CHAPTER 7

The effects of TPA023, A $GABA_A$ α2,3 subtype-selective partial agonist, on essential tremor in comparison with alcohol

Journal of Psychopharmacology 2012, Feb; 26(2), 282-91

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ABSTRACT

BACKGROUND Essential tremor (ET) is a relatively frequent neurological disorder that responds in some patients to $GABA_A$ agonists (like benzodiazepines). Partial subtype selective $GABA_A$-agonists may have an improved side effect profile compared to non-selective $GABA_A$ agonists. However, it is unknown which $GABA_A$ subtypes are involved in the therapeutic effects of benzodiazepines in ET.

METHODS The effects of 2 mg TPA023, a $GABA_A$ α2,3 selective partial agonist on ET were compared to the effects of a stable alcohol level (0.6 $g \cdot L^{-1}$) and placebo in nine ET-patients. Tremor evaluation included laboratory accelerometry and a performance-based scale. Additional measurements were performed to evaluate other effects on the central nervous system (CNS).

RESULTS Alcohol significantly diminished tremor symptoms in the postural and kinetic condition, as assessed by laboratory accelerometry, but the performance-based rating scale was unaffected. Tremor was also reduced after TPA023 treatment in the kinetic condition, albeit not significantly. Additionally, TPA023 decreased saccadic peak velocity, while alcohol decreased subjective feelings of alertness.

CONCLUSIONS This study showed that alcohol reduced maximum tremor power, as assessed by laboratory accelerometry, unlike TPA023, which decreased tremor symptoms to some extent, but not significantly. This study showed that treatment with an α2,3 subunit selective $GABA_A$ partial agonist was less effective compared to a stable level of alcohol in reducing ET-symptoms. These results provide no support for a therapeutic role of TPA023 in the suppression of ET-symptoms.

INTRODUCTION

Essential tremor (ET) is one of the most common movement disorders (Pahwa and Lyons, 2003). The age- and gender-adjusted prevalence of ET is estimated to be 3 to 4 per 1000, with an annual incidence of 23.7 per 100,000. Approximately 4% of adults in the age group above 40 years are affected by ET (Zesiewicz *et al.*, 2010). ET has a 4-12 Hz frequency that predominantly affects the upper extremities, may also affect the head and voice, and rarely affects the legs. In contrast to resting tremor in Parkinson disease, essential tremor is characterised by postural and kinetic components (Elble, 2000). Although its cause is unknown the central oscillatory systems at the level of the inferior olivary nucleus of the medulla oblongata seem to play an important role in the pathophysiology of ET (Deuschl and Elble, 2000). Current therapy for ET includes beta blockers, phenobarbital (a primidone metabolite), benzodiazepines, and some antiepileptic agents. Treatment is symptomatic, effective in no more than roughly half of the patients, and often limited by side effects (Chen and Swope, 2003).

Preclinical and clinical studies suggest that a GABAergic pharmacologic agent could be effective in the treatment of ET (Louis, 1999). Double-blind studies have demonstrated efficacy of alprazolam versus placebo in treating ET (Gunal *et al.*, 2000; Huber and Paulson, 1988). Different $GABA_A$ receptor subtypes have been identified, and pre-clinically linked to well-known effects of $GABA_A$ agonists like sedation (α1) (Rudolph *et al.*, 1999), muscle relaxation (α2) (Rowlett *et al.*, 2005), anxiolysis (α2,3) (Atack *et al.*, 2005; Rudolph *et al.*, 2001) and memory impairment (α5) (Collinson *et al.*, 2002). Among these subtypes, the α2-receptor seems the most promising target for anti-tremor activity, although the exact mechanism of the therapeutic activity of GABA agonists in essential tremor is unknown. TPA023 is being developed as a $GABA_A$ α2,3 selective partial agonist. Based on the selective properties of this compound, it is believed to be a muscle relaxant (and anxiolytic) without causing sedation and instability. Healthy volunteer studies have shown the selective effect profile in comparison with lorazepam (de Haas *et al.*, 2007). Investigating the effects of this novel selective partial $GABA_A$ agonist on essential tremor might reveal new information about the pathophysiology of this disease, and possibly identify a new treatment modality.

