Pharmacological differences of GABAergic compounds: a pharmacodynamic characterization

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SECTION 1

THE ASSESSMENT OF PHARMACODYNAMIC EFFECTS OF NEWLY DESIGNED GABAAERGIC AGENTS IN EARLY PHASE DRUG DEVELOPMENT

CHAPTER 2

Pharmacodynamic and pharmacokinetic effects of TPA023, a GABAA α2,3 subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers

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ABSTRACT

TPA023, a $GABA_A$ α2,3 subtype-selective partial agonist, is expected to have comparable anxiolytic efficacy as benzodiazepines with reduced sedating effects. The compound lacks efficacy at the α1 subtype, which is believed to mediate these effects. This study investigated the effects of 0.5 and 1.5 mg TPA023 and compared them with placebo and lorazepam 2mg (therapeutic anxiolytic dose). Twelve healthy male volunteers participated in this placebo controlled, double blind, double dummy, four-way, crossover study. Saccadic Eye Movements and Visual Analogue Scales (VAS) were used to assess the sedative properties of TPA023. The effects on postural stability and cognition were assessed using body sway and a standardised battery of neurophysiological memory tests. Lorazepam caused a significant reduction in saccadic peak velocity (SPV), the VAS alertness score and impairment of memory and body sway. TPA023 had significant dose dependent effects on saccadic peak velocity (85 deg/sec maximum reduction at the higher dose) that approximated the effects of lorazepam. In contrast to lorazepam, TPA023 had no detectable effects on saccadic latency or inaccuracy. Also unlike lorazepam, TPA023 did not affect VAS alertness, memory or body sway. These results show that the effect profile of TPA023 differs markedly from that of lorazepam, at doses that were equipotent with regard to effects on saccadic peak velocity. Contrary to lorazepam, TPA023 caused no detectable memory impairment or postural imbalance. These differences reflect the selectivity of TPA023 for different $GABA_A$ receptor subtypes.

INTRODUCTION

Generalised anxiety disorder (GAD) is a severe, chronic, and distressing illness that often requires long-term management. The lifetime prevalence is approximately 4 to 6 percent in the general population and is more common in women than in men [1]. Benzodiazepines are the most frequently prescribed pharmacological treatment for GAD [2,3]. Although benzodiazepines are relatively safe drugs and are widely used in the treatment of anxiety, they may produce untoward side effects such as memory impairment, sedation, and muscle relaxation. Particularly in the elderly, these adverse effects are associated with higher incidences of falls [4] and cognitive impairment [5,6].

The anxiolytic effect of benzodiazepines is thought to be mediated by $GABA_A$ $\alpha2$ receptors [7,8], although more recently more emphasis is given to $GABA_A$ $\alpha3$ [9-11]. TPA023 is a $GABA_A$ $\alpha2,3$ subtype-selective partial agonist with higher efficacy at the $\alpha2$ and $\alpha3$ subtypes, compared to antagonist efficacy at the $\alpha1$ and $\alpha5$ subtype [12,13]. The $\alpha1$ subtype appears to be involved in the sedative effects [8,14-17]. TPA023 is therefore expected to result in comparable anxiolytic efficacy as clinically used benzodiazepines, with reduced sedation at therapeutically equivalent dosages. Pre-clinical studies in rodents and primates have already shown that TPA023 has anxiolytic effects without showing sedation [12,13]. Based on tolerability findings in healthy volunteers, two doses of TPA023 were selected for this study: 0.5 mg and 1.5 mg. Both doses were within the range expected to be anxiolytic. Lorazepam 2 mg, which is known to be therapeutically relevant [18,19], was chosen for comparison. Benzodiazepines typically impair memory, alertness and postural stability [20-23]. It is expected that therapeutic doses of partial subtype-selective $GABA_A$ agonists will not show these side effects to the same extent. The aims of this study were to identify the side effect profiles of a TPA023-dose that was expected to be anxiolytic, and compare them to those of a therapeutic dose of lorazepam. It was hypothesised that for at least one of the two dose levels of TPA023 administered, the sedating effects of a single oral dose in healthy male subjects would be similar to placebo.

