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

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AZD6280, A NOVEL PARTIAL γ-AMINOBUTYRIC ACID A RECEPTOR MODULATOR, DEMONSTRATES A PHARMACODYNAMICALLY SELECTIVE EFFECT PROFILE IN HEALTHY MALE VOLUNTEERS

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ABSTRACT

Objective: AZD6280 is a novel GABA-A receptor modulator with higher *in vitro* efficacy at the $\alpha_{2,3}$-subtypes as compared to the α_1- and α_5-subtypes. This study compared the pharmacodynamic effects of single-oral-dose AZD6280 10 mg and 40 mg on the central nervous system (CNS) with lorazepam 2 mg. **Methods:** Sixteen healthy males were enrolled into the double-blind, randomized, 4-way crossover study. Two validated CNS test-batteries, Neurocart and CogState, were administered to measure drug effects on cognition, neurophysiologic function, psychomotor and subjective feelings. Statistical analysis was performed using mixed model analysis of variance, with fixed factors of treatment, period, time and treatment by time, and random factors of subject, subject by treatment and subject by time and the average pre-value as covariate. **Results:** Most pharmacodynamic parameters were affected by lorazepam. AZD6280 induced dose-dependent smaller-than-lorazepam effects on saccadic peak velocity (SPV) (AZD6280 10 mg vs. AZD6280 40 mg vs. lorazepam [degree/second, deg/sec]: -22.6 vs. -50.0 vs. -62.9, $p<0.001$), while the impacts on adaptive-tracking, body-sway, smooth-pursuit and the one-card-learning tests were significant but much smaller than lorazepam. Thus the slopes of regression lines for the ΔLog(Sway)-ΔSPV, ΔTracking-ΔSPV, and ΔSmooth-ΔSPV relations were flatter with AZD6280 than with lorazepam. AZD6280 caused a distinct electro-encephalography signature from that of lorazepam. **Conclusion:** The SPV responses to AZD6280 suggest potential concentration-related anxiolytic effects, while the smaller SPV-normalized effects of AZD6280 on various non-SPV pharmacodynamic parameters suggest a more favorable side-effect profile compared to lorazepam. Overall, the pharmacodynamic profile of AZD6280 matches the pharmacological specificity and selectivity of this compound at the $\alpha_{2,3}$ GABA-A receptor subtypes.

INTRODUCTION

The different anxiety disorders together constitute one of the most prevalent groups of psychiatric disorders [1]. Evidence supports the use of the selective serotonin reuptake inhibitors (SSRIs) and the tricyclic antidepressant drugs (TCAs) in the pharmacological treatment of virtually all anxiety disorders. In addition, the non-selective GABA-A receptor potentiating benzodiazepines (BZDs) are particularly effective in the management of acute forms of anxiety due to their robust anxiolytic effects and rapid onset of action. However, patients frequently discontinue SSRIs and TCAs prematurely due to a delayed onset of action and unacceptable side effects, and the widespread application of BZDs is restricted by untoward effects such as day-time sedation, fatigue, deleterious effects on cognition, memory impairment, tolerance and concerns regarding dependence liability [2,3]. These limitations of existing anxiolytic drugs underlie the pressing need for the development of efficacious innovative anxiolytic agents with more favorable side-effect profiles.

It is well-established that BZDs act through modulation of GABA-A receptors. A range of GABA-A receptor subtypes, defined by their subunit composition, mediate these effects. The use of knock-out and knock-in techniques in rodents has helped to characterize the physiological role of various GABA-A receptor subtypes as candidates for mediating the clinical effects of BZDs [4]: GABA-A receptor subtypes that contain GABA-A α_2 and α_3 subunits may mediate anxiolytic effects [5,6], while GABA-A α_1 and α_5 subunits account for sedation and cognitive impairment [4,7,8,9,10], respectively.

AZD6280 (4-Amino-8-(2,5-dimethoxyphenyl)-*N*-propylcinnoline-3-carboxamide) [11,12] is a novel, subtype-selective GABA-A receptor modulator, which in contrast to BZDs, exerts minimal efficacy at α_1-subunit containing GABA-A receptors. Although AZD6280 has relatively high ($\pm$standard deviation, SD) binding affinity to the α_1 (Ki=0.5$\pm$0.2 nM), α_2 (Ki=21$\pm$5 nM), and α_3 (Ki=31$\pm$17 nM) GABA-A subunits, its affinity for the α_5 subunit is much lower (Ki=1680$\pm$650 nM). On the other hand, the *in vitro* efficacy of AZD6280 at the GABA-A α_2 (32%) or α_3 (34%) receptor subtypes is 4-5-fold higher than that at the GABA-A α_1 (8%) or α_5 (7%) receptor subtypes relative to the corresponding maximal responses to diazepam. This profile is distinct from previously characterized $\alpha_{2,3}$ preferring compounds TPA-023 and AZD7325 in that AZD6280 has greater intrinsic activity at $\alpha_{2,3}$ subunits. Clinical relevance of these pharmacological characteristics has been tested in several pre-clinical animal models, where the compound demonstrated potential anxiolysis with reduced motor and cognitive side effects.

The objectives of this study were to investigate the pharmacodynamic (PD) effects of single oral doses of AZD6280 on the central nervous system (CNS), and compare those effects to lorazepam, a commonly used BZD.

AZD6280 10 mg and AZD6280 40 mg were selected as the investigational doses. In the AZD6280-ascending-dose study single doses up to 60 mg were tested. This dose was associated with increased rate of sedation and one event of transient depersonalization. The 10 mg and 40 mg doses were predicted to lead to peak plasma concentrations above minimally efficacious concentrations in animal models of anxiety, and provide GABA-A receptor occupancy levels 50% or higher of the maximal displaceable binding as determined by [^{11}C]flumazenil. These data demonstrate that AZD6280 crosses the blood brain barrier, interacts with the target, and has the potential to produce anxiolytic activity in humans.

For the current study, the Neurocart CNS test battery [13] and the CogState cognitive test battery were used. Components of these two batteries provide biomarkers for CNS function(s) that have been shown to be sensitive to the effects of BZDs and/or $\alpha_{2,3}$-selective GABA-A agonists [13,14,15,16]. Recent studies have suggested that partial selective $\alpha_{2,3}$ GABA-A agonists exhibit distinct effect profile in the central nervous system, which is characterized by a preserved effect on the saccadic peak velocity (SPV) but relatively reduced impairment of subjective alertness, postural balance and memory, compared to BZDs [13,14,15,16].

