Human pharmacology of current and novel gaba(a)-ergic treatments for anxiety

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NS11821, A PARTIAL SUBTYPE-SELECTIVE GABA-A AGONIST, ELICITS SELECTIVE EFFECTS ON THE CENTRAL NERVOUS SYSTEM IN RANDOMIZED CONTROLLED TRIAL WITH HEALTHY SUBJECTS

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ABSTRACT

NS11821 is a partial GABA-A agonist with relatively dominant $\alpha_{2,3}$ and α_5 subtype efficacy but negligible α_1 agonism. This first-in-human study was performed in healthy male subjects using a single-dose, parallel, double blind, placebo-controlled, randomized, dose-escalation study design. In total six cohorts (n=48) were enrolled. The eight subjects of each cohort received NS11821 (10 mg, 30 mg, 75 mg, 150 mg, 300 mg or 600 mg) or placebo in a 6:2 ratio. At low dose levels, NS11821 had a relatively low exposure and a more-than-proportional increase of AUC and C_{max}, probably due to poor solubility. Saccadic peak velocity (SPV) decreased in a dose-related manner while limited impairments were seen on body sway and the visual analogue scale (VAS) for alertness. The most common adverse events were somnolence and dizziness, which were more prominent with the higher doses. Although no positive control was used in this study, the results were compared *post hoc* to a CHDR dataset for lorazepam 2 mg. The maximum SPV effects seemed comparable to the typical effects lorazepam, whereas the other CNS effects were smaller. These results support the pharmacological selectivity of NS11821 and show that pharmacodynamic effective doses of NS11821 were safe and well tolerated in healthy subjects.

INTRODUCTION

Benzodiazepines (BZDs) are one of the most commonly prescribed anxiolytic drugs, although therapeutic guidelines generally limit their use to several weeks. The use of BZDs is restricted by tolerance and dependence, as well as concomitant psychomotor impairments. In general, the effect profile of BZDs is attributed to their non-selective agonism at the α_1, α_2, α_3, and α_5 subunit-containing GABA-A receptors. Preclinical studies have linked these subtypes to different pharmacological aspects of BZDs: 1) α_1-containing receptors are associated with sedative and motor effects (McKernan et al., 2000; Rowlett et al., 2005); 2) α_2- and α_3-containing receptors are related to anxiolysis and analgesia (Knabl et al., 2008; Knabl et al., 2009); and 3) α_5-containing receptors are involved in amnesic effects (Atack et al., 2006; Ballard et al., 2009). In order to minimize the untoward depressive effects on the central nervous system (CNS), several novel $\alpha_{2,3}$-subtype selective GABAergic compounds are being developed in preclinical and clinical phases and they are expected to deliver anxiolytic effects with less adverse effects.

According to the *in vitro* two-electrode voltage-clamp electrophysiological assessments performed on receptors expressed in oocytes, although the *in vitro* binding affinity is generally comparable (Ki [nM] = 1.6, 9.7, 3.8, 2.5 for α_1, α_2, α_3 and α_5 subunits, respectively, for different human GABA-A receptor subtypes expressed in HEK cells), NS11821 has relatively higher maximum efficacy for GABA-A α_2, α_3 and α_5 over GABA-A α_1 receptors: compared to diazepam, NS11821 showed 17%, 40% and 41% relative efficacy at the α_2, α_3 and α_5 subunits, but 4% relative efficacy for the α_1 subunit, respectively (Neurosearch data on file). The EC50 of NS11821 was 59, 73 and 44 nM for the *in vitro* pharmacological effects on human GABA-A α_2ß2γ2s, α_3ß2γ2s and α_5ß2γ2s receptors, respectively. (Neurosearch data on file). Such a profile is translated to low propensity for sedative effects with retained anxiolytic activity: in both the rat conditioned emotional response (CER) test and the plus-maze task (Davis, 1990; Rodgers and Dalvi, 1997), NS11821 dose dependently reduced anxiety-like behavior with a minimum effective dose (MED) of 3 mg/kg, corresponding to a human equivalent dose (HED) of 29 mg. On the other hand, NS11821 was found to increase exploratory motility at doses between 3-30 mg/kg in mice, but significantly reduce motor activity in rats at doses higher than 30 mg/kg. In the rotarod test, NS11821 had no significant effect on rotarod performance in rats up to 100 mg/kg. In contrast, diazepam significantly reduced exploratory motility and rotarod performance at doses≤ 3 mg/kg. In addition, the passive avoidance memory test in mice has been shown sensitive to BZD-induced anterograde amnesia when the drug was administered prior to the learning session. Compared with a single dose of chlordiazepoxide, NS11821 demonstrated little effect on memory in mice with doses from 10 to 100 mg/kg, while chlodiazepoxide did impaire memory as measured by the test. With regard to safety, the no-observed adverse-effect level

(NOAEL) in rats was 10 mg/kg after repeat doses, corresponding to a HED of 1.61 mg/kg (97 mg) (Neurosearch data on file). Taken together, the above findings inferred potential anxiolytic effect of NS11821, with reduced sedative and memory-impairing effects at its pharmacolocally active doses.

