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The core symptoms of autism spectrum disorder (ASD) include impaired social communication, repetitive behaviors, and restricted interests. No effective pharmacotherapy for these core deficits exists. Within the domain of social communication, the vasopressin system is implicated in social cognition and social signaling deficits of ASD, and represents a potential therapeutic target. We assessed the effects of a single 20 mg intravenous dose of the arginine vasopressin receptor 1A (V1a) antagonist, RG7713, on exploratory biomarkers (eye tracking), behavioral and clinical measures of social cognition and communication (affective speech recognition (ASR), reading the mind in the eyes, olfactory identification, scripted interaction), and safety and tolerability in a multicenter, randomized, double-blind, placebo-controlled, cross-over study of 19 high-functioning adult male subjects with DSM-IV Autistic Disorder (age 18–45 years; full scale IQ > 70; ABC-Irritability subscale ⩽ 13). Eye-tracking showed an increase in biological motion orienting preference with RG7713 (ES = 0.8, $p = 0.047$) and a non-significant improvement in the composite score (ES = 0.2, $p = 0.29$). RG7713 reduced ability to detect lust (ES = − 0.8, $p = 0.03$) and fear (ES = − 0.7, $p = 0.07$) in ASR. However, when all eight individual emotion subscales were combined into an overall ASR performance score, the reduction was non-significant (ES = − 0.1, $p = 0.59$). Thirteen adverse events were reported in 10 subjects; all were of mild (11/13) or moderate (2/13) severity. Although interpretation should be cautious due to multiple comparisons and small sample size, these results provide preliminary evidence from experimental and behavioral biomarkers, that blockade of the V1a receptor may improve social communication in adults with high-functioning ASD. ClinicalTrials.gov identifier: NCT01474278 A Study of RO5028442 in Adult Male High-Functioning Autistic Patients. Available at: https://clinicaltrials.gov/ct2/show/NCT01474278
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INTRODUCTION

Autism spectrum disorder (ASD) is a heterogeneous developmental condition characterized by impairment in reciprocal social behavior and communication (social communication), and repetitive behaviors and restricted interests (restricted, repetitive behaviors) (American Psychiatric Association, 2013). In the American Psychiatric Association's most recent diagnostic guidelines (DSM-V), the term ASD unifies former separate diagnostic categories of Autistic

Disorder with Asperger's Disorder, and Pervasive Developmental Disorder—Not Otherwise Specified (PDD-NOS), capturing the spectrum of features and impairment (American Psychiatric Association, 2013). The estimated prevalence of ASD is 7.6 per 1000 (1/132) persons worldwide, equivalent to 52 million cases in 2010 (Baxter *et al*, 2015; Centers for Disease Control, 2014). Current pharmacological treatments for ASD commonly include antipsychotics, antidepressants, and psychostimulants, targeting associated symptoms such as aggression, self-injurious behavior, impulsivity and irritability, stereotypies, hyperactivity, anxiety and mood symptoms, but no efficacious pharmacotherapy for the core symptoms of ASD exists (Ji and Findling, 2015). Consequently, there is a critical need for treatments that target the lifelong burden of core deficits of social communication and restricted repetitive behaviors.

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Social cognition is an integral part of social communication, comprising perception and processing of socially salient, visual and auditory information, and also higher order integrative functions including empathy and theory of mind (Baron-Cohen et al, 1985; Frith and Frith, 2005). ASD-related differences have been identified at multiple levels of social cognition and communication. The oxytocin and vasopressin systems play a critical role in social cognition and social signaling deficits of ASD, and may be promising therapeutic targets (Andari et al, 2010; Hollander et al, 2007). The importance of the vasopressin system in social cognition and behavior is highlighted by studies of variants of the arginine vasopressin receptor 1A (AVPR1A) gene, which encodes the V1a receptor. These studies have shown AVPR1A gene variants to be associated with autism (Kim et al, 2002; Wassink et al, 2004; Yirmiya et al, 2006; Yang et al, 2010) and to modulate amygdala activation during emotional face processing (Meyer-Lindenberg et al, 2009). AVPR1A gene variants have also been associated with specific personality traits such as pair-bonding behavior in healthy volunteers (Meyer-Lindenberg et al, 2009; Walum et al, 2008). Furthermore, intranasal administration of vasopressin modulates a network involved in processing emotional information (the medial prefrontal cortex–amygdala circuitry) (Zink et al, 2010), and has been shown to increase threat perception in healthy volunteers (Thompson et al, 2004), and to impair emotion recognition in males (Uzefovsky et al, 2012). Taken together, modulation of vasopressin signaling represents a potential therapeutic target in ASD.