METHODS

DESIGN

This was a single-center, four-way crossover, randomized, double blind, double-dummy, placebo-controlled study in 16 healthy male volunteers.

SUBJECTS

Healthy male volunteers, aged 18 to 55 years, with a body mass index (BMI) between 18 and 30 kg/m^2, were medically screened after provision of written inform consent. Eligible subjects were advised not to use alcoholic beverages from 24 hours preceding each study day and refrain from smoking and using caffeine-containing products from 22:00 prior to each study day. Keeping a normal diurnal pattern was also required from two weeks before the first study day until the last study day.

TREATMENTS

All subjects arrived in the research unit at around 08:00hr on the dosing day of each study period. Study medication was administered orally between 09:00 and 11:00hr in the morning, when all pre-dose assessments were completed. Either capsules containing AZD6280 or placebo, or tablets containing lorazepam (identical to the clinically available formulation of lorazepam) or placebo were orally administered.

On each study day, subjects received one of the four treatments according to a randomly allocated treatment schedule: AZD6280 10 mg, AZD6280 40 mg, lorazepam 2 mg, and placebo. The study days were separated by washout periods of 7 days minimum. The order of the treatments was defined by a Williams Latin Square design that led to sixteen completely different sequences of four treatments.

SAFETY

Safety and tolerability were assessed by the incidence and severity of adverse events, abnormalities in vital sign assessments, clinical laboratory parameters, and electrocardiograms (ECG). Twelve-Lead ECG recordings were made, using Cardiofax V equipped with ECAPS12 analysis program (Nihon Kohden, Tokyo, Japan) or Marquette 5000/5500. Supine blood pressure and pulse were measured using a semi-automatic blood pressure recording device (a Nihon-Kohden BSM-1101K monitor or a Colin Pressmate BP 8800 or a Dash 4000). Subjects were required to rest in a supine position for at least 5 minutes prior to these measurements. Safety laboratory tests on blood or urine samples were assayed in the Central Clinical Laboratories of Leiden University Medical Centre.

PHARMACOKINETIC MEASUREMENTS

Venous blood samples (6 mL) for determination of AZD6280 or lorazepam in plasma 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. The plasma concentrations of AZD6280 and lorazepam were measured using two validated methods at Bioanalytical Systems, Inc., West Lafayette, IN, USA. Plasma concentrations of AZD6280 was determined with a solid-phase extraction / liquid chromatography coupled with tandem mass spectrometry [(SPE)/LC-MS/MS] method and lorazepam was measured using a liquid/liquid extraction/LC-MS/MS method. In brief, the plasma samples of AZD6280 were pre-purified by solid-phase extraction (SPE) and analyzed using gradient chromatographic separation on an Atlantis T3 column with a gradient mobile phase, while the plasma samples of lorazepam were pre-purified by liquid/liquid extraction and analyzed using gradient chromatographic separation on an XBridge C18 column with a formic acid/acetonitrile/water mobile phase. The bioassay method for AZD6280 was validated over the concentration range of 0.150 to 120 ng/mL. The lower limit of quantification (LLOQ) was 0.150 ng/mL, utilizing a 50.0 µL sample aliquot with a validated dilution of 50-fold with human plasma. The bioassay method for lorazepam was validated over the concentration range of 0.300 to 100 ng/mL. The lower limit of quantification (LLOQ) was 0.300 ng/mL, utilizing a 150 µL sample aliquot with a validated dilution of 20-fold with human plasma. Inter- and intra- batch precision of both methods were less than 15% and the accuracy was within 85-115%. A matrix-effect

test indicated that the determination was not affected by the matrix. In addition, AZD6280 and lorazepam in plasma were proven to be stable under the storage condition of -80 °C for at least 205 days and 144 days, respectively.

PHARMACODYNAMIC MEASUREMENTS

A collection of computerized neurophysiologic and neuropsychological tests was performed during the study. Most of these assessments were given during the CNS training session to familiarize subjects with the tests and reduce learning effects. The Neurocart battery was performed in the following chronological order: body sway, visual analogue scale (VAS) Bond & Lader, VAS Bowdle, saccadic eye movements, smooth pursuit eye movements, adaptive tracking and electro-encephalogram (EEG). In each treatment period, this battery was assessed at pre-dose (twice) and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 6, 8, and 12 hours post-dose. The CogState early phase battery was carried out before dosing (three times) and at 1.25, 2.25, 3.25, and 4.25 hours post-dose. This cognitive test battery contains the Groton Maze Learning Task (GMLT), Detection task (DET), Identification Task, and One Card Learning Task [17,18]. Each abovementioned Neurocart or Cogstate test lasted 1 to 5 minutes, thus the total duration of these pharmacodynamic assessments was 15-30 minutes at different time points. At 1.75 hours post-dose subjects also completed the International Shopping List Task, a word verbal list learning test presented three times, each time followed by an immediate recall trial. Delayed recall was tested 21 hours later. At each of these assessments, one subject at a time was tested in a quiet room with ambient illumination.

NEUROCART

BODY SWAY

Body sway was measured with an apparatus similar to the Wright ataxiameter [19], which integrates the amplitude of unidirectional body sway. Two-minute measurements were made in the anterio-posterior direction with eyes closed. The subject was asked to stand comfortably on a floor with his/her feet slightly apart. Body sway measures postural (in)stability. It has demonstrated considerable sensitivity to the effect of benzodiazepines [20].

VISUAL ANALOGUE SCALES OF BOND & LADER (VAS B&L) 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 [21,22]. Dutch versions of the scales have been frequently employed at the Centre for Human Drug Research (CHDR), for a variety of sedative agents [23] and circumstances [24]. During the test, the subjects 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 [25] for subjective alertness, contentedness, and calmness.

The Bowdle Psychotomimetic Effects Scores have been used to quantify the psychotomimimetic effects of ketamine [26]. A translated Dutch version of the scale originally developed by Bowdle et al. has been computerized and used at the CHDR to study glutamatergic drug effects. 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.

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, An Astro-Med, Inc. Product Group, 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, deg/sec), latency (i.e. reaction time, msec) 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 effect of benzodiazepines [22] and has been found to be closely related to the anxiolytic component of benzodiazepines and some newly developed compounds with potential anxiolytic effect [14,15,16].

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 step 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 in healthy volunteers dosed with benzodiazepines at the CHDR by van Steveninck *et al.* [23] based on the work of Bittencourt *et al.* [28] and the original description of Baloh *et al.* [29]. 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 parameter.

