4-week placebo were administered in a crossover manner. The primary outcome was the mean difference in the reciprocity score (range: 0–14, higher values represent worse outcomes) on the Autism Diagnostic Observation Schedule between the baseline and end point of each administration period. This trial with two administration periods and eight groups was conducted at seven university hospitals in Japan, enrolling adult males with high-functioning autism spectrum disorder. Enrolment began from June 2018 and ended December 2019. Follow-up ended March 2020.

Of 109 males with high-functioning autism spectrum disorder who were randomized, 103 completed the trial. The smallest *P*-value, judged as the dose–response relationship, was the contrast with the peak at TTA-121 6 U/day, with inverted U-shape for both the full analysis set ($P = 0.182$) and per protocol set ($P = 0.073$). The Autism Diagnostic Observation Schedule reciprocity score, the primary outcome, was reduced in the TTA-121 6 U/day administration period compared with the placebo (full analysis set: $P = 0.118$, mean difference = –0.5; 95% CI: –1.1 to 0.1; per protocol set: $P = 0.012$, mean difference = –0.8; 95% CI: –1.3 to –0.2). The per protocol set was the analysis target population, consisting of all full analysis set participants except those who deviated from the protocol. Most

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dropouts from the full analysis set to the per protocol set occurred because of poor adherence to the test drug (9 of 12 in the first period and 8 of 15 in the second period). None of the secondary clinical and behavioural outcomes were significantly improved with the TTA-121 compared with the placebo in the full analysis set.
A novel intranasal spray of oxytocin with enhanced bioavailability enabled us to test a wide range of multiple doses, revealing an inverted U-shape dose–response curve, with the peak at a dose that was lower than expected from previous studies. The efficacy of TTA-121 shown in the current exploratory study should be verified in a future large-scale, parallel-group trial.

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Abbreviations: ADOS = Autism Diagnostic Observation Schedule; ASD = autism spectrum disorder; FAS = full analysis set; PPS = per protocol set

Introduction

Oxytocin is expected to be a novel therapy for the core symptoms of autism spectrum disorder (ASD),[1,2] such as deficits in social interaction and communication, which are currently untreatable using any established medication.[3] Although previous studies have consistently shown the positive effects of a single oxytocin administration on neurobehavioural measures associated with ASD,[4–8] discrepancies regarding the efficacy of repeated oxytocin administrations on clinical measures of ASD core symptoms[9–15] impede its clinical application.

These discrepancies suggest that the optimal dose for a single administration of oxytocin is not optimal for repeated

administration.[16] A potential deterioration in the efficacy of oxytocin by repeated administration, suggested by previous human[17–19] and animal studies,[20–22] supports this possibility. An inverted U-shape dose–response relationship of oxytocin has been suggested by previous human single-dose[23–25] and animal studies,[26] and both too-high and too-low dose settings can lead to a failure to detect the efficacy of oxytocin. To identify the optimal dose for repeated administrations of oxytocin, a wide range of multiple doses should therefore be used in trials. However, the low bioavailability of administered oxytocin, which is a critical issue for its clinical application,[27] limits the possible range of doses.

Although intranasal administration improves central bioavailability compared with oral or intravenous administrations, doses are difficult to regulate or replicate via this route.[16] TTA-121 is a novel oxytocin nasal spray with a high bioavailability (3.6 times the area under the concentration–time curve (AUCt) of the existing Syntocinon® spray in rabbit brains) that allows increased oxytocin delivery to the brain by adjusting the osmolality and viscosity of the formulation (WO 2017/073798 A1).[28] TTA-121 has been developed to obtain the optimal dose with just one puff because in our preliminary study, participants with ASD complained about the difficulty of administering 24 units (U) intranasal oxytocin in Syntocinon® with six puffs, and difficulty was associated with efficacy. Toxicity studies in animals[29,30] and the phase I trial of TTA-121 have been completed.[31]

The current study aimed, by testing the effect of TTA-121 on ASD social core symptoms and determining the dose–response relationship, to explore the optimal dose for repeated administrations of oxytocin to improve the core social symptoms of ASD. Taking advantage of the enhanced bioavailability of TTA-121, the current trial involved a wide range of oxytocin doses. Additionally, to suppress the placebo effect as much as possible,[10,15,32–34] a crossover design[17,35] and outcome measures with high objectivity[9,16,17,19,34] were used.

Materials and methods

The present double-blind, placebo-controlled, multicentre, crossover trial with two administration periods was conducted at seven university hospitals in Japan. The recruitment process and screening, primary outcome scoring and data and drug management were performed at the main site (Hamamatsu University School of Medicine Hospital). Testing eligibility, confirmation of diagnosis, registration, oxytocin/placebo treatments and clinical assessments were performed at each site (further details and the and prespecified full protocol are provided in the Supplementary material). The study protocol was approved by the institutional review boards for each site and was registered (ClinicalTrials.gov Identifiers: NCT03466671/UMIN000031412). Written informed consent was obtained from all participants at each site.