METHODS

Design

This study was a placebo controlled, randomised, double blind, double-dummy, four-way, crossover, single-centre study in twelve healthy male volunteers. Subjects visited the research unit in the morning of each study period and stayed at the site until ten hours postdose. The next morning they visited the unit again for the last measurements.

Subjects

Twelve healthy non-smoking volunteers were recruited from the Centre for Human Drug Research database. All volunteers gave written informed consent and were medically screened before entry to the study. Subjects were asked not to drink alcohol 48 hours prior to the study, abstain from caffeine-containing products 8 hours prior to the study and from grapefruit (juice) and St John's Wort at least 2 weeks prior to study start until completion of the study. The study was approved by the Medical Ethics Review Board of Leiden University medical Centre, and performed according to the principles of the Helsinki Declaration and the International Conference on Harmonisation/Good Clinical Practice (ICH/GCP).

Treatments

Each subject received a single oral dose TPA023 0.5mg, TPA023 1.5mg, lorazepam 2mg and placebo in a randomized order with at least a five-day washout period. Medication was administered with 250 ml of water in a fasted state at approximately 8 to 9 AM. As it was a double-dummy study, subjects always received three tablets of TPA023 or matching placebo and two capsules of lorazepam or matching placebo. The treatment sequences were determined using 4x4 Latin Squares, balanced for first order carry-over.

Safety

Adverse events, ECG, blood pressure and heart rate measurements were assessed throughout the study. ECGs were assessed with a Cardiofax, equipped with ecaps12 analysis program (Nihon Kohden, Japan). Blood pressure and heart rate were measured with an automated blood pressure monitor (MPV1072, Nihon Kohden, Japan), which displays an average value for two sequential (duplicate) measurements at each time point. All ECG, blood pressure and heart rate measurements were made after the subject had been sitting in a semi-recumbent position for at least 5 minutes.

Pharmacokinetics

Blood samples (5ml) were taken during each study period within 30 minutes predose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 24 hours postdose and were processed to obtain plasma for assay of TPA023 and lorazepam concentrations.

Plasma was separated from heparinized blood samples by centrifugation (2000 gs, 10 min, 4°C) to 4.5 cc Nunc cryotubes and stored at -20°C within 30 minutes after sampling. TPA023 analysis was accomplished by solid phase extraction of the analyte and an internal standard from plasma using a 96-well plate format followed by reversed phase HPLC and ms/ms detection. Lorazepam and its stable-isotoped labeled internal standard were extracted from basified plasma into methyl-t-butyl ether with an automated procedure using a Tomtec Quadra 96 Model 320. Extracts were evaporated under nitrogen, reconstituted and analyzed by lc/ms/ms using positive ion Turbo Ionspray with multiple reaction monitoring.

Pharmacodynamics

Pharmacodynamic measurements were performed predose (within 30 minutes prior to dosing) and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 10 hours postdose. Pharmacodynamic tests were performed in a quiet room with ambient illumination with only one subject in the same room per session. Each session consisted of the following sequence of tests: saccadic eye movements body sway eyes open body sway eyes closed VAS. Cognitive function tests were performed in the 1-3 hours-postdose period between the other measurements.

Saccadic Eye Movements

Saccadic eye movements were recorded using a micro-computer-based system for data recording (Cambridge Electronics Design, Cambridge, UK), Nihon Kohden equipment for stimulus display, signal collection and amplification (Nihon Kohden Corporation, Tokyo, Japan), and disposable surface electrodes (Medicotest N-00-S, Olstykke, Denmark) [24]. Average values of latency (= reaction time), peak saccadic velocity and inaccuracy (difference between stimulus angle and corresponding saccade in %) were calculated for all artefact-free saccades. Saccadic peak velocity has been validated as the most sensitive measure for the sedative effects of benzodiazepines [25-28].

Visual Analogue Scale

Visual analogue scales as originally described by Norris [29] were previously used to quantify subjective effects of benzodiazepines [27]. From the set of sixteen scales three composite factors were derived as described by Bond and Lader [30], corresponding to alertness, mood and calmness. A higher score on these scales indicates a negative effect (sedation, excitation and decrease in mood respectively). These factors were used to quantify subjective drug effects.

Body Sway

Body sway was measured with an apparatus similar to the Wright ataxia meter [31], which integrates the amplitude of unidirectional body movement transferred through a string attached to the subject's waist. Two-minute measurements were made in the antero-posterior direction with eyes open and eyes closed, with subjects standing comfortably on a firm surface with their feet slightly apart.

