Challenging the cholinergic system : ageing, cognition & inflammation

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PHARMACOKINETICS AND PHARMACODYNAMICS OF ORAL MECAMYLAMINE – DEVELOPMENT OF A NICOTINIC ACETYLCHOLINE RECEPTOR ANTAGONIST COGNITIVE CHALLENGE TEST USING MODELLING AND SIMULATION

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ABSTRACT

A pharmacologic challenge model with a nicotinic antagonist could be an important tool not only to understand the complex role of the nicotinic cholinergic system in cognition, but also to develop novel compounds acting on the nicotinic acetylcholine receptor. The objective was to develop a PK-PD model using non-linear mixed effects (NLME) methods to quantitate the pharmacokinetics of three oral mecamylamine doses (10, 20 and 30 mg) and correlate the plasma concentrations to the pharmacodynamic effects on a cognitive and neurophysiologic battery of tests in healthy subjects. A one-compartment linear kinetic model best described the plasma concentrations of mecamylamine. Mecamylamine's estimated clearance was 0.28 ± 0.015 $L \cdot min^{-1}$. The peripheral volume of distribution (291±5.15L) was directly related to total body weight. Mecamylamine impaired the accuracy and increased the reaction time in tests evaluating short term working memory with a steep increase in the concentration-effect relationship at plasma concentrations below 100 $\mu g \cdot L^{-1}$. On the other hand, mecamylamine induced a decrease in performance of tests evaluating visual and fine motor coordination at higher plasma concentrations (EC_{50} 97 $\mu g \cdot L^{-1}$). Systolic and diastolic blood pressure decreased exponentially after a plasma mecamylamine concentration of 80 $\mu g \cdot L^{-1}$, a known effect previously poorly studied in healthy subjects. The developed mecamylamine PK-PD model was used to quantify the effects of nicotinic blockade in a set of neuro-physiologic tests in humans with the goal to provide insight into the physiology and pharmacology of the nicotinic system in humans and the possibility to optimize future trials that use mecamylamine as a pharmacological challenge.

INTRODUCTION

Integrity of the cholinergic system is essential for maintaining adequate cognitive functions. Impairment of the system is seen in both neurodegenerative and psychiatric conditions such as Alzheimer's disease (AD) and schizophrenia and has become an important therapeutic target.

Scopolamine, a selective competitive muscarinic antagonist, has been widely used as a challenge drug to induce temporary disturbances resembling those of Alzheimer's disease (AD) (Ebert and Kirch, 1998). Scopolamine administration induces mainly disturbances in visuo-spatial memory and orientation, short-term verbal, numeric and episodic memory, attention and acquisition (Flicker *et al*, 1992; Molchan *et al*, 1992; Ray *et al*, 1992; Snyder *et al*, 2005; Zemishlany and Thorne, 1991). These cognitive effects were also confirmed by different methods in which scopolamine also induced a diminished hippocampal activation in the MRI (Sperling *et al*, 2002), increased slow frequency waves on EEG (Ebert and Kirch, 1998) and magnetoencephalographic band specific functional brain connectivity disturbances observed in young healthy subjects, similar to those of patients with Alzheimer's disease (Bajo *et al*, 2015). However in the last decade, interest has increased towards understanding the nicotinic acetylcholine receptor (nAChR) and its role in different cognitive functions (Levin, 2002), consequences of functional abnormalities (Court *et al*, 2000) and possible uses as therapeutic target (Hurst *et al*, 2013). The use of a muscarinic agonist as scopolamine would seem less appropriate to investigate cognitive functions involved with nicotinergic agonists and compounds with activity on the nicotinergic system in general.

Mecamylamine (a selective nicotinic acetylcholine receptor antagonist) has slowly regained attention amongst neuroscientists after being an almost obsolete and forgotten drug to treat hypertension (Shytle *et al*, 2002). In the past two decades several studies have explored the neuro-physiological effects of mecamylamine in healthy subjects. Mecamylamine 10 mg induced significant impairment in learning in healthy elderly (Newhouse *et al*, 1994a). In younger subjects, however, mecamylamine doses below 20 mg generally do not produce significant cognitive deterioration (Ellis *et al*, 2009; Little