Using the Food and Drug Administration guidelines for first-in-man studies, a starting dose of NS11821 10 mg was selected. In this study, NS11821 was orally administrated to healthy male volunteers in six single ascending doses (10 mg, 30 mg, 75 mg, 150 mg, 300 mg and 600 mg) and compared to placebo. The objective was to evaluate the safety, tolerability and pharmacokinetic (PK) profile of the compound as well as to estimate the maximum tolerable dose. The study included a validated battery of CNS measurements, including saccadic eye movement, smooth pursuit, body sway, adaptive tracking, tapping, visual analogue scales (VAS) and memory tests, to evaluate the pharmacodynamic (PD) profile of NS11821. In previous studies conducted in healthy volunteers, BZDs have demonstrated robust effects on VAS-alertness, postural stability, memory and neurophysiological functions. These diverse effects across a wide range of different CNS-regions are thought to account for the widespread distribution of GABA-A receptors throughout the brain, and the non-selective, full agonism of BZDs on these receptors.

Out of the many tests used to evaluate the CNS effects of BZDs, saccadic peak velocity (SPV) and the visual analogue scale (VAS) for alertness were identified as the most sensitive parameters for BZDs (de Visser et al., 2003). Both tests showed consistent effects to various BZDs at different doses. Studies with different $\alpha_{2,3}$ subtype selective GABA-A agonists suggest that impairments of subjective alertness and body sway have been primarily attributed to α_1 stimulation, reduction of SPV seems related to mainly reflect α_{2-3} stimulation (Chen et al., 2012), and memory effects could be related to α_5 stimulation (Collinson et al., 2002). As a result, we employed this battery to measure the pharmacodynamic effects of NS11821 in healthy volunteers. In addition, a *post hoc* comparison was performed with a historic data set based on a considerable number of similar studies conducted at CHDR with a therapeutic dose of the full benzodiazepine lorazepam.

METHODS

DESIGN

This was a single-dose, parallel, double blind, placebo-controlled, randomized, dose escalation study in healthy male subjects. There were 8 subjects per dose cohort. Decisions for dose escalation were based on investigator blinded interim assessments of PK, PD and safety results.

SUBJECTS

Forty eight healthy male volunteers were recruited from the Centre for Human Drug Research (CHDR) database. All volunteers gave written informed consent and were medically screened before entry into the study. Subjects were not allowed to smoke more than five cigarettes per day and had to refrain from smoking during the study days. In the 48 hours prior to the study days they were asked not to drink alcohol and to avoid xanthine- containing drinks until the end of the study days. The use of medication was not allowed during the study period (except occasional use of paracetamol, up to 1.5 g per day). The study was approved by the Medical Ethics Review Board of Leiden University medical Centre, the Netherlands.

TREATMENTS

A total of six study cohorts (n=48) received capsules containing 10 mg, 30 mg, 75 mg, 150 mg, 300 mg and 600 mg NS11821 or placebo with 250 mL of water. In each cohort, six subjects were randomized to receive a single dose of NS11821 and two subjects received placebo. A standard lunch was served 2 hr post-dose together with approximately 200 mL water.

SAFETY

Adverse events, electrocardiogram (ECG), blood pressure and heart rate measurements were collected throughout the study. Twelve lead ECG recordings were made using Electrocardiograph Marquette 5000/5500 (USA). Continuous real time telemetry (1 lead ECG) and pulse oxymetry were performed with GE Marquette (USA) and DASH 4000 (USA) respectively. Blood pressure and heart rate were assessed using a Nihon Kohden BSM 1101K monitor (Japan) or a Dash 4000 (USA). All ECG, blood pressure and heart rate measurements were made after the subject had been resting in a supine position for at least 5 minutes. In addition, for the evaluation of orthostatic blood pressure and heart rate, subjects were required to stand for 3 minutes prior to a second assessment.

PHARMACOKINETICS

Whole blood samples and urine samples were taken for assay of the parent drug NS11821 and its metabolite NS14606. Blood samples were taken 1 hour pre-dose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 15, 22, 34 and 48 hours post-dose. The blood was drawn in 10 mL K2 EDTA tubes and then centrifuged (2000 G, 10 minutes, at 2-8°

C), transferred to 3.5 mL Sarsted tubes and stored at -80° C within 30 minutes after sampling. All urine voids were collected throughout the study days and combined in intervals: last 2 hours pre-dose, first 10 hours post-dose, 10-12 hours post-dose and 12-22 hours post-dose. Two 6 ml samples (duplicate) from each time interval were stored at -40° C. Analysis of the blood and urine samples was performed at PRA International (Assen, the Netherlands), using LC MS/MS and validated bio analytical methods. The lower limit of quantification (LLOQ) for plasma NS11821 and NS14606 was set to 0.1 ng/mL.

PHARMACODYNAMICS

All subjects were trained to be familiarized with the psychometric tests during the screening sessions to minimize learning effects preceding the study. During the treatment period, pharmacodynamic measurements were performed twice pre-dose and 0.5, 1, 1.5, 2, 3, 4, 6 and 10 hours post-dose. The CNS tests were performed in a quiet room with subdued illumination with only one subject in the same room per session. Each session consisted of the following sequence of tests: saccadic eye movements; smooth pursuit; pharmaco-EEG, body sway with eyes closed; VAS Bond and Lader; VAS Bowdle; adaptive tracking and tapping. Cognitive function tests were performed at 2 and 6 h post-dose.

SACCADIC EYE MOVEMENT

Measurements of saccadic eye movements were recorded as previously described (de Haas et al., 2008; de Haas et al., 2009). Average values of saccadic peak velocity (SPV), latency (= reaction time) and inaccuracy were calculated for all artefact free saccades. SPV is closely related to their anxiolytic properties (de Visser et al., 2003) and its measurement has been validated as the most sensitive biomarker for the effects of BZDs (de Visser et al., 2003; van Steveninck et al., 1991; van Steveninck et al., 1992; van Steveninck et al., 1999).