Cognitive function tests

Memory testing was performed using the validated FePsy program (The Iron Psyche), an automated system containing a battery of computerised tests for cognitive (neuropsychological) functions [32,33]. Word and picture recognition and recall tests were performed to assess reaction time and number of correct and incorrect answers. The Corsi block tapping test, constructed according the principles of the original Corsi block tapping task [34], assessed the nonverbal memory span.

ANALYSIS

Pharmacokinetics

The pharmacokinetics of TPA023 were investigated using non-linear mixed effect modelling as implemented in NONMEM version V software (NONMEM Project Group, University of California, San Francisco, CA), applying the first order conditional estimation (foce) method with the 'interaction' option. A series of PK models was attempted and compared using the likelihood ratio test [35]. Ultimately, a two-compartment model with first-order absorption and a lag-time was used to describe the pharmacokinetics of TPA023. Intra-individual error was modelled using a constant coefficient of variation error model. No pharmacokinetic parameters were calculated for lorazepam.

Pharmacokinetic/pharmacodynamic relationships

The observed pharmacodynamic effects were plotted against the predicted TPA023 concentrations for each individual. Because the average placebo profile for saccadic peak velocity showed a small diurnal decrease, the average placebo profile was subtracted from all saccadic peak velocity data at corresponding protocol time points and the result was subjected to PK/PD analysis. PK/PD modelling was performed using non-linear mixed effect modelling as implemented in NONMEM. Empirical Bayes pharmacokinetics estimates were generated and used to describe the concentration profile for investigation of the PK/PD relationship between TPA023 and saccadic peak velocity. A linear concentration-effect model was estimated without an effect compartment. Individual graphs indicated that no improvement could be obtained using either a more complex concentration-effect model or an effect compartment and further analysis was not attempted.

Statistics

Treatment response was characterised for continuously measured variables by calculating the area under the effect curve (AUEC) relative to baseline over 6 hours. The pre-values were averaged and set at time = 0 hr. Change from average pre-value (delta) was calculated. The AUECs were calculated using the linear trapezoidal rule up to 6 hours on the basis of protocol (planned) time points and were subsequently divided by the corresponding time span resulting in weighted average change from pre-value. All variables were analysed untransformed except for body sway because only body sway clearly indicated an increase in variability in response with an increase in average response. As cognitive function test results were assessed only once for each treatment, raw scores were analysed. Statistical analysis was initially performed using analysis of variance with factors treatment (4 levels) subject (12 levels) occasion (4 levels) and carry-over (5 levels, coded as the treatment preceding the current treatment, including 'no preceding treatment'). If the carry-over effect was found to be non-significant, the analysis was rerun without the carry-over factor. The four treatments were compared within the ANOVA model using the following contrasts: placebo - TPA023 0.5mg, placebo - TPA023 1.5mg, lorazepam 2mg - TPA023 1.5mg and placebo - lorazepam 2mg. Overall p-value for the treatment effect was reported along with the specified contrasts with 95% confidence intervals and p-values. The current study had a >0.99 a-priori probability (α=0.05, two-tailed, MSE=331), in a sample size of 12 subjects, to detect a larger than 45 deg/sec difference in average saccadic peak velocity between the treatments and placebo. A previous study showed that this difference corresponds to the average change after one night of sleep deprivation [25]. There was a 0.80 a-priori probability to detect a 21 deg/sec mean difference between the treatments. All calculations were performed using SAS for Windows V8.1 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Subjects

Twelve subjects, judged to be in good health on the basis of medical history, physical examination and routine laboratory data, participated in the study after giving written informed consent. Two subjects dropped out one was repeatedly unable to swallow the capsules and another withdrew after the second occasion for personal reasons. Two other healthy male subjects, using the same randomisation sequence, replaced these two subjects. Twelve subjects therefore completed the study. Subjects were on average 25 years of age (range 20-29 yrs), average weight of 82 kg (range 75-90 kg) and average height of 184 cm (range 178-192 cm).