Eligibility criteria for participants

Inclusion criteria were as follows: (i) males aged 18–55; (ii) ASD diagnosis based on the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5)[36] with a score exceeding the cut-off value of 10 for qualitative abnormalities in social reciprocity on the Autism Diagnostic Interview Revised[37]; and (iii) Full Scale Intelligence Quotient above 80, measured using the Wechsler Adult Intelligence Scale-III.[38]

Exclusion criteria were as follows: (ii) diagnosis of bipolar disorder or schizophrenia spectrum disorder; (ii) primary diagnosis of psychiatric disorders other than ASD; (iii) instability in symptoms of comorbid mental disorders; (iv) history of changes in medication or doses of psychotropics within 1 month before registration; (v) current treatment with more than one psychotropic; (vi) history of hypersensitivity to oxytocin; (vii) history of seizures or traumatic brain injury with loss of consciousness for longer than 5 min; (viii) history of alcohol-related disorders, substance abuse or addiction; (ix) family history of male breast cancer; and (x) severe complications or known hypersensitivity to some drugs and foods.

Interventions

The interventions with crossover design included the following eight groups [i.e. two doses (TTA-121 3/10U) × two orders (TTA-121→placebo/placebo→TTA-121) × two frequencies (once/twice per day); Fig. 1]. Groups 1–4: four weeks of TTA-121 at 3 U or 10 U once per day in the morning and placebo once per day in the evening in the first/second period (3 or 10 U/day). After 4 weeks washout, 4 weeks of placebo twice per day in the second/first period. Groups 5–8: four weeks of TTA-121 at 3 or 10 U twice per day in the morning and evening in the first/second period (6 or 20 U/day). After 4 weeks washout, 4 weeks of placebo twice per day in the second/first period.

To avoid any subjective effects of the substances other than oxytocin, the placebo contained all of the inactive ingredients from the oxytocin spray. All participants underwent training regarding intranasal administration. Identical instructions and training materials (Supplementary material) were used at each site before the trial initiation, and the effectiveness of training was confirmed at each 2-week assessment point. Treatment adherence was assessed using a self-reported daily record (Supplementary material).

Randomization and masking of drug administration

Each participant was randomized to one of eight interventions. Randomization was performed by an unblinded randomization manager who was not a research team member using a machine-generated treatment schedule, which allocated each participant to an intervention using a randomly permuted block (block size 8). This protocol allowed the participants, clinicians and research team members to remain blinded throughout the trial's duration.

Outcomes

The primary outcome measure was changes in the social core symptom of ASD assessed using the social reciprocity score of Autism Diagnostic Observation Schedule (ADOS)[39] module 4 (range: 0–14, higher values represent worse outcomes) between the baseline and end point of each administration period. ADOS is a standard diagnosis tool for ASD, but has also repeatedly been adopted as a primary outcome in ASD-related trials.[17,34,40–43] The baseline was just before first drug administration on the same day, and the end point was from ∼15 min after the drug last administration. The ADOS administration were conducted by trained psychiatrists or psychologists who had completed a training course regarding the research use of ADOS and whose credentials had been validated by another certified administrator (Mi.K.). The four ADOS evaluations for each participant were conducted by the same administrator. To minimize interrater variability, all final ADOS scores were rated by one of seven certified administrators (Mi.K., A.Y., C.K., N.I., H.K., T.H., or K. M.) via video recordings. The four ADOS evaluations for each participant were finally scored by the same certified administrator, blinded to the intervention and order of administration (i.e. baseline or end point). The interrater reliability between two certified administrators who rated each case independently was >90%.

The secondary outcomes were items other than reciprocity in ADOS module 4 and general clinical assessments including

