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

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THE CENTRAL NERVOUS SYSTEM EFFECTS OF THE PARTIAL GABA-A α2,3-SELECTIVE RECEPTOR MODULATOR AZD7325 IN COMPARISON WITH LORAZEPAM IN HEALTHY MALES

British Journal of Clinical Pharmacololy 2014; 78(6): 1298-1314

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ABSTRACT

Aims: AZD7325 is a novel $\alpha_{2,3}$-subtype-selective partial GABA-A-receptor modulator. This study investigated the pharmacodynamics of single-oral-dose AZD7325 2 mg and 10 mg on the central nervous system (CNS) compared to placebo and lorazepam 2 mg. **Methods:** This double-blind, randomized, 4-way-crossover study enrolled sixteen healthy males and administered two validated CNS-test-batteries to measure drug effects on cognitive, neurophysiologic and psychomotor function, and subjective feelings. The pharmacological selectivity of AZD7325 was compared to lorazepam by plotting saccadic peak velocity change from baseline (ΔSPV) against body sway (Δsway) and visual analogue scale for alertness ($\Delta VAS_{alertness}$). This analysis has previously been used to identify $\alpha_{2,3}$-subtype-selectivity. **Results:** In contrast with the robust impairment caused by lorazepam (all $p<0.05$ *vs.* placebo), neither dose of AZD7325 induced statistically significant effects on any pharmacodynamic measurements. Lorazepam-induced SPV-reduction was linearly related to changes in other neurophysiologic biomarkers. In contrast, the slopes of the regression lines were flatter for AZD7325, particularly for the ΔLog(Sway)-ΔSPV relation (estimate slope, AZD7325 10 mg vs. lorazepam, difference [95% confidence interval], p-value: -0.00036 vs. -0.00206, 0.001704 [0.000639, 0.002768], p=0.0018) and the $\Delta VAS_{alertness}$-ΔSPV relationship (0.01855 vs. 0.08216,-0.06360 [-0.1046, -0.02257]; p=0.0024). AZD7325 10 mg and lorazepam induced different response patterns on VAS 'feeling high' and electro-encephalography. **Conclusion:** The characteristic ΔSPV-relative effect profiles of AZD7325 versus lorazepam suggests anxio-selectivity related to $\alpha_{2,3}$-selective GABA-A agonism. However, exploration of higher doses may be warranted. The paucity of effects on most CNS-PD parameters also indicates a mitigated side-effect pattern, with potentially lower cognitive and neurophysiological side-effect burden than non-selective benzodiazepines.

INTRODUCTION

Benzodiazepines (BZDs) are widely used in the treatment of anxiety disorders and for symptomatic relief of various anxiety states related to diverse psychiatric disorders, including mood-, psychotic- and personality disorders. However, concerns have been raised regarding the untoward effects of these drugs, which include movement/balance disorders, cognitive impairment, as well as problems with tolerance and abuse liability. All these facts limit the usefulness of BZDs as a long-term therapy in vulnerable patient populations.

Benzodiazepines elicit their pharmacological effects through allosteric modulation of GABA-A receptors. These compounds have non-selective binding affinities and present full *in vitro* efficacy at the GABA-A receptors that contain subunits α_1, α_2, α_3 or α_5. A collection of loss-of-function studies were performed to compare the BZD-mediated behavior between wild-type animals and knock-in animals [1] and the pharmacological role of each GABA-A subtype. These studies in experimental animals have suggested that the GABA α_1 subtype is associated with sedation [2,3]; α_2/α_3 receptors are responsible for the anxiolytic properties of BZDs [4,5], where α_2 is found more correlated to anxiolysis than α_3 [1]; and the α_5 subunit is related to modification of memory and cognition [6,7]. Based on these findings, the adverse effects of benzodiazepines are attributed to the pharmacological effects of these compounds on GABA-A receptors other than the $\alpha_{2,3}$ subtype. Compounds with relatively high efficacy at the $\alpha_{2,3}$ subunits but reduced efficacy at the α_1 and/or α_5 subunits are classified as subtype-selective GABAergic compounds and expected to be potential anxioselective treatments with reduced sedation and no impact on cognition and psychomotor performance.

AZD7325, 4-amino-8-(2-fluoro-6-methoxy-phenyl)-N-propyl-cinnoline-3-carboxamide [8], is a novel partial subtype-selective GABA-A $\alpha_{2,3}$ receptor modulator, which is in development for anxiety disorders. *In vitro* AZD7325 demonstrated functional specificity for the GABA-A α_2 and GABA-A α_3 receptor subtypes. AZD7325 exerts neutral antagonism at the α_1-subunit and partial efficacy at the $\alpha_{2,3}$-subunits over the α_5-subunit ($\alpha2\sim\alpha_3$ vs. α_5: 18%~15% vs. 8%, percentage compared to maximal diazepam response). Meanwhile, the compound has much higher binding affinity (mean Ki [nm], $\alpha_1\sim\alpha_2\sim\alpha_3$ vs. α_5: 0.3~1.3 vs. 230) and larger relative efficacy at the $\alpha_{2,3}$-subunits over the α_5-subunit (AstraZeneca data on file). Selective *in vitro* properties have also been confirmed in preclinical biomarker studies using EEG and PET imaging in rodents and primates, which revealed that exposures that result in 50% occupancy produce robust anxiolytic effects without benzodiazepine-like side effects (AstraZeneca data on file). However, translation of the effects of GABA(A) $\alpha_1$modulation from pre-clinical studies into human has been unpredictable, with some weak partial GABA(A) α_1 modulator showing persistence of sedative properties [9], whereas some non-subtype selective GABA-A-ergic compounds, such

as ocinaplon [10] and alpidem [11,12], were found anxiolytic but less sedating or less psychomotor- and cognition-impairing in the clinic [13]. In addition, the ideal degree of modulation at each of the two preferred subtypes is not known since the behavior of non-sedating benzodiazepines has not been extensively investigated in clinical settings. Since preclinical data that have been obtained on the pharmacology, pharmacokinetics and toxicology of AZD7325 support the conduct of clinical studies in humans, the current phase I trial was designed to provide an initial assessment of the side-effect profile of AZD7325.

The present study aimed to investigate the pharmacodynamic (PD) effects and evaluate the safety and tolerability of single oral doses of AZD7325 in healthy subjects, in comparison with placebo and lorazepam. Lorazepam is clinically effective as an anxiolytic at a dose of 2 mg. Single oral doses of lorazepam 2 mg have demonstrated robust effects on saccadic peak velocity (SPV), smooth pursuit, body sway, and visual analogue scale (VAS) of alertness in healthy volunteers [14,15,16]. These effects reflect the typical effect profile of benzodiazepines on different central nervous system (CNS) functions [17]. More importantly, SPV is very sensitive to the effect of BZDs, and the drug-induced changes of SPV seem to reflect the anxiolytic potency of different anxiolytic compounds [17]. For this study, the doses of AZD7325 were determined at 2 mg and 10 mg. A previous single-ascending-dose (SAD) study in healthy volunteers indicated that AZD7325 has an acceptable safety profile in oral doses up to 100 mg (AstraZeneca data on file). PET study using [^{11}C]-flumazenil suggested that AZD7325 2 mg is associated with approximately 50% occupancy of the GABA-A receptors and 10 mg causes maximal (>80%) displacement of flumazenil at peak concentration of the compound in occipital cortex (AstraZeneca data on file). Compared to the low receptor occupancy of lorazepam 1 mg [18] as well as the *in vitro* $\alpha_{2,3}$-subtype modulation of AZD7325, AZD7325 2 mg and 10 mg are expected to fall within the anticipated clinical therapeutic window of this compound.

METHODS

DESIGN

The trial was designed as a randomized, double-blind, double-dummy, placebo- and comparator- controlled study in sixteen male healthy volunteers, where the positive control was used to benchmark the effects of the investigational product.