SMOOTH PURSUIT

Smooth pursuit was measured as previously described (de Haas et al., 2009). The time in which the eyes were in smooth pursuit of the target was calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was the target parameter.

BODY SWAY

Two minute body sway measurements were performed as previously described (de Haas et al., 2009). Body sway is a measure of postural stability that has previously been shown to be sensitive to BZDs (van Steveninck et al., 1996).

VISUAL ANALOGUE SCALE

The visual analogue scales (VAS) in this study were used as previously described by Norris (Norris, 1971). The Bond and Lader VAS was performed to measure subjective alertness, mood and calmness (de Haas et al., 2009). The Bowdle VAS evaluates psychedelic effects clustered into three distinct total sum scores: internal perception (reflects inner feelings that do not correspond with reality, including mistrustful feelings), external perception (reflects a misperception of an external stimulus or a change in the awareness of the subject's surroundings) and feeling high (Zuurman et al., 2008).

ADAPTIVE TRACKING

The adaptive tracking test will be performed as originally described by Borland and Nicholson (Borland and Nicholson, 1975; Van Steveninck AL, 1993), using customised equipment and software (based on TrackerUSB hard-/software (Hobbs, 2004, Hertfordshire, UK)). The average performance and the standard deviation of scores over a 3.5-minute period will be used for analysis. This 3.5-minute period is including a run in time of 0.5 minute, in this run in time the data is not recorded. Adaptive tracking is a pursuit-tracking task. A circle moves randomly about a screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle.

TAPPING

The test has been adapted from the Halstead Reitan Test Battery (Andrew, 1977), and evaluates motor activation and fluency. The volunteer is instructed to tap as quickly as possible with the index finger and to rest the wrist on the table. The space bar is used as tapping device and each session contains five performances of 10 seconds. The mean tapping rate and the standard deviations for the dominant hand are used for statistical analysis.

PHARMACO-EEG

Pharmacoelectroencephalography (pharmaco-EEG) recordings were performed as previously described (de Haas et al., 2010). EEG recordings were made at Fz, Cz, Pz and Oz. For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta, theta, alpha and beta frequency ranges. The duration of EEG measurements was 64 s per session. Change in amplitudes in the beta frequency band of the EEG is found to be a relevant measure of the pharmacological effect intensity of BZDs (Mandema et al., 1992).

VISUAL VERBAL LEARNING TESTS (VVLT)

Short term and long term memory was similarly tested as recently described in another publication (de Haas et al., 2009). The 30 word memory learning test was performed at 2 h post-dose with 'Immediate Recall' immediately hereafter. Approximately 4 h after start of the Immediate Recall test, 'Delayed Recall' was performed and followed by the 'Delayed Recognition'.

STATISTICAL ANALYSIS

PHARMACOKINETICS

The plasma PK parameter estimates were calculated in WinNonlin Version 5.2 (Pharsight Corporation, USA) using non compartmental analysis of the individual plasma concentrations of NS11821 and NS14606. The area under the curve (AUC) was calculated using the linear trapezoidal method. Terminal rate constants were estimated by fitting a linear regression of log concentration against time. Other parameters determined were: maximum plasma concentrations (C_{max}), time to maximum plasma concentration (T_{max}) and elimination half-life ($T_{1/2}$).

PHARMACODYNAMICS

The PD parameters were analyzed by mixed model analyses of covariance (ANCOVA) with treatment, time and treatment by time as fixed effects, with subject as random effect, and with the baseline value as covariate, where baseline was defined as the average of the available values obtained prior to dosing. The six NS11821 treatments (10 mg, 30 mg, 75 mg, 150 mg, 300 mg and 600 mg) were compared to placebo and used as contrasts within the ANCOVA model. The cognitive function test resulted in single measured pharmacodynamic data for which the fixed factor treatment ANOVA model was used. All variables were analyzed untransformed except for body sway and EEG results, which were log transformed prior to analysis to correct for the expected log normal distribution. Treatment effects were reported as contrasts where the average of the measurements up to last time point, were calculated within the statistical model. Contrasts were reported along with 95% confidence intervals (CIs) and analyses were two sided with a significance level of 0.05. All calculations were performed using SAS for windows V9.1.2 (SAS Institute, Inc., Cary, NC, USA).

Pre-study power calculation based on previous studies conducted at CHDR (de Haas et al., 2007; de Haas et al., 2008; de Haas et al., 2009) with lorazepam 2 mg, revealed that 6 subjects in each group, should provide 80% power, to detect a reduction in SPV of at least 50.3 degrees per second after active treatment compared to placebo, assuming a common standard deviation of 15.0 degrees per second using a two group t-test with a 5% two sided significance level. Similarly, seven subjects

in each group should provide an 80% power to detect a difference of -11.2 mm in VAS-alertness assuming that the common standard deviation is 6.5 mm using a two group t-test with a two-sided significance level of 5%.

POST HOC COMPARISON WITH HISTORIC LORAZEPAM DATA SET

No active control, e.g. benzodiazepine, was used in this first in man study for NS11821. Such a control might have complicated the blinded effect assessments and decisions on dose escalations. Recently, we reported a comparative study of several different subtype selective GABA-A partial agonists with lorazepam, which had been used as a positive control in a number of trials that basically employed the same methods that were also used in this first-in-man study of NS11821 (Chen et al., 2012). The combined lorazepam data from this historic data set were used for a *post hoc* comparison of the pharmacodynamic effects of NS11821 with those of lorazepam.

