CHAPTER 5

Optimizing the glutamatergic challenge model for psychosis: using S(+)-ketamine to induce psychomimetic symptoms in healthy volunteers

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ABSTRACT

The psychomimetic effects that occur after acute administration of ketamine can constitute a model of psychosis and antipsychotic drug action. However, the optimal dose / concentration has not been established and there is a large variety in outcome measures. In this study, a total of 36 healthy volunteers (21 males and 15 females) received infusions of S(+)-ketamine or placebo to achieve pseudo-steady state concentrations of 180 and 360 ng/mL during two hours. The target of 360 ng/mL induced more intense effects than expected, after which the targets were reduced to 120 and 240 ng/mL, which were considered tolerable. There was a clear, concentration-dependent psychomimetic effect as shown on all subscales of the positive and negative syndrome scale (PANSS) and different visual analogue scales (VAS). The startle reflex was inhibited (prepulse inhibition, PPI) by both main target concentrations to a similar extent, suggesting a maximum effect. More general measures of drug effect (including eye movements and prolactin concentrations) were also affected by ketamine in a concentration-related manner. Ketamine was found to constitute a robust model for induction of psychomimetic symptoms and the optimal concentration range for a drug interaction study would be between 100 and 200 ng/mL.

Introduction

All currently available pharmacologic treatments for schizophrenia and psychosis are related to the dopamine hypothesis of schizophrenia (Kapur and Mamo, 2003). Many agents also target other receptors, such as the serotonergic 5-HT_{2A} receptor, but contrary to these targets the occupancy of the dopamine D_2 receptor is comparable for all drugs at clinically relevant doses (Farde et al, 1988; de Visser et al., 2001; Seeman, 2002; Agid et al., 2007). However, the dopamine hypothesis alone does not seem sufficient to explain all signs and symptoms of schizophrenia, and other neurotransmitter systems, including the glutamatergic system, have been implicated in the pathophysiology of schizophrenia and psychosis (Ackenheil and Weber, 2002; Sodhi et al., 2008; Moghaddam and Javitt, 2012).

There are currently several drugs in development with non-dopaminergic mechanisms of actions (Miyamoto et al., 2012). These include drugs with glutamatergic (e.g. glycine transport inhibitors; Umbricht et al., 2010; and $mGlu_{2,3}$ receptor agonists; Patil et al., 2007), serotonergic (e.g. 5-HT_6 antagonists; Liem-Moolenaar et al., 2011), nicotinergic (e.g. α_7 nicotinic receptor agonists; Freedman et al., 2008), tachykinin (e.g. NK_3 antagonists; Meltzer et al., 2004; Liem-Moolenaar et al., 2010a) and cannabinoid targets (e.g. CB_1 antagonists; Kelly et al., 2011). Where the currently available drugs mainly affect the positive symptoms of schizophrenia (such as hallucinations and delusions), these novel pharmacotherapeutic strategies aim to improve other aspects (such as cognition and negative symptoms) as well (Carpenter and Koenig, 2008).

The pharmaceutical industry has found it very difficult to test drugs for psychiatric diseases, resulting in a recent drop in compounds that are being developed. Some companies have announced that they would cease research in psychiatry completely. This trend is referred to as "the shrinking pipeline". The reasons for this drop in development can be found in the lack of understanding of pathophysiology, the difficulty to predict clinical outcome, the lack of experimental disease models and biomarkers and the

increasing costs of drug development (van Gerven and Cohen, 2011; Nutt and Goodwin, 2011). Drug development in psychiatry requires a tailor-made approach, which includes testing for efficacy early in clinical development (Macaluso et al., 2011). Improving the predictability of models early in drug development would improve the risk / benefit ratio for drug development. This tailor-made approach would include the development of specific biomarkers and pharmacologic challenges.

Most models that are currently in use are more predictive of dopamine antagonism, rather than reduction of psychotic symptoms per se (Carpenter and Koenig, 2008). The United States National Institute of Mental Health (NIMH) suggests that psychiatric drug development should be based on an understanding of pathophysiology, rather than mimicking the mechanism of action of current drugs (Brady and Insel, 2012). As an alternative to preclinical models, several models have been developed that involve the induction of psychomimetic symptoms in healthy volunteers. These models have used administrations of THC or cannabis (e.g. D'Souza et al., 2004; Liem-Moolenaar et al., 2010b; Kleinloog et al., 2012), LSD or psilocybin (e.g. Vollenweider et al., 1998; Carhart-Harris et al., 2012), ecstacy or amphetamines (e.g. Strakowski et al., 1996) and ketamine (e.g. Krystal et al., 1994; Malhotra et al., 1996; Vollenweider et al., 2000; Duncan et al., 2001; Abel et al., 2003; Gouzoulis-Mayfrank et al., 2005; Covington et al., 2007; Stone et al., 2008) to induce psychomimetic effects. When used in early phase clinical trials (phase I-II), these models have great potential in predicting clinical efficacy of novel drugs.

However, the currently available literature that focuses on the use of ketamine as a model for psychosis and antipsychotic drug action is limited by several factors. First of all, there is no consensus regarding the optimal dose or concentration for induction of psychomimetic symptoms. Different studies use different dosing schemes and most studies do not use different dose levels to detect a dose-effect relation. Concentrations of ketamine in the blood are useful to explain variation within and between subjects and between studies, but are generally not reported. Whether racemic ketamine

or the S(+)-isomer is used is not always clearly described, while S(+)-ketamine has been shown to be up to four times as potent as R(-)-ketamine (Øye et al., 1992). There is also much variety in the outcome measures that are used to quantify psychomimetic effects.