SUBJECTS

Following the approval of the Medical Ethics Review Board of Leiden University Medical Centre (LUMC), subjects who provided written informed consents received medical screening at the Centre for Human Drug Research (CHDR). The eligibilities

of sixteen healthy male subjects were confirmed before their entry into the trial. These subjects should be aged between 18 and 55 years, with a body mass index (BMI) of 18 to 30 kg/m^2. All subjects were required to refrain from alcoholic beverages, smoking and caffeine-containing products during study days. A normal diurnal rhythm was advised from minimally two weeks before the first study day until the last visit.

SAMPLE SIZE DETERMINATION

Based on power calculations using data from previous studies [14], a sample size of 16 was determined to have equal to or greater than 80% power to detect the mean differences of 1.24 mm in $VAS_{alertness}$ and 20.6 degree/second in SPV, respectively, assuming standard deviations of 1.66 mm (VAS alertness) and 27.4 degree/second (SPV) between placebo and lorazepam 2 mg using a paired t-test with a 0.050 two-sided significance level.

TREATMENTS

The study treatments were randomly allocated based on a 4×4 William's Latin Square. The treatment sequence was unique for each subject. Each subject received 1) AZD7325 2 mg (two capsules of AZD7325 1 mg and two tablets of lorazepam placebo), 2) AZD7325 10 mg (one capsule of AZD7325 10 mg, one capsule of AZD7325 placebo and two tablets of lorazepam placebo), 3) lorazepam 2 mg (two capsules of AZD7325 placebo and two tablets of lorazepam 1 mg), or 4) placebo (two capsules of AZD7325 placebo and two tablets of lorazepam placebo) on the morning of each study day. A washout period of at least 7 days was arranged between treatments.

PHARMACODYNAMIC MEASUREMENTS

A standard Neurocart battery of neurophysiologic and neuropsychological tests included the following validated pharmacodynamic assessments: body sway, visual analogue scale (VAS) of Bond & Lader, VAS Bowdle, saccadic eye movements, smooth pursuit eye movements, adaptive tracking and electro-encephalograms (EEG). The repeatable measurements were presented to the subjects during a pre-dose visit in order to familiarize subjects with the CNS tests and prevent potential learning effects during the post-dose measurements. In each study period, the Neurocart battery was performed twice at baseline and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 6, 8, and 12 hours post-dose. In the meantime, the CogState Early Phase Battery (described below) was carried out thrice pre-dose and four times (i.e. at 1.25, 2.25, 3.25, and 4.25 hours) post-dose. Moreover, subjects completed the Cogstate International Shopping List task, which required them to memorize a shopping

list of 16 words at 1.75 hours post-dose and recall these items both immediately and at 21 hours post-dose without being read the words again. All assessments were performed by one subject at a time, in a quiet room with subdued ambient illumination.

NEUROCART BATTERY

SACCADIC EYE MOVEMENTS

Saccadic eye movements were evaluated using a computer-based system composed of 1) stimulus display and signal collection (Nihon Kohden Corporation, Tokyo, Japan), 2) signal amplification (Grass-Telefactor, Astro-Med, Inc., Braintree, USA), 3) data recording (Cambridge Electronics Design, Cambridge, UK), 4) disposable silver-silver chloride electrodes (Medicotest N-00-S, Olstykke, Denmark), as well as 5) the sampling and analysis scripts developed by CHDR (Leiden, the Netherlands). The parameters of this test were the average values of saccadic peak velocity (SPV, degree/sec), latency (i.e. reaction time, sec) and inaccuracy (%) of all artefact-free saccades that were calculated on each session. Saccadic peak velocity appears to be the most sensitive measure for the sedative effect of benzodiazepines [17], which has been found to be related to the anxiolytic component of benzodiazepines [17] and to be selectively affected by some newly developed GABAergic compounds with potential anxiolytic effects [14,15,16].

BODY SWAY

Body sway was measured with an apparatus similar to the Wright ataxia meter, which integrates the amplitude of unidirectional body sway. Two-minute measurements were made in the antero-posterior direction with eyes closed. The subject was asked to stand comfortably on a stable floor with his/her feet slightly apart. Body sway measures postural (in)stability. It has demonstrated considerable sensitivity to the effect of benzodiazepines [19].

VISUAL ANALOGUE SCALES (VAS) OF BOND & LADER AND BOWDLE

Visual analogue scales as originally described by Norris have often been used previously to quantify subjective effects of a variety of sedative agents [20]. Dutch versions of the scales have been frequently employed at the CHDR, for a variety of sedative agents [21] and circumstances [22]. During the test, the subject indicated (with a mouse click on the computer screen) on horizontal visual analogue scales how he/she feels. From the sixteen measurements of VAS Bond & Lader, three main factors are the calculated [23] for subjective alertness, contentedness, and calmness.

The Bowdle Psychotomimetic Effects Scores have been developed to quantify the psychotomimimetic effects of ketamine [24]. A translated Dutch version of the

original scales has been computerized and used at the CHDR to study the effects of cannabinoids [25] and zolpidem [26], among others. This scale has thirteen 10 cm visual analogue lines ranging from 0 ('not at all) to 100 mm ('extremely') [27], addressing various abnormal states of mind. From the thirteen measurements of VAS Bowdle, three distinct total sum scores are calculated: 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 [28].

SMOOTH PURSUIT EYE MOVEMENTS

The same system as used for saccadic eye movements was also used for measurement of smooth pursuit. For smooth pursuit eye movements, the target moved sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponded to 22.5 degrees eyeball rotation to both sides. Four cycles were recorded for each stimulus frequency. The method has been validated at the CHDR by van Steveninck *et al.* [21,28] based on the work of Bittencourt *et al.* [29] and the original description of Baloh *et al.* [30]. The time in which the eyes were in smooth pursuit of the target were calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies were used as the test parameter. Smooth pursuit is a measure of neurophysiological function and has been shown sensitivity to the effects of BZDs [16], zolpidem [31], and some $\alpha_{2,3}$-subtype selective GABA-A receptor modulators [16].

ADAPTIVE TRACKING

Adaptive tracking is a pursuit-tracking task that measures drug effect on visuo-motor coordination. The adaptive tracking test was performed as originally described by Borland and Nicholson [32], using customised equipment and software. After a 0.5-minute run-in time without data-recording, the average performance over 3.0 minutes was scored and was used as the test parameter. The subject was required to operate a joystick and try to keep a dot inside a circle moving randomly on the computer screen. If he/she succeeded, the speed of the moving circle increased, or vice versa.

EEG

Pharmaco-electroencephalography (Pharmaco-EEG) was used to monitor any drug effects, which can be interpreted as evidence of penetration and activity in the brain [33]. EEG recordings were made using gold electrodes, fixed with EC2 paste (Astromed) and using standard pharmaco-EEG lead placement, with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were kept below 5 kOhm. The signals were

amplified with a Grass 15LT series Amplifier Systems, using a time constant of 0.3 seconds and a low pass filter at 100 Hz. Data collection and analysis were performed using customized CED and Spike2 for Windows software (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of eight seconds were recorded. The signal was AD-converted using a CED 1401 Power (Cambridge Electronics Design, Cambridge, UK) and stored on hard disk for subsequent analysis. Data blocks containing artifacts were identified by visual inspection and these were excluded from analysis. For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta- (0.5-3.5 Hz), theta- (3.5-7.5 Hz), alpha- (7.5-11.5 Hz), beta- (11.5-30 Hz) frequency ranges. Frequency band above 30 Hz was also recorded for exploratory pharmaco-EEG analyses, in order to test whether the findings of an effect of AZD7325 on gamma-frequency band (> 30 Hz) in animals translate to humans.