Clinical observations

No serious adverse reactions occurred following any of the treatments. The most frequently reported adverse event after administration of lorazepam, the high and low doses of TPA023 and placebo were sedation (including drowsiness) by eight, five, three and two subjects respectively. Other reported adverse events were, dizziness after TPA023 1.5mg administration (four subjects), sleepiness and headache after lorazepam 2mg administration (seven and three subjects, respectively) and fatigue and headache after placebo administration (six and five subjects, respectively).

Pharmacokinetics

The average plasma concentration-time curves for both doses of TPA023 and lorazepam are shown in figure 1. Both doses of TPA023 and lorazepam showed maximum concentrations after approximately 2 hours. The average pharmacokinetic model based parameters (with inter-individual variation coefficients (CV) of TPA023 were: apparent clearance (clearance divided by bioavailability) of 246 mL/min (CV 29%), initial half-life of 142 min (CV 6%), terminal half-life of 437min (CV 0%, fixed), apparent central volume of distribution (volume divided by bioavailability) of 71.1 L (CV 20%), absorption half-life of 33.6 min (CV 39%) and a lag-time of 27.4 min (CV 19%).

Pharmacodynamics

Saccadic Eye Movements

Saccadic peak velocity (SPV), which for benzodiazepines relates to sedative and anxiolytic properties [28], demonstrated significant effects with lorazepam and both doses of TPA023 (figure 2 and table 1). There was a dose-dependent increase of SPV with TPA023 0.5 and 1.5 mg (AUEC0-6hr decrease of 22 deg/sec and 45 deg/sec). No changes were observed in saccadic latency and saccadic inaccuracy for either doses of TPA023, in contrast to the significant increases with lorazepam. The high dose of TPA023 and lorazepam caused similar average maximum effects on SPV relative to baseline. However, the effects of lorazepam lasted slightly longer, leading to a significant difference in time-corrected AUEC0-6hr (table 1).

Visual Analogue Scale

The VAS score of alertness, which was used to estimate subjective sedative effects, only showed a significant average effect after lorazepam (table 1). The lower dose of TPA023 did not show any effects on any of the subscales. The average curve for the high dose of TPA023 was in between the average curves of lorazepam and placebo (figure 3), and consequently, the AUC 0-6hr of the high dose of TPA023 did not differ significantly from either lorazepam or placebo. Subjective calmness was reduced after the high dose of TPA023, while none of the other treatments showed any effect. No significant effects were observed for the VAS contentedness subscale.

Body Sway

No postural instability was observed after either dose of TPA023 compared to placebo (figure 4). Lorazepam, however, caused a profound and highly significant increase in body sway (table 1).

Cognitive Function Tests and Corsi Block Tapping Task

Three of the four recognition tests revealed that lorazepam caused significant memory impairment, compared to placebo (figure 5). In contrast, neither dose of TPA023 showed any significant effect on memory. Aside from the effects of lorazepam on the ability to answer correctly, it also significantly increased the reaction times to the correct answers of all memory tests with a range of 0.5-1.3 sec from placebo (figure 5). These significantly higher reaction times were not found with TPA023. No treatment effects were observed on the Corsi block tapping task.

Pharmacokinetic/pharmacodynamic relationships (PK/PD)

Concentration-effect-relationships were only determined for statistically significant pharmacodynamic effects of TPA023 (ie only for SPV). The average PK/PD relationship between the changes in SPV from baseline and the predicted concentration for both doses of TPA023 is represented in figure 6. A linear concentration-effect model was estimated without an effect compartment for both doses of TPA023. Both slope and intercept for SPV did not differ significantly between the two doses of TPA023. There were no obvious signs of hysteresis or maximum effects. Individual graphs indicated that no improvement could be obtained using either a more complex concentration-effect model or an effect compartment.

DISCUSSION

The current placebo-controlled study in healthy male volunteers investigated the effects of two doses of TPA023, a $GABA_A$ α2,3 subtype-selective partial agonist. The benzodiazepine lorazepam was used in a therapeutic anxiolytic dose, as a positive control. As expected, lorazepam caused sedation (shown by SPV-decreases and VAS-effects), and impairments of memory and postural stability. These effects are typical for benzodiazepines, and are often used as indicators for the drugs' effects [26,27]. TPA023 caused dose dependent SPV-effects of a similar magnitude as lorazepam, but TPA023 had no detectable effects on VAS alertness score, memory or postural stability. A comparison between the two drugs is dependent on the relative efficacies of the used therapeutic equipotency. This cannot be proven at this stage, because the clinical effects of TPA023 have not yet been determined in patients with anxiety. However, lorazepam 2 mg and the highest dose of TPA023 caused similar reductions in SPV, and in this respect the two treatments were equipotent. At these SPV-equipotent doses, effects on VAS alertness, body sway and cognitive function differed markedly between both drugs. These differences may have implications for the pharmacological activities of the two drugs, and their therapeutic effect profiles.