Alcohol, which is an indirect agonist of the $GABA_A$-receptor (Santhakumar *et al.*, 2007) relieves tremor symptoms in an estimated 50 to 90% of ET patients (Rajput *et al.*, 1975; Deuschl, 1999) by reducing tremor

amplitude without affecting frequency (Koller and Biary, 1984). It most likely acts via a reduction of cerebellar over-activity, which results in reduced tremor amplitude, whereas the frequency is not altered (Koller and Biary, 1984; Koller *et al.*, 1994; Koller, 1991). In this study, alcohol was chosen as a positive control, using a novel clamping technique that was based on earlier publications (O'Connor *et al.*, 1998). Ideally, a novel therapy for ET would closely approach the tremor reducing effects of alcohol. Since the duration of action of TPA023 was estimated to be four hours, the clamping procedure took place during a similar period. To maximize the power of alcohol as a positive control, only patients who were familiar with the positive effects of alcohol on their symptoms were included in this project.

In this exploratory study, the novel α2,3-selective $GABA_A$ partial agonist TPA023 was compared with intravenous alcohol and placebo for their effects on tremor symptoms. A battery of Central Nervous System (CNS) function tests including body sway, eye movements, Visual Analogue Scales and adaptive tracking was also performed. The objectives were to distinguish between general CNS pharmacodynamic effects of the $GABA_A$ α2,3-selective drugs and tremor-specific effects in the accelerometry recordings, in a relatively small study.

METHODS

Design

This exploratory study was performed in a double-blind, double-dummy, randomized, placebo-controlled, 3-period, crossover fashion in nine patients diagnosed with essential tremor, with at least a five-day washout period.

Subjects

Nine patients with essential tremor were recruited from the hospital database of the Leiden University Medical Centre, the Dutch patients' association and by advertising in local newspapers. Subjects were informed about the contents of the study during an information visit. When they had decided to participate, subjects visited the research unit for a medical screening. After signing informed consent, they were medically screened to evaluate eligibility for study participation. A neurologist (JvG) diagnosed essential tremor (ET) of the hands and forearms according to the diagnostic criteria for 'classic ET' defined by the consensus statement of the Movement Disorder Society (Deuschl *et al.*, 1998), adapted from previous criteria established by the Tremor Research Investigation Group (TRIG) (Deuschl *et al.*, 1995), as well as a more recent study indicating the importance of kinetic tremor (Brennan *et al.*, 2002). An isolated head tremor was not allowed.

Only subjects who stated a positive effect of alcohol on their tremor symptoms were included in the study. Subjects had to refrain from alcohol 48 hours prior to a treatment day and caffeine-containing products for at least 12 hours before treatment. Subjects were not allowed to use their own anti-tremor medications and had to abstain from grapefruit (juice) and St John's Wort for at least 2 weeks before the start until completion of the study, because these substances have stimulating and inhibiting effects on CYP3A4, respectively. They were not allowed to drink more than six units of caffeine-containing products or three units of alcohol per day or smoke more than five cigarettes per day during the total study period. On treatment days, the use of caffeine-containing products or smoking was not allowed. The study was approved by the Medical Ethics Review Board of Leiden University Medical Centre, and performed according to their standards.

Study treatments

Each patient received a single oral dose of TPA023 2 mg or matching placebo. Also on each study day, an alcohol (10% in 5% glucose) or sham placebo (5% glucose) clamping procedure was performed. Only the person who was responsible for the clamping method was unblinded for alcohol infusion due to measurement of the breath alcohol concentrations (BrAC). This person was not involved in any other part of the study.

Alcohol was infused using a recently developed clamping method, in which the BrAC was used to guide intravenous dosing (Zoethout *et al.*, 2008). An alcohol level of 0.6 g·L^{-1} was chosen, because this was expected to cause a significant tremor reduction in the majority of patients. Previous studies have shown that mean blood alcohol levels of 0.35 and 0.50 g·L^{-1} had tremor diminishing effects, but this was after a single oral dose (Zeuner *et al.*, 2003). Levels of 0.6 g·L^{-1} are routinely achieved during social drinking, without causing too many adverse effects.

In previous studies, the 0.6 g·L^{-1} alcohol clamp was well-tolerated and produced statistically significant pharmacodynamic CNS effects (Zoethout *et al.*, 2009). It was estimated that the duration of action of TPA023 would be approximately four hours (de Haas *et al.*, 2007). To optimize the value of alcohol as a positive control, a clamping period of four hours was chosen for this study.

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), showing an average value for two sequential (duplicate) measurements at each time point. All safety measurements were made after sitting in a semi-recumbent position for at least 5 minutes.