RG7713 is a potent and highly selective V1a receptor antagonist, which may provide a novel approach to treat core deficits of ASD (Ratni et al, 2015). A phase I single-ascending dose (SAD) study of RG7713 (intravenous administration) was previously conducted in healthy volunteers in order to provide safety, tolerability, and pharmacokinetic information. The SAD study confirmed brain penetration of RG7713 by measuring its concentration in the cerebrospinal fluid (CSF), and indicated that the highest dose tested, 20 mg, provides V1a receptor occupancy in the CNS of approximately 90% for at least 8 h, with no safety concerns (a summary of pharmacokinetic data is presented in Supplementary Table S1) (F. Hoffmann-La Roche, 2015). Dose selection for clinical studies was based on the results of dose–response studies in the rat valproate model of autism, which demonstrated a maximum efficacy at 86% receptor occupancy. In addition, PET studies of different marketed GPCR antagonists have demonstrated that a receptor occupancy of 60–80% is needed for clinical efficacy (Grimwood and Hartig, 2009). Thus, we chose a single intravenous 20 mg dose of RG7713 to explore its effects on exploratory behavioral biomarkers of social cognition and behavioral and clinical measures of social cognition and communication in high functioning adult subjects with autistic disorder. Safety and tolerability of RG7713 were also assessed.

MATERIALS AND METHODS

This study (NCT01474278) was a multicenter (three sites), randomized, double-blind, placebo-controlled, two period crossover study of a single dose of RG7713 in adult male subjects with high-functioning ASD ($n = 19$). The study was conducted under an experimental IND (which limited the number of participants and the duration of treatment) and in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines (International Conference on Harmonisation, 1996; World Medical Association, 2008). The study protocol and all accompanying documentation were approved by institutional review boards (IRBs) at UCLA (UCLA IRB, Los Angeles, CA, USA), Yale University (Yale Human Investigation Committee, New Haven, CT, USA), and Albert Einstein College of Medicine (Albert Einstein West Campus IRB, Bronx, NY, USA). All participants provided written informed consent for involvement in the study.

A screening examination was performed within 5 weeks before the start of the study and included electrocardiograms (ECG), vital signs, physical examination, laboratory testing (hematology, chemistry, urinalysis, serology, and alcohol and drugs of abuse), clinical evaluation by clinicians with expertise in ASD diagnosis and treatment, and assessments with the Autism Diagnostic Observation Schedule (ADOS) (Lord et al, 1989), Wechsler Abbreviated Scale of Intelligence-Second Edition (WASI-2) (Wechsler, 1999), Columbia-Suicide Severity Rating Scale (C-SSRS) (Posner et al, 2011), Aberrant Behavior Checklist (ABC) (Aman et al, 1985), and Vineland Adaptive Behavior Scales-Second Edition (VABS-II) (Sparrow et al, 2005). Key inclusion and exclusion criteria are presented in Supplementary Table S2.

A single 20 mg dose of RG7713 or placebo was administered intravenously as a 2-h infusion to each subject on two different days (treatment visits 1 and 2) separated by a 7–14-day washout period (Figure 1). Based on the pharmacokinetics observed in the SAD study (half-life = 7.5–9 h), this washout period between administration of RG7713 and placebo was considered sufficient to avoid carryover effects. Subjects received RG7713 and placebo in one of two sequences: Sequence 1—subjects received 20 mg RG7713 during treatment visit 1 followed by placebo during treatment visit 2 and Sequence 2—subjects received placebo followed by RG7713. Subjects were randomized to sequence 1 or 2 using a randomized treatment schedule that was developed by the study sponsor for each site and incorporated into double-blind (investigator and subject) treatment labeling.

During each treatment visit, the investigator carried out baseline assessments and checks before administration of study drug. Efficacy assessments—key exploratory behavioral biomarkers, behavioral and clinical measures of social cognition and communication, an abbreviated version of ABC, Clinical Global Impression-Improvement scale (CGI-I) (Guy, 1976) and State-Trait Anxiety Inventory (STAI) (Spielberger and Gorsuch, 1983)—were performed before and after, or after drug administration, as shown in Figure 1. Safety assessments included physical examination, review of adverse events (AEs), C-SSRS, ECG, vital signs and laboratory tests, also obtained pre- and post-infusion.

