CHAPTER 8

The effects of a novel histamine-3 receptor inverse agonist on essential tremor in comparison to stable levels of alcohol

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ABSTRACT

BACKGROUND Essential tremor (ET) is a common movement disorder. Animal studies show that histaminergic modulation might affect the pathological processes involved in the generation of ET. Histamine-3 receptor inverse agonists (H3RIA) have demonstrated attenuating effects on ET in the harmaline rat model.

METHODS In this double-blind, three-way cross-over, single-dose, double-dummy study the effects of 25 mg of a novel H3RIA (MK-0249) and a stable alcohol level (0.6 $g \cdot L^{-1}$) were compared to placebo, in 18 ET-patients. Tremor was evaluated using laboratory tremorography, portable tremorography and a clinical rating scale. The Leeds Sleep Evaluation Questionnaire (LSEQ) and a choice reaction time (CRT) test were performed to evaluate potential effects on sleep and attention, respectively.

RESULTS A steady state of alcohol significantly diminished tremor as assessed by laboratory tremorography, portable tremorography and clinical ratings compared to placebo. A high single MK-0249 dose was not effective in reducing tremor, but caused significant effects on the LSEQ and the CRT-test.

CONCLUSIONS These results suggest that treatment with a single dose of MK-0249 does not improve tremor in alcohol-responsive patients with ET, whereas stable levels of alcohol as a positive control reproduced the commonly reported tremor diminishing effects of alcohol.

INTRODUCTION

Essential tremor (ET) is one of the most common neurological disorders among adults, and is the most common tremor disorder (Louis *et al.*, 1998; Louis, 2001; Louis, 2005). In contrast with the resting tremor in Parkinson's disease, ET is provoked by postural and kinetic movements and has a dominant frequency of 4-12 Hz (Pahwa and Lyons, 2003). The upper extremities are predominantly affected, but the head, the neck and the voice may also be involved, either separately or in combination. The prevalence of ET is estimated to be 4% within the general population (Pahwa and Lyons, 2003), and approximately 5% in the population above 65 years (Louis *et al.*, 1998).

Current pharmacological treatment, including beta blockers, benzodiazepines and primidone, act symptomatically and have variable effectiveness (Chen and Swope, 2003). Moreover, the occurrence of side effects, like sedation, weight gain and cognitive impairment, limit their use. Estimations indicate that alcohol is effective in approximately 70% of ET patients (Lou and Jankovic, 1991; Koller *et al.*, 1994). This finding is confirmed by controlled studies where alcohol was administered acutely (Growdon *et al.*, 1975; Koller and Biary, 1984). It most likely acts via a reduction of central over-activity, which results in reduced tremor amplitude, whereas the frequency remains unaffected (Koller and Biary, 1984; Koller, 1991). ET can also be treated surgically. Stereotactic thalamotomy and continuous deep-brain stimulation are promising techniques for patients with severe functional disability who are unresponsive to drug therapy (Koller, 1991; Louis, 2005). These two procedures are equally effective, but thalamic stimulation has fewer adverse effects and results in a greater improvement in function (Schuurman *et al.*, 2000).

Because little is known about the pathophysiology of ET, the search for novel pharmacological treatment options has been challenging. Recent studies indicate that ET might be attributed to a defect in the central oscillatory systems at the level of the inferior olivary nucleus of the medulla oblongata (Deuschl and Elble, 2000). Since histaminergic neurons project to the inferior olivary nucleus in the brainstem, the histaminergic system might play a role in the modulation of pathological processes involved in the generation of ET. In addition, animal models for ET show that modulating histaminergic tone in the central nervous systems (CNS) neurons might be a fruitful strategy in the development of future pharmacological treatment (Merck Research Laboratories (MRL) – data not shown).

The histaminergic system is widely disseminated in the CNS and regulates multiple functions including, arousal, satiety, attention and cognition. Histaminergic neurons originate in the tuberomammillary nucleus of the hypothalamus and project throughout the CNS, including the inferior olivary

nucleus in the brainstem. Currently, four types of histamine receptors have been identified. Postsynaptically localized histamine subtype-1 (H1) and histamine subtype-2 (H2) receptors mediate the preponderance of CNS effects, while the histamine subtype-3 (H3) receptors are localized on the presynaptic membrane as autoreceptors, and regulate the production and synaptic release of histamine as a part of a negative feedback mechanism. H3-receptors are mainly distributed in the CNS and function to modulate histaminergic effects in the brain, making them a potential target for pharmacological manipulation. H3-receptors signal constitutively, which serves to tonically suppress histamine production at baseline.

Recent preclinical findings indicate a potential role for the histaminergic system in the treatment of ET. Rats treated with the β-carboline harmaline, a toxin from the Syrian Rue plant, develop an 8-12 Hz postural and kinetic tremor that is clinically similar to ET (Martin *et al.*, 2005). The toxin also activates and synchronizes firing in the olivocerebellar system (Martin *et al.*, 2005). Harmaline has been used to develop a rat model of ET that responds in a dose dependent fashion to several of the currently available treatments for the disease, including propranolol, alcohol and benzodiazepines (Martin *et al.*, 2005). An increase in brain histamine induced by single doses of histamine modulating agents at the H3-receptor reduced tremor significantly in a dose-dependent fashion in the harmaline rat model of ET (MRL – data not published). Thus, histaminergic modulation might also contribute to the treatment of tremor seen in ET.