Alcohol concentrations

Breath alcohol (BrAC) samples were performed using the hand-held Alco-Sensor IV meter (Honac, Apeldoorn, The Netherlands). To reduce fatiguing of the Alco-Sensor meter, a minimum interval of approximately 10 minutes was maintained between BrAC samplings, by alternating two different measurement devices. A recent study using the described alcohol clamping technique shows similar findings for breath alcohol and blood alcohol levels (Zoethout *et al.*, 2008). Therefore, no pharmacokinetic blood samples were obtained for alcohol and only the breath alcohol samples were used for further pharmacokinetic analysis.

Pharmacodynamics

TREMOR EVALUATION Tremor evaluations were performed at screening, predose (within 60 minutes prior to dosing) and 60, 150, 240, 330, 420 minutes postdose on each study day.

QUESTIONNAIRE/EVALUATION SCALE The Tremor Disability Questionnaire was only performed at screening and was executed to assess the level of disability due to ET according to the patients' opinion. This is a 36-item, 10 minute questionnaire that was designed in 1997 for the CADET study (Wendt *et al.*, 2000). It has shown substantial test-retest reliability and was validated against multiple other endpoints, including a neurologist's clinical ratings, the performance-based test of function, and quantitative computerized tremor analyses (Louis *et al.*, 2000). The total score of this questionnaire ranges from 0 (no disability) to 100 (completely disabled).

Additionally, a Performance-Based Tremor Evaluation (PBTE) was performed (Louis *et al.*, 1999). The test included the performance of 15 activities that were scored by a trained measurement assistant from 0 (no difficulty) to 4 (unable to perform), and the total score was converted to a percentage ranging from 0 (no disability) to 100 (maximally impaired) (Louis *et al.*, 1999).

LABORATORY TREMOGRAPHY Tremor was evaluated according the methodology of Gironell *et al.* (Gironell *et al.*, 1999) using three miniature linear piezo-electric accelerometers (Nihon Kohden, MT-3T), which were attached to the distal end of a clamp, above the fingertips of the dominant arm. The accelerometers were placed at right angles to one another to enable three-dimensional analysis of movement (Gironell *et al.*, 1999; Van Hilten *et al.*, 1991). An EMG recording of the flexor and extensor forearm muscles

was also obtained with silver-silver chloride electrodes applied 2cm apart at the belly of the muscles. The signals were amplified by use of a Grass 15LT (15A54/15A94), with a time constant of 1 second and a low pass filter at 100 Hz. For the fast Fourier analysis, data collection and analysis were performed using customized CED software (Cambridge Electronics Design, Cambridge, UK). The upper limb tremor was recorded in three positions, each held for a 60-second interval: (1) at rest, with the arm hanging relaxed along the body, (2) postural, with the arm held in an outstretched, horizontal, prone position and (3) kinetic, moving the hand from a set point to the nose (back and forth). Tremor was quantified by a power spectrum analysis to determine the dominant frequency peak (Hz) and the magnitude of the accelerometer signal (absolute power of the dominant frequency peak in μV).

CNS MEASUREMENTS

Pharmacodynamic measurements were performed predose (within 60 minutes prior to dosing) and 30, 120, 210, 300, 390 and 480 minutes postdose. Subjects underwent pharmacodynamic tests individually in a quiet room with ambient illumination. All subjects were thoroughly trained and familiarized with the tests within 14 days preceding study start to minimize learning effects before proceeding to the study.

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) (van Steveninck *et al.*, 1989). 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 (SPV) has been validated as the most sensitive measure for the sedative effects of benzodiazepines (van Steveninck *et al.*, 1991; van Steveninck *et al.*, 1992; van Steveninck *et al.*, 1999; de Visser *et al.*, 2003) Previous healthy volunteer studies showed that TPA023 caused SPV reductions, while alcohol did not (de Haas *et al.*, 2007; Zoethout *et al.*, 2009).

VISUAL ANALOGUE SCALES Visual analogue scales as originally described by Norris (Norris, 1971) were previously used to quantify subjective effects of benzodiazepines (van Steveninck *et al.*, 1991). From the set of sixteen scales three composite factors were derived as described by Bond and Lader (Bond and Lader, 1974), corresponding to alertness, mood and calmness. These factors were used to quantify subjective treatment effects. In contrast to TPA023, alcohol has previously shown to affect the VAS alertness scale (de Haas *et al.*, 2007; Zoethout *et al.*, 2009).

BODY SWAY Body sway was measured with an apparatus similar to the Wright ataxia meter (Wright, 1971), 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 closed, with subjects standing comfortably on a firm surface with their feet slightly apart. In contrast to TPA023, alcohol has previously shown to increase postural instability (de Haas *et al.*, 2007; Zoethout *et al.*, 2009).