In the *post hoc* analysis the relationship of individual changes from baseline against the change from baseline of SPV (ΔSPV) was done for: body sway (Δsway), tracking (ΔTracking), VAS-alertness (ΔVAS-alertness), and smooth pursuit (Δsmooth). These data were then compared to the profile from a full, non-selective GABA-A agonist (Lorazepam 2 mg). The slopes of these regression lines are thought to reflect the relation between the effect profile of anxiolysis and sedation (Chen et al., 2012). A mixed effect model was used, where the fixed factors were treatment and treatment by SPV, whereas the random factors were subject slope and intercept. The estimate of the slopes of the regression lines of these ΔSPV-relative effect profiles were compared between three high doses of NS11821 (150 mg, 300 mg, 600 mg) and lorazepam.

RESULTS

SUBJECTS

Sixty seven male volunteers were medically screened after given written informed consent and forty eight were randomized. None of these subjects dropped out of the study; all randomized subjects completed all study days and had a follow up visit. On average (min-max) subjects were 23 (18-41) years old with a weight of 75 (57-101) kg, and a body mass index of 22.9 (18.1-29.7) kg/m^2. The mean body measures were generally comparable among the seven treatment groups, Table 1.

CLINICAL OBSERVATIONS / SAFETY

All forty-eight subjects were included in the safety analysis. No serious adverse events occurred during the study. Neither did subjects withdraw from the study due to adverse events. No clinically significant changes in vital signs or ECG

characteristics were noted for any of the NS11821-treated groups. The highest dose of NS11821 (600 mg) caused the most gastrointestinal and neurological events, with ≥50% subjects experiencing nausea, vomiting, fatigue, dizziness, and/or somnolence. The incidences of these AEs were higher than those reported in the placebo group and were all judged 'probably' treatment-related by the investigator. None of the AEs required medical intervention. Except for two AEs occurring in one subject dosed with NS11821 600 mg, all AEs were classified mild in intensity. This subject suffered from moderate dysphagia, nausea and subsequent vomiting, while trying to swallow the study medication (twelve capsules).

PHARMACOKINETICS

One subject in the 600 mg NS11821 dose group vomited shortly after taking the study medication and the pharmacokinetic data from this subject were excluded from the PK analysis. Figure 1 depicts the average plasma concentration-time curves for NS11821 and its metabolite NS14606 for all 6 doses. Table 2 summarizes the pharmacokinetic parameters of NS11821 and NS14606 by dose. The absorption time was dose dependent; T_{max} increase from 0.5 to 4.0 hr following doses of 10 to 600 mg. A more than proportional increase in exposure was seen; AUC_{inf}/dose increase with a factor 10 from 0.9 at 10 mg to 9.7 at 600 mg. There was considerable variability between subjects was seen for all the pharmacokinetic parameters.

PHARMACODYNAMICS

A total of three subjects, who were administered with NS11821 600 mg, vomited post-dose during performance of the PD tests. Their pharmacodynamics data were excluded from the PD datasets before the statistical analysis.

Compared to placebo, NS11821 600 mg elicited statistically significant effects on a variety of pharmacodynamic parameters, Table 3. At a dose level of 150 mg NS11821 affected EEG ß-band power of the frontal-central leads statistically significantly, a trend was seen at lower doses. NS11821 300 mg was associated with significant effects on SPV, tracking, and EEG ß-band power of the frontal-central leads and the parietal-occipital leads. NS11821 300 mg also reduced the frequency of tapping, with an effect size similar to NS11821 600 mg. No significant effects were observed on body sway or smooth pursuit with any dose of NS11821.

The effects of lorazepam 2 mg are also presented in Figure 2. These results were based on historic data for a number of studies with GABA-A subtype selective partial agonists, in which lorazepam 2 mg was used as a positive control (Xia-Chen et al. 2012). Effects of 2 mg lorazepam on SPV appear to be in line with the effects of higher doses of NS11821 (300-600 mg). Apart from the effect of 600 mg NS11821 on VAS-alertness, lorazepam effects on body sway and VAS-alertness clearly distinct from the effects observed from NS11821.

In addition to the above mentioned psychomotor effects, NS11821 reduced the response accuracy of immediate recall in a dose-dependent manner (600 mg: -7.2 words [-10.7; -3.7], p=0.0001; 300 mg: -3.0 [-5.7, -0.3], p=0.0305). Doses of 75-600 mg NS11821 decreased the number of delayed recalled words in a dose dependent manner without reaching statistical significance, while 75 and 300 mg of NS11821 impaired delayed recognition statistically significantly; 75mg: -5.6 [-9.7,-1.4], p=0.0095 and 300mg -5.2 [-9.4,-1.1], p=0.0143; respectively (Figure 1, Panel D). A historic comparison between the memory effects of lorazepam 2 mg and NS11821 300 mg and 600 mg was made with a previous study that used the same word memory test (De Haas et al. 2009). This analysis suggests that the average percentage decrease relative to placebo is in the same order of magnitude among lorazepam 2 mg, NS11821 300 mg and 600 mg, for immediate recall (-61%, -78% and -48%, resp.), delayed recall (-46%,-67% and -48%) and delayed recognition (-76%,-79% and -82%).