An increase in histamine concentration in the brain by pharmacological interference at the H3-receptor would avoid peripheral histamine effects. While a classical antagonist would only interfere with histamine-mediated negative feedback, H3-receptor inverse agonists have been demonstrated to decrease constitutive H3-receptor signalling, thus blocking tonic inhibition of histamine synthesis and release and further potentiating histaminergic effects (Arrang *et al.*, 2007).

The primary objective of this study was to evaluate the effects of a single dose of MK-0249, a recently developed H3-receptor inverse agonist (shown in figure 1), on average maximum tremor power in ET-patients. The chemical and biological characteristics of this class of H3-receptor inverse agonists have recently been published (Nagase *et al.*, 2008). Since most ET-patients experience attenuating effects of alcohol on their symptoms (Rajput *et al.*, 1975), the effects of MK-0249 on ET were compared to a steady state level of alcohol as a positive control, versus placebo. The study design, methodologies for measuring tremor and data analysis plan were based upon a prior, yet unpublished pilot study.

METHODS

Design

Eighteen patients diagnosed with ET, were included in the study. The effects of MK-0249 were investigated and compared to alcohol (as an active comparator) and placebo in a randomized, double-blind fashion. Since ethanol was delivered as an intravenous infusion and MK-0249 was administered orally, a double-dummy design was adopted to maintain the double-blind character of the study. On each study day, patients received one of three different treatment combinations: active ethanol with MK-0249 placebo, ethanol placebo (glucose 5% vehicle) with active MK-0249 and ethanol placebo with MK-0249 placebo. The washout period between treatments was at least seven days.

Patients

Men and women of at least 18 years of age with documented ET for at least six months were recruited from the databases of the Leiden University Medical Centre, University Medical Centre St. Radboud in Nijmegen and from a number of local surrounding hospitals. Patients were informed about the contents of the study during an information visit. Once having agreed to participate and having signed informed consent, patients visited the research unit for a medical screening, at which time they were medically

screened to evaluate eligibility for study participation. A neurologist (J.G.) with experience in movement disorders clinically confirmed the diagnosis of 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). This statement was adapted from previous criteria established by the Tremor Research Investigation Group (TRIG) (Deuschl *et al.*, 1995) as well as from a more recent study indicating the importance of kinetic tremor (Brennan *et al.*, 2002). An isolated head tremor was not allowed. Patients were also selected for having symptoms that, by history, were relieved with alcohol consumption.

Only patients who stated a positive effect of alcohol on their tremor symptoms were included to maximize the power of alcohol as a positive control. Patients had to refrain from alcohol 48 hours prior to a treatment day and from caffeine-containing products for at least 12 hours before treatment. They 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 of the study until completion of the study. They were not allowed to drink more than six units of caffeine-containing products or three beverages 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. The study was performed in compliance with the standards of Good Clinical Practice and the Declaration of Helsinki.

Study treatments

On study days, the effects of a single oral dose of 25 mg MK-0249 or an intravenous alcohol infusion (10% w/v in 5% glucose) were examined. This MK-0249 dose was the highest single dose that was previously administered to healthy elderly male and female subjects. Elsewhere, single doses of MK-0249 up to 150 mg were safely administered to healthy young male volunteers. A yet unpublished positron emission tomography (PET) study in healthy young males suggests that the peak brain H3-receptor occupancy (after a single dose of 25 mg) would be approximately 90%, and be reached after approximately 6 hours. Given the long half life of this compound (approximately 14 hours), we would expect this level of receptor occupancy to be maintained during the entire observation period.

To diminish variability due to changing alcohol levels, alcohol was infused using a recently developed clamping method (Zoethout *et al.*, 2008). Based on a procedure for clamping breath alcohol concentrations (BrAC) previously described by O'Connor *et al.* (O'Connor *et al., 1998)* a spreadsheet-based alcohol clamping paradigm was developed. Changes in BrAC were used to adapt the intravenous infusion rate of alcohol (10% w/v in 5% glucose). This resulted in stable alcohol levels with minimal variability (Zoethout *et al.*, 2008). The alcohol clamp has previously been well-tolerated and produced statistically significant pharmacodynamic CNS effects (Zoethout *et al.*, 2009).

An alcohol level of 0.6 $g \cdot L^{-1}$ was chosen for this study, because this level is routinely achieved during social drinking, without causing too many adverse effects. Moreover, a prior study showed tremor reducing effects of alcohol at a mean level of 0.55 $g \cdot L^{-1}$, after a single oral dose (Zeuner *et al.*, 2003). Considering its pharmacokinetic profile, it was estimated that the action of MK-0249 would peak at about four hours and be sustained at high levels for at least seven hours. Consequently, to optimize the value of alcohol as a positive control, a level of 0.6 $g \cdot L^{-1}$ was maintained for seven hours . The research assistant who was responsible for performing breath samples was unblinded to the alcohol infusion, but this person was not involved in any other assessments, to preserve the double-blind character of the study.

This was a double-dummy study, so each active treatment was accompanied by administration of placebo for the alternative treatment. Thus, a matching placebo capsule for MK-0249 was given during the active alcohol occasion. On the day of administration of MK-0249 25 mg, a sham clamp was performed with glucose 5%, including preprogrammed pump rate changes and breath sampling (without revealing the results to the patient or the study team).