Efficacy assessments were selected on the basis of two key considerations. Firstly, to provide an evaluation of potential drug effects at different system levels of social cognition and communication, including a basic level of screening and acquiring socially relevant information (eye tracking),
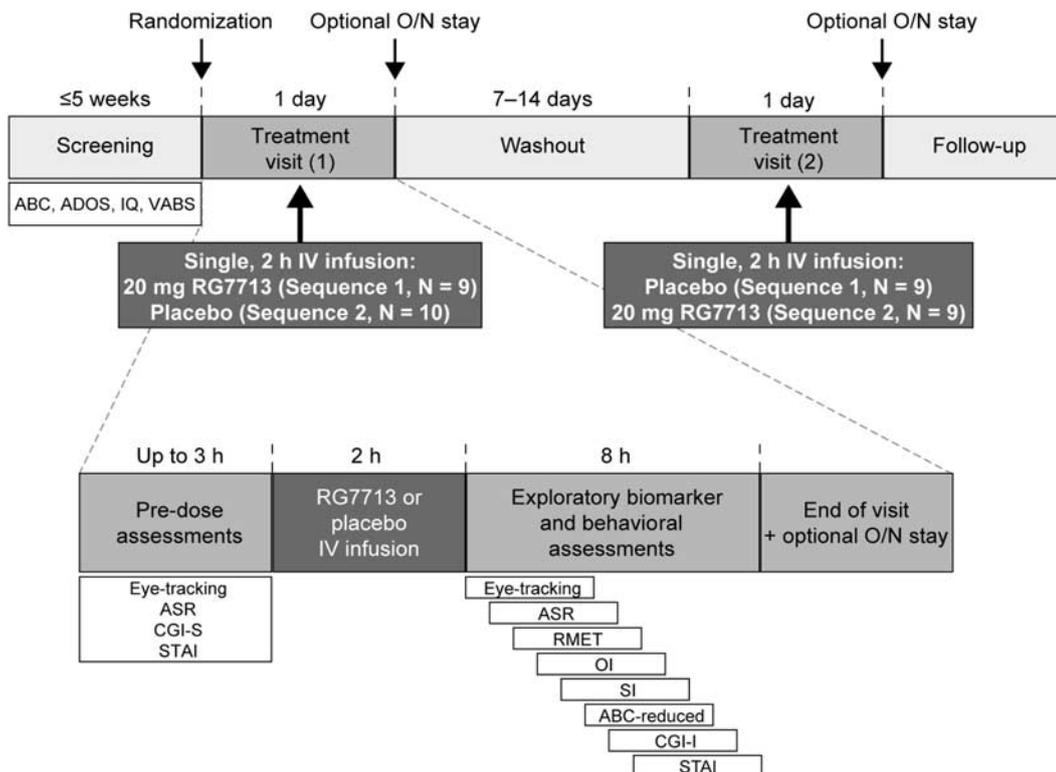
Figure 1 Study design. Abbreviations: ABC, Aberrant Behavior Checklist; ADOS, Autism Diagnostic Observation Schedule; ASR, affective speech recognition; CGI-I, Clinical Global Impressions—improvement; CGI-S, Clinical Global Impressions—severity; h, hour; IV, intravenous; IQ, intelligence quotient; OI, olfactory identification; O/N, overnight; RMET, reading the mind in the eyes test; SI, scripted interaction; STAI, State-Trait Anxiety Inventory; VABS, Vineland Adaptive Behavior Scale.

and intermediate levels corresponding to the ability to capture composite information that is critical for social communication (ASR, RMET) which would be mirrored in behavioral aspects of social communication (ABC, scripted interaction). Secondly, the choice of assessments was based on previous evidence of sensitivity to pharmacological intervention. This second consideration was only fulfilled for eye tracking, with these measures previously shown to be sensitive to administration of oxytocin (Andari et al, 2010).

An abbreviated version of the ABC was used as some of the items of this scale pertain to behaviors that can only be observed in a social context and were deemed inappropriate to assess the short-term effects of a pharmacological intervention. The items were selected by the investigators in this study (EH, JTM, LS) and included items 2, 4, 9, 10–12, 14, 16, 17, 19, 20, 22, 23, 27, 29, 33, 35–37, 40–43, 45–47, 49, and 58 from the 58-item full scale (Aman et al, 1985).

Eye-Tracking

A Tobii T60XL 60 Hz eye-tracking system was used to measure gaze position and pupil size in the following paradigms shown to differ between subjects with ASD and healthy volunteers: patterns of scanning during free-viewing of two actresses engaged in simple play activities (activity monitoring) (Shic et al, 2011); detection of biological motion vs phase-scrambled biological motion (biodetection) (Kaiser et al, 2010); preference for biological motion over phase-scrambled biological motion or repetitive motion

(biomotion) (Klin et al, 2009); gender discrimination; gaze direction discrimination (Andari et al, 2010); preference for dynamic human activities over dynamic geometric patterns (human activity) (Pierce et al, 2011); patterns of scanning during free-viewing of complex social scenes (clips from the movie Who's Afraid of Virginia Woolf?; WAVW) (Klin et al, 2002). An a priori defined composite eye-tracking score was derived from key parameters of all administered eye-tracking tasks, as a weighted sum of subcomposite scores (each subcomposite score indexing a different eye-tracking task). Further details of the components and calculations used for the composite score are provided in Supplementary Table S3.