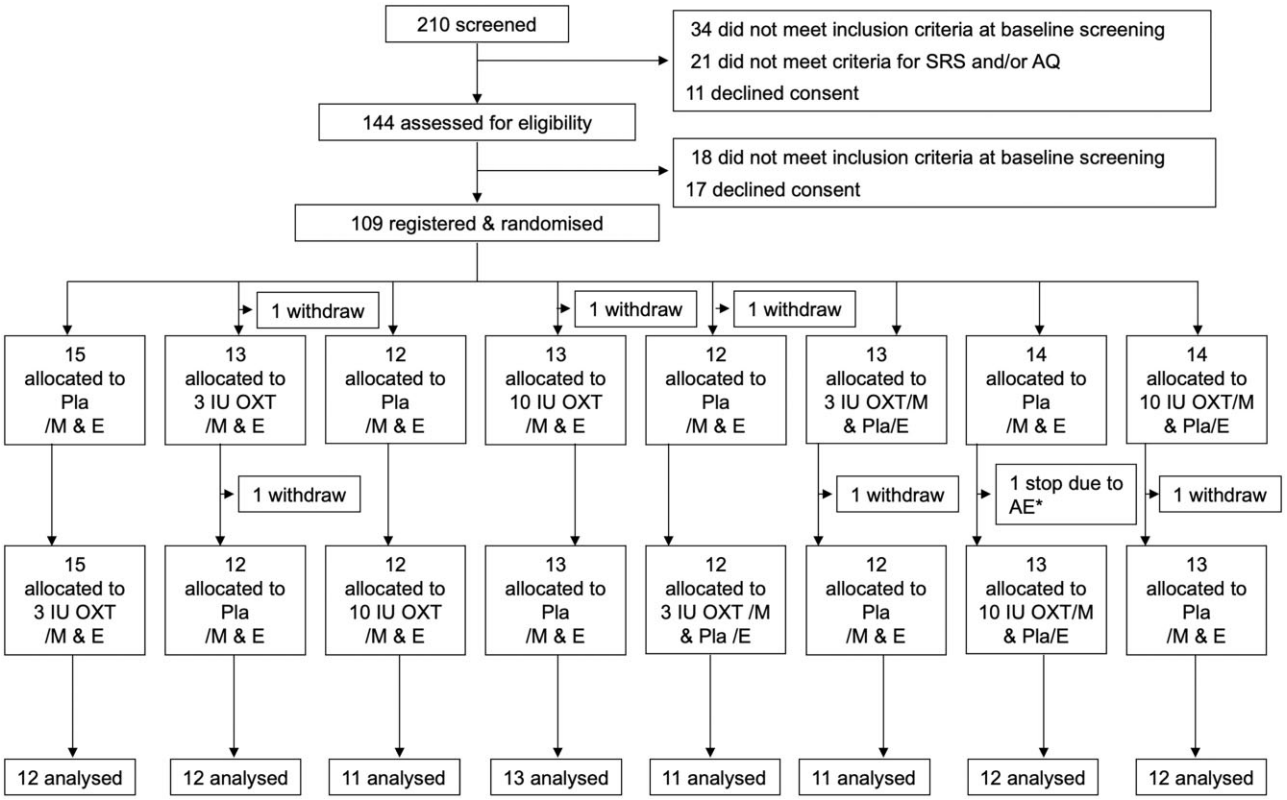

Figure 1 Participant flow diagram of the clinical trial. AE = adverse event; AQ = autism-spectrum quotient; E = evening M = morning OXT = oxytocin; Pla = placebo; SRS = Social Responsiveness Scale.

Clinical Global Impression[44] and Global Assessment of Functioning (GAF).[45] Quantitative measurements of social behaviours that had significant improvements in our previous trial of intranasal oxytocin (Syntocinon®),[19,34] including fixation time of eye gaze on social region and facial expressions during social interactions in an ADOS activity, were also included as secondary outcomes. Exploratory outcomes included items in quantitative measurements of social behaviours that did not have significant improvements in our previous trial of Syntocinon®,[19,34] including fixation time of eye gaze on social regions and facial expressions during social interactions. Quantitative measurements of speech prosody that characterize ASD compared with typically developed individuals[46] were also included as exploratory outcomes (details in the Supplementary material).

Sample size

Our previous placebo-controlled, double-blind, crossover trial in which 24 U of intranasal oxytocin in Syntocinon® spray were administered twice per day for 6 weeks in 18 participants with ASD showed changes in the reciprocity scores of ADOS.[17] The mean of amount of change was –0.5 [standard deviation (SD): 1.42] in the oxytocin group, 0.78 (SD: 1.63) in the placebo group and the difference between groups was 1.28 (SD: 1.64), which was a significant result ($P = 0.034$). On the other hand, we examined the data collected from 104 participants with ASD in another previous clinical trial[34] to test the relationship between the amount of change in the ADOS reciprocity score and clinical function in daily life. The clinically significant amount of change in the GAF score that reflects clinical function in daily life was reported to be 10 points. Based on the data of the above clinical trial, as the GAF score and the ADOS reciprocity score were significantly correlated (correlation coefficient = –0.35, $P < 0.0001$), it was shown that the amount of change in the GAF score of 10 points corresponds to the change in the ADOS reciprocity score of 1.2 points. Based on the above results, the difference between oxytocin and placebo treatment at the dose at which the maximum effect observed was set to 1.3. In addition, the SD of each treatment group was expected to be 1.5, and the multiple dose–response relationships listed in Supplementary Table 1 were assumed for the ADOS reciprocity score.