ADAPTIVE TRACKING The adaptive tracking test was first performed by Borland and Nicholson (Borland and Nicholson, 1984), using customised equipment and software (Hobbs, 2000, Hertfordshire, UK). The average performance and the standard deviation of scores were used for analysis. Adaptive tracking is a pursuit-tracking task. A circle moves randomly about a screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. Adaptive tracking was scored over a 3-minute period. Each test was preceded by a run-in period. The adaptive

tracking test has proved to be useful for measurement of CNS effects of alcohol (van Steveninck *et al.*, 1996), various psychoactive drugs (Gorman *et al.*, 1986), and sleep deprivation (van Steveninck *et al.*, 1999).

Statistical analyses

The study was performed to explore the effects of TPA023 on ET compared to an alcohol infusion. Because of the exploratory character of the study, no formal power calculation could be performed.

Most PD parameters 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 is defined as the average of the available values obtained prior to dosing. Treatment effects were reported as the contrasts specified below where the average of all post-dose measurements was calculated within the statistical model. Contrasts were reported along with 95% confidence intervals (95%CI) and analyses were two-sided with a significance level of 0.05.

Body sway and tremor parameters were analyzed after log-transformation due to skewed response distribution. All other parameters were analyzed untransformed. Log-transformed parameters were back-transformed after analysis where the results may be interpreted as percentage change. All calculations were performed using SAS V9.1.2 for Windows (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Subjects

Seventeen patients were medically screened after giving written informed consent. Seven patients did not qualify for the study mainly because their tremor was too mild to be quantified reliably with the laboratory tremography. Nine patients (two female, seven male) fulfilled the study criteria and completed the study. The remaining patient was standby to replace any discontinuations, but never participated in the study. Apart from their tremor, they were judged to be in good health on the basis of medical history, physical examination and routine laboratory data. Subjects were on average 47 years of age (range 18-80), had an average weight of 77 kg (range 62-103 kg) and average height of 175 cm (range 159-189 cm).

The mean (range) dominant tremor frequency at screening was 7.7 (95%CI 5.4-9.4) Hz. Mean (standard deviation) scores on the Tremor Disability Questionnaire and Performance-Based Tremor Evaluation were 30 (18.2)% and 34 (30)% respectively. Two patients stopped their propranolol treatment two weeks before participation in this trial. The other patients did not use any treatment.

Clinical observations

No serious adverse reactions occurred following any of the treatments. Frequently reported adverse events include headache, sleepiness, dizziness and a painful arm during infusion. Headache was the most common adverse event, occurring in five, one and two patient(s) after administration of alcohol, TPA023 and placebo, respectively. Dizziness and sleepiness occurred in four, two and one subject(s) after administration of alcohol, TPA023 and placebo, respectively. Five patients reported a painful arm just after the start of the alcohol infusion. All adverse events were single occasions and considered mild of intensity.

Pharmacodynamics

TREMOR EVALUATION

LABORATORY TREMOGRAPHY During the postural condition of the measurement, alcohol infusion reduced maximum power of both the left-right (-31.4% (-45.9, -13.0%)) and backward-forward (-37.6% (-60.4, -1.7%))

tremor direction compared to placebo. For the kinetic condition, similar results were obtained (table 1). The maximum power of the left-right direction was reduced by 19.2% (-31.7, -4.3%) and that of the backward-forward direction by 29.5% (-44.8, -9.9%) after alcohol infusion compared to placebo. These changes resulted in a decrease in average power in both conditions (figure 2). In the resting condition, no effects were seen in any tremor direction for any treatment. TPA023 did not affect tremor symptoms as effectively as a stable level of alcohol.

PERFORMANCE-BASED TREMOR EVALUATION Mean total tremor score decreased shortly after alcohol infusion had begun (figure 3). However, mean scores during placebo and TPA023 treatment also decreased. No differences compared to placebo were present after alcohol (-2.2 (-6.3, 1.9)) and TPA023 (-1.0 (-5.1, 3.0)) treatment (table 1).