Several studies have examined the effect of the known antipsychotics haloperidol and clozapine on ketamine-induced psychomimetic symptoms in either healthy volunteers (Malhotra et al., 1997b; Krystal et al., 1998 and 1999; Oranje et al., 2000 and 2002; Lahti et al., 2003) or patients with schizophrenia (Lahti et al., 1995 and 2003; Malhotra et al., 1997a and 1997b). In some of these studies, a mild effect of treatment was seen, although this effect in general is inconclusive, possibly due to the previously described limitations of the application of the ketamine model. Prior to performing an interaction study using the ketamine model of psychosis and a known antipsychotic, we wanted to optimize the model. Therefore, this study aimed to explore the relation between the concentration of ketamine and the psychomimetic, general and adverse effects of ketamine. This would allow the selection of a target concentration that induces psychomimetic effects at a sufficient level to allow for a reduction in effect by an antipsychotic drug, whilst still being tolerable for the subjects. This information could be used for decisions on study designs and ketamine infusion rates. Furthermore, different outcome measures for psychomimetic effects were compared on sensitivity and variability.

Methods

Participants

Healthy subjects aged between 18 and 45 years (inclusive) and with a body mass index between 18 and 30 kg/m^2 (inclusive) were recruited by the Centre for Human Drug Research. Recreational experience with ketamine was not required, but subjects had to be mild cannabis users to ensure they

were familiar with induced psychomimetic symptoms. Mild cannabis use was defined as use of cannabis at least four times in the last year and no more than once a week on average. After providing written informed consent, subjects received a medical screening within 3 weeks prior to study participation. Clinically relevant abnormalities, in particular a personal or family history of clinically relevant psychiatric illness and/or abnormalities on psychiatric examination were considered reason for exclusion. The use of medication and agents that were expected to affect central nervous system performance or the pharmacokinetics of the study medication was not allowed during the study period. Female subjects were tested for pregnancy (in urine) and all subjects were tested for the use of recreational drugs (in urine) and alcohol (in breath). All subjects were required to have negative drug screens on all occasions. Following the medical screening, subjects were trained for the study procedures.

Study design

This was a randomized, double-blind, placebo-controlled, three-way cross-over trial with a washout period of minimally two days. The study was performed in accordance with Good Clinical Practice and the Dutch Medical Research Involving Human Subjects Act and was approved by the Independent Ethics Committee of the Leiden University Medical Centre.

Interventions

To achieve a pseudo-steady state in effect, S(+)-ketamine (Ketanest®, Pfizer) or matching placebo (0.9% sodium chloride) was administered using individualized two-hour intravenous infusion regimens to achieve pseudo-steady plasma concentrations. Target concentrations were selected based on the dosing schedules of previous studies using ketamine as a model for psychosis (Vollenweider et al., 2000; Duncan et al., 2001; Abel et al., 2003;

Gouzoulis-Mayfrank et al., 2005; Covington et al., 2007; Stone et al., 2008). As these studies did not describe the pharmacokinetic profile of S(+)-ketamine or the active metabolite S(+)-norketamine, plasma concentrations were simulated to select target concentrations using the deSolve package for R (www.r-project.org), as shown in Figure 1. This simulation was based on the pharmacokinetic model described by Sigtermans et al. (2009). Two target concentrations were selected, based on the simulated pharmacokinetic profiles, to examine a concentration-effect relation. Subsequently, individualized infusion regimens were calculated based on the pharmacokinetic model described by Sigtermans et al. (2009), adapted to sex and weight (which were the covariates in the model). In order to create optimal blinding circumstances, the S(+)-ketamine concentration within the syringes prepared by the pharmacy was different for each study day (2.50 mg/mL, 1.25 mg/mL or placebo), which allowed for the use of the same infusion rates during each study day and thus aided study blinding.

Outcome measures

The effect of S(+)-ketamine was measured using the positive and negative syndrome scale (PANSS), prepulse inhibition of the startle reflex (PPI) and an extensive test battery for CNS effects (NeuroCart) consisting of several visual analogue scales (VAS), saccadic and smooth pursuit eye movements, pupillometry, adaptive tracking and body sway. All tests were measured repeatedly throughout each study day, including two baseline measurements before the start of infusion, two measurements during infusion and one measurement several hours after infusion. For the PANSS, only one baseline measurement was performed and for the PPI an additional measurement was performed 45 minutes after the stop of infusion. In addition, blood samples for cortisol and prolactin concentrations in serum and concentrations of S(+)-ketamine and the active metabolite S(+)-norketamine in plasma were taken repeatedly throughout the study day.

Positive and negative syndrome scale

The primary method to measure psychomimetic effects was the PANSS, described by Kay et al. (1987). The PANSS is a clinically validated scale to longitudinally measure changes in psychotic symptoms and is based on a structured clinical interview. To adjust for the repetition of interviews, time frames for symptoms evaluation were limited to 'since this morning' for the baseline assessment or 'since the last interview' for all other assessments. All interviews were recorded on video and rated independently by a second blinded person. For the analysis, the geometric mean of the two scores was used. All team members performing the interviews and rating the videos were certified PANSS raters™ (by the PANSS Institute LLC, New York). The PANSS consists of 30 items that are scored on a seven-point scale. The PANSS is subdivided into three subscales: positive, negative and general. The positive subscale, which consists of 7 items resulting in a total score ranging from 7 to 49, was predefined as the main evaluation endpoint.

Prepulse inhibition

As a more objective measure of psychomimetic symptoms, PPI was used as described by Braff et al. (2001). The startle reflex consists of a contraction of the skeletal and facial muscles in response to a sudden, relatively intense stimulus. Subjects were exposed to a background noise level of 70 dB starting 600 ms before the first trial and lasting until 400 ms after the last trial, presented binaurally through stereo headphones. Each trial consisted of either a pulse alone or a prepulse followed by a pulse. The startle-eliciting stimulus (pulse) was 115 dB during 40 ms. If used, the prepulse was 85 dB during 20 ms, with a prepulse-to-pulse interval of 120 ms or 240 ms. Each measurement consisted of 32 trials, presented in a random order and separated by random inter-trial intervals of 10-20 s (average 15 s). Randomisation was subdivided in two blocks of 16 trials to allow for an analysis of the sensitivity of test blocks of different length. The eyeblink component of the startle was

measured using electromyography (EMG) of the orbicularis oculi muscle. PPI was calculated as the percentage of inhibition of the area under the curve (AUC) of the startle response following prepulse plus pulse, compared to the AUC of the startle response of pulse alone. PPI is a ubiquitous and robust experimental phenomenon that is present even at the first exposure to the stimulus and is not affected by habituation or extinction following multiple trials (Braff et al., 2001).