ADAPTIVE TRACKING

The adaptive tracking test was performed as originally described by Borland and Nicholson [30], using customised equipment and software. After a 0.5-minute

run-in time without data-recording, the average performance over the rest 3.0 minutes was scored and was used as the test parameter. Adaptive tracking is a pursuit-tracking task. 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 increases, and vice versa.

ELECTROENCEPHALOGRAPHY

Pharmaco-electroencephalography (pharmaco-EEG) was used to monitor any drug effects, which can be interpreted as evidence of penetration across the blood brain barrier and changes in the activity of the brain [31,32]. EEG provides non-specific measures of CNS functions. EEG recordings were made using gold electrodes, fixed with EC2 paste (Astromed) and using standard pharmacoEEG lead placement, with the same common ground electrode as for the eye movement registration (international 10/20 system for EEG electrode placement [33]). The electrode resistances were kept below 5 kOhm. The signals were amplified by use of a Grass 15LT series Amplifier Systems with 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 pharmacoEEG analyses in order to test whether the findings of an effect of AZD6280 on gamma-frequency band (> 30 Hz) in animals translate to humans.

COGNITION MEASURES

The computerized CogState Early phase Battery, consists of four tasks with demonstrated sensitivity to cognitive change associated with drug effects [34]. These tests are listed below, in order of administration. In addition Cogstate's International Shopping List Task immediate and 21 hour delayed recall were administered once per dosing period.

GROTON MAZE LEARNING TASK (GMLT)

This is an executive problem solving and spatial learning task which requires the subject to find a 28-step pathway that is hidden under a 10 x 10 grid of tiles displayed 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) while adhering to

several rules (no diagnonals, no skipping, no retracing movements). 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. 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 aspect of the task 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 possible. If subjects responded before the card flipped over or did not respond to a card that had flipped over, an error sound was emitted. After a brief practice test, the real test began. The test measures attention and psychomotor function.

IDENTIFICATION TASK

A playing card was presented face down 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 (black) 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 working memory and learning ability.

INTERNATIONAL SHOPPING LIST TASK (ISLT)

At 1.75h post-dose, the test supervisor read a shopping list of 16 words to the subject as they appeared 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. The list was read (in the same order) and the immediate recall session were repeated two more times for a total of three trials. The task measures verbal learning ability. At 21h post-dose, subjects were required to recall the shopping list without being read them again, while the test supervisor clicked / touched the appropriate button on the screen with the stylus or mouse. This part of the ISLT task measures long term memory and retrieval (delayed recall).

STATISTICAL ANALYSIS

Statistical analysis was carried out using SAS (version 9.1.3). The primary variables for pharmacodynamic evaluation were outcome parameters from VAS assessments for alertness, CogState battery and tests about other CNS functions. The secondary variables for safety and tolerability evaluation were adverse events (AEs), vital signs assessments (blood pressure, heart rate, respiratory rate and [auricular] temperature), laboratory parameters, and ECGs. The variables for pharmacokinetic evaluation were C_{max}, T_{max}, AUC_{0-t}, $T_{1/2\lambda z}$, and CL/F.

The pharmacokinetic analyses were performed by Clinical Pharmacology, AstraZeneca Wilmington, DE, USA using the WinNonlin program (Pharsight Corporation, MountainView, California, USA) and descriptive statistics of the PK parameters were summarized by treatment.

In total, twenty-nine pharmacodynamic (PD) parameters and their change from baseline were analyzed by mixed model analyses of variance (using SAS PROC MIXED) with treatment, period, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effects, and with the baseline value as covariate, where baseline was defined as the average of the available values obtained prior to dosing. Treatment effects were reported as the contrasts of each active treatment vs. placebo, each dose of AZD6280 vs. lorazepam, as well as AZD6280 40 mg vs. AZD6280 10 mg. No multiplicity adjustment was used for this study. The least square means (LSMs) of the measurements up to 8h post-dose were calculated within the statistical model. Contrasts were reported along with 95% confidence intervals. The EEG, body sway, and VAS Bowdle subscales were analyzed after log-transformation, while the other parameters were analyzed without transformation. Log-transformed parameters were back-transformed after analysis where the results were interpreted as percentage change.

Adverse events were listed and summarized by system organ class and treatment. Vital sign assessments, parameters of the 12-lead ECG recordings and safety laboratories, along with their changes from baseline, were summarized using descriptive statistics for each scheduled measurement by treatment.

Previous studies demonstrated good sensitivity of SPV to the effect of BZDs [13,22] and $\alpha_{2,3}$ subtype-selective GABA-A agonists [13,14,15,16]. As $\alpha_{2,3}$ subunits are the common pharmacological targets shared by these compounds, SPV is hypothesized to be a biomarker for GABA-A $\alpha_{2,3}$ subunit modulation. Moreover, early clinical findings with TPA023 [4] also link the pharmacodynamics effect on SPV to therapeutic anxiolysis. Body sway, tracking, $VAS_{alertness}$, and smooth pursuit, on the other hand, are thought to reflect the sedative and adverse properties of GABA-ergic compounds. As such, we performed a regression analysis to explore the relationship of individual changes from baseline in body sway (Δsway), tracking (ΔTracking), VAS alertness ($\Delta VAS_{alertness}$), and smooth pursuit (Δsmooth), relative

to the changes from baseline in SPV (ΔSPV). The slopes of these regression lines are thought to reflect the relations between drug-induced anxiolysis and CNS-depression. A mixed effect model was used, where the fixed factors were treatment and treatment by SPV, whereas the random factors were subject and subject by SPV. The estimate of the slopes of the regression lines of these ΔSPV-relative effect profiles were compared between each dose of AZD6280 and lorazepam.

The pharmacodynamic effects (i.e., the changes from baseline) on SPV were listed with plasma drug concentrations obtained at the same post-dose time points with AZD6280 and lorazepam, respectively. Based on the effect profile of SPV (Figure 2), the median effect size was summarized from all negative values, that is, the same direction of effect as the maximal effect of lorazepam and AZD6280. Plasma drug concentrations of AZD6280 and lorazepam that correspond to 40% to 60% percentile of the overall PD effect size were summarized. The two resultant geometric mean concentrations, termed as 'pharmacodynamically equivalent concentrations' in this case, were used to normalize the actual concentration measurements of each compound. Subsequently, the post-dose PD effect values were plotted with the normalized drug concentrations.

RESULTS

SUBJECTS

A total of seventeen male healthy volunteers participated in the study. Sixteen subjects completed the study. One subject was withdrawn from the study due to positive THC result in urine drug screen test on his 2nd treatment period. This drop-out was replaced by a subject receiving the same order of study treatments. Subjects had an average age of 31.7±12.6 years, and BMI of 23.4±2.0 kg/m^2. Data from all treated subjects were used in the analyses of safety and pharmacokinetics. Subjects who completed the study per protocol were included in the pharmacodynamic analysis.