It has been suggested that tremor symptoms could be enhanced before dosing, e.g. by stress associated with the start of the study (Whitney, 2006) To by-pass this problem a 90-minute, a single-blind saline infusion (0.9%) preceded the actual alcohol/placebo infusion. After this 90 min 'run-in phase', MK-0249 (or placebo) was administered orally at t = 0 min and at the same time we started the alcohol (or placebo) clamping procedure. The run-in phase was single blind, and patients were told that the experiment started at t = - 90 min. The data collected between t = –90 min and t = 0 min were discarded.

Safety

Adverse events, ECG, blood pressure and heart rate measurements were assessed throughout the study. During study days, a parallel glucose infusion (glucose 5%) was administered to all patients during the first ten minutes post-start, to minimize pain and discomfort in the infusion arm, caused by the alcohol infusion.

Drug concentrations

Breath alcohol samples (BrAC) were performed using the hand-held Alco-Sensor IV meter (Honac, Apeldoorn, the Netherlands). The pharmacokinetic profile of MK-0249 had already been extensively investigated, and it was therefore decided not to analyze the pharmacokinetic samples of MK-0249 in this study.

Tremor evaluation

Tremor evaluations were performed at screening, predose (within 60 minutes prior to dosing) and at 60, 150, 240, 330, 420 and 510 minutes postdose on each study day.

LABORATORY TREMOROGRAPHY Tremor evaluation was measured according to the methodology of Gironell *et al.* (Gironell *et al.*, 1999). Tremor was evaluated with three miniature linear piezo-electric accelerometers (Nihon Kohden, MT-3T), which were attached to the distal end of the 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 (Van Hilten *et al.*, 1991; Gironell *et al.*, 1999). 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 an outstretched position to the nose, as accurately as possible.

PORTABLE TREMOROGRAPHY Tremor was also evaluated using a portable accelerometer (Dynaport MiniMod3) that consisted of three linear accelerometers placed in a perpendicular array, a data logger and a power source. The portable accelerometer is the size of a match-box, and was affixed to the fingertips of the dominant hand. The portable tremorography assessment was performed immediately following each laboratory tremorography assessment on the same hand, in exactly the same way.

PERFORMANCE-BASED CLINICAL RATING SCALE (CRS) Clinical rating scales are often described as useful methods to assess tremor severity in ET patients (Bain *et al.*, 1993; Louis *et al.*, 2000). A performance-based clinical rating scale (Louis *et al.*, 1999) was used in this study to investigate the practical implications of alcohol and MK-0249 treatment on 15 simple, 'daily-life' activities (e.g. carry a tray with two filled glasses, thread a needle or pour liquid from a milk carton into a glass). Performance on the CRS was videotaped to permit post-hoc analysis by a blinded independent expert. Performance on each item was scored from 0 (no difficulty) to 4 (unable to perform). The total sum of activity scores was calculated.

Additional measurements

CHOICE REACTION TIME (CRT) TEST To investigate the possible effects of MK-0249 (and alcohol) on psychomotor function, a CRT test was administered. Choice reaction time was chosen for its known sensitivity to the sedative effects of alcohol and other drugs (Grant *et al.*, 2000), as well as to stimulant effects of drugs like caffeine (Lieberman, 2007). During this test, either the word 'left' or the word 'right' was presented on a computer screen. All patients were instructed to press a corresponding button as quickly as possible. There were 32 trials for which each stimulus word was chosen randomly with equal probability. The duration of the interstimulus interval varied randomly. Both the amount of correct scores and the reaction time were assessed. CRT was tested at screening, predose (within 60 minutes prior to dosing) and at 60, 150, 240, 330, 420 and 510 minutes postdose on each study day.

LEEDS SLEEP EVALUATION QUESTIONNAIRE (LSEQ) Since MK-0249 is thought to increase CNS histamine levels, it is expected to be associated with alerting effects. Therefore the LSEQ was administered, to investigate the possible effects of MK-0249 on a wide scale of sleep parameters (Parrott and Hindmarch, 1980). The LSEQ is a standardized self-reporting instrument comprising ten 100 mm visual analogue scales that score the ease of getting to sleep, quality of sleep, ease of awakening from sleep and alertness and behavior following wakefulness (Parrott and Hindmarch, 1980). The LSEQ was administered at screening and pre dose on the morning of day 1 and at 24 hours post dose. To maximize compliance, patients were contacted by telephone the next morning, 24 hours after dosing, to check whether the LSEQ was completed.

Statistical analyses

Changes in maximum kinetic tremor power (especially backward-forward movements), as assessed by laboratory accelerometry were of primary concern. Given their pharmacologic profiles, tremor measurements between 240 and 510 minutes post dose were specifically hypothesized to be affected to the largest extent by treatment. A mean treatment-induced reduction of 25% (or more) compared to average baseline tremor scores was considered clinically meaningful. With 18 patients, there was more than 99% power to detect such a difference between MK-0249 and placebo or between alcohol and placebo.