COGSTATE BATTERY

The CogState Early Phase Battery is a computer administered cognitive test battery that takes about 12 minutes to perform. It is designed to provide objective information regarding possible drug effects on cognitive function [34]. The CogState Early Phase Battery consists of the following tests that address pharmacodynamic effects on different cognitive domains [35]. The tests were presented in the order listed below. In addition, the 16-word CogState International Shopping List Task and its delayed recall session were presented once per dosing period, respectively [36].

GROTON MAZE LEARNING TASK (GMLT)

A 28-step pathway was hidden among the 100 possible locations of a 10 x 10 grid of tiles showing on a computer touch screen. Subjects were instructed to move from the start location (top left), one tile at a time, toward the end (bottom right). The entire 28-step pathway could be figured out based on the computer's feedbacks. Once completed, subjects returned to the start location and repeated the task for 4 more times. Twenty well-matched alternate forms of this task were cycled among measurements so that subjects would not take a same trial during one dosing period. The GMLT is a measure of executive functioning. During a 'timed chase' part of the test, the subject was asked to quickly follow a moving tile around in a 10 x 10 grid of tiles on a computer touch screen with a stylus pen for 30 seconds. This test measures attention and psychomotor function.

DETECTION TASK

During the test, a playing card was presented in the center of the screen. Subjects were required to press the 'Yes' key whenever the card flipped over and faced up.

Subjects were encouraged to work as quickly and accurately as they could, but try not to press the 'Yes' key before a card flips over. If subjects did this or did not respond to a card that had flipped over, they would hear an error sound. After a short period for practice, the real test began. The test measures attention and psychomotor function.

IDENTIFICATION TASK

A playing card was presented in the center of the screen and flipped over from time to time. When the card faced up, the subject should press 'Yes' for a red card but 'No' for a non-red one. An error sound would appear when the subject pressed a key before a card flipped over or made a mistake. The real test began after practice. The test measures speed of mental processing and attention.

ONE CARD LEARNING TASK

Subjects were asked to identify whether the playing card presented on the screen had been shown during the current test trial. They responded by pressing the 'Yes' or 'No' key. An error noise would appear when there was an incorrect or missing response. The real test began after practice. The test measures spatial learning.

INTERNATIONAL SHOPPING LIST TASK (ISLT)

At 1.75 h post-dose, the test supervisor read a shopping list of 16 words forthe subject as they appear on the computer screen at a rate of one word every two seconds. Subjects were instructed to memorize and recall as many words as possible, while the test supervisor clicked / touched the appropriate button on the screen with the stylus or mouse. As such, the entire word list reading session (in the same order) and the immediate recall session were repeated for another two times. The task measures verbal learning ability. At 21 h post-dose, subjects were required to recall the previous shopping list without being read them again, while the test supervisor clicked / touched the appropriate button on the screen with the stylus or mouse. Performance on this test reflects long term storage, memory consolidation and retrieval.

SAFETY

Safety and tolerability were evaluated using clinical laboratory tests, 12-lead electrocardiograms (ECGs), and records of adverse events and vital signs. Twelve-Lead ECG recordings were assessed with Cardiofax V (Nihon Kohden, Tokyo, Japan) or Marquette 5000/5500. After a 5-minute rest in supine position, blood pressures and pulses were taken with a semi-automatic blood pressure recording device (a Nihon-Kohden BSM-1101K monitor or a Colin Pressmate BP 8800 or a Dash 4000) at supine and, 2 minutes later, at standing position. The Central Clinical Chemistry

and Haematology Laboratories of LUMC were responsible for the safety laboratory assays on blood or urine samples.

PHARMACOKINETIC MEASUREMENTS

In order to acquire the plasma concentrations of AZD7325 or lorazepam, venous blood samples (6 mL) were collected at pre-dose and 0.5, 1, 1.25, 1.5, 2, 2.5, 3.25, 4, 4.5, 6, 8, 12, and 21 hours post-dose into ethylenediamine tetra-acetic acid (EDTA K2) spray-dried tubes. These tubes were immediately ice-bathed and centrifuged for 10 minutes at 2°C to 8°C at a relative centrifugal force of 2000g within 30 minutes from collection. Thereafter, the plasma was transferred to two 2 mL Sarstedt tubes and immediately frozen upright at or below -70°C within 15 minutes of plasma preparation and stored at this condition until bioassay.

STATISTICAL ANALYSIS

The pharmacodynamic parameters (short names are written in parentheses) of the Neurocart consist of amplitude of body sway (Sway), saccadic peak velocity (SPV), percentage of smooth pursuit (Smooth), performance of adaptive tracking (Tracking), visual analogue scale for alertness ($VAS_{alertness}$), feeling high (VAS_{high}), internal perception ($VAS_{internal}$), and external perception ($VAS_{external}$), power of various EEG bands (delta, theta, alpha, beta, gamma bands in the frontal-central [Fz-Cz] and the parietal-occipital [Pz-Oz] areas, respectively). EEG parameters, body sway and VAS Bowdle sub-scales were log-transformed prior to analysis and thus corrected for the expected log-normal distribution of the data. Safety variables were frequency and incidence of adverse events (AEs) and related information, vital signs (blood pressure, heart rate, respiratory rate and auricular temperature), laboratory parameters, and ECG outputs. All statistical analyses were performed with SAS (version 9.1).

ANALYSIS OF THE SPV CHANGE FROM BASELINE (ΔSPV)-RELATIVE EFFECT PROFILES

Previous studies suggested good sensitivity of SPV to the effect of BZDs [17] and $\alpha_{2,3}$ subtype-selective GABA-A receptor modulators [14,15,16]. Based on these results, SPV is hypothesized as a biomarker indicative of clinical anxiolysis associated with GABA $\alpha_{2,3}$ activation, and the predictability of SPV was supported by early clinical findings with TPA023 [6]. BZDs also affected body sway and $VAS_{alertness}$, suggesting balance impairment and subjective sedation, respectively [14,15,16,17]. Given the clinical relevance of these pharmacodynamic parameters, the SPV-relative effect

profiles of both body sway and $VAS_{alertness}$ have been shown to differentiate the pharmacological selectivity of $\alpha_{2,3}$ subtype-selective GABA-A receptor modulators from the non-selective GABA-A agonism with BZDs [37]. As such, we performed a regression analysis to demonstrate the relationship of individual changes from baseline on body sway (Δsway) and VAS alertness ($\Delta VAS_{alertness}$) against the change from baseline of SPV (ΔSPV). The slopes of these regression lines are thought to correspond with the relation between off-target sedating effects and anxiolysis [37]. 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 estimates of the slopes of the regression lines of these ΔSPV-relative effect profiles were compared between each dose of AZD7325 and lorazepam.

Repeated pharmacodynamic measurements were also compared with a mixed model analysis of variance with fixed factors of treatment, period, time and treatment by time, and random factors subject, subject by treatment and subject by time, and the average pre-value (average over all measurements at or before time=0) as covariate. The least square means (LSMs) of the measurements up to 8h post-dose were calculated within the statistical model. Contrasts of placebo *vs* each active treatment and between each two active treatments were reported along with 95% confidence intervals. The log-transformed parameters were back-transformed after analysis, where the results were interpreted as percentage change.

Moreover, the ΔSPV-relative effect profiles of adaptive tracking (ΔTracking) and smooth pursuit (Δsmooth) were explored to gain further insights about the pharmacological selectivity of AZD7325.

The pharmacokinetic analysis was performed at Clinical Pharmacology, AstraZeneca Wilmington, DE, USA using the WinNonlin program (Pharsight Corporation, MountainView, California, USA) using non-compartmental analysis. The resultant PK parameters were summarized with descriptive statistics by treatment. The frequency and incidence of adverse events were summarized based on preferred terms by system organ class (SOC) and treatment. Parameters of vital signs, 12-lead ECGs and safety laboratories, along with their changes from baseline, were summarized using descriptive statistics by treatment.