RELATIONSHIP BETWEEN LABORATORY TREMOGRAPHY AND PBTE Tremor amplitude and tremor rating scales are logarhithmically related and one can be estimated if the other parameter is known, using a set of formulas proposed by Elble (Elble *et al.*, 2006). We used these formulas to investigate to what extent the PBTE serves as a predictor for the effect on tremor amplitude. Based on the mean reduction in PBTE, which was measured after alcohol treatment (compared to placebo) a 15% decrease in tremor amplitude was expected, while a 20% reduction was observed (for the kinetic condition). Additionally, the mean reduction in PBTE scores, which was measured after TPA023 treatment in this study resulted in an estimated reduction in tremor amplitude of 7%, while a 6% reduction was observed in the kinetic condition.

CENTRAL NERVOUS SYSTEM TESTS

SACCADIC EYE MOVEMENTS TPA023 significantly decreased saccadic peak velocity (SPV) with 45.4 deg/sec (-65.6, -25.1deg/sec) compared to placebo. TPA023 also increased inaccuracy with 1.0% (0.3, 1.6) but not latency. Alcohol did not show significant effects on eye movements (table 2).

BODY SWAY Neither treatment significantly affected body sway compared to placebo (table 2).

ADAPTIVE TRACKING Adaptive tracking performance and SD of performance were not significantly affected compared to placebo (table 2).

VISUAL ANALOGUE SCALES Alcohol decreased the VAS alertness scale by 8.0 mm (-13.2, -2.7mm) compared to placebo. VAS mood and VAS calmness were not affected significantly by alcohol or TPA023 treatment (table 2).

Pharmacokinetics

Mean breath alcohol concentrations increased in approximately 15 minutes to 0.58 g/L and stabilized for 4 hours, after which the infusion was stopped and concentrations returned to baseline (figure 1).

Post-hoc power calculation

In this exploratory study, the potential tremor reducing effects of TPA023 were compared to the effects of alcohol that were identified in this study. A post-hoc power calculation revealed that a sample size of 139 ET-patients would have been necessary to have 80% power to detect the same average difference between TPA023 and placebo (i.e. 0.068 μV) that was observed in nine ET-patients during alcohol treatment.

DISCUSSION

This placebo-controlled study explored the effects of a novel $GABA_A$ α2,3 selective partial agonist in patients with essential tremor. Results showed that only alcohol, which was used as a positive control, reduced tremor power, as assessed by the laboratory tremography.

Several studies have shown that alcohol is effective in reducing tremor amplitude in patients with essential tremor (Growdon *et al.*, 1975; Zeuner

et al., 2003; Rajput *et al.*, 1975; Koller and Biary, 1984). It is thought that alcohol acts within the central nervous system and not by affecting peripheral tremorogenic mechanisms (Growdon *et al.*, 1975; Zeuner *et al.*, 2003). Previous studies showed tremor reductions after a single oral intake of alcohol or an infusion bolus, causing mean blood alcohol levels of 0.35-0.55 $g \cdot L^{-1}$ (Zeuner *et al.*, 2003). The current study was able to keep alcohol levels stable at 0.6 $g \cdot L^{-1}$ for approximately four hours. Tremor power was reduced as long as the alcohol levels were stable and returned to baseline after the infusion was stopped.

Allthough tremor reducing effects were observed after TPA023 treatment in the kinetic condition, these effects did not reach significance in this relatively small study. It is unlikely that this was due to doses that were too low. There were clear effects of TPA023 2 mg on saccadic eye movements in this study, and similar finding were obtained with the same dose in healthy volunteers (de Haas *et al.*, 2007). The dose was predicted to be therapeutically active for the treatment of anxiety. A positron emission tomography study (Atack *et al.*, 2010) demonstrated that the dose of TPA023 used in this study produces substantial and sustained occupancy (47 to 64% at ~2 hours and 34 to 59% at ~7 hours postdosing). Although the required receptor occupancy for the postulated GABAergic effect in ET is unknown, these levels are consistent with the occupancy associated with the minimum effective dose in animal models of anxiolysis (44 to 76%, 46% and 89%, for the elevated plus maze, fear-potentiated startle and conditioned suppression of drinking paradigms, respectively), indicating that they are likely to be centrally active (MSD, data on file). In addition, the preliminary results of a fear-potentiated startle paradigm in healthy subjects support the anxiolytic efficacy of single dose TPA023 2.0 mg (MSD, data on file). In the latter study, startle amplitude during threat conditions was significantly reduced when subjects received TPA023 2.0 mg compared to placebo ($p<0.0035$).