Because of their skewed response distribution all tremor parameters were analyzed after log-transformation. Log transformed changes from baseline for accelerometry tremor endpoints were analyzed with mixed effects models (using SAS PROC MIXED). This resulted in least square means estimates for each treatment that indicate the change from baseline on the log scale. Subsequently, mean treatment effects were calculated as the contrasts between these least square means for placebo and either MK-0249 or alcohol after back transformation from the log scale. The average of the measurements at 240, 330, 420 and 510 minutes was calculated within the statistical model. All results are presented on the original scale and reported as percent changes (all analyses were two-sided, with a significance level of 0.05). Between treatment comparisons were made for LSEQ, CRT and CRS on the original scale. All calculations were performed using SAS V9.1.2 for Windows (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Patients

Nineteen ET-patients were found eligible for study participation. Apart from their tremor, they were judged to be in good health on the basis of medical history, physical examination and routine laboratory data. One patient dropped out after the first period because of the need to resume antidepressive medication. This patient was replaced by another patient, who received the same treatment randomization order. Eighteen patients (11 men, 7 women) completed the study per protocol. Patients were on average 47 years of age (range 19-81), had an average weight of 75 kg (range 61-98 kg) and an average height of 178 cm (range 161-191 cm).

Alcohol concentrations

Figure 2 shows the mean graph of breath alcohol values for all 18 patients. The average target level of 0.60 $g \cdot L^{-1}$ was achieved within 30 minutes. The steady state level was maintained until approximately 7 hours post-dose, after which alcohol infusion was stopped. Thereafter, BrAC returned to baseline.

Tremor evaluation

LABORATORY TREMOROGRAPHY Baseline tremor scores are presented in table 1. Treatment effects on maximum tremor power obtained by laboratory tremorography are summarized in table 2. MK-0249 treatment had no significant effect on the laboratory tremorography in any direction. For the kinetic condition, the mean maximum power of the backward-forward direction (primary endpoint) was reduced by 34.9% (95% CI: -52.7%, -17.3%) and that of the left-right direction was reduced by 42.0% (95% CI: -59.9%, -24.5%) after alcohol infusion compared to placebo. Also, mean average maximum power was reduced by 33.4% (95%CI: -49.8%, -17.2%) compared to placebo (figure 3). During the postural condition of the laboratory tremorography, alcohol infusion significantly reduced mean maximum power of both left-right (-38.9% (95% CI: -67.2%, -11.6%)) and backward-forward (-38.4% (95% CI: -66.1%, -11.9%)) tremor direction compared to placebo. The mean average maximum power was also significantly reduced after alcohol treatment (-37.0% (95% CI: -62.7%, -12.0%)) for the postural condition compared to placebo (figure 4). During the rest condition, alcohol infusion significantly reduced mean maximum power of both the up-down (-26.7% (95% CI: -53.8%, 0.0%)) and left-right condition (-46.1% (95% CI: -73.5%, -19.4%)) compared to placebo. Alcohol also reduced the mean average maximum power by 37.6% (95% CI: -62.5%, -13.4%) in the rest condition compared to placebo. Table 2 also shows additional contrasts (e.g. up-down for kinetic and postural and back-forward for rest) for which statistical significance was not noted.

PORTABLE TREMOROGRAPHY Treatment effects on tremor power variables obtained by portable accelerometry are summarized in table 3. No effects of MK-0249 were observed in the rest, postural or kinetic condition during portable accelerometry measurements. Alcohol decreased mean maximum power in the up-down (-25.7% (95% CI: -42.9%, -8.8%), left-right (-27.8% (95% CI: -43.2%, -12.6%) as well as in the backward-forward (-32.7% (95% CI: -55.5%, -10.3%) directions compared to placebo, during the kinetic assessment. Mean average maximum power was also significantly reduced by 27.9% (95% CI: -44.2%, -11.8%) compared to placebo (figure 5). During postural measurements, alcohol reduced mean maximum tremor power in both the left-right and the backward-forward direction compared to placebo by 28.4% (95% CI: -56.6%, -1.1%) and 35.9% (95% CI: -69.4%, -3.8%) respectively. Mean average maximum power was also significantly reduced (-29.0% (95% CI: -58.2%, -0.8%) during postural assessments compared to placebo (figure 6). No significant changes were observed during the rest condition for any treatment, compared to placebo.

PERFORMANCE-BASED CLINICAL RATING SCALE The videotaped clinical rating scales were analysed by a blinded independent expert (E.D.L.). While all assessment time points were videotaped, performance scores obtained at t = 240 minutes were selected (the expected T_{max} for MK-0249) for comparison between treatments. This analysis showed a significant effect of alcohol. Alcohol scores were on average 17% lower (95% CI: 3, 28%) compared to placebo, when both treatments were corrected for baseline values. There was no apparent effect of MK-0249.

CLINICAL RATING SCALE VS. TREMOROGRAPHY
To estimate the predictive value of the experimental tremor registrations (tremorography) for clinical outcome, correlation coefficients of the clinical rating scale *vs.* tremorography were calculated at 240 minutes post dose. The Pearson correlation coefficient for the clinical rating scale *vs.* laboratory tremorography was 0.33 (p=0.018). The Pearson correlation coefficient for the clinical rating scale *vs.* portable tremorography was 0.38 (p=0.006).

Additional measurements

CHOICE REACTION TIME (CRT) During the CRT test both reaction time (msec) and the number of correct responses were determined. Both alcohol and MK-0249 affected the reaction time significantly during this test. Alcohol infusion resulted in a mean increase of 43.2 msec (95% CI: 18.0, 68.5 msec) and MK-0249 resulted in a mean increase of 27.6 msec (95% CI: 1.8, 53.4 msec), as compared to placebo. The mean number of correct reactions after MK-0249 treatment (31.49) improved slightly but statistically significantly compared to placebo treatment (31.23) by 0.26 trials (95% CI: 0.00, 0.52; $p < 0.05$). There was no apparent effect of alcohol on the number of correct responses.