RESULTS

SUBJECTS

Eighteen healthy male subjects, aged 24.6±7.6 years, were eligible for the trial. Two subjects withdrew their informed consents for personal reasons after completion of the first treatment period and were replaced by another two male subjects who received the same sequences of treatments. Sixteen subjects completed the study

per protocol. The mean (standard deviation, SD) body weight and body mass index (BMI) of the completers were 74.3 (7.2) kg and 22.6 (2.4) kg/m^2, respectively. Safety analyses were performed on data from all treated subjects. Valid data from subjects who completed at least one dosing period per protocol were included into the pharmacokinetic and pharmacodynamic analyses.

PHARMACODYNAMIC (PD) RESULTS

The profiles of various pharmacodynamic parameters were obtained with each study treatment and graphically presented in Figure 1-4. In general, the maximal effect of lorazepam 2 mg appeared around 3 hours post-dose, which was slightly behind the time to peak plasma concentration, whereas the EEG effects of lorazepam and AZD7325 reached their peak level around T_{max}.

An overview of the regression analyses for the slopes of effects relative to SPV is plotted in Figure 5, in combination with the calculated population regression lines (Table 1). In figure 5, each dot represents the average change from baseline of the y-axis PD parameter versus that of the x-axis PD parameter (i.e. ΔSPV) of 17 subjects at a certain time point. There are in total 12 dots per treatment arm in each graph panel. Each dot refers to one post-dose time-point pre-scheduled in the study. The connecting lines represent the time line, which suggest there was no obvious time-shift between the effect on SPV and any of the other CNS-PD effects. The straight lines indicate the regression lines for the ΔPD-ΔSPV relations. These regression lines are not based on the average dots but on the underlying individual values that are not shown in the graphs.

As is can be seen the figure, the slopes of the regression lines are generally flatter for either dose of AZD7325 than for lorazepam. 10 mg demonstrated statistically significant difference from lorazepam 2 mg in most ΔSPV-relative relations, except the Δsmooth-ΔSPV relation. The effects of AZD7325 2mg were too small for a reliable determination of effect slopes.

The effects of AZD7325 10 mg also failed to reach statistical significance for $VAS_{alertness}$, SPV, body sway, smooth pursuit, or adaptive tracking. In contrast, lorazepam 2 mg induced robust and significantly larger effects on these pharmacodynamic parameters compared to either dose of AZD7325 or placebo.

There was a trend towards a short-lasting small increase in VAS_{high} after AZD7325 10 mg, without significant alteration in either internal ($VAS_{internal}$) or external ($VAS_{external}$) perceptions. The only statistically significant effect of AZD7325 10 mg was an EEG power reduction in the delta (2-4 Hz) and theta (4-7.5 Hz) bands of the frontal-central area. These EEG profiles of AZD7325 differed from the characteristic benzodiazepine EEG signature induced by lorazepam, which was associated with increased power in delta, beta (13.5-35 Hz) and gamma (35-48 Hz) bands, as well as reductions in theta and alpha (7.5-13.5 Hz). The pharmacodynamic

(PD) effects of each active treatment and the results of statistical comparisons are summarized and tabulated in Table 2 and Table 3.

Results of the CogState Early Phase Battery are presented in Figure 4. As expected, repeated exposure to the test paradigms resulted in no significant learning effects in the placebo group. Neither dose of AZD7325 showed statistically significant effects on any individual CogState variable. In contrast, lorazepam induced statistically significant impairments on the following cognitive parameters compared to placebo (lorazepam *vs.* placebo, [unit], *p*-value): reaction time of correct responses in the detection task (2.59 *vs.* 2.52 [log(msec)], p<0.0001), reaction time of correct responses in the identification task (2.78 *vs.* 2.70 [log(msec)], p<0.0001), response accuracy in the one card learning task (0.70 *vs.* 0.86 [arc(%)], p<0.0001), moves per second (mps) in the chase test (1.58 vs. 1.84 [mps], p<0.0001), and the sum of errors in GMLT (62.0 vs. 33.1, p=0.0003), as well as reduced the number of words recalled in both the ISLT and the ISL delayed-recall task.

SAFETY

Single oral dose of AZD7325 2 mg, AZD7325 10 mg or lorazepam 2 mg were generally safe and well-tolerated in the eighteen selected healthy male participants. A majority of subjects reported adverse events after administration of lorazepam 2 mg (AE frequency, incidence%: 14, 87.5%), whereas the high dose of AZD7325 was associated with relatively fewer adverse events (12, 70.6%), and even lower incidences of AEs were observed after AZD7325 2 mg (4, 23.5%) and placebo (9, 56.3%). As was observed with lorazepam 2 mg (14, 87.5%), most AEs that occurred in subjects receiving AZD7325 10 mg (11, 64.7%) were classified as 'nervous system disorders', but fewer subjective somnolence (AZD7235 10 mg: 7, 41.2%; AZD7325 2 mg: 2, 11.8%) and dizziness (AZD7235 10 mg: 3, 17.6%; AZD7325 2 mg: 1, 5.9%) were reported with either dose of AZD7325 than with lorazepam (somnolence: 9, 56.3%; dizziness: 5, 31.3%). On the other hand, the incidence of somnolence and dizziness was higher with AZD7325 10 mg than with placebo (3, 18.8%). The frequency of gastrointestinal events was also less with AZD7325 10 mg (2, 11.8%) than with lorazepam (6, 37.5%). No changes or individual abnormalities of vital signs or laboratory or ECG results were judged clinically important by the investigator.

PHARMACOKINETIC (PK) RESULTS

Both AZD7325 2 mg and AZD7325 10 mg were quickly absorbed after oral administration, with a short lag time of maximally 0.5 hours. Mean (SD) peak plasma concentration (C_{max}) arrived at 14.2 (5.36) ng/ml between 1 hours and 3.25 hours after AZD7325 2 mg, and at 67.4 (33.5) ng/ml between 0.5 hours and 3.25 hours after AZD7325 10 mg, with a median time to C_{max} (T_{max}) of 1.75 hours and 2 hours,

respectively. The area under the concentration-time curve from zero to the last detectable concentration (AUC_{0-t}) was 51.9 (18.9) h·ng/mL for AZD7325 2 mg and 259 (77.6) h·ng/ml for AZD7325 10 mg. As is shown in Figure 6, drug elimination seemed to exhibit roughly three phases after T_{max}. The mean elimination half-life was 8.5 to 9.0 hours for both doses of AZD7325 (ranging from 5.09 to 15.4 hr). The apparent oral clearance (CL/F) of AZD7325 was 38.3 L/hr on average (ranging from 9.87 to 89.9 L/hr). No statistically significant differences were found between AZD7325 2 mg and AZD7325 10 mg with respect to T_{max}, $T_{1/2}$, or CL/F ($p>0.05$). In comparison, lorazepam reached a mean (SD) C_{max} of 20.7 (4.86) ng/ml in a longer median T_{max} of 2.50 hr (range 0.50-6.00 hr) and was eliminated with a mean $T_{1/2}$ of 14.6 hr (range 8.31-25.1 hr). The AUC_{0-t} for lorazepam was 233 (35.8) h·ng/mL. The average levels of AUC and C_{max} increased in a dose proportional manner with similar dose-normalized values of C_{max} and AUC_{0-t} between AZD7325 2 mg and AZD7325 10 mg.

DISCUSSION

In vitro, AZD7325 exhibits relatively potent positive modulation at the $\alpha_{2,3}$ subunits together with neutral α_1-antagonism and weak α_5-affinity. Based on these properties, the compound was expected to have a rapid onset of anxiolysis with less untoward effects at its therapeutic dose(s) in healthy volunteers. Prior to initiating phase II trials, the present study aimed to provide support for the pharmacological selectivity of AZD7325 in healthy volunteers by comparing its pharmacodynamic profile to the non-selective GABA-A receptor modulator, lorazepam.