DISCUSSION

This study investigated the pharmacokinetic, pharmacodynamics as well as safety and tolerability profiles of NS11821 after single oral doses. As a novel subtype selective GABA-A agonist, NS11821 was administered from a lower-than-MED dose to a dose 6 times higher than its human equivalent dose of the NOAEL with a dose escalation factor between 2 and 3.

The systemic exposure of NS11821 (AUC and C_{max}) increased approximately by a factor 10 following administration of 10 to 600 mg, a considerable variability between subjects was seen, Coefficient of Variation (CV) for the AUC and C_{max} was more than 30%. The half-life increased with increasing dose levels. In addition, the time to maximum plasma concentration was dose dependent and increased with increasing dose levels. This complex absorption profile may be due to low solubility properties of the compound in gastric fluid.

NS14606, the hydroxyl metabolite of NS11821, accounts for 30-50% of the parent compound. The ratios of active metabolite to parent compound are generally similar among the six doses. As is shown in Figure 1, the plasma concentration of NS11821 and NS14606 underwent multi-phase declines after T_{max}. Taken together, all these factors contribute to the complex non-linear pharmacokinetic profile of NS11821/NS14606 pharmacokinetics. Future studies of NS11821 should explore additional formulations, as for example acidic solutions of NS11821 to investigate how solubility affects the bioavailability of NS11821.

The study did not show clear pharmacodynamic effects with the lower doses of NS11821 (i.e. 10 mg, 30 mg, and 75 mg). However, single doses of NS11821 150 mg and higher caused statistically significant effects on several pharmacodynamic parameters in a dose dependent manner. NS11821 600 mg demonstrated the most extensive and robust effects, with impairments on SPV, VAS-alertness, and adaptive

tracking, as well as increase on VAS internal, VAS feeling high and the spectrum power of EEG beta bands. However, this high dose required the administration of a large number of tablets, which was associated with nausea and vomiting in a substantial proportion of subjects. Consequently, the results were affected by low numbers of observations, and by adverse events. NS11821 300 mg showed smaller effects on SPV and adaptive tracking and did not differ from placebo on VAS internal perception or VAS-alertness. Studies with other GABA-A subtype selective partial agonists have suggested that effects on VAS-alertness and adaptive tracking are closely linked to GABA-A α_1 subunit modulation (de Haas et al., 2010, Chen et al., 2014, Chen et al., 2015), whereas SPV is primarily sensitive to drug effects on the GABA-A $\alpha_{2,3}$ subunits (Chen et al., 2012). NS11821 300 mg and NS11821 600 mg are both effective on SPV, indicating potential anxiolysis of the two doses.

GABA-A α_5-agonism is considered to be associated with the memory-impairing effects of lorazepam (Chen et al., 2014, Chen et al., 2015). NS11821 has comparable effect potency at the GABA-A α_5-subunit and the GABA-A $\alpha_{2,3}$ subunits. This could cause memory deficits with clinically anxiolytic doses of NS11821. This seems to be corroborated by a historic comparison with lorazepam 2 mg, which suggests that the two highest doses cause roughly similar memory deficits. However, the memory effects need to be interpreted with caution. In particular, dose-relatedness wasn't always very consistent. For instance, two doses (i.e. 75 mg and 300 mg) of NS11821 were linked to considerable reduction in delay word recall compared to placebo. The study also showed similar average responses between NS11821 10 mg and NS11821 300 mg in immediate recall, and demonstrated comparable responses among NS11821 10 mg, 150 mg and placebo in delay recall (Figure 2, Panel D). These findings support the contribution of inter-subject variation to the observation of inter-treatment differences in this parallel-group study. Consequently, quantitative conclusions about memory effects of NS11821 are currently not warranted, and more dedicated studies with larger sample size are needed to further investigate these effects.

One limitation of this study is the lack of positive control to confirm the sensitivity of the pharmacodynamic measurements and benchmark the effect size of NS11821 at different doses. To compensate this deficiency, we calculated the sample size on a power level of 80% based on previous studies with benzodiazepines (Chen et al., 2012, Chen et al., 2014, Chen et al., 2015) and compared the results of this study with those studies in the statistical analysis. Figure 3 provide further information regarding the ΔSPV-relative pharmacodynamic profiles. The three high doses of NS11821 (150 mg, 300 mg, 600 mg) showed similar flatness in the ΔSPV-Δlog(Sway) relation, the ΔSPV-ΔVAS-alertness relation, and the ΔSPV-ΔSmooth relation. A certain reduction of SPV is accompanied with smaller change of body sway, VAS-alertness or smooth pursuit, compared to the ΔSPV-relative responses of these PD parameters after lorazepam 2 mg. For the relation between ΔSPV and ΔTracking, the slope of the regression line is marginally smaller with NS11821 300

mg versus lorazepam, but comparable between either 150 or 600 mg and lorazepam (Table 4). Normalized by the effect size on SPV, the ΔPD-ΔSPV relations compare the anxiolytic potency of a treatment versus its effect potency on one of other CNS regions. Based on the ΔPD-ΔSPV profiles of NS11821 at different doses, NS11821 300 mg showed the most prominent SPV effect against the other CNS-PD effects. As such, NS11821 300 mg is thought to be an effective anxiolytic dose with minimal off-target CNS-effects. With NS11821 150 mg, the compound only had marginal effect on saccadic peak velocity, and adaptive tracking performance was even better than during placebo at some time points. These observations may provide some explanation for the lack of significant difference between NS11821 150 mg and lorazepam 2 mg. When subjects are dosed with NS11821 300 mg, both α_1-containing receptors and $\alpha_{2,3,5}$-containing receptors are modulated, but pharmacological selectivity is preserved, causing minimal effects on body sway and VAS-alertness. Such SPV-relative pharmacodynamic effect profiles are distinct from those of a non-selective partial GABA-A agonist, where the slopes of the ΔPD-ΔSPV regression lines are theoretically comparable between the partial agonist and the full agonist. The effects of NS11821 600 mg seemed non-selective. These observations should be interpreted carefully since the occurrence of nausea and vomiting may have interfered with the pharmacodynamic measurements.