Affective Speech Recognition (ASR)

Subjects were played a recording of four sentences of neutral content (eg 'The boy went to the store') with eight different emotional intonations (angry, disgusted, fearful, happy, lustful, neutral, sad, and surprised) (Hollander et al, 2007). Each emotional intonation was repeated six times for a total of 48 sentences with a fixed time interval for presentation and subject scoring. Subjects indicated the emotion by pointing to the corresponding word.

Reading the Mind in the Eyes Test (RMET)

Subjects were presented with 36 pictures of the eye region of different people, and instructed to choose which of four

alternative labels best described what the person might be thinking or feeling (Baron-Cohen et al, 2001).

Olfactory Identification

The 'Sniffin' Sticks' smell identification test is a commercially available test of olfaction (Burghard Medical Technology, Wedel, Germany), which uses 12 pen-like devices, each containing a single odorant (Hummel et al, 2007). The devices were uncapped one at a time for 3 s and then placed 1–2 cm in front of the subject's nostrils. Subjects indicated the odorant among a field of four choices.

Scripted Communication and Interaction Test

The Scripted Communication and Interaction Test (SCIT) assessed change in communication domains in short-term treatment. The measure has two forms that are suitable for repeated administration with short intervals (in the order of weeks). A trained examiner conducts a structured 15–20-min conversational interaction using explicit and scripted prompts that focus on the following domains: social awareness and response to others; verbal and nonverbal responsiveness to the examiner; initiation of communication;

Table 1 Baseline Characteristics

Characteristics	Mean (SD), N = 19
Age, years	23.4 (5.1)
Weight, kg	79.5 (19.6)
BMI, kg/m^2	25.7 (5.5)
Full scale IQ (Wechsler)[a]	100 (14.5)
Verbal IQ	98 (17.4)
Performance IQ	101 (11.1)
ADOS total score	12 (4.8)
Communication score	3 (1.7)
Reciprocal social interaction score	6 (2.4)
Communication+social interaction score	10 (3.7)
Vineland II Adaptive Behavior score	62 (12.9)
Adaptive behavior composite	63 (12.5)
Communication	65 (21.8)
Daily living skills	66 (9.6)
Socialization	65 (13.0)
ABC-full total score	27 (19.9)
Irritability	3 (3.9)
Lethargy/social withdrawal	9 (6.7)
Stereotypic behavior	3 (2.6)
Hyperactivity	9 (9.3)
Inappropriate speech	3 (2.7)
CGI-Severity	4[b] (0.8)

Abbreviations: ABC, Aberrant Behavior Checklist; ADOS, Autism Diagnostic Observation Schedule; BMI, body mass index; IQ, intelligence quotient; SD, standard deviation; WASI, Wechsler Abbreviated Scale of Intelligence.
[a]Full-scale IQ was derived from subsets of WASI.
[b]Moderately ill.

conversational turn-taking; appropriateness during interaction; and emotional insight. At the end of the interview, the social and interactional skills are scored from 1 (behavior is absent, deficient, or odd) to 5 (typical for age) for each of the six domains; higher scores correspond to better social communication and interaction.

Order of Assessments

Owing to the number of measures, eye-tracking tasks and the ASR were conducted before and after drug administration (as a 2-h intravenous infusion), while the other assessments were performed after completion of the infusion (between 2 and 8 h; CNS exposure was shown to remain constant up to 8 h (the last time-point assessed) post-infusion in the SAD study).

Statistical Analysis

Behavioral and questionnaire end points were analyzed using mixed model analysis of variance (ANOVA), with baseline measurement fitted in the model as a covariate if a pre-dose value was available. For each end point, the treatment difference (RG7713 – placebo) was estimated together with two-sided 90% confidence interval (CI). Effect sizes (ES; standardized mean differences) were calculated to allow comparison of effects across the different assessments and were considered as small (0.2), medium (0.5), or large (0.8) (Cohen, 1988; Faraone, 2008).

In this crossover study, a sample size of approximately 20 subjects was considered sufficient to detect major tolerability and safety issues with RG7713 vs placebo. In addition, assuming a one-sided significance level of 5%, a sample size of 18 would provide a power of more than 80% to detect an ES of 0.7.

In line with the exploratory nature of this proof of mechanism study, no statistical control for type-I error from the multiple comparisons was pursued, and p-values should be interpreted with care, as descriptive weights of evidence rather than as confirmatory claims.