PHARMACOKINETICS (PK)

As demonstrated in Figure 1, both AZD6280 10 mg and AZD6280 40 mg were rapidly absorbed after oral administration with no absorption lag time. The median time to maximum plasma-concentrations (T_{max}) was 1.50 hr (range 0.50 hr-3.23 hr) with AZD6280 10 mg and 1.38 hr (range 0.50 hr-4.00 hr) with AZD6280 40 mg. The mean termination half-life ($T_{1/2}$) was comparable between the two dose levels of AZD6280 (7.10 hr for 40 mg and 6.65 hr for 10 mg) Lorazepam 2 mg had similar T_{max} (median 1.50 hr, range 1.00 hr-4.50 hr) but longer $T_{1/2}$ (mean 12.9 hr, range 8.28 hr-18.4 hr) when compared to AZD6280. The dose-normalized PK parameters appeared to be independent from the dose of AZD6280 (see Table 1).

PHARMACODYNAMICS

Table 2 summarizes the pharmacodynamic (PD) effects of each active treatment compared to placebo. $VAS_{alertness}$, was not significantly impaired by either AZD6280 10 mg or AZD6280 40 mg compared to placebo (Table 2). However, alertness was significantly decreased after lorazepam 2 mg. Sedation caused by lorazepam was significantly larger than that of AZD6280 10 mg (mean change from baseline: -5.9 mm *vs.* -0.8 mm, p=0.0051), but was marginally distinguishable from the effect of AZD6280 40 mg (-5.9 mm *vs.* -3.1 mm, p=0.1055).

Saccadic peak velocity (SPV) was significantly reduced by the three active treatments (Table 2), respectively, compared to placebo (Figure 2). SPV reductions differed between lorazepam and AZD6280 [estimated difference in SPV, p-value] (lorazepam vs. AZD6280 10 mg: -40.3 deg/sec, p<0.0001; lorazepam vs. AZD6280 40 mg: -12.9 deg/sec, p=0.0367) and were dose-dependent (AZD6280 40 mg vs. AZD6280 10 mg: -27.3 deg/sec, p<0.0001).

Body sway, smooth pursuit, tracking and VAS 'feeling high' were significantly affected by lorazepam 2 mg and AZD6280 40 mg, compared to both placebo and AZD6280 10mg. The effects of lorazepam 2 mg on body sway, smooth pursuit, and tracking were significantly larger than those of AZD6280 40 mg (Sway: 89.04% vs. 21.12%, p<0.0001; Smooth: -10.8% vs. -4.0%, p=0.0003; Tracking: -9.53% vs. -2.65%, p<0.0001). VAS 'feeling high' [estimated difference between two treatments, 95% CIs, p-value] was significantly increased by AZD6280 40 mg and lorazepam 2 mg (Table 2). Although the effect size of AZD6280 40 mg was comparable to that of lorazepam 2 mg (0.02 [log(mm)], [0.08, -0.12], p=0.6282), the effect lasted considerably shorter with AZD6280 40 mg (Figure 3). Moreover, lorazepam also significantly distorted internal and external perceptions compared to placebo, but the effects of AZD6280 did not differ from placebo (Table 2).

The three active treatments had a different spectrum of pharmaco-EEG effects. All frequencies of EEG bands were statistically significantly affected by lorazepam 2 mg. In comparison, AZD6280 40 mg induced lower effects on alpha bands, no effect on gamma band, opposite effects on delta band (estimated difference versus placebo, 95% confidence interval, p-value: AZD6280 40 mg: -6.7% (-12.3%, -0.6%), p=0.0325; lorazepam 2 mg: 8.6% (1.9%, 15.8%), p=0.0129) and affected only the theta and beta bands in the Fz-Cz (i.e. frontal-central) leads. AZD6280 10 mg only induced changes in theta and alpha bands and the extent and distribution of these effects were similar to those of AZD6280 40 mg.

Results of the CogState early battery are presented in Figure 4. Neither dose of AZD6280 affected the individual CogState variables, except for the statistically significant effect of AZD6280 40 mg associated with worse performance on one-card learning accuracy compared to placebo (AZD6280 40 mg vs. placebo: -0.09 vs. -0.02, [acr(%)], p=0.0018). However, the effect size of AZD6280 was significantly

less than that of lorazepam 2 mg (lorazepam vs. placebo: -0.14 vs. -0.02, [acr(%)], p<0.0001). Moreover, lorazepam was also associated with extensive impairments on the other CogState cognitive parameters compared to placebo (lorazepam *vs.* placebo, [unit], *p*-value): reaction time of correct responses in the detection task (0.12 *vs.* 0.03, [log(msec)], p<0.0001), reaction time of correct responses in the identification task (0.08 *vs.* 0.02, [log(msec)], p<0.0001), moves per second (mps) in the chase test (-0.20 vs. 0.03, [mps], p<0.0001), and the sum of errors in GMLT (22.2 vs. 4.5, p=0.0003). Lorazepam also reduced the number of words recalled in both the ISLT immediate-recall (lorazepam *vs.* placebo: 25.2 vs. 35.3, p<0.001) and the ISLT delayed-recall (lorazepam *vs.* placebo: 4.8 vs. 10.1, p<0.001).

Several CNS effects of the different compounds are plotted against their impact on SPV. The regression analyses for ΔLog(Sway)-ΔSPV and ΔTracking-ΔSPV are plotted in Figure 5, in combination with the calculated population regression lines. The absolute slopes of the regression lines were significantly smaller for the relations of ΔLog(Sway)-ΔSPV (AZD6280 10 mg vs. lorazepam 2 mg: -0.00056 vs. -0.00157, p=0.0099; AZD6280 40 mg vs. lorazepam 2 mg: -0.00080 vs. -0.00157, p=0.0135), ΔTracking-ΔSPV (AZD6280 10 mg vs. lorazepam 2 mg: 0.03474 vs. 0.06453, p=0.0168; AZD6280 40 mg vs. lorazepam 2 mg: 0.03080 vs. 0.06453, p=0.0006), and Δsmooth-ΔSPV (AZD6280 10 mg vs. lorazepam 2 mg: 0.06146 vs. 0.01083, p=0.0232; AZD6280 40 mg vs. lorazepam 2 mg: 0.06106 vs. 0.01083, p<0.0001) for either dose of AZD6280 than for lorazepam 2 mg, but the relations of $\Delta VAS_{alertness}$-ΔSPV are comparable between AZD6280 and lorazepam.