In conclusion, the effects of NS11821 on the pharmacodynamic biomarkers indicate entry of the compound into the central nervous system and a concentration dependent effect profile. The dose related effect of NS11821 on SPV suggests potential anxiolytic effect; while the minimal effects of NS11821 150 mg, 300 mg and 600 mg on subjective alertness, postural balance, psychomotor and cognitive functions imply reduced side effects of this compound. Pharmacological subtype selectivity is further confirmed by the response relation of various CNS pharmacodynamic biomarkers versus SPV as compared to lorazepam 2 mg. The absence of pharmacodynamic effect and the placebo-like AE profile seen with NS11821 10 mg, 30 mg, and 75 mg may be caused by low exposure of this compound. Single oral doses of NS11821 10-600 mg were safe and well tolerated in the healthy male participants of this study. Modifying the formulations of NS11821 may result better bioavailability and dose-proportionality.

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Table 1 · Summary of subject characteristics

Cohort	n	Age (yrs)*	BMI (kg/m^2)*	Height (m)*	Weight (kg)*
Placebo	12	21.8 (18-29)	22.1 (18.1-28.1)	1.81 (1.72-1.93)	72.7 (59.2-88.2)
1: 10 mg NS11821	6	25.2 (20-41)	23.8 (21.5-28.7)	1.83 (1.78-1.88)	79.7 (68.6-101.4)
2: 30 mg NS11821	6	26.2 (19-39)	24.3 (21.1-29.7)	1.78 (1.71-1.85)	77.6 (61.7-98.1)
3: 75 mg NS11821	6	22.5 (18-31)	22.9 (20.6-25.2)	1.80 (1.67-1.89)	74.5 (57.5-89.0)
4:150 mg NS11821	6	20.0 (19-22)	23.3 (20.8-25.5)	1.81 (1.76-1.87)	76.2 (68.4-89.2)
5:300 mg NS11821	6	20.3 (18-24)	23.0 (19.6-25.6)	1.83 (1.73-1.90)	76.5 (65.0-87.5)
6:600 mg NS11821	6	22.7 (18-26)	22.1 (19.2-24.9)	1.84 (1.77-1.92)	74.5 (64.5-83.0)

**mean (min-max)*

Table 2 · Summary of pharmacokinetic parameters of NS11821

Cohort	T_{max} (h)	C_{max} (ng/ml)	CV% for C_{max}	$C_{max/D}$	$T_{1/2}$* (h)	AUC_{inf} (h*ng/ml)	CV% for AUC_{inf}	AUC_{inf}/D	M/P Ratio	CL_R (l/h)
1: 10 mg	0.50	9.37	53	0.94	0.62	8.98	23	0.90	0.39	-
2: 30 mg	1.0	22.8	59	0.76	0.95	38.8	44	1.3	0.32	0.021
3: 75 mg	0.99	77.2	147	1.0	1.9	110	77	1.5	0.42	0.021
4:150 mg	0.75	236	111	1.6	3.2	419	90	2.8	0.31	0.0067
5:300 mg	2.5	477	49	1.6	5.0	1440	50	4.8	0.44	0.0061
6:600 mg	4.0	1430	83	2.4	4.4	5790	93	9.7	0.49	0.0046

*For C_{max}, AUC, CL_R the geometric mean values are presented, for T_{max} the median and for all other parameters the mean value is presented; * The half-life of the elimination phases;* M/P *ratio* = $AUC_{NS14606}/AUC_{NS11821}$

Table 3 · Pharmacodynamic effect on saccadic peak velocity, smooth pursuit, body sway, visual analogue scales and tapping