Compared with lorazepam, both doses of AZD7325 demonstrated smaller absolute slopes of the regression lines in the ΔSPV-ΔLog(Sway) relation and the ΔSPV-$\Delta VAS_{alertness}$ relation. Thus, AZD7325 is associated with a ΔSPV-dominant response profile, whereas the pharmacodynamic responses to lorazepam are more comparable and balanced among the same set of CNS parameters. This has also been observed with other subtype-selective GABA-A $\alpha_{2,3}$ receptor modulators [14,16]. Since SPV was found sensitive [17] and functionally specific to the effect of anxiolytic drugs acting on the GABAergic system [37], the distinction between AZD7325 and lorazepam suggests that the $\alpha_{2,3}$-selective agonist may cause less sedation than the benzodiazepine, at doses with a similar anxiolytic effect.

An alternative explanation for the non-significant SPV effects of AZD7235 could be that the doses of this compound may have been too low to be pharmacologically equipotent to lorazepam 2 mg. In the human PET study with [^{11}C]flumazenil, a 50% receptor occupancy was linked to a free plasma concentration of approximately 4 ng/ml [AstraZeneca data on file] which corresponded to estimated maximal concentrations achieved after AZD7325 1.3 mg orally. Doses greater than 5 mg were linked with high levels of occupancy (> 70%). In the present study, the 10 mg dose

resulted in average peak plasma concentration of 67.4 ng/ml and average plasma concentration of 12.3 ng/mL over 21 hours, which are expected to produce GABA-A occupancy levels accounting for 80-90% and 60-70%, respectively, of the maximal occupancy level. It remains unknown whether higher doses of AZD7325 would have more profound effects on SPV. As another member of the family of GABA-A $\alpha_{2,3}$ subtype-selective partial agonist, TPA023 also produced average receptor occupancies over 70% at a dose of either 3 mg in immediate-release (IM) formulation or 8-12 mg in a controlled release (GEM) formulation [1]. However, a relatively small single dose of TPA023 (IM 1.5 mg) is required to produce comparable SPV reduction as lorazepam 2 mg in healthy volunteers [14]. The development of TPA023 was discontinued due to toxicity findings in rodents following long-term administration. Nevertheless, limited clinical efficacy data that were accumulated before termination suggest an anxiolytic-like effect of TPA023 with flexible-doses (1.5-4.5mg b.i.d. or 3-8mg b.i.d.) of the extended-release (GEM) formulation of TPA023 [1]. *In vitro*, TPA023 exerts 11% and 21% modulation at the α_2 and α_3 subunits relative to chlordiazepoxide [38], whereas the α_2- and α_3-agonism of AZD7325 are equivalent to 18% and 15% of the full efficacy of diazepam, respectively. The combination of these information suggests that relative to the doses of AZD7235 used in this study, either stronger partial agonism at the α_2 or α_3 subunits or higher exposure (with larger or repeated dosing) are necessary for clinical anxiolysis.

Subsequent to the current study, two double-blind placebo-controlled proof-of-concept studies were performed in patients with GAD. AZD7325 doses 2 mg BID, 5 mg BID, or 10 mg QD were investigated in study NCT00808249 (register identifier in ClinicalTrial.gov) [39] and doses 5 mg BID or 15 mg BID and lorazepam 2 mg BID were investigated in study NCT00807937 [40]. Both studies were of 28 days duration. These studies were designed when the results of this study were available. Given that the AZD7325 10 mg dose was well tolerated in the present study and the SPV-effects of AZD7325 10 mg were not equipotent with the lorazepam effects, it was decided that a higher dose of AZ7325 could be tested in the GAD study (i.e. 15 mg BID). Since the incidence of CNS side-effects appeared to be dose-dependent in the current study and the use of SPV as a benchmark for anxiolytic efficacy is still experimental, the dose of AZD7325 15 mg BID was selected as the highest dose to be tested with predicted positive benefit to risk ratio. Although AZD7325 demonstrated some anxiolytic activity in selected secondary end-points in these two studies, none of the AZD7325 doses met the statistical significance for the primary end-point of improvement in Hamilton Rating scale for Anxiety (HAM-A) at 4 weeks. Lorazepam 2mg BID was shown to be marginally anxiolytic at 4 weeks based on the improvement in HAM-A (mean change from baseline $\pm$ standard error: -10.8 $\pm$ 0.88 vs. -9.5 $\pm$ 0.88 [with placebo] vs. -10.4 $\pm$ 0.89 [with AZD7325 15mg BID]) [40]. In line with our observations described here, lower incidence of 'fatigue', 'somnolence' and 'sedation' occurred in the AZD7325-treated groups compared with lorazepam 2 mg BID.

AZD7325 up to doses of 15 mg BID was generally well tolerated in GAD patients. The most common adverse event associated with AZD7325 was dizziness. In addition, more adverse events of euphoric mood were seen with AZD7325 in comparison to placebo.

The high dose of AZD7325 elicited a transient increase of VAS_{high}, with the maximal back-transformed amplitude (2.01 mm) similar to that after lorazepam (1.71 mm). In contrast to AZD7325, however, lorazepam also caused concomitant enhancement of VAS internal ($VAS_{internal}$) and external ($VAS_{external}$) perception. This is in line with the findings in the phase-II studies, in which adverse event 'euphoric mood' was more frequently reported by GAD patients dosed with AZD7325 than those taking placebo [39,40] or lorazepam [40].

The main pharmacodynamic effects of AZD7325 were on EEG parameters, which were distinct from the EEG effects of lorazepam. The decrease in delta-activity contrasts with the increase in delta-power seen with lorazepam and is consistent with the lack of effect of AZD7325 on measures of sedation and alertness. A reduction of theta activity was seen with both AZD7325 and lorazepam and may relate to a common effect independent of sedation. Whatever their physiological or clinical meaning, these findings demonstrate a central pharmacodynamic effect of AZD7325. The EEG effects exhibited dose-response relationships and a close temporal link to the plasma concentrations. T_{max} was short and associated with a rapid peak effect, which may reflect a potential of quick-onset clinical effect after AZD7325.

In conclusion, the pharmacodynamic profile of AZD7325 differed from that of a typical benzodiazepine. At doses up to 10 mg, AZD7235's SPV-effects were non-significant by themselves, but showed preference over other CNS-effects. Since the doses of AZD7325 were not equivalent to lorazepam 2 mg, the lack of effects on subjective alertness, visuo-motor coordination, postural balance, and psychomotor and cognitive functions cannot be directly extrapolated as reduced clinical side-effects. Therefore, further clinical evaluations with higher doses are warranted, but the dose-dependent side-effects on the central nervous system should be considered to balance dose selection. The effects of AZD7325 10 mg on EEG spectrum and VAS_{high} suggest entry of the compound into the central nervous system and a rapid onset of pharmacodynamic effect.