To maximize the power of alcohol in detecting a treatment effect, only patients who were familiar with the positive effects of alcohol on their symptoms were included in this project. Patients could also have been selected on their (prior) responses to benzodiazepines or barbiturates. However, the clinical acceptability of such treatments was expected to be determined not only by the effect on ET-symptoms but also by the individual tolerability to side effects. Moreover, benzodiazepine withdrawal can also induce tremor, which can be difficult to distinguish from recurrence of ET-symptoms. It was considered less problematic to use a positive alcohol response as a potential predictor of an effect of subtype-selective partial $GABA_A$-agonist. Many patients report a positive effect of alcohol, but still do not routinely use alcohol to suppress their tremor. The half-life of alcohol is shorter than for most tremor-reducing medications. This diminishes the chance of tolerance and withdrawal after stopping alcohol. Since this drug also acts as an allosteric $GABA_A$-agonist, subjective ET-suppression with alcohol was also expected to increase the chance of finding effects with a subtype-selective $GABA_A$-agonist.

For treatment of ET and anxiety, similar clinical doses of benzodiazepines are used (Chouinard *et al.*, 1982; Pahwa and Lyons, 2003). Early clinical studies also indicate that TPA023 has an anxiolytic effect (Atack *et al.*, 2006). Therefore, the lack of effects of TPA023, which has a selective activity at the $\alpha 2,3$ subunit of the $GABA_A$ receptor, suggests that effects of GABAergic treatments of tremor are probably not mediated via the $\alpha 2,3$ subunit. However, we cannot rule out the possibility that the $\alpha 2$ and/or $\alpha 3$ subtypes *are* involved, but that TPA023 does not have enough intrinsic efficacy to produce clinical efficacy. It is uncertain which other $GABA_A$-receptor subtypes are involved. The $\alpha 5$ subunit is mainly located in the hippocampus, and has been shown to be involved in memory processes (Wendt *et al.*, 2000). The $\alpha 1$ subunit is probably the best candidate, since it is the most widely distributed $GABA_A$-receptor subtype (Benke *et al.*, 2004). Recently, the $\alpha 1$ subunit knockout mouse has been introduced as an animal model for essential tremor as it exhibits postural and kinetic tremors that clearly reproduce the features of essential tremors (Jankovic and Noebels, 2005; Kralic *et al.*, 2005). This suggests that the expression of $\alpha 1$ subunits in the brains of patients with ET may be abnormal. A pilot study in ET patients,

however, could not demonstrate any relation between ET and variants in the gene coding for this α1 subtype receptor (Deng *et al.*, 2006). Another recent paper confirms this statement (Garcia-Martin *et al.*, 2011). Nevertheless, other defects in the α1 subunit may still play a role in the pathophysiology of ET. Since this receptor subtype is also involved in sedation, it may be difficult to find GABAergic tremor treatment that is completely devoid of this side effect, which is particularly cumbersome in elderly patients.

It appeared that tremor power was largely reduced in all treatment groups in the first hour after treatment had started, as shown in figure 2. After this initial decrease, tremor remained stable during placebo treatment. This suggests that tremor was enhanced at baseline, e.g. by stress associated with the start of the study day (Whitney, 2006), or that there is a substantial initial placebo effect in treating ET. The alcohol effects were still clearly present on top of these significant placebo effects. Nonetheless, a wash-in placebo infusion would be useful in future studies, to ensure a reduction to stable baseline tremor levels in this patient group.

Although clinical rating scales have proven their effectiveness in the assessment of ET severity (Bain, 2000; Bain, 1998), the performance-based tremor evaluation scale used in this study was not able to measure a statistically significant effect of alcohol on ET, compared to placebo. The scoring of the test was performed by four trained persons, which might have caused too much inter-rater variability to detect a significant effect. Interestingly, the relationship between PBTE and tremor amplitude in this relatively small study approximately complied with the logarhithmic criteria, which were proposed earlier (Elble *et al.*, 2006).

In healthy volunteers, alcohol causes readily detectable increases in body sway and decreases in adaptive tracking (Zoethout *et al.*, 2009), but this could not be found in our study. This could have been caused by two opposing effects of alcohol in this patient group. On the one hand, alcohol has positive effects on the tremor itself, counterbalancing the negative performance on this task caused by alcohol (Zoethout *et al.*, 2009). Similar factors could play a role in the lack of effects on body sway. Previous studies have shown that patients with essential tremor had higher baseline ataxia scores compared to healthy controls, which diminished after alcohol ingestion (Klebe *et al.*, 2005). As for general performance, the lack of a net effect of alcohol on body sway could be explained by a reduction of ataxia in ET, combined with alcohol-induced postural instability. This might be a reason for the difference of alcohol effects between healthy volunteers and this patient group.