Visual analogue scales

VAS are widely used tools to quantify subjective effects. VAS are 100 mm long lines with two extreme subjective states on each end (e.g. 'drowsy' and 'alert' or 'not at all' and 'extremely'). Subjects indicate their current feelings on these lines, resulting in a score between 0 and 100 mm. The current study included the composite scales for mood, calmness and alertness (described by Bond and Lader, 1974), the composite scales for psychedelic effects (described by Bowdle et al., 1998), three scales for general drug effect ('feel drug', 'like drug' and 'dislike drug' with 'not at all' to 'extremely' as extremes, referred to as VAS drug rating) and a newly developed set of scales for psychedelic effects (the development and evaluation of this new VAS will be described elsewhere).

Saccadic and smooth pursuit eye movements

Both saccadic and smooth pursuit eye movements were recorded through three electrodes placed on the forehead and next to both lateral canthi. The stimulus for saccadic eye movements had a fixed amplitude of approximately 15 degrees to either side, with interstimulus intervals varying randomly between 3 and 6 seconds. Smooth pursuit eye movements were stimulated in a sinusoidal manner at frequencies ranging from 0.3 to 1.1 Hz with amplitude of 22.5 degrees to either side. Eye movements are described in greater detail by Zuurman et al. (2008). Saccadic peak velocity is one of the most

sensitive parameters for sedation by a wide range of different causes (van Steveninck et al., 1991). The percentage time in which the eye movements are in smooth pursuit of the target is a parameter for motor coordination.

Pupillometry

The ratio between the diameter of the pupil and the iris is determined by the autonomous nervous system activity. Diameters were determined using digital photography with flash after adaptation in ambient lighting (Twa et al., 2004).

Adaptive tracking

For the adaptive tracking test, subjects had to keep a dot inside a moving circle using a joystick. If the subject was successful, the speed of the moving circle increased and if a subject was unsuccessful, the speed decreased. Adaptive tracking was performed using customised equipment and software (as described by Borland and Nicholson, 1984) and was measured during three minutes (after a run-in period of 30 seconds). Subjects received three training sessions to minimize learning effects. Adaptive tracking is a pursuit task that is very sensitive to drug effects.

Body sway

The body sway meter records body movements in a single (sagittal) plane during two minutes, while the subjects close their eyes, providing a measure of postural stability, which can be used as a biomarker for drug effect (Liem-Moolenaar et al., 2010a).

Hormones

Serum prolactin and cortisol concentrations were measured using electrochemiluminescence immunoassay (ECLIA) as a biomarker for dopaminergic activity.

Pharmacokinetics

Concentrations of S(+)-ketamine and its main active metabolite S(+)-norketamine were determined in plasma, using high performance liquid chromatography with ultraviolet detection (HPLC-UV), with a lower limit of quantification of 10 ng/mL.

Safety

In addition to the pharmacokinetic and pharmacodynamic measurements, several tests were performed for safety reasons. Subjects were tested for drugs (urine), pregnancy (urine, females only) and alcohol (breath) at the start of each study day. Electrocardiograms (ECGs) and vital signs (blood pressure, heart rate and oxygen saturation) were repeatedly recorded throughout the study day. All subjects were frequently asked if they had any symptoms and all symptoms were recorded, whether assumed to be related to the study drug or not.

All subjects had breakfast two hours prior to the start of infusion and lunch half an hour after the infusion. Subjects remained in the research unit until approximately four and a half hours after the stop of infusion. Prior to infusion, subjects were informed that they could ask for the premature termination of the infusion at any time if they had negative experiences. During the study period (from two weeks prior to the first dose), subjects were not allowed to use recreational drugs or any medication that has a potential influence on the pharmacokinetics of ketamine. From 24 hours before the study day until the end of the study day, subjects were instructed not to smoke, exert heavily physical exercise or use alcohol, xanthine or grapefruit juice.

Sample size

As this was an exploratory study, no formal power calculation has been performed. For equal randomisation, the number of subjects had to be a

multiple of six and males and females were distributed in an even fashion. A total of 12 male and 12 female subjects were required to complete the study.

Statistical analyses

All pharmacodynamic outcome measures were analysed within SAS (version 9.1.3) using a mixed model analysis of covariance (ANCOVA) with treatment, study period, time, gender, treatment by time, treatment by gender, gender by time and treatment by gender by time as fixed factors and subject, subject by treatment and subject by time as random factors and the average baseline value as covariate. The body sway was analysed without taking gender into account and without treatment by time as random factor, because of many missing observations (the body sway was performed at the end of each testing round and was cancelled if there was a delay more than 10%). Results of the body sway, PANSS scores, cortisol and prolactin concentrations were log-transformed prior to analysis, based on expected log-normal distribution of the data. VAS Bowdle items 8 (voices), 9 (meaning) and 10 (suspicious) did not show any variation under any condition and could not be analysed. VAS Bowdle items 1 (body), 2 (surroundings), 6 (colours), 7 (sound), 12 (drowsy) and 13 (anxious) were log-transformed after the addition of 2 points (to prevent log-transformation from 0). VAS Bowdle items 3 (time), 4 (reality), 5 (thoughts) and 11 (high) did not need transformation. As all items from both VAS Bowdle and VAS drug rating did not show any variation under placebo, the placebo condition was not included in the analysis of these scales.