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Table 1 · Results of the linear model for the Δlog(Sway)-ΔSPV, $\Delta VAS_{alertness}$-ΔSPV, ΔTracking-ΔSPV, and ΔSmooth-ΔSPV relations

Slope	AZD7325 2 mg	AZD7325 10 mg	Lora 2mg	AZD-2mg vs. Lorazepam		AZD-10mg vs. Lorazepam	
				Estimate of difference [95% CI]	*p*-Value	Estimate of difference [95% CI]	*p*-Value
ΔLog(Sway)-ΔSPV	-0.00124	-0.00036	-0.00206	0.000826 [-0.00024, 0.001892]	0.1286	0.001704 [0.000639, 0.002768]	0.0018
$\Delta VAS_{alertness}$-ΔSPV	0.003046	0.01855	0.08216	-0.07911 [-0.1209, -0.03735]	0.0002	-0.06360 [-0.1046, -0.02257]	0.0024
ΔTracking-ΔSPV	0.04789	0.01547	0.04791	-0.00001 [-0.02190, 0.02187]	0.999	-0.03244 [-0.05390, -0.01098]	0.0031
ΔSmooth-ΔSPV	0.000958	0.03297	0.06075	-0.5979 [-0.1040, -0.01557]	0.0081	-0.02778 [-0.07115, 0.01559]	0.2087

CI=confidence interval

Table 2 · Summary of pharmacodynamic (PD) effect of single doses of lorazepam 2 mg, AZD7325 10 mg, and AZD7325 2 mg, compared to placebo as estimated difference (95% confidence interval)

PD Parameter	Lorazepam-2mg vs. Placebo	AZD7325-2mg	AZD7325-10mg
$VAS_{alertness}$ (mm)	-7.9(-11.0, -4.7)	-1.6(-4.8,1.6)	-1.6(-4.7,1.6)
	p<0.0001	p=0.3111	p=0.3225
$VAS_{calmness}$ (mm)	3.5 (0.6, 6.4)	-1.7 (-4.6,1.2)	1.1 (-1.8,4.0)
	p=0.0195	p=0.2417	p=0.4452
VAS_{mood} (mm)	0.2 (-2.9,3.2)	-2.0 (-5.0,1.1)	-1.4 (-4.5,1.7)
	p=0.9146	p=0.2012	p=0.3593
Sway (%)	89.13(60.99,122.2)	1.03(-13.9,18.50)	-10.1(-23.4,5.52)
	p<0.0001	p=0.8968	p=0.1869
SPV (deg/sec)	-40.4 (-58.6,-22.1)	-14.1 (-32.2,4.1)	-15.2 (-33.3,2.9)
	p<0.0001	p=0.1240	p=0.0974
SacInacc (%)	1.1 (0.5, 1.6)	0.3 (-0.3, 0.8)	-0.1 (-0.6, 0.4)
	p=0.0002	p=0.3385	p=0.6511
Sac RT (sec)	0.014 (0.001,0.026)	-0.009 (-0.021,0.003)	-0.009 (-0.021,0.003)
	p=0.0305	p=0.1337	p=0.1389
Smooth (%)	-10.5 (-14.3,-6.7)	-3.2 (-7.0,0.6)	-1.9 (-5.7,1.9)
	p<0.0001	p=0.0997	p=0.3094
Tracking (%)	-7.26 (-8.98,-5.54)	-0.59 (-2.32,1.13)	-0.15 (-1.91,1.61)
	p<0.0001	p=0.4890	p=0.8648
$VAS_{external}$ log(mm)	0.13 (0.06, 0.19)	0.01 (-0.05, 0.08)	0.04 (-0.03, 0.10)
	p=0.0003	p=0.6941	p=0.2242
$VAS_{internal}$ log(mm)	0.06 (0.03, 0.10)	0.00 (-0.04, 0.04)	0.02 (-0.02, 0.06)
	p=0.0009	p=0.9964	p=0.2613
VAS_{high} log(mm)	0.25 (0.09, 0.41)	0.00 (-0.16, 0.16)	0.16 (-0.00, 0.32)
	p=0.0028	p=0.9889	p=0.0570

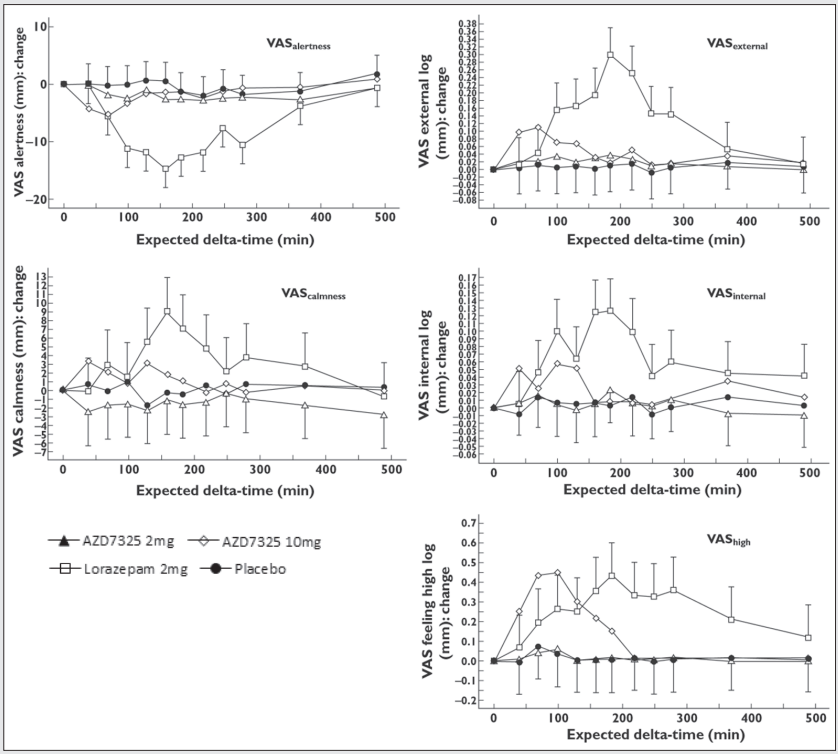
VAS=visual analogue scale; Smooth=Smooth pursuit; Tracking=Adaptive Tracking; SPV=saccadic peak velocity; SacRT=saccadic reaction time; SacInacc=saccadic inaccuracy

Table 3 · Summary of electroencephalogram (EEG) effect of single doses of lorazepam 2 mg, AZD7325 10 mg, and AZD7325 2 mg, compared to placebo as estimated difference (95% confidence interval)

EEG Parameters(%)	Lorazepan-2mg	AZD-2mg	AZD-10mg
	vs. Placebo		
Alpha Fz-Cz	-19.5(-27.2,-11.0)	2.89(-6.96,13.80)	0.19(-9.40,10.80)
	p<0.0001	p=0.5700	p=0.9696
Alpha Pz-Oz	-41.4(-51.4,-29.4)	0.31(-16.8,20.97)	10.33(-8.58,33.15)
	p<0.0001	p=0.9732	p=0.2937
Beta Fz-Cz	12.15(3.94,21.00)	-0.84(-8.11,7.00)	2.14(-5.32,10.19)
	p=0.0040	p=0.8240	p=0.5760
Beta Pz-Oz	-12.9(-22.3,-2.34)	-4.26(-14.7,7.43)	3.09(-8.21,15.79)
	p=0.0194	p=0.4487	p=0.5987
Delta Fz-Cz	10.21(2.42,18.58)	-3.66(-10.5,3.67)	-18.7(-24.5,-12.4)
	p=0.0108	p=0.3087	p<0.0001
Delta Pz-Oz	7.64(-2.54,18.88)	1.16(-8.39,11.70)	-15.9(-23.8,-7.11)
	p=0.1421	p=0.8157	p=0.0011
Gamma Fz-Cz	8.57(2.39,15.13)	-0.68(-6.52,5.52)	1.73(-4.09,7.90)
	p=0.0073	p=0.8207	p=0.5593
Gamma Pz-Oz	1.02(-12.6,16.75)	-8.20(-20.8,6.34)	-1.80(-15.2,13.65)
	p=0.8885	p=0.2469	p=0.8033
Theta Fz-Cz	-7.75(-13.8,-1.33)	-2.45(-8.81,4.36)	-13.5(-19.1,-7.43)
	p=0.0200	p=0.4624	p<0.0001
Theta Pz-Oz	-15.0(-24.2,-4.80)	1.55(-9.43,13.86)	-10.3(-19.9,0.53)
	p=0.0062	p=0.7874	p=0.0610