As is shown in Figure 6, the effect size on SPV generally grows with the increase of concentration with either AZD6280 or lorazepam. The range of effect size is generally comparable between lorazepam and AZD6280 on SPV. However, when the effect size is higher than 30 deg/sec (i.e. SPV reduction > 30 deg/sec), the corresponding range of normalized drug concentration is much larger with AZD6280 than that with lorazepam. Moreover, lorazepam produces a relatively sharp decline of SPV with concentration elevation, while the concentration-effect profile of AZD6280 is less steep. The same concentration-effect profile also applies to the profiles of body sway and VAS alertness, except that AZD6280 had much lower maximal effect on the two PD parameters within the observed range of plasma concentrations (Figure 6). These findings indicate that AZD6280 is pharmacologically less potent than lorazepam, and that higher concentrations of the new compound are needed to reach the same effect as the benzodiazepine.

SAFETY

The administration of single dose AZD6280 10 mg or 40 mg were safe and well tolerated in the 17 healthy male subjects. In general, Treatment-emergent AEs (frequency [incidence]) occurred more frequently with AZD6280 40 mg (25 [75.0%])

than with AZD6280 10 mg (7 [43.8%]), but were milder and less frequent than with lorazepam 2 mg (39 [93.8%]). The frequency and severity of AEs with low dose (10 mg) AZD6280 were comparable to those with placebo (8 [47.1%]). Compared to lorazepam 2 mg, AZD6280 40 mg (lorazepam vs. AZD6280 40mg) caused fewer gastrointestinal (2 [12.5%] vs. 1 [6.3%]) and less frequent and less intensive neurological events (particularly indicative of sedation) (14 [87.5%] vs. 11 [68.8%]). No clinically significant abnormalities in vital signs, laboratory or ECG results were identified during the study.

DISCUSSION

The current study compared the CNS effects of AZD6280, a novel $\alpha_{2,3}$ subtype-specific GABA-A receptor modulator intended for anxiolytic use, with those of lorazepam. A comprehensive selection of neurophysiological and neuropsychological tests was employed to address the pharmacodynamic effects of the two GABA-ergic compounds on various brain domains. As a positive control, therapeutic dose of lorazepam was designed to benchmark the effect of clinically relevant GABA-A agonism on each pharmacodynamic measure.

Single doses of AZD6280 10 mg, AZD6280 40 mg, and lorazepam presented distinct pharmacodynamic effect profiles on the central nervous system. These effect profiles are likely to result from differences in selectivity and potency of the compounds to modulate the subtypes of the GABA-A receptor complex (AZD6280 vs. lorazepam) as well as from differences in exposure levels of the same drug (AZD6280 10 mg vs. 40 mg). The highest dose of AZD6280 in this study caused SPV reductions that on average were somewhat smaller than those of lorazepam. This indicates that the treatments might not be fully equipotent. SPV has been suggested as a pharmacodynamic biomarker for clinical anxiolysis of benzodiazepines and novel $\alpha_{2,3}$-subtype selective GABA-A receptor agonists [4,13,22]. The concentration-effect profiles of lorazepam and AZD6280 on SPV provide a better approach for the evaluation of dose equivalence, because they show the entire range of drug plasma levels and effects. Noteworthy, the range of effect size is comparable between lorazepam 2 mg and AZD6280 40 mg on SPV, although fewer individual SPV measurements reach the level that is regularly attained after lorazepam. Also, a larger range of AZD6280 concentrations was linked to significant effect on SPV (i.e. >30 deg/sec SPV reduction) compared with lorazepam (Figure 6). These findings and the known relevance of SPV effect suggest that the anxiolytic effect of peak concentrations of AZD6280 40 mg may be similar to the effects of lorazepam 2 mg. Moreover, the SPV effects of AZD6280 diminish at relatively high plasma levels, which are compatible with partial agonism, with a maximum effect that seems to be smaller than with high doses of the full agonist lorazepam.

The CNS-PD parameters other than SPV are linked to various clinical side-effects of benzodiazepines, including postural instability, visuo-motor coordination, hypnotic effects and cognitive impairments. In Figure 6, the ranges of AZD6280 and lorazepam concentrations are identical to the concentration-effect plots delineated for SPV; however, larger relative differences are seen in the maximally attainable effect on sway between lorazepam and AZD6280, versus the difference seen in peak SPV effects. The smaller maximum effect of AZD6280 on body sway indicates less impairment of postural stability despite of equal or even higher normalized concentrations of the new compound compared to lorazepam. These findings can be interpreted by the superior pharmacological selectivity of AZD6280 over the non-selective GABA-A agonism of lorazepam.

Moreover, the relations between drug-induced SPV reduction and the drug effects on these side-effect-related CNS-PD parameters were proposed to quantitatively compare the pharmacodynamic selectivity of a GABA-A-ergic drug with benzodiazepine, regardless of dose or dose equivalence. In this study, AZD6280 demonstrated relatively flat regression lines in the scatter graphs for body sway, tracking or smooth pursuit against SPV. The slopes for these regression lines were significantly different from those observed after lorazepam. Such a SPV-dominant effect profile is distinguishable from that of benzodiazepines and is consistent with our previous reports regarding other GABA $\alpha_{2,3}$-selective partial agonists [13]. On the other hand, the similar slopes of the regression lines between the two doses of AZD6280 support that such SPV-relative indices are independent from dose or dose equivalence.

In line with their distinct pharmacodynamic profile, lorazepam and AZD6280 differed considerably in their profiles of CNS depression. Fewer treatment-emergent adverse events, mostly 'neurological disorders' as defined in MedDRA-ergic, occurred with the highest dose of AZD6280 than with lorazepam. Lorazepam caused remarkable concentration-dependent response profiles on adaptive tracking, body sway, smooth pursuit, visual analogue scales for alertness, 'feeling high', external and internal perceptions, and various cognitive functions. The effects of AZD6280 40 mg on these parameters were smaller and often not statistically significant. All these findings suggest that lorazepam induces substantial impairments in a wide range of CNS domains, but the effects of AZD6280 40 mg were less extensive. These results are consistent with those reported in previous single-dose studies with lorazepam and other $\alpha_{2,3}$-selective GABA-A partial agonists [14,15,16]. Thus the abovementioned pharmacodynamic effect profiles probably reflect the characteristics of the entire family of $\alpha_{2,3}$-selective GABA-A partial agonists, which may translate into a reduced propensity to cause sedative side effects.