The different effects of TPA023 in the current study and those observed in a previous study investigating the effects of TPA023 (de Haas *et al.*, 2007) are approximately similar. TPA023 accounted for significant decreases in SPV in both studies and VAS alertness and body sway were not affected by TPA023 treatment in both sessions. The only observed difference between the two studies is an increase in saccadic inaccuracy in the current study, which was not found in the previous study. The higher dose in the current study might be an explanation for this phenomenon.

This study has shown that treatment with an α2,3 subunit selective $GABA_A$ partial agonist was less effective compared to a stable level of alcohol in reducing ET-symptoms. This suggests that the α2,3 subunit of the $GABA_A$ receptor is probably of less importance in the pathophysiology of essential tremor or in the beneficial effects of non-selective benzodiazepines and barbiturates. Additionally, the study has shown that the alcohol clamp is a reliable method for studies in patients with essential tremor.

FIGURE 1 AVERAGE GRAPH OF BREATH ALCOHOL CONCENTRATIONS (G/L) WITH SD ERROR BARS

Maximum and minimum values are shown with thin lines.

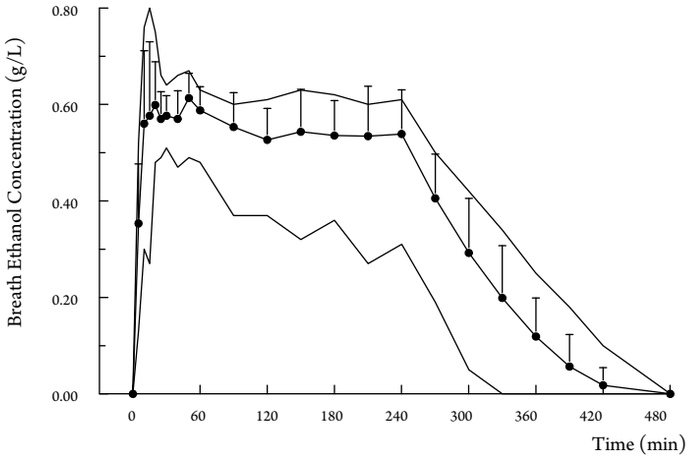

FIGURE 2 GRAPHS SHOW CHANGES FROM BASELINE OF AVERAGE MAXIMUM POWER OF LABORATORY ACCELEROMETRY IN POSTURAL AND KINETIC CONDITION WITH 95%CI ERROR BARS

Closed circle is TPA023; square is alcohol; open circle is placebo.

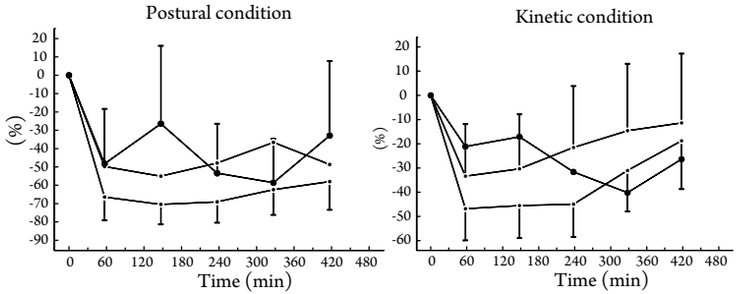

FIGURE 3 GRAPH SHOWS CHANGE FROM BASELINE OF AVERAGE SCORE ON PERFORMANCE-BASED TREMOR EVALUATION SCALE WITH 95%CI ERROR BARS

Closed circle is TPA023; square is alcohol; open circle is placebo.

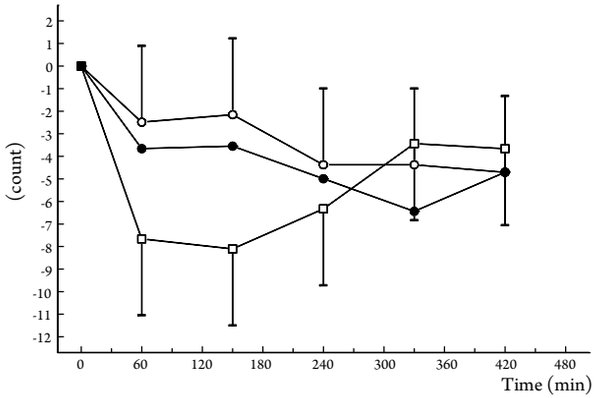

TABLE 1 LEAST SQUARE MEANS AND TREATMENT DIFFERENCES RELATIVE TO BASELINE FOR DIFFERENT TREMOR VARIABLES

ANOVA results are shown as contrasts (%), p-values and 95% CI.