RESULTS

Demographics

Nineteen subjects with a diagnosis of autistic disorder (DSM-IV-TR) were randomized between December 2011 and February 2013 to receive study drug and placebo in Sequence 1 (9 subjects) and Sequence 2 (10 subjects) (Supplementary Figure S1). All subjects completed the study. One subject was wrongly administered placebo during Period 2 after already receiving placebo during Period 1; this subject's data from Period 2 were excluded from the per-protocol analysis population. Another subject was wrongly administered study drug and placebo in reverse order; this subject's data were assigned to the treatment actually received and included in all analysis populations.

Subjects had a mean (SD) age of 23 (5.1) years, full scale IQ of 100 (14.5), VABS-II score of 62 (12.9), and CGI-Severity score of 4 (0.8), that is, were moderately ill. Further details of baseline characteristics are presented in Table 1.

Pharmacokinetics

Plasma concentrations following the intravenous infusion of 20 mg RG7713 over 2 h were comparable in adult male subjects with ASD (mean $\pm$ SD concentration of 129 ± 64.4 ng/ml at 2 h post-dose; and 45.6 ± 12.9 ng/ml at 8 h post-dose) and healthy subjects in the SAD study (130 ± 16.8 ng/ml at 2 h post-dose and 46.9 ± 8.44 at 8 h post-dose; Supplementary Table S1).

Efficacy

Eye-tracking results showed that administration of RG7713 was associated with a large and statistically significant increase in biological motion orienting preference (ES = 0.8, $p = 0.047$) and a non-significant improvement in the composite score (ES = 0.2, $p = 0.29$) (Table 2). RG7713 was associated with a significantly reduced ability to detect lust in ASR (ES = -0.8, $p = 0.03$) and a non-significant reduction in the ability to detect fear (ES = -0.7, $p = 0.07$). However, when all eight individual emotion subscales were combined into an overall ASR performance score, the change was non-significant (ES = -0.1, $p = 0.59$) (Table 2). Treatment with RG7713 was also associated with small, non-significant improvements in RMET performance, olfactory identification, and overall assessment of ASD with the CGI-I (RG7713: mean $\pm$ SD = 3 ± 0.8, minimally improved *vs* placebo: mean $\pm$ SD = 4 ± 0.5, no change). A summary of results for all efficacy end points is provided in Table 2; results for other efficacy endpoints did not reach statistical significance.

Safety

Four AEs were reported in five subjects after treatment with RG7713, but not after placebo: dizziness ($n = 2$), disturbance in attention ($n = 1$), infusion site rash ($n = 1$), and anxiety ($n = 1$). All AEs reported (13 AEs in 10 subjects) were of mild or moderate severity, with the moderate AEs (2 AEs in 2 subjects) all reported during the placebo treatment period. Most AEs (10/13) resolved within 1 day of onset, and all resolved within 2 weeks. There were no serious AEs reported, no early terminations, and no clinically relevant changes in laboratory parameters, ECG, or vital signs were observed (Supplementary Table S4).

Post-dose anxiety levels measured by the STAI showed small improvements from baseline. However, no treatment differences were observed (RG7713 period pre-dose: mean $\pm$ SD = 31 ± 7.8, $n = 18$; RG7713 period post-dose: mean change from baseline $\pm$ SD = -2 ± 4.8 points, $n = 18$, *vs* placebo period pre-dose: mean $\pm$ SD = 31 ± 8.4, $n = 19$; placebo post-dose: mean change $\pm$ SD = -4 ± 8.1 points, $n = 19$).

DISCUSSION

We used exploratory biomarkers and behavioral assessments comprising eye-tracking paradigms, ASR, RMET, and olfactory identification along with the SCIT to investigate the effects of the novel, potent and highly selective V1a receptor antagonist, RG7713, on measures associated with communication and social cognition in high functioning adult subjects with ASD. The subjects in this study presented

diagnostic profiles, and impairments in RMET performance and olfaction identification similar to those of subjects with ASD reported in previous studies (Hus and Lord, 2014; Baron-Cohen *et al*, 2001; Suzuki *et al*, 2003; Bennetto *et al*, 2007; Galle *et al*, 2013; Ashwin *et al*, 2014; Wicker *et al*, 2016), but displayed higher ASR performance (57% *vs* ~ 41%) than previously reported (Hollander *et al*, 2007). Although statistically significant effects were limited to increased orienting preference for biological motion in eye tracking and decreased ability to detect lust in ASR, this study provides preliminary evidence of a positive effect of RG7713 on social cognition. No safety signals associated with administration of RG7713 were identified.

The goal of this study was to gather evidence for V1a receptor antagonism as a potential treatment for deficits in social communication in ASD. Targeting complex behavioral domains such as social cognition and communication in ASD in a single-dose, proof-of-mechanism study is a challenging but necessary first step. Typical rating scales used in ASD target relatively heterogeneous groups of behaviors and were not originally developed to sensitively assess social communication or more narrow components of social responsiveness in response to a single dose of a drug, and were therefore considered unsuitable as primary pharmacodynamic variables in this study.