Variable	10 mg NS11821 - Placebo	30 mg NS11821 - Placebo	75 mg NS11821 - Placebo	150 mg NS11821 - Placebo	300 mg NS11821 - Placebo	600 mg NS11821 - Placebo
Saccadic peak velocity (deg/s)	-5.4 (-25.7; 14.9) p=0.5940	6.3 (-14.1; 26.7) p=0.5364	-7.8 (-28.1; 12.6) p=0.4439	-16.8(-37.2; 3.5) p=0.1023	**-23.1 (-43.5; -2.7) p=0.0279**	**-41.4 (-68.7; -14.1) p=0.0040**
Smooth pursuit (%)	-0.6 (-4.1; 3.0) =0.7468	-2.3 (-5.9; 1.2)p=0.1916	-3.2 (-6.8; 0.4) p=0.0785	-0.5 (-4.0; 3.1)p=0.7853	-3.4 (-7.1; 0.2)p=0.0643	-1.1 (-5.7; 3.6)p=0.6458
Body Sway (mm)	11.05% (-9.78; 6.70) p=0.3133	7.44% (-12.5; 31.98) p=0.4842	19.14% (-2.99; 46.34) p=0.0926	8.11% (-12.5; 33.57) p=0.4599	15.84% (-5.84, 42.53) p=0.1590	24.68% (-5.24, 64.04) p=0.1119
VAS-Alertness (mm)	-0.2 (-1.9; 1.5) p=0.8047	-0.6 (-2.3; 1.1) p=0.4738	-1.2 (-2.9; 0.6) p=0.1830	-1.5 (-3.2; 0.2) p=0.0868	-0.5 (-2.3; 1.2) p=0.5422	**-3.6 (-5.8; -1.4) p=0.0023**
VAS Internal (log(mm))	0.001 (-.008; 0.009) p=0.9057	-.000 (-.009; 0.008) p=0.9283	0.005 (-.003; 0.013) p=0.2324	0.004 (-.004; 0.012) p=0.3510	0.002 (-.007; 0.010) p=0.6910	**0.012 (0.001; 0.023) p=0.0281**
VAS Feeling high (log(mm))	0.006 (-.067; 0.080) p=0.8636	0.000 (-.073; 0.073) p=1.0000	0.016 (-.057, 0.090) p=0.6573	0.047 (-.026; 0.121) p=0.1986	0.048 (-.025; 0.121) p=0.1941	**0.095 (0.000; 0.190) p=0.0491**
Adaptive tracking (%)	0.38 (-1.66; 2.43) p=0.7054	-0.49 (-2.58; 1.60) p=0.6363	-1.67 (-3.71; 0.37) p=0.1052	-1.47 (-3.53; 0.59) p=0.1565	**-2.97 (-5.00; -0.94) p=0.0054**	**-8.55 (-11.2; -5.93) p=<.0001**
Mean of 5 tapping trials (taps/10 sec)	-2.24 (-5.85; 1.38) p=0.2174	-1.57 (-5.02; 1.89) p=0.3641	2.16 (-1.27; 5.59) p=0.2097	1.16 (-2.32; 4.64) p=0.5039	**-3.60 (-7.05; -0.15) p=0.0413**	-3.07 (-7.55; 1.41) p=0.1734
Pharmaco-EEG EEG Beta Fz-Cz (uV)	3.59% (-6.09; 14.27) p=0.4711	8.36% (-1.51; 19.21) p=0.0968	6.78% (-2.91; 17.44) p=0.1708	**16.20% (5.58; 27.89) p=0.0030**	**16.79% (5.92; 28.78) p=0.0027**	**58.31% (38.41; 81.06) p=<.0001**
EEG Beta Pz-Oz (uV)	3.72% (-8.87; 18.05) p=0.5712	13.59% (-0.19; 29.27) p=0.0532	5.25% (-7.52; 19.79) p=0.4279	8.98% (-4.32; 24.13) p=0.1889	**15.20% (1.09; 31.29) p=0.0346**	**36.64% (14.52; 63.02) p=0.0010**

Differences in LSM values are shown for each contrasts with (95% CI) and P-value; LSM=Least square mean; CI=Confidence interval; NS=NS11821; PBO=placebo; SPV=Saccadic Peak Velocity; VAS=Visual Analogue Scale

Table 4 · Results of the linear model for Saccadic Peak Velocity change from baseline and other pharmacodynamic parameter change from baseline by treatment

Relation	Slope of NS11821	NS11821 vs. Lorazepam	P-value
ΔSmooth/ΔSPV			
NS11821 150 mg	0.01118	-0.09867	0.0449
NS11821 300 mg	0.02222	-0.08764	0.0680
NS11821 600 mg	0.03822	-0.07164	0.1321
ΔSway/ΔSPV			
NS11821 150 mg	-0.00084	0.00221	0.0276
NS11821 300 mg	0.000347	0.0034	0.0004
NS11821 600 mg	-0.00053	0.002525	0.0077
ΔVAS-alertness/ΔSPV			
NS11821 150 mg	0.03906	-0.08692	0.1285
NS11821 300 mg	-0.01465	-0.14062	0.0108
NS11821 600 mg	0.00655	-0.11942	0.0279
ΔTracking/ΔSPV			
NS11821 150 mg	0.04592	-0.01128	0.6112
NS11821 300 mg	0.01835	-0.03885	0.0681
NS11821 600 mg	0.04672	-0.01049	0.6112

Figure 1 · Mean (SD) drug concentrations profile of NS11821 (panel A) and NS14606 (panel B) following oral administration of 10-600 mg NS11821 (logarithmic scale).