AZD6280 and lorazepam also showed different effects on the EEG power spectrum. The effect of AZD6280 40 mg on EEG Delta band was opposite to that of lorazepam and may relate to differences in the sedative effects of the compounds. In

the EEG bands between 4 Hz and 35 Hz, the EEG responses to AZD6280 were generally consistent with but smaller than those to lorazepam. Lorazepam was associated with an increase in EEG power in the Gamma band, while the effect of AZD6280 was negligible with either dose. The similarities of EEG signatures between AZD6280 and lorazepam in the medium frequency bands, as well as those between the two doses of AZD6280, may reflect common pharmacological properties underlying an anxiolytic action. Furthermore, the relatively low effects of AZD6280 on the EEG parameters may be explained by the partial GABA-A modulation of this new compound, compared with the full agonism of lorazepam.

In conclusion, the SPV responses to AZD6280 suggest potential anxiolytic effect of the compound, while the absent or smaller effects of AZD6280 on subjective alertness, visuo-motor coordination, postural balance, psychomotor and cognitive functions indicates a more favorable and concentration-related side-effect profile compared to that of lorazepam. Overall, the pharmacodynamic profile of AZD6280 is consistent with the specificity and selectivity of this compound at the $\alpha_{2,3}$ GABA-A receptor subtypes. This pharmacodynamic profile, combined with a good tolerability profile, support further clinical development of AZD6280 as a potential fast-onset anxiolytic at doses around 10 to 40 mg.

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Table 1 · Mean (SD) of the dose-normalized pharmacokinetic parameters after administration of AZD6280 10 mg and AZD6280 40 mg (n=16)

	Unit	AZD6280 10mg	AZD6280 40mg	Lorazepam 2mg
C_{max},norm*	ng/mL/mg	9.9 (2.4)	8.2 (2.4)	NC
AUC_{0-t},norm	h·ng/mL/mg	41.0(8.6)	37.9 (10.0)	NC
$AUC_{0-\infty}$,norm	h·ng/mL/mg	46.0 (10.8)	42.2 (11.9)	NC
C_{max}	ng/mL/mg	98.5(24.3)	328(97.5)	21.9(4.67)
AUC_{0-t}	h·ng/mL/mg	397(100)	1515(400)	235(47.8)
$AUC_{0-\infty}$	h·ng/mL/mg	444(125)	1686(477)	360(103)
$AUC_{1/2}$	h	7.0(1.2)	6.7(0.9)	13.0(2.6)
CL/F	L/hr	24.9(9.55)	25.8(8.09)	5.96(1.56)

*NC=not calculated; *'norm' indicates dose-normalized pharmacokinetic parameter*

Table 2 · Treatment contrasts as Estimated difference for the PD parameters

Parameters	Lorazepam 2mg	AZD6280 10mg	AZD6280 40mg
	vs. Placebo		
SPV (deg/sec)	-62.9 (-75.2, -50.6)	-22.6 (-34.6, -10.6)	-50.0 (-62.1, -37.8)
	p<0.0001	p=0.0005	p<0.0001
SacInacc (%)	0.7 (0.1, 1.3)	-0.4 (-0.9,0.2)	-0.4 (-1.0, 0.2)
	p=0.0206	p=0.2191	p=0.1590
SacRT (sec)	0.029 (0.023, 0.035)	0.003 (-0.003, 0.009)	0.004 (-0.002, 0.010)
	p<0.0001	p=0.2964	p=0.1548
Sway (%)	89.0%(62.8%, 119.6%)	-1.0%(-14.8%, 15.1%)	21.1%(4.3%, 40.7%)
	p<0.0001	p=0.8965	p=0.0134
Smooth (%)	-10.8 (-14.2, -7.3)	-1.1 (-4.6, 2.3)	-4.0 (-7.5, -0.6)
	p<0.0001	p=0.5103	p=0.0238
Tracker (%)	-9.53 (-11.9, -7.21)	-0.63 (-2.95, 1.69)	-2.65 (-4.97, -0.33)
	p<0.0001	p=0.5806	p=0.0266
$VAS_{alertness}$ (mm)	-5.6 (-9.1, -2.1)	-0.5 (-3.9, 2.9)	-2.7(-6.1, 0.7)
	p1=0.0024	p=0.7691	p=0.1178
$VAS_{calmness}$ (mm)	2.2(-0.8, 5.3)	1.4 (-1.6, 4.5)	1.2 (-1.8, 4.3)
	p=0.1429	p=0.3402	p=0.4114
VAS_{mood} (mm)	-0.7 (-2.5, 1.2)	0.5 (-1.4, 2.4)	0.0 (-1.8, 1.9)
	p=0.4733	p=0.5847	p=0.9723
$VAS_{external}$ log(mm)	0.10 (0.05, 0.16)	0.01 (-0.04,0.07)	0.05 (-0.00, 0.10)
	p=0.0004	p=0.5931	p=0.0719
$VAS_{internal}$ log(mm)	0.07 (0.03, 0.11)	0.01 (-0.02,0.05)	0.01 (-0.03, 0.05)
	p=0.0007	p=0.4781	p=0.5087
VAS_{high} log(mm)	0.12 (0.02, 0.22)	-0.01 (-0.11,0.08)	0.10 (0.00, 0.19)
	p=0.0168	p=0.7881	p=0.0474

1. The p-value represent the result of statistical comparison between the corresponding active treatment (i.e. AZD6280 40 mg, AZD6280 10 mg, or lorazepam 2 mg) and placebo for each pharmacodynamic parameter

Figure 1 · Mean (standard error as error bars) concentration-time profiles of AZD6280 10 mg, AZD6280 40 mg and lorazepam