We expected that any therapeutic effects of RG7713 could be identified and quantified more accurately as effects on the component processes underlying social cognition and communication—analogous to results reported with single-dose administration of oxytocin (Andari *et al*, 2010). Thus, we sought to identify effects of RG7713 with exploratory behavioral biomarkers and behavioral assessments relating to these component processes, combined with higher-order behavioral and clinical ratings. These biomarkers and assessments included eye-tracking paradigms, as a measure of attunement to and extraction of socially relevant information, and ASR and RMET, as measures of the ability to detect and process socially relevant information in human communication. We also explored effects on olfaction as a sensory modality assumed to play a role in social interaction, and a novel clinical assessment, the SCIT, to directly evaluate separate domains of social communication.

The observed effects of RG7713 treatment on eye tracking in this study were mostly subtle and their interpretation and extrapolation to behavioral outcomes is challenging. While the overall effects on eye tracking (composite score) suggested an improvement in abnormal eye gaze patterns, statistical significance was not reached. The largest signal (ES = 0.8) was seen in the biomotion paradigm in which subjects administered RG7713 oriented more often (orienting preference) to biological information. If replicated, this may suggest that RG7713 could have positive effects on a behaviorally 'primitive' attentional response to biologically relevant information (Klin *et al*, 2009; Simion *et al*, 2008). It is important to note that reduced attention to biological motion is one of the most highly replicated biomarker findings in ASD (Chita-Tegmark, 2016). Such reductions in social attention have been shown to be specific to ASD, as they are not seen in subjects with other developmental disabilities (Klin *et al*, 2009; Pierce *et al*, 2011). Indeed, this marker has been proposed by some as a possible screening test for ASD risk. It is theorized that reduced attention to

Table 2 Efficacy Results

Assessment	Component	LS mean after RG7713 treatment[a]	>LS mean after placebo[a]	Estimated treatment difference RG7713 − placebo (90% CI)	p-value	Effect size
Eye-tracking	*Activity monitoring (ratio)*					
	Activity	0.38	0.41	−0.03 (−0.08, 0.01)	0.23	−0.3
	Background	0.11	0.11	0.00 (−0.02, 0.02)	0.74	0.1
	Background with distractors	0.25	0.24	0.01 (−0.02, 0.03)	0.75	0.1
	Body	0.12	0.13	−0.01 (−0.03, 0.01)	0.30	−0.2
	Distractors	0.14	0.13	0.01 (−0.02, 0.03)	0.58	0.1
	Head	0.25	0.23	0.02 (−0.03, 0.07)	0.46	0.2
	Person	0.37	0.36	0.01 (−0.03, 0.05)	0.62	0.1
	Biodetection					
	D-prime (masked condition)	1.23	1.5	−0.31 (−0.77, 0.16)	0.27	−0.2
	Biomotion					
	Latency (ms)	256.14	275.45	−19.31 (−57.96, 19.35)	0.37	−0.4
	Looking preference (ratio)	0.60	0.60	−0.01 (−0.05, 0.04)	0.73	−0.1
	Orienting preference (ratio)	0.52	0.47	0.05 (0.01, 0.08)	0.05	0.8
	WAVW (ratio)					
	Background	0.12	0.13	−0.01 (−0.03, 0.01)	0.35	−0.2
	Body	0.12	0.10	0.02 (−0.00, 0.03)	0.13	0.3
	Eyes	0.38	0.38	0.00 (−0.21, 0.21)	0.10	0.0
	Head	0.75	0.75	−0.00 (−0.03, 0.02)	0.80	0.0
	Mouth	0.25	0.29	−0.05 (−0.11, 0.02)	0.23	−0.4
	Gaze discrimination (ratio)					
	Eyes	0.39	0.39	−0.00 (−0.06, 0.06)	0.97	0.0
	Inside face	0.93	0.95	−0.02 (−0.04, 0.00)	0.11	−0.4
	Mouth	0.07	0.07	0.00 (−0.02, 0.02)	0.83	0.0
	Nose	0.32	0.36	−0.05 (−0.09, −0.00)	0.09	−0.3
	Gender discrimination (ratio)					
	Eyes	0.22	0.26	−0.04 (−0.08, −0.01)	0.07	−0.4
	Inside face	0.93	0.96	−0.03 (−0.05, −0.00)	0.08	−0.5
	Mouth	0.10	0.08	0.02 (−0.01, 0.05)	0.26	0.2
	Nose	0.45	0.47	−0.02 (−0.10, 0.06)	0.63	−0.2
	Human activity (ratio)					
	Preference (ratio)	0.67	0.66	0.00 (−0.12, 0.13)	0.95	0.0
	Composite score	0.19	−0.01	0.20 (−0.12, 0.52)	0.29	0.2
	Pupillometry (mm)					
	Activity monitoring	3.50	3.56	−0.06 (−0.16, 0.05)	0.34	−0.1
	Biodetection	4.12	4.33	−0.21 (−0.53, 0.10)	0.25	−0.4
	Biomotion	4.22	4.26	−0.05 (−0.24, 0.14)	0.67	−0.1
	WAVW	4.00	4.03	−0.03 (−0.24, 0.18)	0.77	−0.1
	Gaze discrimination	3.48	3.48	0.01 (−0.14, 0.15)	0.93	0.0
	Gender discrimination	3.50	3.51	−0.01 (−0.11, 0.10)	0.87	0.0
	Human activity	3.57	3.65	−0.08 (−0.21, 0.05)	0.30	−0.2
ABC reduced	Total score	9.35	8.39	0.96 (−0.34, 2.25)	0.25	0.1
	Inappropriate speech	3.39	2.83	0.56 (−0.03, 1.51)	1.15	0.2
ASR	Emotion (% responses)					
	Angry	52.90	51.65	1.24 (−14.02, 16.51)	0.89	0.0
	Disgusted	65.39	65.26	0.13 (−12.37, 12.63)	0.99	0.0
	Fearful	55.82	75.47	−19.64 (−36.92, −2.37)	0.07	−0.7
	Happy	65.85	61.57	4.28 (−8.74, 17.29)	0.57	0.2