The contrasts corresponding to the above dose-response relationships were considered as follows: (i) [2, 1, 0, –1, –2]; (ii) [9, 4, –1, –6, –6]; (iii) [8, 3, –2, –7, –2]; (iv) [7, 2, –3, –3, –3]; (v) [4, –1, –6, –1, 4]; (vi) [1, 0, –1, 0, 0]; (vii) [4, –1, –1, –1, –1]; and (viii) [1, –1, 0, 0, 0]. If a one-way ANOVA was used with a two-sided significance level of 5%, the powers at which the respective comparisons were significant under the setting of 120 cases were as shown in Supplementary Table 2. Using the corresponding contrasts, a power of greater than ∼80% was ensured in all dose–response relationships (diagonal contrasts correspond to true dose–response relationships).

Because this clinical trial was a crossover trial, it was expected that the SD of the difference between the groups would be smaller in the pairwise comparison with placebo treatment. Therefore, 120 cases would ensure sufficient power. Based on the above rationale, the required number of participants was 120 in this clinical trial. However, assuming that 20% of the cases were dropped during the trial, the target number of cases was set to 144 cases.

Statistical analysis

Further details of statistical analyses are provided in the Supplementary material.

Table 1 Characteristics of each administration group at baseline (safety analysis set)

Characteristics	Placebo (total) n = 103	Oxytocin 3 U n = 25	Oxytocin 6 U n = 28	Oxytocin 10 U n = 27	Oxytocin 20 U n = 25
Age, mean (SD), years	28.3 (8.4)	29.1 (7.7)	28.0 (8.8)	29.0 (9.2)	28.0 (8.2)
Height, cm	170.6 (5.4)	171.5 (5.3)	170.4 (6.5)	171.6 (5.0)	169.2 (5.0)
Body weight, kg	68.2 (14.6)	71.7 (15.8)	68.3 (14.0)	67.9 (14.1)	67.1 (13.9)
Self SES: 1/2/3/4/5	8/37/20/25/13	3/10/7/1/4	1/8/4/7/8	1/13/1/10/2	3/7/8/7/0
Parental SES: 1/2/3/4/5	12/54/29/8/0	3/17/4/1/0	4/11/9/4/0	2/13/11/1/0	2/15/6/2/0
Autism Diagnostic Interview-Revised					
Social	19.9 (5.4)	21.2 (5.0)	19.3 (5.4)	18.9 (5.6)	20.2 (5.4)
Communication	14.2 (4.4)	15.2 (3.5)	13.7 (4.3)	13.7 (4.8)	14.3 (4.9)
Repetitive	5.4 (2.7)	5.8 (3.1)	5.5 (2.8)	4.6 (2.5)	5.6 (2.7)
Wechsler Adult Intelligent Scale-III					
Full IQ	107.5 (14.3)	109.5 (14.1)	107.0 (15.1)	108.3 (14.1)	104.8 (13.8)
Verbal IQ	111.7 (15.6)	113.8 (18.2)	111.0 (12.0)	113.7 (15.4)	110.4 (13.1)
Performance IQ	97.8 (18.1)	97.3 (16.7)	100.4 (20.0)	99.0 (14.6)	96.0 (17.9)
Current psychotropic medications	29/103	3/25	9/28	9/27	8/25

IQ = intelligence quotient; SES = socioeconomic status, where a lower value indicates a higher socioeconomic status.

Primary outcome

The change in ADOS reciprocity score during 4 weeks of administration was calculated for each subject and summary statistics were calculated for each administered group. As the fixed effects on the change in each administration period, the content of administration (TTA-121/placebo), order of crossover administration (TTA-121→placebo/placebo→TTA-121) and interaction between content and order were included in a mixed-effects model in which the individual effects were included as random effects. The Kenward–Roger method was used to calculate the degrees of freedom. In addition, for the dose contents [3 U once daily (3 U), 3 U twice daily (6 U), 10 U once daily (10 U), and 10 U twice daily (20 U)], contrasts were set to test dose–response relationships,[47] and the *P*-value for each contrast was calculated. Contrasts for placebo, 3 U, 6 U, 10 U and 20 U were as follows: [2, 1, 0, −1, −2], [9, 4, −1, −6, −6], [8, 3, −2, −7, −2], [7, 2, −3, −3, −3], [4, −1, −6, −1, 4], [1, 0, −1, 0, 0], [4, −1, −1, −1, −1], [1, −1, 0, 0, 0]. Without correcting for multiple testing, we considered the contrast with the minimum *P*-value as the dose–response relationship.

The dose that was considered to be most effective based on the identified dose–response relationship was compared with the placebo. The differences between groups and their 95% confidence intervals (CIs) and *P*-values were calculated using paired *t*-tests. Similarly, for other doses, pairwise comparisons with placebo were performed.