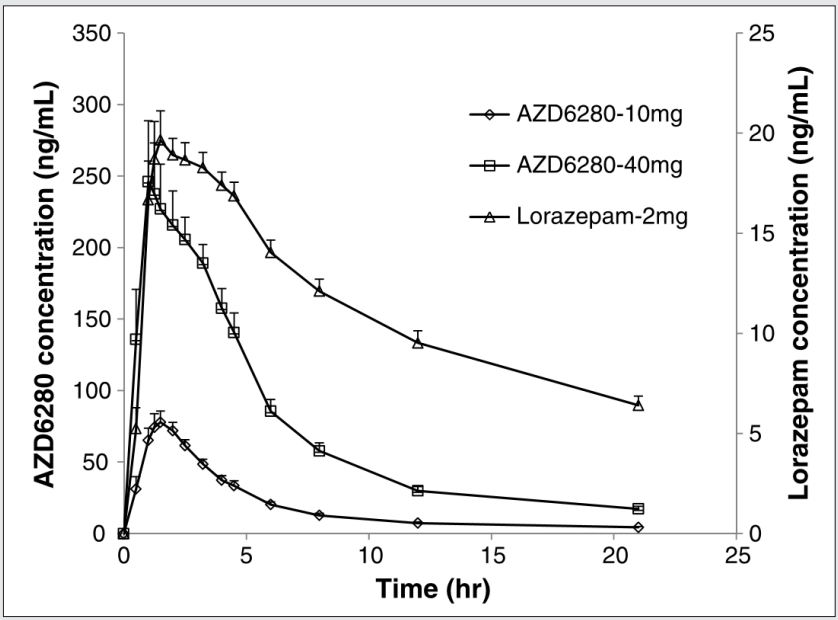


Figure 2 · Mean (standard error) profiles of objective pharmacodynamic parameters after the treatments of placebo, lorazepam 2 mg, AZD6280 10 mg, and AZD6280 40 mg

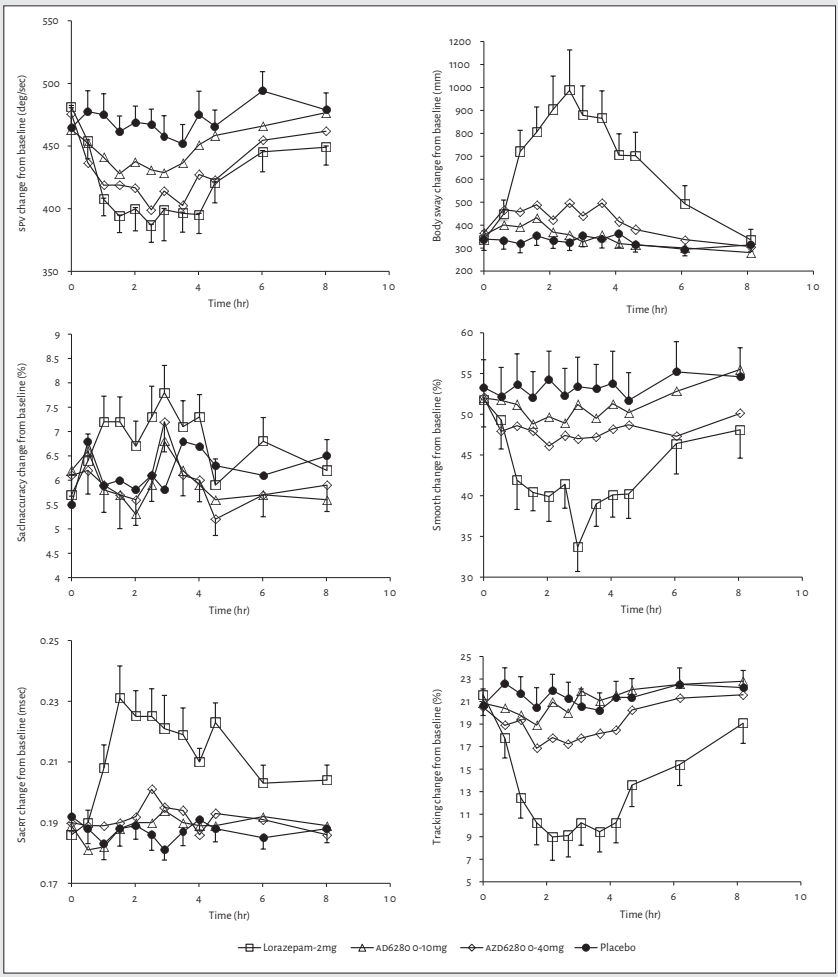


Figure 3 · Arithmetic mean (standard error) profiles of subjective pharmacodynamic paramters (i.e. visual analogue sub-scales) after the treatments of placebo, lorazepam 2 mg, AZD6280 10 mg, and AZD6280 40 mg