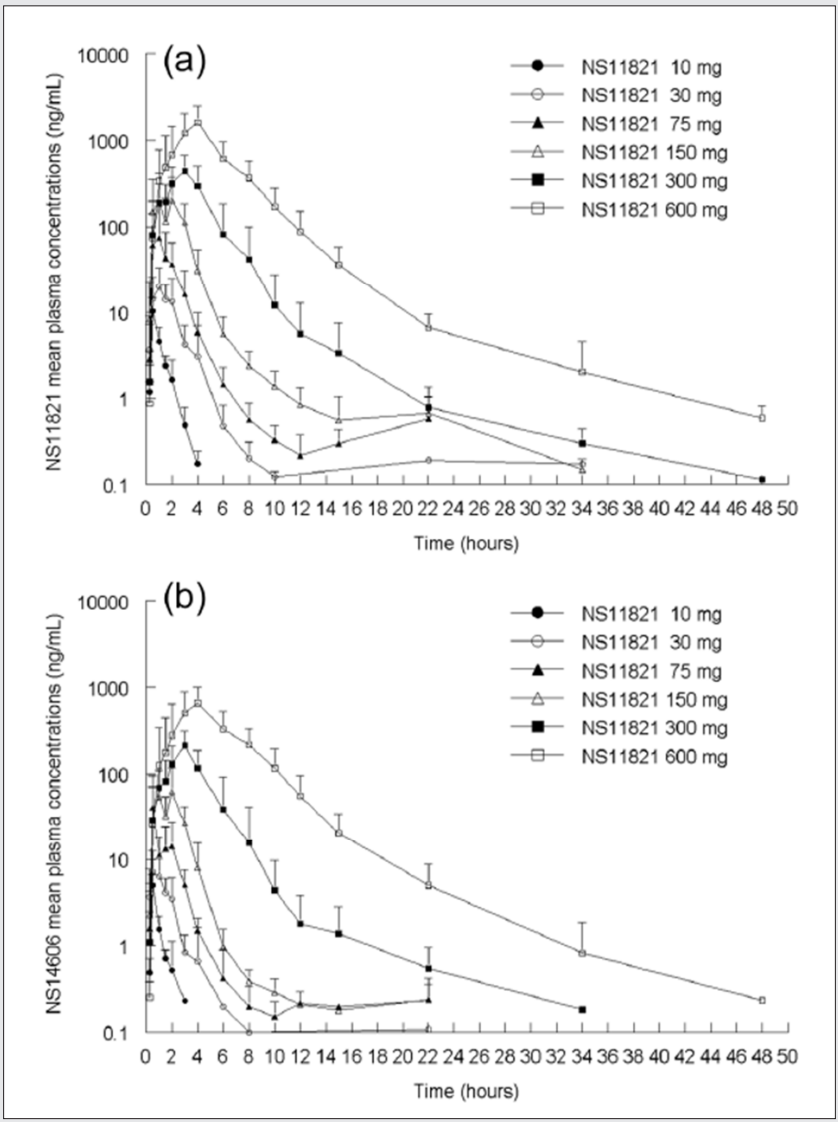
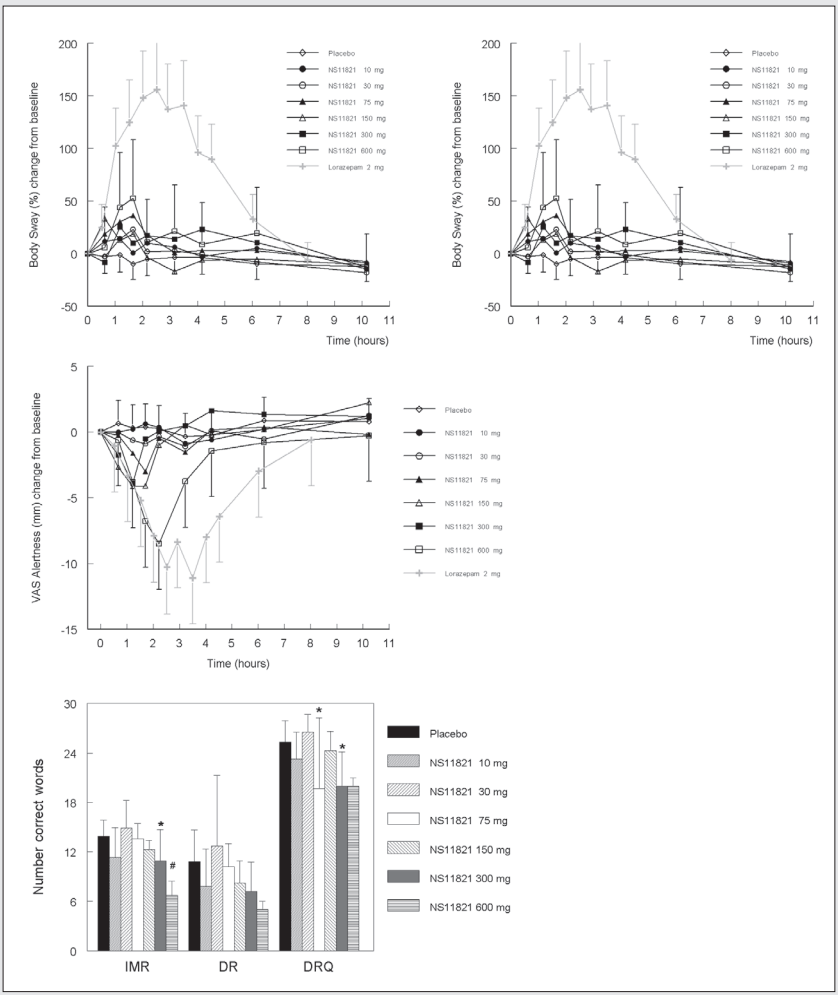


Figure 2 · Pharmacodynamic profile for NS11821 with additional historic lorazepam 2 mg data for: saccadic peak velocity (panel A), body sway (panel B), $VAS_{alertness}$ (panel C), and visual verbal learning test (panel D) (the y-axis presents the LSMs change from baseline profile with 95% CI error bars)



$p < 0.05$ and # $p = 0.001$: comparing NS11821 to placebo. IMR=Immediate Recall, DR= Delayed Recall and DRQ= Delayed Recognition for number of correct words

Figure 3. ΔPD-ΔSPV relative effect profile of NS11821 150 mg, NS11821 300 mg, and NS11821 600 mg vs. lorazepam 2 mg, respectively.