The question arises, how the effect selectivity of TPA023 was observed in this study, relates to the preclinical binding profile to the different α subunit subtypes [8,16,36]. In pre-clinical experiments, TPA023 is a $GABA_A$ partial α2,3 agonist and an antagonist at the α1and α5 subtype. The α1 subunit is believed to primarily mediate the sedative properties and as a consequence, to contribute to memory impairment caused by non-selective $GABA_A$ agonists. Alpha-2 and more recently also α3 activity is held responsible for the anxiolytic effects [7-11]. Preclinical evidence also suggests that the α2, α3 and α5 subunits mediate myorelaxation and motor impairment. If both the anxiolytic and motor effects of TPA023 are attributed to α2 efficacy, the compound shows a surprising lack of motor impairment in healthy volunteers. There are several explanations. First, the preclinical binding profile to the different α-subunit subtypes is characterized by maximal activity, not by measures of sensitivity. The maximal effects of TPA023 were not determined in the current study. Thus, different subtypes may show differences in sensitivity, and motor impairment may become more apparent at higher TPA023-doses that were not evaluated in this study. The preclinical binding profile would predict that even high TPA023-concentrations would still cause less body sway than a full agonist. Alternatively, receptor subtype selectivity may show different patterns in humans than in preclinical models. In this case, different studies with a variety of subtype-selective $GABA_A$ agonists would be needed to define distinct effect profiles that are predictive for the different desired and undesired effects of this new drug class. Finally, the results of this study may be chance findings. However, this is unlikely, because effect profiles as different as for TPA023 are not found among full-agonist benzodiazepines.

Although direct comparative studies are rare, non-selective benzodiazepines, like diazepam [37,38], zopiclone [39], flurazepam [39], lormetazepam [39], triazolam [39], temazepam [40] and lorazepam [19,40,41], usually show comparable effects on memory, alertness and postural stability. Other GABAergic anxiolytic agents, that are non-selective partial agonists at all $GABA_A$ receptor subtypes, also show less differentiating effects than TPA023 [42,43]. Bretazenil, which is less potent on all α-subtypes compared to a full agonist like diazepam [44], showed little evidence of a dissociation between sedative effects and effects on VAS alertness and saccadic eye movements at a dose of 0.5 mg [38]. Ro 41-3696, reported to be a partial agonist, induced fewer effects on psychomotor performance and memory than 10 mg zolpidem at 1.5 h after intake [45], but the effects were still significantly larger than after placebo. Abecarnil, another non-selective partial agonist, also did not show significant effects compared to placebo [46,47]. However, it is unknown whether these doses were equipotent, an important requisite for comparison of partial agonism and subtype selectivity. True subtype-selective agonists are novel agents and mostly still experimental. For compounds like L-838417 [15], NGD 91-2, NGD 91-3 [43], quinolone 'compound 4' [42,48] and SL-651498 [14,49], no clinical data are available. Only for SL-651498 it was reported that different Phase IIa/b trials for GAD and muscle spasms were conducted with this compound [43], but results have not been provided. Comparative studies with full agonists have not been published. Thus, experience suggest that non-selective $GABA_A$ and benzodiazepine agonists cause a general depression of alertness, memory and motor stability, although the overall level of these reductions is dose-dependent, and probably different between full and partial agonists.