Leeds Sleep Evaluation Questionnaire (LSEQ)

There were no significant effects of alcohol on the LSEQ. On the night following administration, MK-0249 decreased the 'speed of getting to sleep' (i.e. getting to sleep more slowly than usual) by 12.4 mm (95% CI: -23.9, -0.9 mm) compared to baseline (pre-dose). MK-0249 also changed 'restlessness during sleep' (i.e. more restless than usual) and the 'periods of wakefulness' (i.e. more periods of wakefulness than usual) with 30.4 mm (95% CI: -38.9, -21.8 mm) and 30.2 mm (95% CI: -38.8, -21.6 mm) respectively. By comparison, in a study by Hindmarch *et al.* (Hindmarch *et al.*, 2000), 150 mg caffeine increased the time to sleep onset and was associated with deterioration in the perceived quality of sleep compared with placebo. Mean changes in the 100 mm scale were 22 and 16 mm for time to sleep onset and quality of sleep, respectively.

Adverse events

All 19 subjects enrolled in the study were included in the assessment of safety and tolerability. Eighteen (18) of the 19 subjects enrolled, reported one or more clinical adverse experiences (AE). No laboratory adverse experiences were reported, no serious adverse experiences were reported, and no deaths occurred. If a subject reported the same AE more than once (per treatment period), this AE was counted only once. A total of 115 clinical adverse experiences were reported and 31 occurred while taking MK-0249. Of the 31 clinical adverse experiences reported while taking MK-0249, 25 were considered to be related to study drug. The most common drug-related adverse experiences during active MK-0249 treatment were perspiration (5 subjects), insomnia (4 subjects), and nausea (3 subjects). The most frequently reported adverse events after alcohol treatment were sleepiness (9 subjects), inebriation (7 subjects), a painful arm at the start of the infusion (7 subjects), dizziness (7 subjects) and headache (6 subjects). Headache was also reported by 6 subjects after placebo treatment. All symptoms were transient and mild in severity. One patient on placebo discontinued the study due to 'moderate lightheadedness', which was considered to be related to discontinuation of his own medication.

DISCUSSION

A stable level of alcohol (0.6 $g \cdot L^{-1}$) for seven hours was associated with a reduction in average maximum tremor power by approximately 30%. The tremor diminishing effects of alcohol were not only observed using laboratory accelerometry, the pre-specified primary endpoint, but we also found that the portable tremorographer detected changes in tremor associated with alcohol treatment. Although it has only been tested under standard experimental conditions here, portable tremorography could be a promising technique for portable, standardized, ambulatory tremor registrations.

The present study confirms the commonly reported attenuating effects of alcohol on ET that have been demonstrated earlier, in both database studies (Lou and Jankovic, 1991; Koller *et al.*, 1994) and in controlled trials (Growdon *et al.*, 1975; Koller and Biary, 1984). However, in contrast to most controlled trials in which the effect of alcohol on ET is being studied, our results were obtained under tightly controlled, stable alcohol levels for a prolonged period of time. These fixed levels accounted for a stable condition that minimized the variability. The steady state level of alcohol clearly showed tremor relieving effects, confirming that the (partly experimental) methods and the design

of this study were sensitive enough to detect tremor reductions. The alcohol effects also set a benchmark for clinically significant tremor reductions, demonstrating that the effects of MK-0249 did not only fail to reach statistical significance, but also that they were much smaller than what is achieved with alcohol.

In contrast to resting tremor in Parkinson's disease, essential tremor is characterized by postural and kinetic components (Elble, 2000), which were both affected by alcohol treatment in this study. Tremor symptoms at rest do occur in some ET patients (Louis, 2006), and this finding may explain the effects of alcohol in the resting condition during laboratory accelerometry measurements. The effects of alcohol on ET were maintained for most of the infusion period. Although acute tolerance to the effects of alcohol is frequently described (Martin and Moss, 1993; Hiltunen *et al.*, 2000), no indications for acute changes in effects of alcohol on tremor power were observed in this study.

Despite the promising results during the preclinical phase, single doses of MK-0249 (25 mg) did not reduce maximum tremor power on any of the tremor measurements in this study of alcohol-responsive ET-patients. On average, most tremor measurements even seemed to deteriorate slightly with the H3-inverse agonist, although this was not statistically significant. The clear improvements with alcohol indicated that the power of the study and the sensitivity of the methods were sufficient to detect a clinically significant tremor reduction. Thus, a single dose of MK-0249 25 mg was not effective in reducing ET symptoms and does therefore not seem to have a beneficial effect on human ET. This was not due to a lack of brain penetration or CNS activity, since clear CNS effects were observed on the CRT and the LSEQ after MK-0249 administration. Moreover, a comparable single dose of MK-0249 was associated with alerting effects in a study of sleep-deprived healthy male volunteers (Iannone *et al.*, 2010). It cannot be excluded that a more sustained exposure of MK-0249 would be needed for amelioration of tremor than would have been achieved with a single dose.