Laboratory accelerometry maximum power variables		**LS means**			**TPA023 - placebo**				**Alcohol - lacebo**			
		TPA023	Alcohol	Placebo	difference	P - value	95%CI		difference	P - value	95%CI	
Rest	Up-Down (uV)	0.192	0.149	0.151	26.8	0.19	-12.2,	83.1	-1.3	0.94	-31.7,	42.7
	Left-Right (uV)	0.316	0.263	0.289	9.3	0.53	-18.8,	47.2	-8.8	0.53	-32.5,	23.3
	Back-Forward (uV)	0.174	0.150	0.139	25.5	0.15	-8.8,	72.6	8.2	0.60	-21.5,	49.1
	Average Maximum power (uV)	0.233	0.191	0.195	19.0	0.26	-13.4,	63.6	-2.3	0.88	-29.0,	34.5
Postural	Up-Down (uV)	0.623	0.390	0.588	6.0	0.77	-31.2,	63.3	-33.6	0.060	-56.8,	2.1
	Left-Right (uV)	0.782	0.495	0.721	8.4	0.48	-15.0,	38.2	-31.4	0.0049	-45.9,	-13.0
	Back-Forward (uV)	0.482	0.317	0.508	-5.1	0.80	-39.7,	49.4	-37.6	0.043	-60.4,	-1.7
	Average Maximum power (uV)	0.652	0.410	0.620	5.2	0.75	-25.6,	48.7	-33.8	0.023	-53.1,	-6.7
Kinetic	Up-Down (uV)	1.296	1.108	1.393	-6.9	0.58	-29.4,	22.7	-20.5	0.099	-39.9,	5.2
	Left-Right (uV)	2.719	2.344	2.899	-6.2	0.41	-20.5,	10.6	-19.2	0.018	-31.7,	-4.3
	Back-Forward (uV)	1.112	0.866	1.228	-9.5	0.40	-29.6,	16.5	-29.5	0.0095	-44.8,	-9.9
	Average Maximum power (uV)	1.741	1.486	1.862	-6.5	0.48	- 23.6,	14.4	-20.2	0.036	-35.2,	-1.7
Performance Based Tremor Evaluation score		15.1	13.9	16.1	-1.0	0.59	-5.1,	3.0	-2.2	0.26	-6.3,	1.9

TABLE 2 LEAST SQUARE MEANS AND PHARMACODYNAMIC DIFFERENCES RELATIVE TO BASELINE FOR SACCADIC EYE MOVEMENTS, VISUAL ANALOGUE SCALES, BODY SWAY AND ADAPTIVE TRACKING

ANOVA results are shown as contrasts, p-values and 95% CI.

CNS Variable	**LS means**			**TPA023 - placebo**				**Alcohol - placebo**			
	TPA023	**Alcohol**	**Placebo**	**difference**	**P - value**	**95%CI**		**difference**	**P – value**	**95%CI**	
Saccadic Peak Velocity (deg/sec)	413.6	443.0	459.0	-45.4	0.0002	-65.6,	-25.1	-16.0	0.095	-35.3,	3.2
Latency (sec)	0.233	0.228	0.224	0.009	0.14	-0.003,	0.021	0.004	0.47	-0.008,	0.016
Inaccuracy (%)	7.5	6.5	6.6	1.0	0.0080	0.3,	1.6	-0.1	0.80	-0.8,	0.6
VAS Alertness (mm)	61.6	56.2	64.2	-2.6	0.33	-8.1,	2.9	-8.0	0.0061	-13.2,	-2.7
VAS mood (mm)	73.8	73.2	73.9	-0.2	0.92	-3.9,	3.6	-0.7	0.68	-4.4,	3.0
VAS Calmness (mm)	64.5	69.4	68.1	-3.6	0.24	-10.0,	2.8	1.3	0.65	-5.0,	7.6
Body Sway Eyes Closed (mm)	426.7	465.9	413.7	3.1	0.64	-10.6,	19.0	12.6	0.11	-3.1,	30.9
Adaptive tracking performance (%)	13.60	14.01	15.03	-1.43	0.065	-2.96,	0.10	-1.02	0.17	-2.54,	0.50
SD of adaptive tracking performance (%)	2.47	2.61	2.53	-0.06	0.71	-0.37,	0.26	0.09	0.54	-0.22,	0.40

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