Table 2 Dose–response relationships for the primary outcome: ADOS reciprocity

Contrasts [Placebo, 3 U, 6 U, 10 U, 20 U]	*P*-value*	
	FAS	PPS
Linear dose–response relationship [2,1,0, −1, −2]	0.599	0.984
Peak at 10 U/morning [9, 4, −1, −6, −6]	0.705	0.710
Peak at 10 U/morning with inverted U-shape [8,3, −2, −7, −2]	0.922	0.378
Peak at 3 U/morning and evening [7, 2, −3, −3, −3]	0.799	0.292
Peak at 3 U/morning and evening with inverted U-shape_1 [4, −1, −6, −1, 4]	0.182**	0.073**
Peak at 3 U/morning and evening with inverted U-shape_2 [1, 0, −1, 0, 0]	0.193	0.082
Peak at 3 U/morning [4, −1, −1, −1, −1]	0.915	0.408
Peak at 3 U/morning with inverted U-shape [1, −1, 0, 0, 0]	0.650	0.983

*P-values calculated from analyses performed by applying the mixed-effects model with the amount of change in each administration period as the response variable, with the content of administration (placebo, 3 U, 6 U, 10 U, 20 U) and the order of administration (active drug→placebo/placebo→active drug) as the fixed effects, with the interaction between the content of administration and the order of administration, and with the effect of the individual as a random effect.
**P-values showing the minimum value.

Secondary and exploratory outcomes

The secondary and exploratory outcomes were used to calculate changes compared with baseline measurements and summary statistics for these outcomes were calculated for each treatment group. The mean differences between treatment groups and their 95% CIs were estimated. The statistical model was similar to that of the primary outcome, and the effect on the outcomes of each dose compared with the placebo was evaluated.

Data availability

Individual participant data after de-identification underlying the results reported in this article are available on request from investigators providing a methodologically sound proposal and whose proposed use of the data has been approved by an independent review committee identified for this purpose. Proposals should be directed to yamasue@hama-med.ac.jp. Maintenance of the dataset in the participants of clinical trials will be ended 5 years following article publication. The data are not publicly available due to them containing information that could compromise research participant privacy or consent.

Results

Baseline characteristics

Before visiting each trial site, 34 of 210 candidates did not meet the eligibility criteria based on background information, 21 did not meet the criteria for Social Responsiveness Scale[48] and/or Autism Spectrum Quotient[49] scores (Supplementary material) and 11 declined to consent to participate. Of the 144 participants assessed for eligibility, consent was obtained from 127 adult males with