Interestingly, an analysis of linkage in a two-generation pedigree of patients with Tourette's syndrome recently identified a rare functional mutation in the HDC gene encoding L-histidine decarboxylase, the rate-limiting enzyme in histamine biosynthesis. These findings pointed to a role for histaminergic neurotransmission in the mechanism and modulation of Tourette's syndrome and tics (Ercan-Sencicek *et al.*, 2010). This suggests that MK 0249 may be beneficial for patients with Tourette's syndrome and related features of compulsive behavior, but this hypothesis has not been tested.

Because neuronal histamine is one of the most important systems that stimulates and maintains wakefulness (Yanai and Tashiro, 2007), and because MK-0249 exerts its action through the histaminergic system, we expected that MK-0249 would be alerting. The significant effect on the LSEQ confirmed that MK-0249 had a pharmacodynamic effect in the CNS in this population of patients. This effect was in agreement with the reported sleep-related adverse events. An H3-inverse agonist was also expected to increase attentiveness. MK-0249 caused a marginal increase in reaction time as measured by the CRT-test, but was also associated with a statistically significant improvement in the number of correct scores. This could signify a shift in performance strategy from speed to accuracy. At the same time, it should be realized that the changes were very small in magnitude. These effects were however quite different from those of alcohol, which caused a significant delay in reaction time on the CRT test and only little effect on the LSEQ.

There was a weak positive correlation between the clinical rating scale and the tremorography data. Hence, experimental tremor registrations used in this study were only slightly predictive for clinical outcome as measured by the clinical rating scale. However, both clinical ratings and portable tremorography improved after alcohol administration, albeit with somewhat less sensitivity than laboratory tremorography, showing convergent evidence for the effects of alcohol on ET. These findings indicate the importance of performing clinical rating scales in addition to portable tremor registration methods, in studies focusing on tremor severity.

A prior tremor study at our centre (CHDR – data on file) showed large pharmacodynamic placebo effects during the first hour following the start of the study. Since tremor increases during anxiety or excitement (Whitney, 2006), this placebo effect was attributed to stress at the start of the experiment. It was therefore decided to precede the actual clamping phase by a single-blinded 90 min saline infusion period, to allow adaptation to the study circumstances before baseline values for each assessment were obtained. In contrast to the previous tremor study (CHDR – data on file), no placebo effect was observed in the present study. An 'adaptation period' therefore seems to be a useful procedure in studies focusing on ET measurements.

MK-0249 showed clear CNS-effects that were compatible with its pharmacological action as a H3RIA, but it did not reduce tremor in this study. In contrast to alcohol, most tremor measurements seemed to increase, although this never reached statistical significance. To the extent that the hypothesis could be tested with exposures from a single dose, these findings suggest that the histamine-3 receptor does not play an important role in the treatment of essential tremor. The harmaline rat model therefore does not seem a valuable predictor for the effects of histaminergic modulation on human ET. Moreover, H3-inverse agonism as a target for treatment of human ET seems of minor relevance. Clearly, more research is needed to investigate the role of histaminergic and other systems in the pathophysiology and possible treatment of this common movement disorder. This study shows that such studies are feasible and informative, using sensitive tremor detection methods in relatively small numbers of patients.

FIGURE 1 CHEMICAL STRUCTURE OF MK-0249

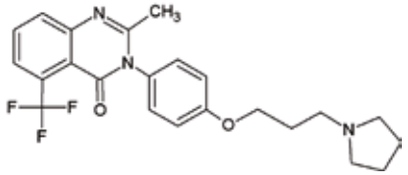

FIGURE 2 AVERAGE GRAPH OF BREATH ALCOHOL CONCENTRATION (BrAC) WITH SD'S AS ERROR BARS

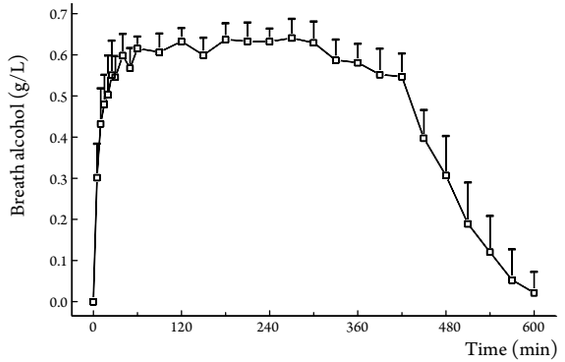

FIGURE 3 LEAST SQUARE MEANS GRAPH OF LABORATORY ACCELEROMETRY IN THE KINETIC CONDITION

Average max power (change from baseline), with 95% CI error bars for MK-0249 (up) and alcohol (down).

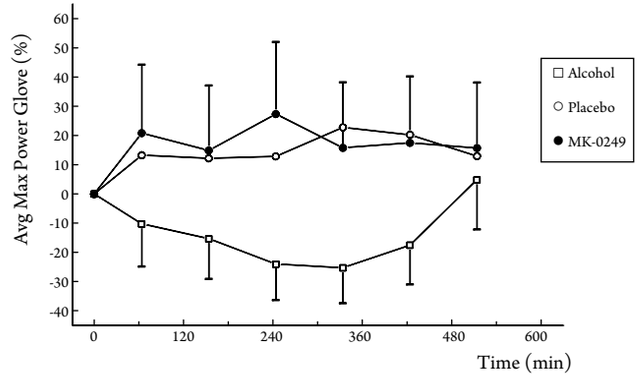

FIGURE 4 LEAST SQUARE MEANS GRAPH OF LABORATORY ACCELEROMETRY IN THE POSTURAL CONDITION

Average max power (change from baseline), with 95% CI error bars for MK-0249 (up) and alcohol (down).