Results are presented as least square means (LSM), which are calculated within the ANCOVA and result from the minimisation of the sum of squared residuals. LSM are based on the data and estimations for missing observations and are less sensitive for an unbalanced design than geometric means. Therefore, LSM are more likely to represent the estimated population means. Data that were log-transformed prior to analysis are presented after back-transformation. Differences are to be interpreted as percentage

change. As two points were added to the VAS Bowdle scores prior to transformation, the percentage difference refers to score (a+2) and score (b+2), rather than (a) and (b).

To test the suitability of the pharmacokinetic model (by Sigtermans et al., 2009) simulations were performed using the deSolve package within R (version 3.0.2). The simulations used the population parameter estimates (theta's), parameters for inter-subject variability (eta's), parameters for intra-subject variability (sigma's) and the uncertainty of the population parameter estimates (covariance matrix). For each individual subject (using body weight, sex and the individualized infusion regimen), a simulation was performed with 1000 permutations, resulting in a mean predicted pharmacokinetic profile and a 95% prediction interval. The actual measurements were presented using standardised scores (actual measurement - mean predicted / standard deviation of prediction) over time. Approximately 95% of the actual measurements are expected to have a standardised score between -2 and 2.

Results

Selection of target concentration

Based on the simulated pharmacokinetic profiles of the available literature (presented in Figure 1), target concentrations of 180 ng/mL and 360 ng/mL were initially selected for the study. These concentrations were chosen to represent a wide range of subanaesthetic concentrations in which psychomimetic effects were expected while subjects would still remain responsive. To achieve the pseudo-steady state, the infusion regimen was determined as a bolus of 32 µL/kg (target concentration x volume of central compartment / concentration in syringe), followed by subsequent infusion rates of 0.56 mL/kg/hr for 7 minutes, 0.36 mL/kg/hr for 23 minutes and 0.24 mL/kg/hr for another 90 minutes. As the clearance within the model by Sigtermans et

al. (2009) was higher for females and the relative contribution of clearance increases over time, the three infusion rates were increased for females by 5%, 10% and 15%, respectively. This infusion regimen was predicted to yield the same pharmacokinetic profile for a wide range of body weights in both males and females. The mean simulated concentration of S(+)-ketamine stayed within 10% of the target concentration throughout the infusion. As the size of effect of the active metabolite S(+)-norketamine on psychomimetic symptoms is unknown, this effect was not incorporated in the determination of the infusion regimen.

Adverse events

Within the first six subjects (all male) who were dosed, three adverse events were seen that were more severe than expected. One subject had a presyncope 2 minutes after the stop of infusion for the 180 ng/mL target concentration. The same subject had altered consciousness after 29 minutes of infusion for the 360 ng/mL target, for which the infusion was stopped immediately (symptoms subsequently improved rapidly and had disappeared after 22 minutes). Another subject experienced anxiety and an altered mood with the 360 ng/mL target after 70 minutes of infusion (the infusion was stopped 10 minutes later, after which symptoms improved rapidly and disappeared completely within 20 minutes after the stop of infusion). Because of these unexpected intense effects, the study was temporarily halted. Other adverse events that occurred were expected and included feeling high, changes in perception, nausea, hypoaesthesia and somnolence, all of mild or moderate intensity. One subject vomited during the 180 ng/mL target and another subject vomited during the 360 ng/mL target. The intensity of adverse effects appeared to increase during the infusion and improved rapidly after the stop of infusion. Due to the temporary interruption of the study, only two of these initial six subjects (33%) completed all three study days according to the protocol, one subject (17%) completed two study days (ketamine 180 and 360 ng/mL) and three subjects (50%) completed one study

day (two subjects received placebo, one ketamine 360 ng/mL). The available data of these subjects is presented graphically, but was not taken along in the statistical analysis due to the limited number and truncation of the observations. The average response is indicated separately in the figures.

Adjustment of target concentration

Based on the experience with the first subjects, the rationale for selection of target concentrations was revisited. As we aimed to establish a concentration that showed maximum psychomimetic effects, whilst being easily tolerated by the subjects, the target concentrations were reduced to 120 ng/mL and 240 ng/mL respectively. The intensity of effects slowly increased during the infusion, which was hypothesized to be related to the formation of the active metabolite S(+)-norketamine. Although the size of the effect in humans is unknown, animal studies suggest that norketamine is roughly one third as potent for CNS effects as ketamine (White et al. 1975). Within the adjusted infusion regimen, the effect of S(+)-norketamine was incorporated by targeting a stable level of [S(+)-ketamine concentration + 1/3 S(+)-norketamine concentration]. The infusion regimen was set to a bolus of 0.21 µL/kg and infusion rates of 0.34 mL/kg/hr for 15 minutes, 0.22 mL/kg/hr for 25 minutes and 0.12 mL/kg/hr for the last 80 minutes. Infusion rates were again increased for females with 5%, 10% and 15% respectively.

Adverse events with adjusted target concentrations

All adverse events that occurred after the adjustment of target concentrations were expected and of mild or moderate intensity. However, the infusion was stopped prematurely due to adverse events (either at the subject's wish or judgement by the investigator) in 15 subjects (56%) on the 240 ng/mL target (after 8 to 105 minutes), 1 subject (4%) on the 120 ng/mL target (after 25 minutes) and 1 subject (3%) on placebo (after 96 minutes). The most important reason for stopping the infusion prematurely on the 240 ng/

mL target was vomiting (7/15, 47%). Other adverse events included feeling high, changes in perception, derealisation, nausea, bradyphrenia, dizziness, hypoaesthesia and somnolence. Of the thirty subjects who were enrolled after the adjustment in target concentrations, 24 subjects (80%) completed all three study days, 2 subjects (7%) completed two study days (both placebo and the 240 ng/mL target; both stopped due to adverse events) and 4 subjects (13%) completed only one study day (3 on placebo and 1 on ketamine 240 ng/mL; 2 stopped due to adverse events and 2 for personal reasons).