Many biomarkers of 'alertness' are used in healthy volunteer studies,

and although there are differences in sensitivity, these markers usually show comparable effects of different sedative drugs or circumstances [25-28]. Previous studies have shown that a decrease in SPV is a highly sensitive indicator of sedation, not only caused by benzodiazepines [26,27] but also by sleep deprivation [25] or compounds that are not particularly anxiolytic, like H1-antagonists [50], α2-agonists [51,52], and anticholinergic agents [53]. All these drugs and circumstances cause reductions in VAS-alertness, saccadic peak velocity, latency and accuracy. In this respect, subjective alertness scores and saccadic eye movements can be considered as largely overlapping ven-diagrams, which both also show a considerable overlap with anxiolysis. Contrary to other compounds that fall into two or three of these categories, TPA023-effects seem to be restricted to SPV alone. The differences compared to lorazepam were quite apparent and could not be attributed to differences in test-sensitivity or statistical type II-errors. We have not been able to find other compounds that cause SPV-decreases without VAS- reductions or vice versa. This separation thus seems to be unique for TPA023. It is tempting to assign these divergent effects to the subtype-selectivity of TPA023, although the exact nature of the relationships between the pharmacological and functional effect profiles cannot be established from this study.
A recent literature review showed clear relationships between anxiolytic doses of benzodiazepines and their SPV-effects [28]. For full benzodiazepines, anxiolytic effects are inseparable from the sedative effects. SPV-reduction is usually (although not always statistically significantly) accompanied by effects on latency and accuracy. But for a subtype-selective GABA agonist, SPV reduction without an effect on latency or any subjective indication for sedation could signify anxiolysis without impairment of alertness. If SPV-reduction is predictive of anxiolysis, TPA023 1.5 mg could be equally anxiolytic as lorazepam, but considerably less sedative. Clearly, this remains to be established in clinical trials.

TPA023 did not cause any effect on memory which was expected since TPA023 has antagonistic effects at the α5-subunit that is believed to be involved in memory and cognition [54,55]. Lorazepam is known to affect memory [19,56], which was also confirmed in this study. Based on lack of effects on memory testing and body sway, TPA023 could also have fewer effects on cognition and postural stability, perhaps leading to a decreased chance of memory impairment or falls.

In conclusion, this study showed a clear differentiation in pharmacodynamic effects for the selective $GABA_A$ agonist TPA023, which was not found for the non-selective benzodiazepine lorazepam. This differentiation seems to reflect the TPA023's selectivity for different $GABA_A$ receptor subtypes, although preclinical pharmacological profiles could not be immediately translated into predictions of clinical effects. TPA023 1.5 mg and lorazepam 2 mg showed equipotent reductions of saccadic peak velocity, which could point to comparable anxiolytic efficacy. Contrary to lorazepam, TPA023 did not have any effect on subjective alertness, memory or postural stability. It remains to be established whether the selectivity of TPA023 is reflected into an improved therapeutic window.

Table 1 Effects on Saccadic Eye Movements, Visual Analogue Scales and Body Sway

Variable	Overall treatment effect (p-value)	Placebo - TPA023 0.5mg	Placebo - TPA023 1.5mg	Lorazepam 2mg - TPA023 1.5mg	Placebo - Lorazepam 2mg
Saccadic Peak Velocity (deg/sec)	<.0001	21.58 (8.40 / 34.76) p = 0.002	45.24 (32.06 / 58.42) p < 0.001	-13.99 (-27.17 / -0.81) p = 0.038	59.23 (46.05 / 72.41) p < 0.001
Saccadic Latency (sec)	0.0003	-0.002 (-0.014 / 0.009) p = 0.672	-0.009 (-0.021 / 0.002) p = 0.116	0.017 (0.006 / 0.029) p = 0.005	-0.027 (-0.039 /-0.015) p < 0.001
Saccadic Inaccuracy (%)	0.0008	-0.09 (-1.27 / 1.08) p = 0.874	-0.03 (-1.21 / 1.14) p = 0.954	2.21 (1.03 / 3.38) p < 0.001	-2.24 (-3.42 / -1.07) p < 0.001
VAS Alertness (ln mm)	0.0082	1.35 (-0.37 / 3.08) p = 0.119	-0.33 (-2.05 / 1.39) p = 0.698	1.47 (-0.25 / 3.19) p = 0.092	-1.80 (-3.52 /-0.08) p = 0.041
VAS Contentedness (ln mm)	0.2630	-0.25 (-0.97 / 0.48) p = 0.492	-0.71 (-1.44 / 0.02) p = 0.055	-0.47 (-1.20 / 0.25) p = 0.193	-0.24 (-0.96 / 0.49) p = 0.510
VAS Calmness (ln mm)	0.0097	-0.14 (-0.46 / 0.17) p = 0.355	-0.53 (-0.84 /-0.22) p = 0.002	-0.43 (-0.74 /-0.12) p = 0.009	-0.10 (-0.41 / 0.22) p = 0.529
Log Body Sway Eyes Closed (log mm)	<.0001	0.009 (-0.087 / 0.106) p = 0.849	-0.001 (-0.098 / 0.095) p = 0.976	0.310 (0.214 / 0.407) p < 0.001	-0.312 (-0.408 /-0.215) p < 0.001
Log Body Sway Eyes Open (log mm)	<.0001	-0.026 (-0.102 / 0.050) p = 0.487	-0.021 (-0.097 / 0.055) p = 0.575	0.267 (0.192 / 0.343) p < 0.001	-0.288 (-0.364 /-0.213) p < 0.001