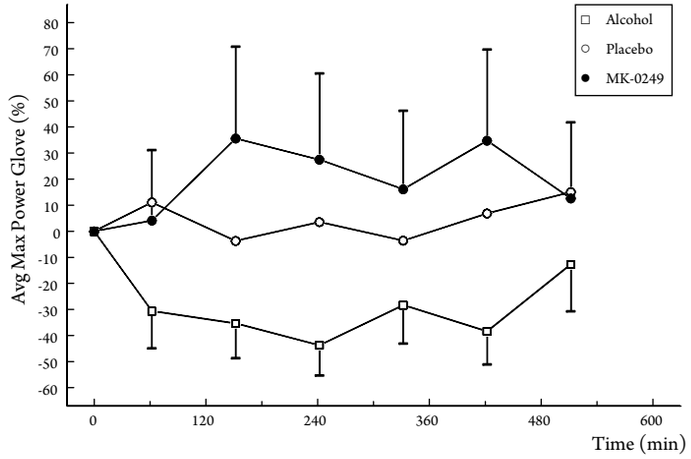

FIGURE 5 LEAST SQUARE MEANS GRAPH OF PORTABLE ACCELEROMETRY IN THE KINETIC CONDITION

Average maximum power (change from baseline), with 95% CI error bars for placebo (up) and alcohol (down).

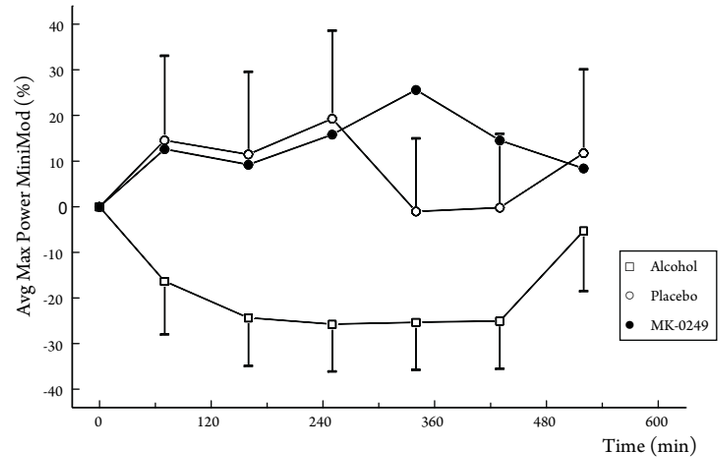

FIGURE 6 LEAST SQUARE MEANS GRAPH OF PORTABLE ACCELEROMETRY IN THE POSTURAL CONDITION

Average maximum power (change from baseline), with 95% CI error bars for MK-0249 (up) and alcohol (down).

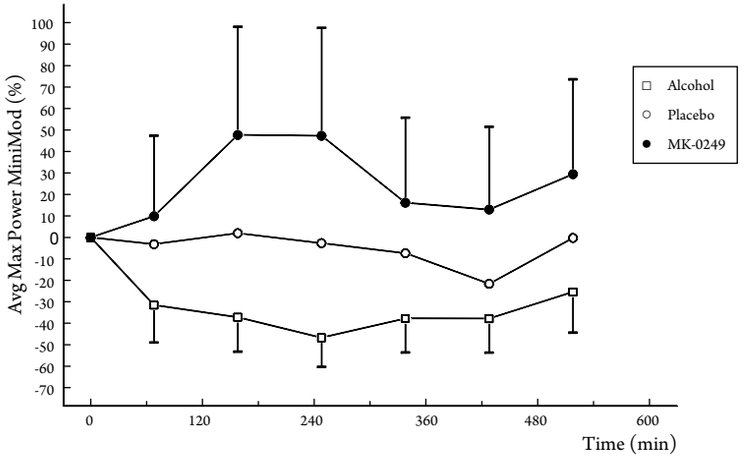

TABLE 1 BASELINE TREMOR VALUES (IN μV) WITH STANDARD DEVIATIONS BETWEEN BRACKETS

		Placebo	MK-0249	Alcohol
Kinetic	Back-Forward	1.11 (0.73)	1.21 (1.15)	1.05 (0.77)
	Average	1.88 (1.11)	1.92 (1.32)	1.89 (1.01)
	Up-Down	1.81 (1.82)	1.84 (2.23)	1.72 (1.63)
	Left-Right	2.70 (1.71)	2.72 (1.90)	2.91 (2.00)
Postural	Back-Forward	0.89 (1.35)	1.05 (2.13)	1.08 (1.86)
	Average	1.21 (1.37)	1.16 (1.13)	1.22 (0.98)
	Up-Down	1.61 (2.31)	1.35 (1.57)	1.44 (1.53)
	Left-Right	1.11 (0.83)	1.07 (0.86)	1.14 (0.63)
Rest	Back-forward	0.15 (0.05)	0.17 (0.10)	0.20 (0.17)
	Average	0.31 (0.19)	0.46 (0.50)	0.50 (0.38)
	Up-Down	0.33 (0.34)	0.50 (0.68)	0.56 (0.77)
	Left-Right	0.46 (0.34)	0.70 (0.92)	0.73 (0.58)

TABLE 2 LEAST SQUARE MEANS AND TREATMENT CONTRASTS OF TREMOR POWER AS ASSESSED BY LABORATORY TREMOROGRAPHY IN THREE DIFFERENT CONDITIONS.