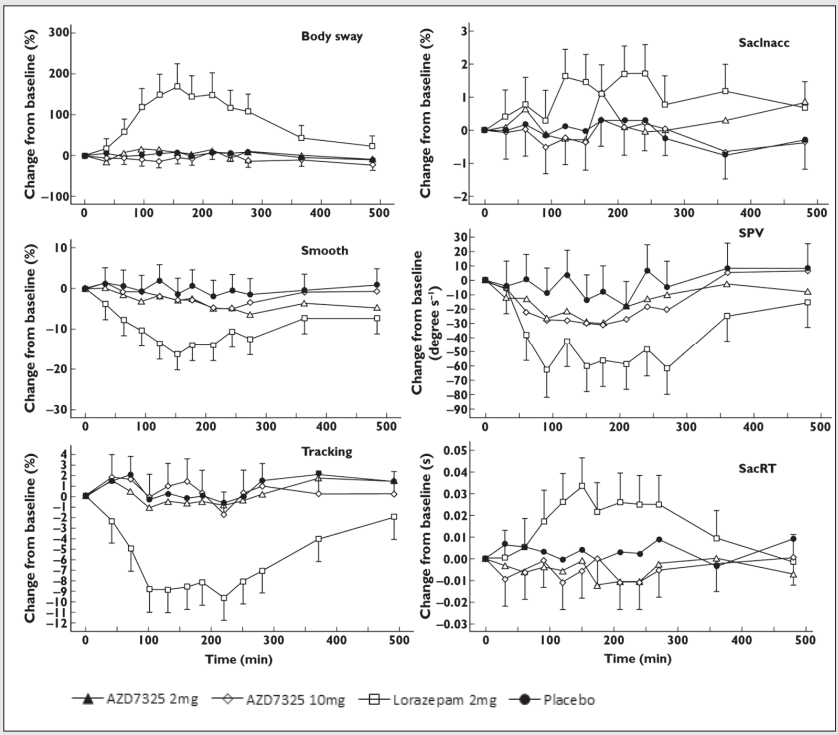
Fz-Cz=Frontal-central area; Pz-Oz=Parietal-occipital area.

Figure 1 · Least square means of change-from-baseline profiles of subjective pharmacodynamic paramters (i.e. visual analogue sub-scales) after the treatments of placebo, lorazepam 2 mg, AZD7325 2 mg, and AZD7325 10 mg



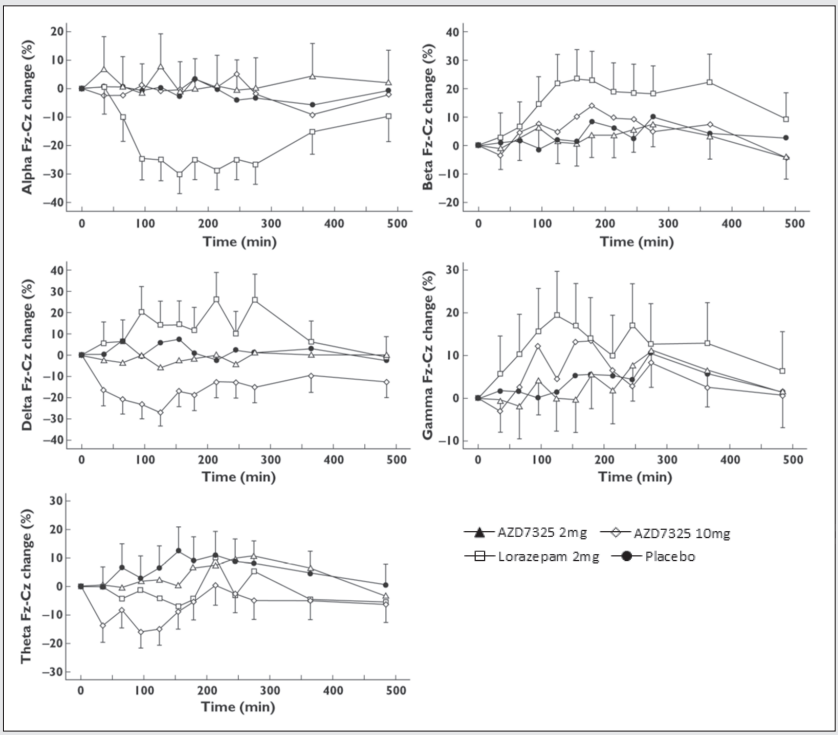
With 95% CI error bars; VAS=visual analogue scale

Figure 2 · Least square means of change-from-baseline profiles of objective pharmacodynamic parameters after the treatments of placebo, lorazepam 2 mg, AZD7325 2 mg, and AZD7325 10 mg



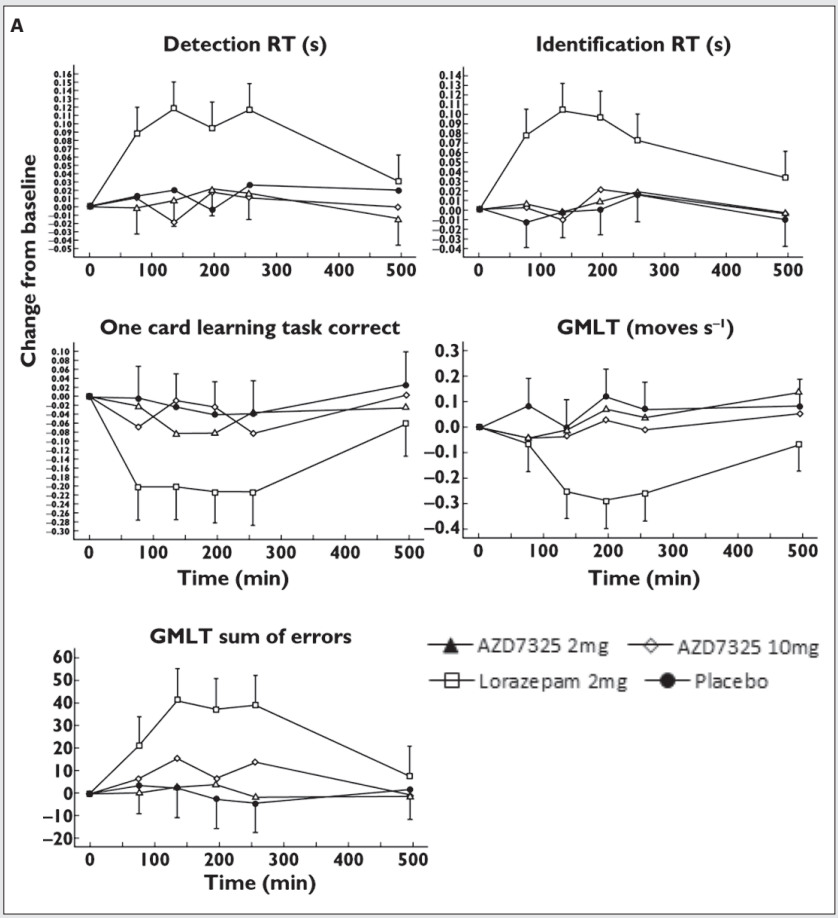
With 95% CI error bars; Smooth=Smooth pursuit; Tracking=Adaptive Tracking; Sac Inacc=Saccadic Inaccuracy; SacRT=Saccadic Reaction Time; SPV=Saccadic Peak Velocity.