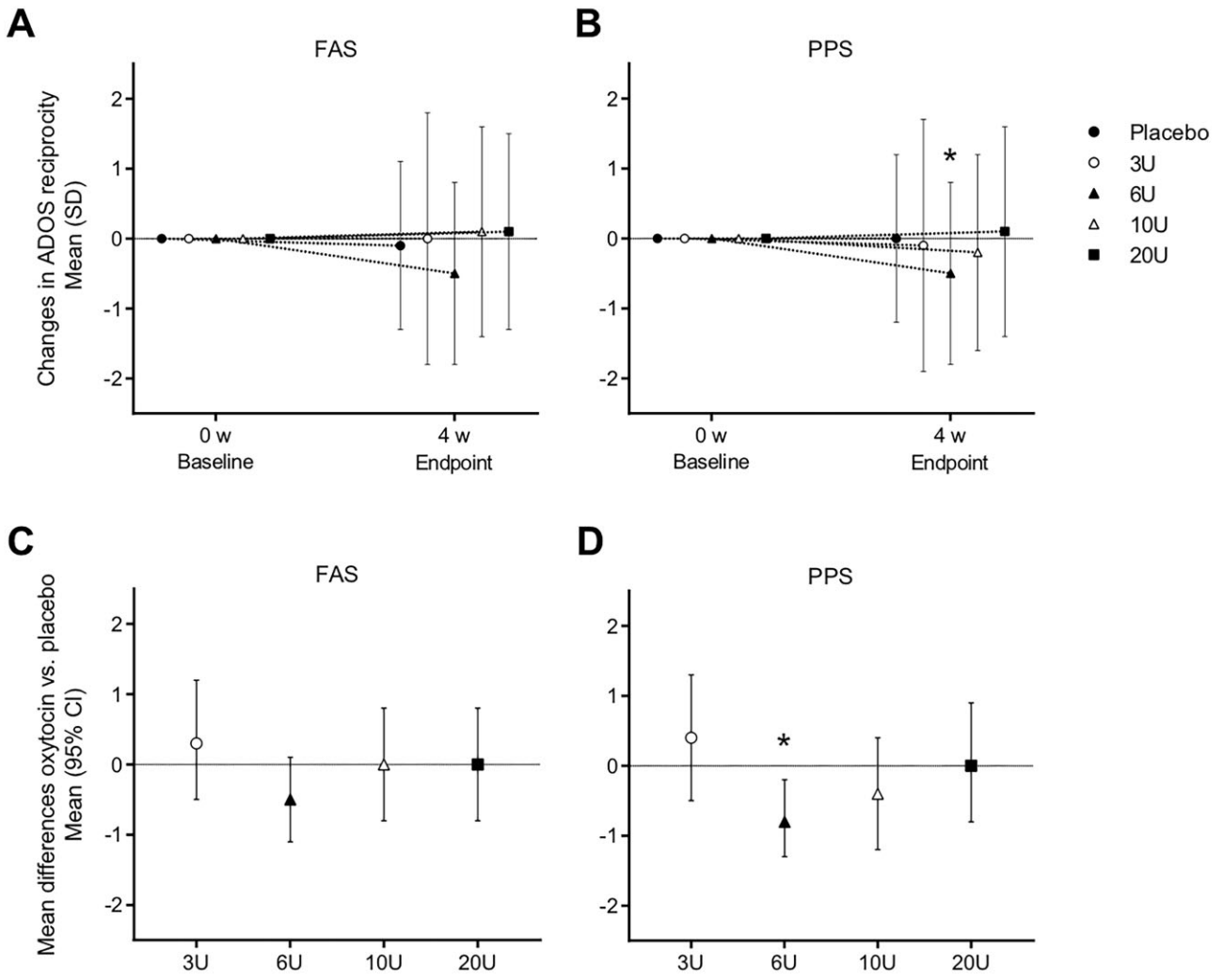

Figure 2 The effects of oxytocin on the primary outcome: ADOS reciprocity. (**A** and **B**) Plots illustrating changes in the means and error bars representing the standard deviations of ADOS reciprocity scores (range of possible scores, 0–14: higher scores indicate greater severity) in the FAS (**A**) and PPS (**B**). (**C** and **D**) Plots illustrating the mean differences between oxytocin and placebo, and error bars representing their 95% CIs, in ADOS reciprocity scores in the FAS (**C**) and PPS (**D**). *Statistically significant as indicated by $P < 0.05$ from paired *t*-test.

ASD. Of these, 18 did not meet the eligibility criteria and 109 males with high-functioning ASD were enrolled between 25 June 2018 and 26 December 2019. End point assessments were performed between 27 September 2018 and 19 March 2020. The actual rate of withdrawal (7 of 109 cases, 6.4%) was lower than expected (i.e. 20%). Because the number of cases needed for analyses was calculated as ∼120, we completed recruitment of 127 participants before registering a target sample size of 144 participants, which was calculated with an expected withdrawal rate of 20%.

Six of the 109 participants did not complete the trial because they withdrew their consent. Of these, three withdrew before the first administered period and three withdrew during the washout period. Another participant in the placebo-TTA-121 10 U group did not complete the trial because of an adverse event (a mild emergence of blasts in the blood) during the washout period (Fig. 1).

Consequently, we analysed 106 participants as the full analysis set (FAS; Table 1). The FAS was the analysis target population, consisting of all participants except for those who met the following criteria among the participants who had been randomized: participants who were not diagnosed with ASD ($n = 0$); participants who have never taken the investigational drug ($n = 3$); participants with no data evaluating efficacy after randomization ($n = 0$). The per protocol set (PPS) was the analysis target population, consisting of all FAS participants except those that met the following criteria: participants who did not meet the inclusion criteria; participants who fulfilled the exclusion criteria; participants who violated the rules for prohibited drugs; participants with an investigational drug compliance rate of 85% or less. For PPS of the first period, nine participants with poor (<85%) drug adherence (Supplementary Table 3), one with visit out of period, one with more than one psychotropic drug and one with deviations from the protocol potentially influencing the assessment of efficacy were excluded; 94 participants were included. For the PPS of the second period, eight participants with poor drug adherence, one with visit out of period, one with more than one psychotropic drug and one with deviations from the protocol potentially influencing the assessment of efficacy were excluded; 91 participants were included. Plasma oxytocin levels were measured in peripheral blood samples collected just before the first and 60 min after the last double-blind administration in each period, using liquid chromatography–mass spectrometry (details in the Supplementary material). These levels were elevated relative to baseline following TTA-121 administration [means (pg/ml): 3 U: from 0.00 to 1.63; 6 U: 0.00 to 1.84; 10 U: 0.04 to 6.92; 20 U: 0.00 to 6.47]; such elevations were not observed

Table 3 Observed changes from baseline to end point for the primary outcome: ADOS reciprocity