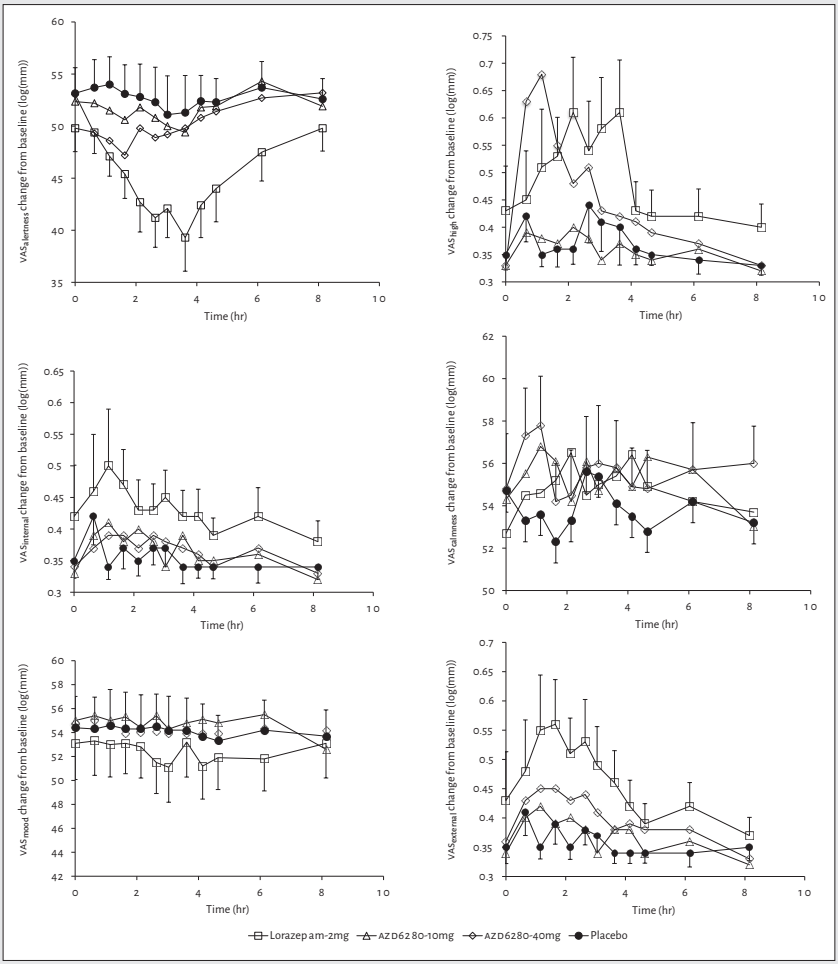
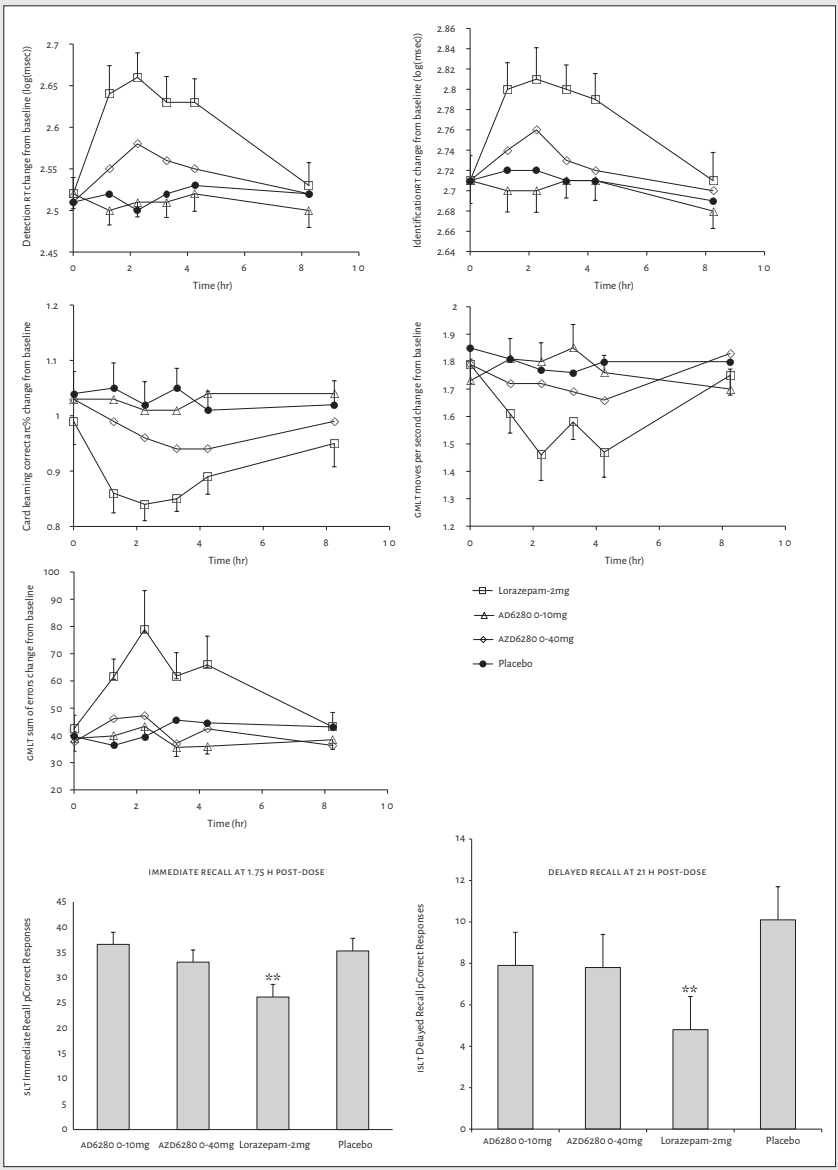


Figure 4 · Means (standard error) profiles of CogState parameters after the treatments of placebo, lorazepam 2 mg, AZD6280 10 mg, and AZD6280 40 mg



*ISLT=International Shopping List Task; ** represent 'p<0.001' compared to the placebo arm.*

Figure 5 · The ΔLOC(Sway)-ΔSPV, $\Delta VAS_{alertness}$-ΔSPV, ΔTRACK-ΔSPV, ΔSMOOTH-ΔSPV relations of AZD6280 10 mg and 40 mg vs. lorazepam 2 mg.

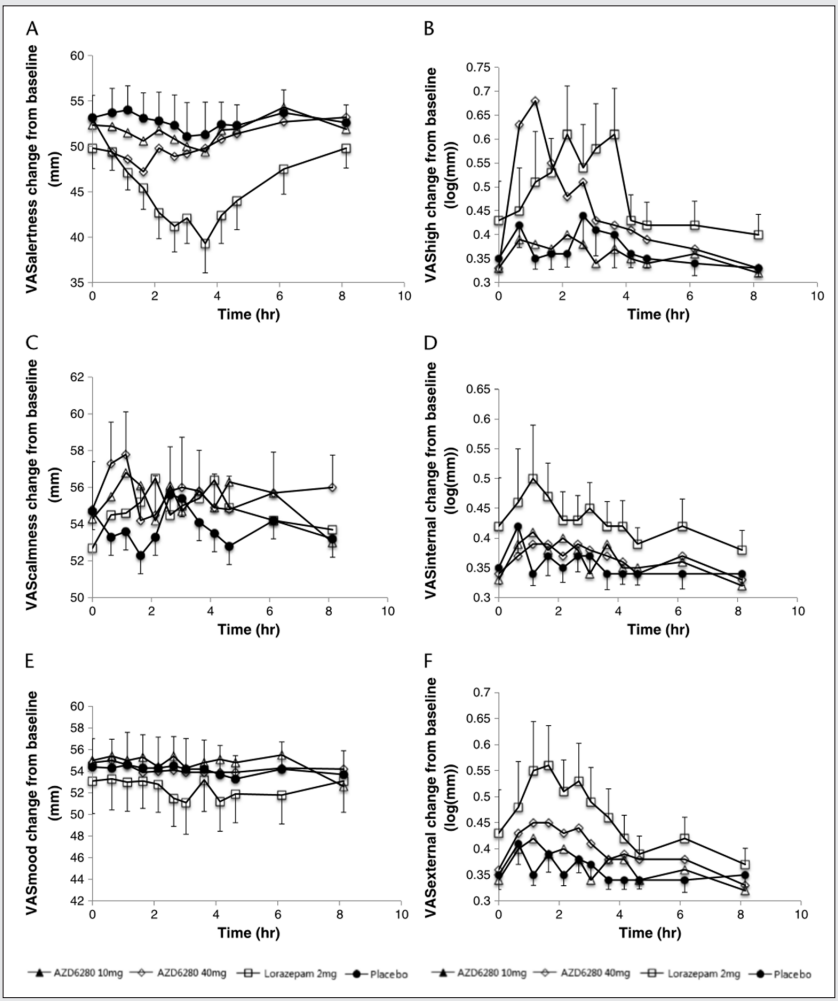


Figure 6 · Normalized concentration-effect profiles of lorazepam and AZD6280 on saccadic peak velocity (SPV), body sway (sway), and adaptive tracking (track)