Demographics

A total of 21 male and 15 female subjects were included in this study (15 males and 15 females for the target concentrations of 120 and 240 ng/mL). The median age was 23 years (range 18-42) and mean body weight 71.6 kg (range 47.6-95.5). 32 subjects (88.9%) were Caucasian. The median frequency of cannabis use was 10 times / year (range 4-52) and 23 subjects (63.9%) were non-smokers. Of subjects who smoked, the median frequency was 3 cigarettes / day (range 1-5).

Pharmacokinetics

Figure 2 provides a standardised representation of the relation between the simulated concentrations and the measured concentrations over time for ketamine and norketamine. The model is able to describe the measured concentrations adequately. All measured concentrations are between the mean predicted concentration (standard score of 0) and one third the standard deviation below the mean predicted concentration (standard score of -0.33). However, the measured concentrations of ketamine were lower than expected and the standard deviation of the predicted values is much larger than the standard deviation of the measured values, suggesting that the model is not able to accurately predict ketamine concentrations.

Pharmacodynamic effects

The results of the pharmacodynamic endpoints are described below and summarised in tables 1 and 2.

Positive and negative syndrome scale

All subscales of the PANSS showed a robust, dose-dependent increase, as presented in Figure 3. The positive PANSS, which was predefined as the main endpoint, was increased by 43.7% (95%CI 34.4-53.7, $p < 0.0001$) for the 120 ng/mL target compared to placebo and 70.5% (95%CI 59.0-82.8, $p < 0.0001$) for the 240 ng/mL target compared to placebo. These increases represent an average absolute increase on the positive PANSS of 3.1 and 5.1 points, respectively.

Prepulse inhibition

For the 240 ms prepulse-to-pulse interval, a clear inhibition of the startle reflex was seen for both target concentrations (an absolute increase in the full version of 28.5% and 23.9% compared to placebo for 120 and 240 ng/mL respectively). There was no difference between target concentrations, which might be because of a maximum effect or differences in baseline values. However, the effect with the higher concentration seems to persist longer after the stop of infusion. The 120 ms prepulse-to-pulse interval did not result in the same changes in PPI. When only the first 16 blocks (short version) of each measurement were analysed, the differences are comparable to the analysis with all 32 blocks (full version). Therefore, a shorter version of the PPI with a prepulse-to-pulse interval of 240 ms is regarded sufficient for future studies. The results of the short version with a prepulse-to-pulse interval of 240 ms are presented in Figure 4.

Visual analogue scale as described by Bowdle

Items 8 (voices), 9 (meaning) and 10 (suspicious) of VAS Bowdle did not show any variation during measurements and could therefore not be analysed. Items 1 (body), 2 (surroundings), 6 (colours), 7 (sound), 12 (drowsy) and 13 (anxious) were not normally distributed and were log-transformed after addition of 2 points (as 0 cannot be log-transformed). The outcome of the ANCOVA can be interpreted as percentage difference between the original scores (after addition of 2 points). The remaining items 3 (time), 4 (reality), 5 (thoughts) and 11 (high) met the criteria for ANCOVA and were not transformed prior to analysis. Differences for these contrasts are presented as absolute differences. Except for the non-responsive items 8, 9 and 10, all items showed a clear response and this response was stronger for the 240 ng/mL target than the 120 ng/mL target. Figure 4 presents the time profile of item 11 (feeling high).

Zuurman et al. (2008) describe three clusters of VAS Bowdle, based on the response to THC: internal perception (items 4, 8, 9, 10 and 13), external perception (items 1, 2, 3, 5, 6 and 7) and feeling high (item 11). To allow comparison with these and other studies, the results on the clusters internal and external perception are also included in Table 2. As these clusters are created by calculating the mean of log-transformed data (after the addition of 2 points), back-transformation is no longer meaningful and the transformed data are presented as 'units'.

Kleinloog et al. (2013) also describe three clusters of subjective effects in response to THC, based on the items included in VAS Bowdle and VAS Bond and Lader: the cluster 'perception' (Bowdle items 3, 5 and 11, see Table 2), the cluster 'relaxation' (Bond and Lader items 1, 4, 9 and 11, see Table 1), and the cluster 'dysphoria' (Bowdle items 8, 9 and 10, all three items were not responsive and therefore not included in the tables).

Visual analogue scale for drug rating

All items of VAS drug rating ("feel drug", "like drug" and "dislike drug") were analysed without transformation and responded strongly to ketamine. The effect of the drug was experienced as more intense, but less pleasant for the 240 ng/mL target than for the 120 ng/mL target. For both target concentrations the score on VAS like drug (44.8 mm and 37.9 mm) was higher than on VAS dislike drug (13.3 mm and 23.8 mm).

Visual analogue scale as described by Bond and Lader

The most prominent effects on VAS Bond and Lader were seen within the "alertness" cluster. Subjects report a dose-dependent decrease in alertness (i.e. an increase in sedation) following administration of ketamine (-3.7 mm and -13.1 mm). Within the "calmness" cluster, an increase was seen for the 120 ng/mL target (+4.9 mm), but not the 240 ng/mL target. Cluster "mood" was not significantly affected.

Saccadic and smooth pursuit eye movements

Saccadic eye movements were impaired in a dose-dependent manner following administration of ketamine (saccadic peak velocity decreased by 33 deg/s and 76 deg/s for the lower and higher target respectively). Smooth pursuit eye movements (displayed in Figure 4) were impaired in a concentration-related manner (absolute decrease of 9.9% and 13.6%).

Other CNS effects

The pupil/iris ratio was affected by the higher, but not the lower target concentration. As the pupil/iris ratio represents the activity of the autonomic nervous system, this might indicate the start of anaesthetic effect. The body sway, which is a measure for general drug effect, was increased in a

dose-dependent manner (relative increase of 25.6% and 76.4%). Adaptive tracking was affected by the higher (absolute reduction of 7.7%), but not the lower target concentration.

Neuroendocrine parameters

Both cortisol and prolactin were increased in a dose-dependent manner following administration of ketamine. Cortisol increased by 46.5% and 89.8% respectively, whereas prolactin increased by 32.3% and 95.6%. Based on the individual graphs, the increase in prolactin for the 240 ng/mL target concentration was largely associated with premature termination of the infusion due to nausea and vomiting.