Dose	Administration	*n*	Baseline	End point	Change	Mean difference	95% CI	*P*-value	Cohen's *d*
FAS									
6 U*									
	6 U	25	8.2 (3.4)	7.7 (3.1)	–0.5 (1.3)	–0.5	–1.1 to 0.1	0.118	–0.384
	Placebo (6 U)	24	7.7 (3.3)	7.5 (3.3)	–0.1 (1.3)				
3 U									
	3 U	25	7.1 (2.4)	7.1 (2.7)	0.0 (1.8)	0.3	–0.5 to 1.2	0.389	0.333
	Placebo (3 U)	23	7.4 (2.4)	6.9 (2.7)	–0.5 (0.9)				
10 U									
	10 U	26	7.2 (3.0)	7.3 (2.6)	0.1 (1.5)	0.0	–0.8 to 0.8	1.000	0.000
	Placebo (10 U)	27	7.4 (2.4)	7.4 (2.6)	0.0 (1.2)				
20 U									
	20 U	24	8.1 (2.0)	8.2 (2.9)	0.1 (1.4)	0.0	–0.8 to 0.8	1.000	0.000
	Placebo (20 U)	23	7.9 (2.3)	8.2 (2.2)	0.3 (1.3)				
PPS									
6 U*									
	6 U	22	8.4 (3.6)	7.9 (3.3)	–0.5 (1.3)	–0.8	–1.3 to –0.2	0.012*	–0.667
	Placebo (6 U)	20	7.9 (3.4)	8.1 (3.3)	0.2 (1.2)				
3 U									
	3U	22	7.2 (2.5)	7.1 (2.9)	–0.1 (1.8)	0.4	–0.5 to 1.3	0.384	0.444
	Placebo (3 U)	21	7.4 (2.5)	6.9 (2.8)	–0.6 (0.9)				
10 U									
	10 U	22	7.8 (2.6)	7.6 (2.6)	–0.2 (1.4)	–0.4	–1.2 to 0.4	0.358	–0.333
	Placebo (10 U)	23	7.8 (2.3)	7.9 (2.3)	0.1 (1.2)				
20 U									
	20 U	23	8.1 (2.1)	8.3 (2.9)	0.1 (1.5)	0.0	–0.8 to 0.9	0.909	0.000
	Placebo (20 U)	22	8.0 (2.4)	8.2 (2.2)	0.2 (1.3)				

Baseline, end point and change data are presented as mean (SD).
*The dose of contrast showing the smallest *P*-value for the dose–response relationship.
**$P < 0.05$ from paired t-tests.

following placebo administration (placebo: 0.02 to 0.09; Supplementary Table 4).

Primary outcome

The contrast with the smallest *P*-value, judged as the dose–response relationship, was [Placebo, TTA-121 3 U, 6 U, 10 U, 20 U: 4, –1, –6, –1, 4]. The contrast of inverted U-shape had its peak at TTA-121 6 U (i.e. 3 U/morning and evening) for both the FAS ($P = 0.182$) and PPS ($P = 0.073$; Table 2). Next, differences between TTA-121 6 U and placebo were calculated using paired *t*-tests; the ADOS reciprocity score was reduced from baseline to end point in the TTA-121-administered period compared with the placebo period (FAS: $P = 0.118$, mean difference = –0.5; 95% CI: –1.1 to 0.1; PPS: $P = 0.012$, mean difference = –0.8; 95% CI: –1.3 to –0.2; Fig. 2 and Table 3). No significant design and hangover effects in crossover design were revealed for any doses (3 U: $P = 0.215$; 6 U: $P = 0.365$; 10 U: $P = 0.476$; 20 U: $P = 0.868$; Supplementary Tables 5 and 6).

Secondary and exploratory outcomes

No secondary outcomes were significantly improved by TTA-121 compared with placebo (Supplementary Tables 7–12). Among the exploratory outcomes, the mean of log F_0 ($P = 0.015$, mean difference = –0.066; 95% CI: –0.117 to –0.014) and correlation of blockwise mean of log F_0 ($P = 0.039$, mean difference = –0.379; 95% CI: –0.736 to –0.023) were reduced from baseline to end point in the oxytocin administration period compared with placebo, while the log pause-to-turn ratio was increased during the oxytocin administration period ($P = 0.028$, mean difference = 0.085; 95% CI: 0.010 to 0.159; Supplementary Table 13). Fixation times on eye regions in the movies of human faces without lip movement and while blinking were reduced from baseline to end point in the oxytocin administration period compared with placebo ($P = 0.042$, mean difference = –0.217; 95% CI: –0.426 to –0.008; $P = 0.046$, mean difference = –0.219; 95% CI: –0.433 to –0.004, respectively; Supplementary Table 14). No other exploratory outcomes were significantly improved by oxytocin administration compared with placebo (Supplementary Table 15).

Safety

No severe adverse events were observed in any doses (Supplementary Tables 16–19). Although one participant discontinued the trial because of an adverse event (mild emergence of blasts in blood), the event was observed during washout after the placebo administration period and recovered after observation. The emergence ratios of adverse events in administration periods were 24.0% in TTA-121 3 U, 46.4% in 6 U, 40.7% in 10 U, 40.0% in 20 U and 36.9% in placebo (Supplementary Table 16).