Table 2 Continued

Assessment	Component	LS mean after RG7713 treatment[a]	>LS mean after placebo[a]	Estimated treatment difference RG7713 − placebo (90% CI)	p-value	Effect size
	Lustful	41.17	64.46	− 23.29 (−39.04, − 7.53)	0.03	− 0.8
	Neutral	67.10	65.92	1.19 (−9.18, 11.55)	0.84	0.0
	Sad	61.81	6.46	1.35 (−16.43, 19.14)	0.89	0.0
	Surprised	69.70	64.73	4.97 (−6.11, 16.04)	0.44	0.2
	Correct answers	53.89	56.59	− 2.70 (−11.33, 5.94)	0.59	− 0.1
CGI	Improvement	3.43[b]	3.52	− 0.08 (−0.40, 0.24)	0.65	− 0.1
RMET	Correct answers (%)	63.72	61.22	2.50 (−1.19, 6.19)	0.25	0.2
Scripted communication and interaction test	*Domain (score)*					
	Social awareness of and responsiveness to other (non-verbal)	3.82	3.80	0.02 (−0.27, 0.31)	0.91	0.0
	Awareness of and responsiveness to other (verbal)	4.23	4.06	0.18 (−0.03, 0.38)	0.15	0.2
	Initiation of communication	3.91	3.85	0.07 (−0.20, 0.33)	0.67	0.1
	Conversational turns	3.76	3.69	0.07 (−0.16, 0.30)	0.60	0.1
	Appropriateness to interaction	3.44	3.64	− 0.21 (−0.48, 0.07)	0.21	−0.2
	Insight/ability to describe emotion	3.08	2.84	0.24 (−0.16, 0.63)	0.31	0.2
Olfactory identification	Correct answers (%)	79.40	76.29	3.12 (−1.97, 8.20)	0.30	0.2
STAI scale	Total score	29.01	26.75	2.27 (−0.95, 5.48)	0.24	0.3

Abbreviations: ABC, Aberrant Behavior Checklist; ASR, affective speech recognition; CGI, Clinical Global Impressions scale; LS, least squared; CI, confidence interval; ms, milliseconds; STAI, State-Trait Anxiety Inventory; RMET, reading the mind in the eyes test; WAVW, *Who's Afraid of Virginia Woolf?*
[a]Behavioral and questionnaire end points were analyzed using mixed model analysis of variance (ANOVA), with baseline measurement fitted in the model as a covariate if a pre-dose value was available.
[b]3 = mildly ill; 4 = moderately ill.

biological motion, especially prominent in testing paradigms of more complex and active social scenes, may create a cascading effect leading to impaired social responsiveness. Andari *et al*, (2010) evaluated eye tracking during face scanning in sex and gaze discrimination tasks in a comparable population of high functioning young adults with ASD ($N = 13$, mean age = 26 years, age range = 17–39 years); after treatment with a single dose of intranasal oxytocin, an improvement in visual scanning of faces and the eyes in gaze direction (ES = 0.6), and gender discrimination tasks (ES = 0.5) was observed. The effects of RG7713 on comparable measures in our study were weaker, and the biomotion paradigm on which the strongest effect was observed was not performed by Andari *et al* (2010). The different effects observed with oxytocin and RG7713 in the eye-tracking tasks could indicate that the vasopressin and oxytocin systems modulate different aspects of the process of acquisition of socially relevant information, rather than having simply antagonistic effects as is sometimes assumed.