Safety

No relevant changes in vital signs, ECG or physical examination were found during the study.

Discussion

The current study aimed to optimise the ketamine model of psychosis and explore the relation between the concentration of ketamine and the psychomimetic, general and adverse effects of ketamine. The target concentrations used in this study represent a broad range of the subanaesthetic level. Very early in the execution of the study, it became clear that the 360 ng/mL target concentration was too high, as it resulted in unwanted adverse effects that can be related to the (anaesthetic) effect of ketamine. The target concentrations between 120 ng/mL and 240 ng/mL were more tolerable for the subjects, although the infusion was terminated prematurely quite frequently (56%) during the 240 ng/mL target. From a point of tolerability, the optimal target concentration would therefore be slightly lower, e.g.

up to 200 ng/mL. All adverse effects that were seen during the study were transient and resolved spontaneously, which is in line with a meta-analysis on the adverse effects of ketamine as a psychosis model (Perry et al., 2007). Within the current study, a total infusion time of two hours was used, during which many tests were performed. Based on the experience of the subjects, the total duration would ideally be shorter and there should be more time in between tests to allow the subjects to experience the effect of ketamine without performing pharmacodynamic tests.

The measured concentrations of ketamine and its metabolite norketamine were within the expected ranges as simulated using the model described by Sigtermans et al. (2009). However, the actual measured concentrations of ketamine were lower than predicted and the standard deviation of the predictions was much larger than the standard deviation of the measured concentrations. It is hypothesized that this is due to uncertainty of the parameter estimation for (intercompartmental) clearance, overestimation of the residual error or the influence of covariates. It is recommended that the pharmacokinetic model will be further improved by adding data from studies with different dosing regimens (including the current study). In that way, the population parameter estimates could be estimated more accurately, which would most likely result in better predictions for future studies.

There was a clear, concentration-dependent psychomimetic effect as shown on all subscales of the PANSS and many VAS scales. As a general rule of thumb, a difference of three points on the positive subscale of the PANSS is considered clinically relevant, although a smaller difference can be acceptable for biomarker research in healthy volunteers. The increase seen for the 120 ng/mL target is similar to this minimum clinically relevant change and the 240 ng/mL target had an even stronger response. Although different study designs make it difficult to compare the dose-related effects with the literature, the absolute increases on the positive and negative subscale that were seen in this study are comparable to previous findings with different exposure levels in healthy volunteers (Krystal et al., 1994, 1998 and 1999;

Malhotra et al., 1996 and 1997b; Breier et al., 1997; Anand et al., 2000; Hetem et al., 2000; Umbricht et al., 2000; Kegeles et al., 2002; Lahti et al., 2003; Aalto et al., 2005; Deakin et al., 2008; Morgan et al., 2006; Nagels et al., 2011; Passie et al., 2005) and findings in patients with schizophrenia (Lahti et al., 1995 and 2003; Malhotra et al., 1997a and 1997b). Chronic treatment with haloperidol (Lahti et al., 1995) and clozapine (Malhotra et al., 1997a) reduces these effects in patients with schizophrenia. In healthy volunteers, ketamine-induced psychomimetic symptoms were reduced by acute treatment with lamotrigine (Anand et al., 2000; Deakin et al., 2008), but not by haloperidol (Krystal et al., 1999), lorazepam (Krystal et al., 1998) or the metabotropic glutamate receptor agonist LY354740 (Krystal et al., 2005). The effects that were seen on the PANSS with the ketamine-model are stronger and more robust than those seen with the THC model of psychosis (D'Souza et al., 2004; Liem-Moolenaar et al., 2010b; Kleinloog et al., 2012) or the psilocybin model of psychosis (Umbricht et al., 2003).

Most other studies that measured VAS feeling high have found an increase of 30-40 mm (Krystal et al., 1998 and 1999; Morgan et al., 2006), similar to the effects of the 120 ng/mL target concentration. Niesters et al. (2012) describe an increase of 60 mm, which is comparable with the 240 ng/mL target concentration.

The startle reflex (PPI) was inhibited by both main target concentrations to a similar extent and consistent with available literature (Oranje et al., 2002; Abel et al., 2003; Heekeren et al., 2007), suggesting a maximum effect. Although PPI is an objective measure, its outcome measure is not as robust as the effects measured on the PANSS and it is more difficult to translate to clinical effect.

Eye movements, in particular non-saccades and anti-saccades, are affected in patients with schizophrenia and their non-affected first-degree relatives (reviewed by Levy et al., 2010). They therefore seem a good, objective biomarker for psychomimetic effect and possibly antipsychotic drug action. In our study, visually guided saccadic and smooth pursuit eye movements were affected by ketamine administration. This effect

was dose-dependent for saccadic eye movements and appeared to have reached a maximum effect for smooth pursuit eye movements. The effect on saccadic eye movements is more likely to be related to sedation. There is also a large body of literature that supports the use of antisaccades as a biomarker for schizophrenia, but antisaccades were not measured in this study.

Both cortisol and prolactin were increased in a dose-dependent manner. The effects on these neuroendocrine parameters were similar to other studies (Krystal et al., 1994, 1998 and 1999; Oranje et al, 2002; van Berckel et al., 1998).

Overall, ketamine administration resulted in a dose-dependent increase in biomarkers for psychomimetic effect (i.e. the PANSS, VAS, PPI, eye movements). This included not only the typical, positive symptoms, but also negative and cognitive symptoms. Most currently available antipsychotic drugs (with the exception of clozapine) are only effective on positive symptoms and therefore, there is a large unmet clinical need to treat the negative and cognitive symptoms of schizophrenia (Carpenter and Koenig, 2008). Many of the drugs that are currently in development for the treatment of schizophrenia have novel mechanisms of action and would potentially influence these symptoms as well. As ketamine induces positive, negative and cognitive psychomimetic symptoms, this model might be able to predict the efficacy of novel antipsychotic drugs in these domains.