Treatment differences in pharmacodynamic measurements in AUC 0-6hr relative to baseline
ANOVA results are shown as contrasts (95% CI) and p-value

Figure 1 Average drug concentration profiles (mean + SD) of TPA023 0.5mg (squares), TPA023 1.5mg (circles) and lorazepam 2mg (triangles) after oral administration.

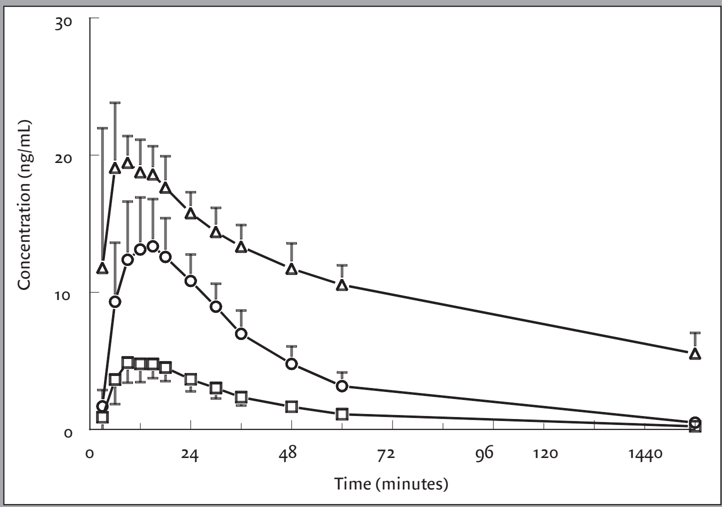


Figure 2 Average time profile (mean + SD) of Saccadic Peak Velocity (change from baseline) after oral administration of placebo (closed circles), TPA023 0.5mg (squares), TPA023 1.5mg (open circles) and lorazepam 2mg (triangles).

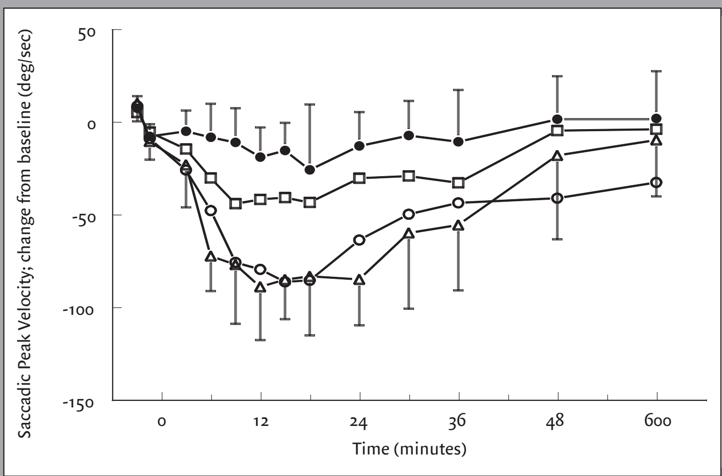


Figure 3 Average time profile (mean + SD) of VAS Alertness (change from baseline) after oral administration of placebo (closed circles), TPA023 0.5mg (squares), TPA023 1.5mg (open circles) and lorazepam 2mg (triangles).