There were small improvements with RG7713 in the identification of emotions in the RMET and identification of smells in the olfactory identification test; performance in both of these tests is impaired in patients with ASD (Baron-Cohen *et al*, 2001; Rozenkrantz *et al*, 2015). While the RMET and olfactory identification results observed in this study were not statistically significant, they may suggest improvements in social communication. Interestingly, we observed a slight worsening of the total performance in the ASR, largely driven by a decreased sensitivity to recognize fear and lust. Such an effect is consistent with a potential anxiolytic effect of a V1a receptor antagonist, bringing subjects' performance closer to levels observed in healthy volunteers (del Valle Rubido *et al*, 2016; F. Hoffmann-La Roche, 2014).

Results of studies in rodents and non-human primates indicate that increases in vasopressin receptor expression enhance social recognition (Liu *et al*, 2001), cooperative behavior, monogamous and con-specific bonding (Insel *et al*. 1994; Young *et al*, 1997) and social communication measured by flank marking and scent marking (Ferris *et al*, 1984, 1985; Albers *et al*, 1986; Irvin *et al*, 1990; Winslow and Insel, 1991; Hennessey *et al*, 1992), and that differences in neuroanatomical distribution of vasopressin V1a receptors can influence social organization and pair-bonding behavior (Young *et al*, 1999; Lim *et al*, 2004; Walum *et al*, 2008;

Albers, 2012; Caldwell and Albers 2016). Thus, it could be argued that a vasopressin receptor agonist, but not antagonist, may be beneficial to treat ASD. However, vasopressin has also been shown to increase brain response to offensive aggression in rodents (Delville *et al*, 1996; Ferris *et al*, 1997; Bester-Meredith *et al*, 1999; Caldwell and Albers, 2004) and to increase maternal aggression (Winslow and Insel, 1991; Bosch, 2011; Bosch and Neumann, 2010). In humans, intranasal vasopressin administration was shown to increase threat perception in healthy volunteers (Thompson *et al*, 2004) to impair emotion recognition in males (Uzefovsky *et al*, 2012) and to modulate social behavior by increasing response to socially threatening stimuli, measured with BOLD magnetic resonance imaging during a face matching task (Zink *et al*, 2010). This may be the result of the effects of vasopressin on a neural network that combines the subgenual and supragenual cingulate, and amygdala through negative feedback loops (Zink *et al*, 2010). These findings along with the results of this study support the concept that vasopressin receptor antagonism could have a prosocial effect by ameliorating aggressive behavior and reducing threat perception, which would be beneficial for treatment of ASD.

We used a crossover design to control for inter-patient variability and improve the likelihood of identifying significant effects. Nevertheless, the study is limited by the small sample size, single drug dose, short timeframe for assessments (the assessment period was completed within 8 h of drug infusion) and potential selection bias (subjects were all high functioning adult males, with ABC-Irritability subscale scores $\leqslant 13$). In addition, a large number of statistical comparisons were performed without correction for multiplicity, which should be taken into account when interpreting the results.

Overall, the observed effects of RG7713 were quantitatively small, and the paucity of statistically significant results may suggest that the study was underpowered to detect them: the study was designed with a sample size sufficient to detect medium/large effect sizes, but most of the trends observed were of small/medium effects. Although the 8-h efficacy assessment window was supported by the drug exposure data (CSF concentrations remained at approximately the same level during that time, as measured in the SAD study), some effects may only emerge with longer continuous treatment, and may not be apparent yet, or only partially so, in a single-dose study. In addition, to simplify analyses, data were analyzed in aggregate, for example, a single total score was used for each eye-tracking outcome measure despite the presence of multiple assessments and embedded experimental conditions within tasks, potentially diminishing statistical power. Nonetheless, this study adds to the evidence that administration of single doses of potential therapeutic compounds for ASD can result in subtle but possibly meaningful changes in biomarkers and behavioral assessments of social cognition, and may constitute an informative approach to establish confidence in a therapeutic target early in clinical development.

In conclusion, we present initial evidence that treatment with the V1a receptor antagonist, RG7713, provides subtle improvements in social communication surrogates such as eye tracking, ASR, RMET, and olfaction identification, in adults with high-functioning ASD. These preliminary findings of the effects of a single 20 mg dose and the use of these biomarkers support further clinical exploration of V1a receptor antagonism as a therapeutic approach to treat core symptoms in ASD.

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