Biomarkers that can be related to the onset of the anaesthetic effect of ketamine (i.e. pupil size ratio and adaptive tracking) were mainly affected by higher concentrations of ketamine, whilst body sway (a biomarker of general drug effect) increased dose-dependently. This supports the use of a maximum target concentration of 200 ng/mL, which is consistent with the suggested limit from a tolerability point of view.

A pharmacologic challenge with ketamine provides a great potential as psychosis model in healthy volunteers. The current study improved the understanding of the optimal range of drug concentrations to induce psychomimetic effects, whilst being tolerable. Also, the use of an infusion

regimen based on a pharmacokinetic model has been shown to be useful in maintaining pseudo-steady state plasma concentrations. However, the model's usefulness to predict antipsychotic drug action remains unclear. Future studies should explore the predictability of clinical antipsychotic effect using the ketamine model of psychosis.

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TABLE 1 Overview of least square means by treatment and differences for contrasts (Δ = absolute or relative difference; sac. = saccadic).

		Least square means			120 ng/mL vs placebo			240 ng/mL vs placebo			240 ng/mL vs 120 ng/mL		
Outcome	unit	pla	120	240	Δ	95% CI	p	Δ	95% CI	p	Δ	95% CI	p
PANSS positive	points	7.2	10.3	12.3	+43.7%	34.4 - 53.7	<0.0001	+70.5%	59.0 - 82.8	<0.0001	+18.6%	10.3 - 27.6	<0.0001
PANSS negative	points	7.7	8.8	11.1	+14.6%	4.0 - 26.4	0.0072	+44.0%	30.3 - 59.1	<0.0001	+25.6%	12.9 - 39.7	<0.0001
PANSS general	points	16.5	20.0	25.1	+21.6%	13.6 - 30.2	<0.0001	+52.3%	41.8 - 63.6	<0.0001	+25.3%	16.4 - 34.8	<0.0001
PANSS total	points	31.4	39.2	48.7	+24.9%	17.4 - 33.0	<0.0001	+55.2%	45.4 - 65.7	<0.0001	+24.2%	16.2 - 32.8	<0.0001
PPI 120 ms, full	%	28.3	38.9	35.0	+10.6	0.0 -21.1	0.0491	+6.7	-4.8 - 18.3	0.2471	-3.9	-16.1 - 8.3	0.5228
PPI 120 ms, short	%	28.0	38.0	28.2	+10.0	-2.9 - 22.9	0.1256	+0.2	-13.2 - 13.7	0.9736	-9.8	-24.2 - 4.7	0.1794
PPI 240 ms, full	%	-8.2	20.3	15.7	+28.5	10.6 - 46.4	0.0025	+23.9	4.6 - 43.3	0.0164	-4.6	-25.1 - 16.9	0.6554
PPI 240 ms, short	%	-5.9	21.6	18.5	+27.5	9.5 - 45.5	0.0037	+24.4	5.2 - 43.7	0.0140	-3.0	-23.8 - 17.7	0.7692
VAS mood	mm	52.5	55.9	54.6	+3.3	-0.3 - 7.0	0.0739	+2.1	-1.9 - 6.1	0.3005	-1.2	-5.5 - 3.0	0.5651
VAS calmness	mm	52.4	57.3	54.3	+4.9	2.3 - 7.5	0.0005	+1.9	-1.0 - 4.9	0.1988	-3.0	-6.1 - 0.1	0.0596
VAS alertness	mm	50.2	46.6	37.2	-3.7	-7.3 - 0.0	0.0503	-13.1	-17.0 - -9.1	<0.0001	-9.4	-13.6 - -5.2	<0.0001
VAS relaxation	mm	49.8	55.7	66.1	+6.0	1.9 - 20.8	0.0052	+16.3	11.9 - 20.8	<0.0001	+10.4	5.7 - 15.0	<0.0001
Sac. peak velocity	deg/s	478	445	402	-33	-45 - -20	<0.0001	-76	-91 - -62	<0.0001	-43	-58 - -28	<0.0001
Sac. reaction time	ms	185	191	203	+6	-1 - 13	0.1027	+18	10 - 27	<0.0001	+12	4 - 21	0.0064
Sac. inaccuracy	%	6.9	6.8	7.7	-0.1	-1.1 - 0.8	0.7673	+0.8	-0.3 - 1.9	0.1681	+0.9	-0.3 - 2.1	0.1229
Smooth pursuit	%	45.1	35.1	31.5	-9.9	-12.7 - -7.1	<0.0001	-13.6	-16.6 - -10.6	<0.0001	-3.7	-6.9 - -0.5	0.0248
Pupil / iris ratio	-	0.47	0.45	0.42	-0.02	-0.06 - 0.02	0.3035	-0.05	-0.10 - -0.00	0.0425	-0.03	-0.08 - 0.02	0.2907
Body sway	mm	295	371	521	+25.6%	2.7 - 53.5	0.0277	+76.4%	28.1 - 143.0	0.0009	+40.5%	0.2 - 97.1	0.0489
Adaptive tracking	%	25.1	23.6	17.4	-1.5	-3.4 - 0.4	0.1259	-7.7	-10.2 - -5.3	<0.0001	-6.3	-8.7 - -3.8	<0.0001
Cortisol	ng/mL	0.30	0.44	0.57	+46.5%	29.4 - 65.8	<0.0001	+89.8%	68.2 - 114.2	<0.0001	+29.6%	13.7 - 47.7	0.0002
Prolactin	ng/mL	6.40	8.46	12.51	+32.3%	14.7 - 52.6	0.0003	+95.6%	69.7 - 125.5	<0.0001	+47.9%	27.1 - 72.1	<0.0001

TABLE 2 Overview of least square means (LSM) by treatment, without placebo (Δ = absolute or relative difference).