Discussion

In this multicentre trial of males with ASD, 4 weeks of treatment with multiple doses of TTA-121 revealed a peak of efficacy at 6 U with inverted U-shape as the dose–response relationship of intranasal oxytocin. The ASD social core symptoms were improved during the TTA-121 6 U-administered period compared with the placebo period in the PPS.

The inverted U-shape dose–response relationship of oxytocin is consistent with previous studies in experimental animals[26] and single administrations in healthy humans.[23–25] In rabbit brains, as TTA-121 has a 3.0 times higher maximum plasma concentration

(C_{max}) and 3.6 times higher AUCt compared with Syntocinon®,[28] based on the assumption that the results from rabbit approximates those from human, the peak dose of TTA-121 6 U approximately corresponds to Syntocinon® 18 to 21.6 U per day, which is lower than the doses in previous studies of repeated oxytocin administration in adults with ASD (i.e. 32 U[11] or 48 U[9,17,34]). The current findings suggest that suboptimal doses of oxytocin may contribute to the inconsistent efficacies of previous studies with repeated administration.

Although there were significant improvements in the PPS, no significant effects were observed in the FAS. Most dropouts from FAS to PPS were because of poor adherence to the test drug (9 of 12 in the first period and 8 of 15 in the second period). It is therefore reasonable to detect significant efficacy in the PPS, without subjects with poor adherence who were included in the FAS. However, because FAS is considered as the sample closest to general clinical situations,[50] future studies with a larger sample size should show the efficacy of TTA-121 in FAS. Considering the robust preclinical data on the social effect of oxytocin in animal models,[51] the lack of robust effects in clinically relevant outcomes in the FAS in the current study may have been related to the context in which the intervention was given. It has been proposed that oxytocin is most effective when combined with specific contexts[52] or with other compounds.[53,54] For example, it has been suggested that intranasal oxytocin might be effective for enhancing the efficacy of behavioural therapies, because animal studies suggest that oxytocin enhances the salience of social cues and therefore could make behavioural therapy more salient and effective.[55] Future studies should examine this issue with a combination of intranasal oxytocin and behavioural therapies.

No secondary outcome results supported the efficacy of TTA-121 that was revealed for the primary outcome, although some exploratory outcomes results supported this efficacy. The differences between the current study and previous studies showing significant improvements of oxytocin on these secondary outcomes[19,34] have several possible causes. First, our previous study with a parallel-group design demonstrated that quantified facial expression and eye gaze were advantageous for controlling the placebo effect compared with ADOS,[19,34] whereas the current crossover-design trial showed an adequately suppressed placebo effect with ADOS. Therefore, although the detectability of ADOS showed some advantages in the crossover trial, the advantages of quantified social behaviours can be expected in a future parallel-group trial rather than a crossover trial. Second, our previous study included longitudinal assessments every 2 weeks showed time-course changes in efficacy as assessed by quantified facial expressions (such as the maximum improvement at 2 weeks and deterioration at 4 and 6 weeks).[19,54] The current study only used 0- and 4-week assessments and therefore cannot rule out the possibility of improvements at 2 weeks. Future studies should address this possibility.

Limitations

First, the current early-phase trial should be verified in a future large trial. The effect size of TTA-121 6 U on ADOS reciprocity in the FAS (i.e. $d = 0.384$) indicates that the sample size should be larger (i.e. $n = 56$ with sufficient power 0.8) to detect the significant effects of TTA-121 on ADOS reciprocity in FAS. Although the current crossover design appears to control the placebo effect, the efficacy of TTA-121 should be verified in a future parallel-group trial. Second, the current early-phase study was confined to Japanese men with high-functioning ASD. Although such homogeneous characteristics enhance the detection of TTA-121 efficacy, the generalizability of current findings regarding the efficacy and safety of TTA-121 should be tested in future trials. Third, considering the exploratory nature of the current study, correction for multiple testing was not used to identify the dose–response relationship among multiple candidate contrasts. A future verification study is also needed to determine the dose–response relationship.

Conclusions

The current multicentre trial investigated 4 weeks of treatment with a wide range of doses of TTA-121, a novel oxytocin nasal spray with enhanced bioavailability, and revealed an inverted U-shape dose–response relationship with a peak at a dose lower than that expected from previous studies in subjects with ASD. The ASD social core symptoms were significantly improved with the TTA-121 6 U compared with placebo in the PPS. Although the current trial supports the efficacy of TTA-121 on ASD core symptoms and its safety, improvement did not reach statistical significance in the FAS. Thus, the efficacy should be verified in a future large-scale, parallel-group trial.

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Competing interest

There are no conflicts of interest. The investigational drugs in this clinical trial, including TTA-121 and placebo, were provided by Teijin Pharma Limited. Neither the funder nor sponsor had any involvement in the data collection, analyses, writing, or interpretation of the study.

Supplementary material

Supplementary material is available at *Brain* online.

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