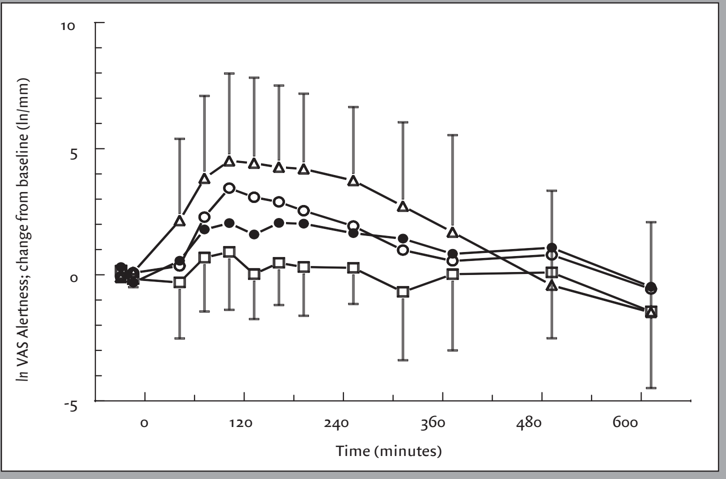


Figure 4 Average time profile (mean + SD) of LOG Body Sway Eyes Closed (change from baseline) after oral administration of placebo (closed circles), TPA023 0.5mg (squares), TPA023 1.5mg (open circles) and lorazepam 2mg (triangles).

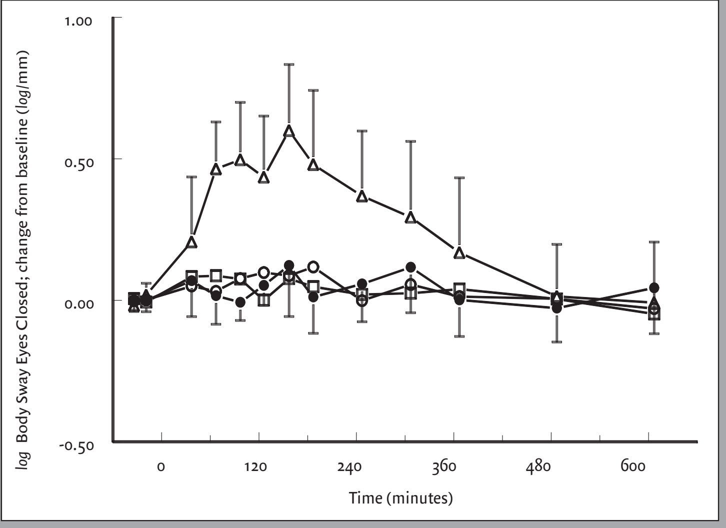
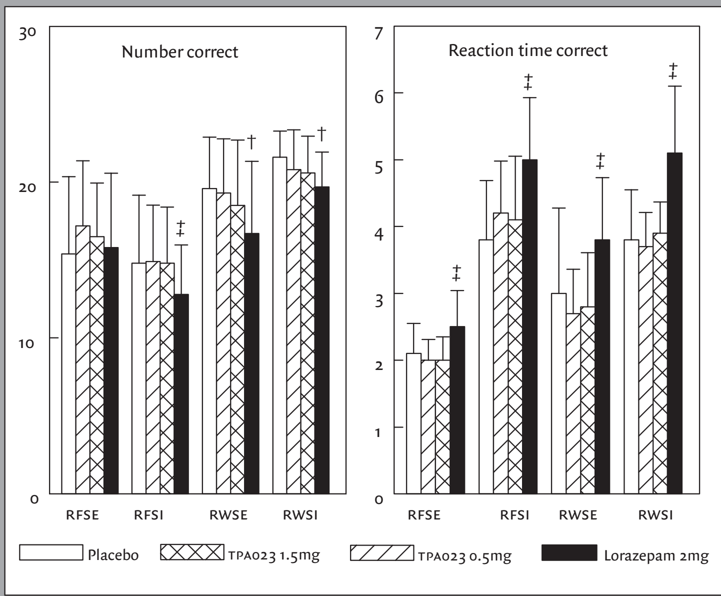


Figure 5 Effects on cognitive function tests (mean + SD).



RFSE = Recognition Figures Serial RFSI = Recognition Figures Simultaneous RWSE = Recognition Words Serial RWSI = Recognition Words Simultaneous. †: p<0.05 compared to placebo, ‡: p<0.05 compared to placebo and TPA023 1.5mg.

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