Outcome	unit	120	240	Δ	95% CI	p
VAS body (1)	mm	1.1	6.7	+182%	68 - 373	0.0005
VAS surroundings (2)	mm	1.9	18.6	+424%	256 - 670	< 0.0001
VAS time (3)	mm	10.5	34.0	+23.5	13.5 - 33.5	0.0001
VAS reality (4)	mm	13.9	48.6	+34.7	24.9 - 44.5	< 0.0001
VAS thoughts (5)	mm	10.6	34.3	+23.8	13.5 - 34.1	< 0.0001
VAS colors (6)	mm	1.3	7.5	+189%	77 - 365	0.0004
VAS sound (7)	mm	1.5	6.9	+155%	46 - 346	0.0023
VAS high (11)	mm	27.4	61.8	+34.4	19.4 - 49.3	0.0001
VAS drowsy (12)	mm	2.8	11.9	+190%	66 - 407	0.0008
VAS anxious (13)	mm	-0.0	1.1	+54%	12 - 113	0.0107
VAS internal	units	0.42	0.60	+0.18	0.12 - 0.24	< 0.0001
VAS external	units	0.60	1.11	+0.50	0.38 - 0.63	< 0.0001
VAS perception	mm	16.0	43.9	+27.8	19.2 - 36.4	< 0.0001
VAS feel drug	mm	52.4	84.0	+31.6	18.9 - 44.2	< 0.0001
VAS like drug	mm	44.8	37.9	-6.9	-19.6 - 5.8	0.2726
VAS dislike drug	mm	13.3	23.8	+10.5	1.9 - 19.1	0.0208

FIGURE 1 Simulated pharmacokinetic profiles of selected literature, including initial target concentrations.

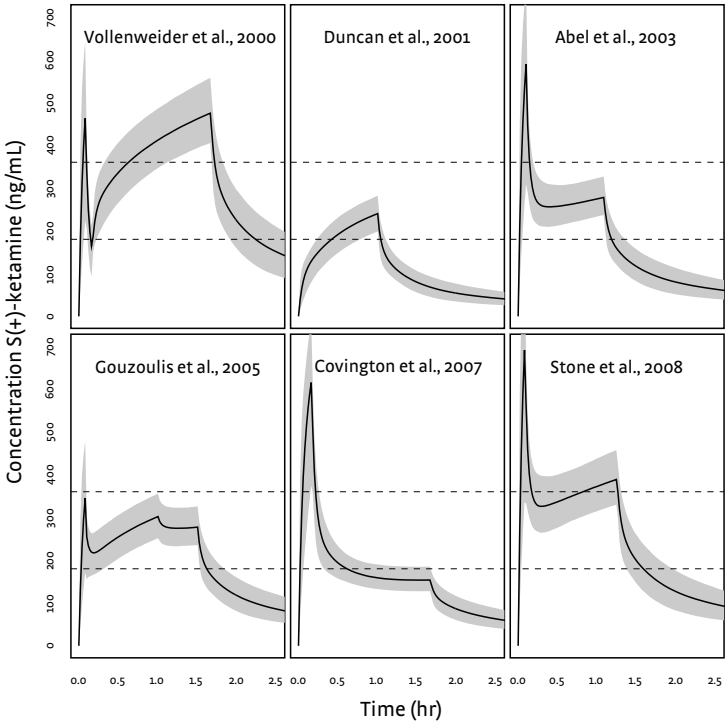

FIGURE 2 Standardized presentation of measured ketamine and norketamine concentrations relative to simulated concentrations.

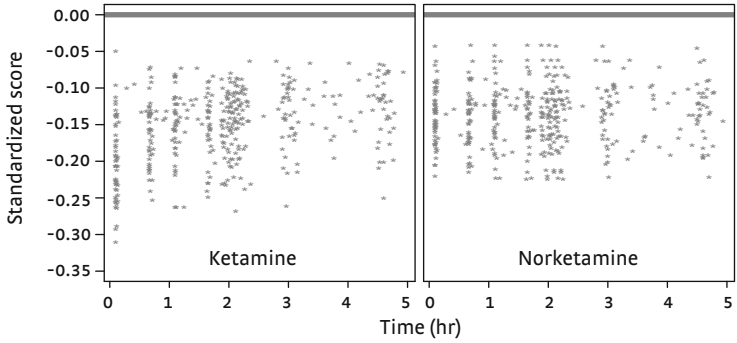

FIGURE 3 Time profile (mean ± SD) for PANSS subscales.

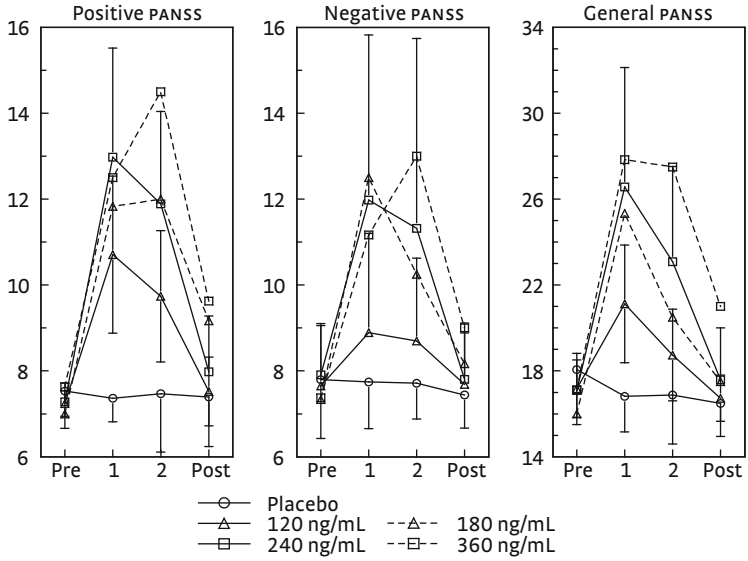

FIGURE 4 Time profile (mean ± SD) for prepulse inhibition (short form, 240 ms interval), VAS feeling high and smooth pursuit eye movements.