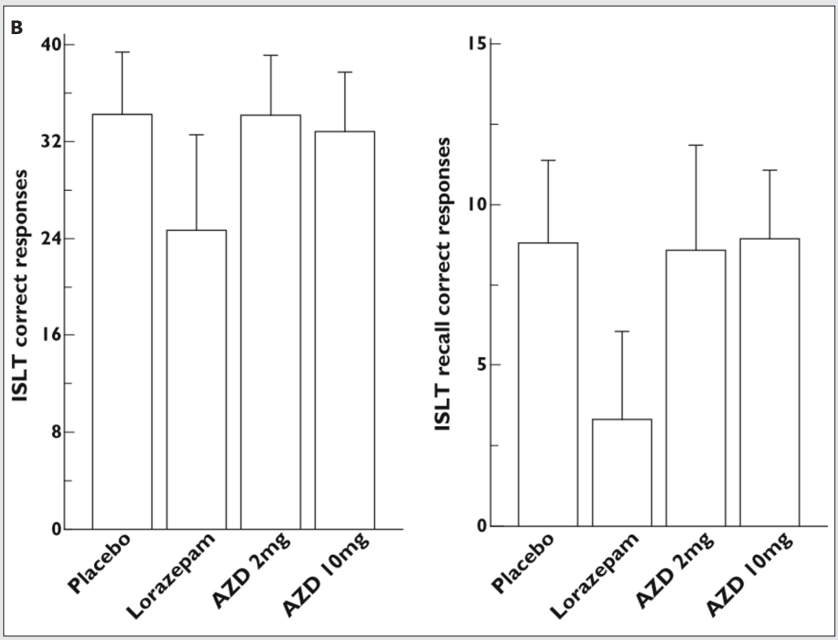
Figure 3 · Least square means of change-from-baseline profiles of electroencephalogram parameters after the treatments of placebo, lorazepam 2 mg, AZD7325 2 mg, and AZD7325 10 mg



With 95% CI error bars

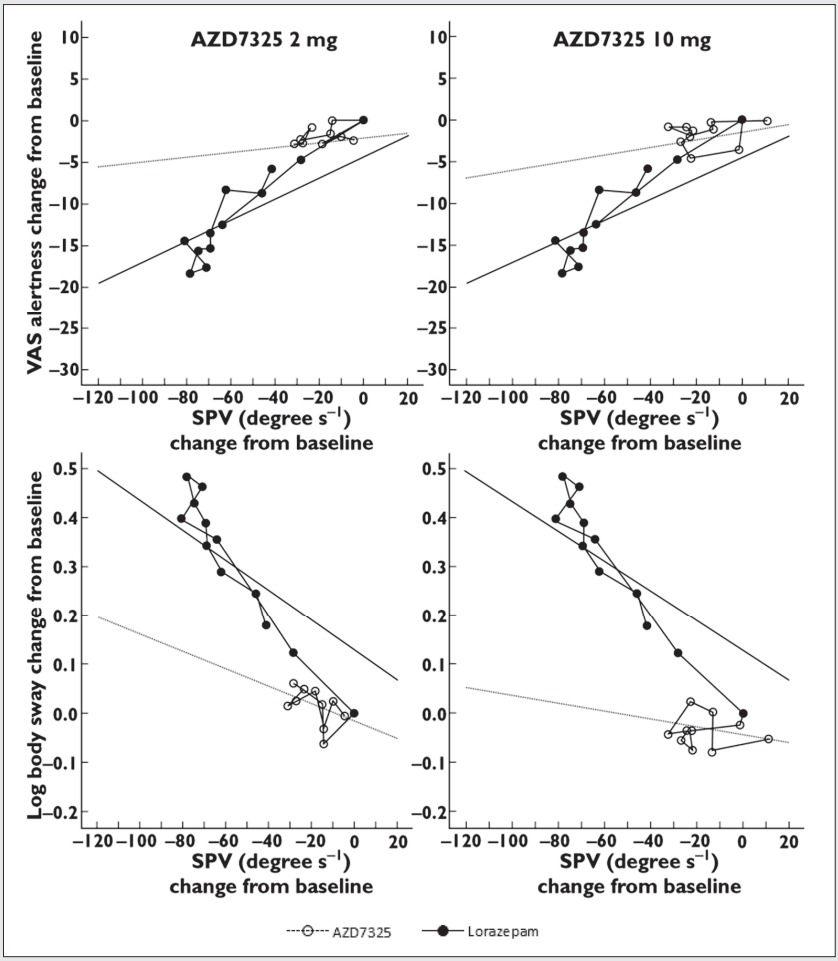
Figure 4 · Least square means of change-from-baseline profiles of CogState parameters after the treatments of placebo, lorazepam 2 mg, AZD7325 2 mg, and AZD7325 10 mg (Panel A); mean number of correct responses in the International Shopping List Test (ISLT) and the delayed recall ISLT (Panel B).

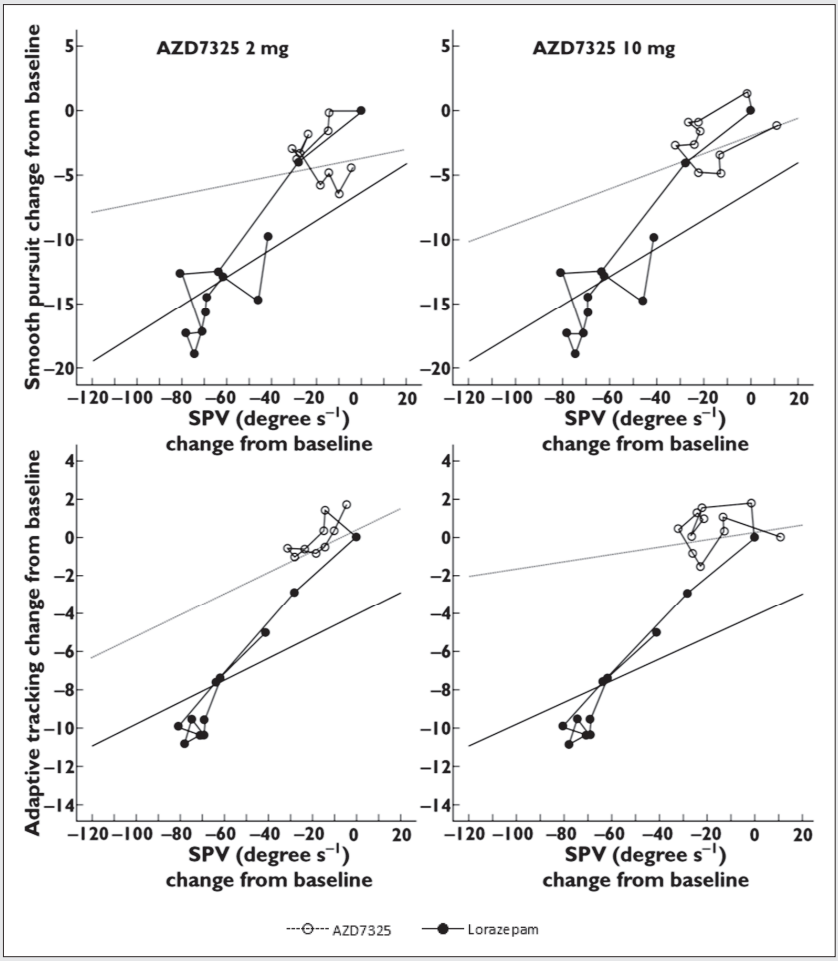




With 95% CI error bars ; RT=Reaction Time; GMLT= Groton Maze Learning Task; ISLT=International Shopping List Task.

Figure 5 · The $\Delta VAS_{alertness}$ (mm)-Δspv (deg/sec), Δsway(logmm)-Δspv (deg/sec), Δsmooth(%)-Δspv (deg/sec), and Δtracking(%)-Δspv(deg/sec) relation profiles of AZD7325 2 mg and AZD7325 10 mg vs. lorazepam 2 mg, respectively.





The AZD7325 2 mg at time=30 min does not have an error bar down, because the value of the average - error bar reaches a below zero value (AVG=3.53, SD=4.169) and cannot be shown on a log based axis.

Figure 6 · Mean concentration-time profiles of AZD7325 2 mg, AZD7325 10 mg and lorazepam with standard deviation as error bars linear (Panel A) and semi-logarithmic coordinates (Panel B)