Laboratory accelerometry maximum power variables		LS Means 4-8½h (change from baseline)			MK-0249 vs. Placebo				Alcohol vs. Placebo			
		Placebo	MK-0249	Alcohol	difference	p-value	95% CI		difference	p-value	95% CI	
Kinetic	Back-Forward (μV)	10.4%	20.1%	-24.4%	9.7%	0.3771	-12.2%	31.7%	-34.9%	0.0003	-52.7%	-17.3%
	Average (μV)	17.1%	19.0%	-16.3%	1.9%	0.8440	-17.3%	21.0%	-33.4%	0.0002	-49.8%	-17.2%
	Up-Down (μV)	10.7%	26.4%	-10.0%	15.6%	0.3527	-18.1%	49.7%	-20.8%	0.1474	-49.6%	7.7%
	Left-Right (μV)	23.6%	19.6%	-18.5%	-4.0%	0.7058	-25.1%	17.1%	-42.0%	<.0001	-59.9%	-24.5%
Postural	Back-Forward (μV)	-1.5%	27.9%	-39.9%	29.4%	0.1284	-8.9%	68.6%	-38.4%	0.0056	-66.1%	-11.9%
	Average (μV)	5.2%	22.4%	-31.7%	17.2%	0.3000	-15.9%	50.6%	-37.0%	0.0047	-62.7%	-12.0%
	Up-Down (μV)	-0.4%	30.9%	-22.3%	31.3%	0.1200	-8.5%	72.1%	-21.9%	0.1558	-53.2%	8.7%
	Left-Right (μV)	8.5%	16.6%	-30.4%	8.0%	0.6452	-27.0%	43.3%	-38.9%	0.0063	-67.2%	-11.6%
Rest	Back-Forward (μV)	1.1%	13.0%	-11.4%	11.9%	0.2048	-6.7%	30.6%	-12.6%	0.1309	-29.1%	3.9%
	Average (μV)	19.9%	5.6%	-17.7%	-14.3%	0.2905	-41.6%	12.8%	-37.6%	0.0033	-62.5%	-13.4%
	Up-Down (μV)	14.0%	-5.6%	-12.7%	-19.6%	0.1588	-47.5%	8.0%	-26.7%	0.0499	-53.8%	0.0%
	Left-Right (μV)	33.3%	13.1%	-12.8%	-20.2%	0.1791	-50.4%	9.7%	-46.1%	0.0012	-73.5%	-19.4%

TABLE 3 LEAST SQUARE MEANS AND TREATMENT CONTRASTS OF TREMOR POWER AS ASSESSED BY PORTABLE ACCELEROMETRY IN THREE DIFFERENT CONDITIONS

Portable accelerometry maximum power variables		LS Means 4-8½h (change from baseline)			MK-0249 vs. Placebo				Alcohol vs. Placebo			
		Placebo	MK-0249	Alcohol	difference	p-value	95% CI		difference	p-value	95% CI	
Kinetic	Back-Forward (mG)	16.8%	26.8%	-15.9%	10.0%	0.4640	-17.4%	37.5%	-32.7%	0.0054	-55.5%	-10.3%
	Average (mG)	7.1%	15.9%	-20.8%	8.8%	0.3620	-10.6%	28.3%	-27.9%	0.0012	-44.2%	-11.8%
	Up-Down (mG)	1.4%	10.6%	-24.3%	9.2%	0.3642	-11.1%	29.6%	-25.7%	0.0039	-42.9%	-8.8%
	Left-Right (mG)	7.4%	16.0%	-20.4%	8.6%	0.3474	-9.7%	27.0%	-27.8%	0.0007	-43.2%	-12.6%
Postural	Back-Forward (mG)	-0.4%	27.9%	-36.3%	28.3%	0.2129	-16.9%	74.6%	-35.9%	0.0294	-69.4%	-3.8%
	Average (mG)	-8.3%	25.8%	-37.3%	34.2%	0.0912	-5.7%	75.2%	-29.0%	0.0443	-58.2%	-0.8%
	Up-Down (mG)	-9.1%	16.1%	-36.1%	25.2%	0.1833	-12.5%	63.8%	-27.0%	0.0587	-55.9%	1.0%
	Left-Right (mG)	-9.6%	27.0%	-37.9%	36.6%	0.0649	-2.4%	76.7%	-28.4%	0.0415	-56.6%	-1.1%
Rest	Back-Forward (mG)	11.1%	3.7%	-6.1%	-7.4%	0.5310	-31.2%	16.3%	-17.2%	0.1285	-39.8%	5.2%
	Average (mG)	-4.7%	3.5%	-7.2%	8.2%	0.4163	-12.0%	28.5%	-2.5%	0.7874	-21.2%	16.2%
	Up-Down (mG)	-13.8%	0.6%	-9.4%	14.4%	0.1456	-5.2%	34.2%	4.4%	0.6257	-13.8%	22.7%
	Left-Right (mG)	-7.1%	4.9%	-7.8%	12.0%	0.2939	-10.8%	34.9%	-0.7%	0.9444	-21.5%	20.0%

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