et al, 1998; Newhouse *et al*, 1994a; Voss *et al*, 2010). Mecamylamine 20 mg in younger healthy subjects cause significant increases in the number of errors in a learning and retrieval task, and an increase in the inspection time during a visual discrimination test, effect that was partially reversed by 5 mg of donepezil (a cholinesterase inhibitor) (Thompson *et al*, 2000). Several authors have suggested that co-administration of scopolamine and mecamylamine would better resemble cognitive impairment observed in AD patients (Ellis *et al*, 2006; Little *et al*, 1998). For a proper characterisation of nicotinic-muscarinic interactions, it is important to first quantify the neuro-physiological effects induced of either compound alone. We have previously described the concentration-effect relationships of scopolamine (Alvarez-Jimenez *et al*, 2016; Liem-Moolenaar *et al*, 2011); and we have now examined the concentrations and effects mecamylamine alone in healthy subjects, in order to determine the plasma concentration-effects (PK-PD) relationship of mecamylamine. PK-PD modelling is a widely used technique that integrates the exposure (measured using the plasma concentrations) and effects in a semi-mechanistic model approach in order to better interpret and understand experimental results and trial outcomes. The technique has gain popularity since it provides a more mechanistic explanation of the studied system and offers the possibility to create hypothetical scenarios by simulating outcomes in different situations offering a confirmatory rather than exploratory approach to clinical trials (Danhof *et al*, 2007).

A PK-PD model of mecamylamine-induced neurophysiological and cognitive effects may be used to optimise pharmacological challenge tests of this compound, to explore the effects of antagonism of the nicotinergic system and possible reversal by selective agonists.

In the current experiments, three mecamylamine doses (10, 20 and 30 mg compared to placebo) were administered to healthy subjects to further correlate the plasma mecamylamine concentrations with the effects while concentrations and effects were frequently measured. We utilized non-linear mixed effects (NLME) methods to quantitatively correlate the pharmacokinetic plasma mecamylamine concentrations to the pharmacodynamic cognitive and neurophysiologic effects in healthy subjects based on two related clinical studies.

METHODS

STUDY POPULATION

Forty-four healthy male subjects between 18 and 45 years of age (under and upper limits included) participated in two clinical studies performed at the Centre of Human Drug Research (Leiden, the Netherlands). Information on demographics and dose levels administered can be found in Table 5.1. A medical ethics committee approved the study protocols. After giving written informed consent, all subjects were medically screened prior to study participation. Exclusion criteria included the use of agents or drugs known to influence cognitive performance and evidence of relevant medical abnormalities including conditions that could cause any kind of cognitive impairment.

STUDY DESIGN

Data for this analysis were obtained from two related clinical studies. The first was an exploratory study intended to describe the cognitive effects of mecamylamine10 and 20 mg to those of scopolamine. In the second study, after performing an interim analysis to determine a safe dose increase, the dose range was expanded to 30 mg, and the effects of two cholinergic agonists (nicotine and galantamine) were examined. Galantamine was chosen as it exerts an allosteric modulatory activity on the nAChR, which other cholinesterase inhibitors lack (Coyle and Kershaw, 2001; Maelicke *et al*, 2001; Maelicke and Albuquerque, 2000). Both studies are in preparation for publication. However, since neither of the manuscripts would allow an integrated description of concentration-effect relationships for mecamylamine across the full (10-30 mg) dose range, we decided to perform a separate dedicated PK-PD analysis that is described in this article.

In both studies mecamylamine was administered orally in fasting conditions. Subjects were fasting for at least 4 hours and administration occurred with water. Mecamylamine capsules (Euticals SpA, Milan, Italy) containing mecamylamine HCl and microcrystalline cellulose as filling agent (used also in the placebo capsules) were administered

orally in blinded conditions. Plasma mecamylamine concentrations were determined using a validated, selective and sensitive liquid chromatography coupled to tandem-mass spectrometry (LC-MS/MS) method (Lower Limit of Quantification for the first trial was 1.54 $\mu g \cdot L^{-1}$ and for the second trial lowered to 0.5 $ng \cdot L^{-1}$).

The NeuroCart battery of tests evaluating different neurophysiological, psychomotor and cognitive tests was performed to quantify mecamylamine pharmacodynamic effects on different domains. The battery of tests has been previously extensively used in clinical drug development, and detailed descriptions can be found in other publications on a range of different compounds (de Haas *et al*, 2008; Liem-Moolenaar *et al*, 2010a, 2010b, 2011; van Steveninck *et al*, 1999; Strougo *et al*, 2008), including anticholinergic challenge tests (Liem-Moolenaar *et al*, 2010a, 2010b, 2011). On each study day, all pharmacodynamic tests were performed frequently at different time points per occasion in a quiet room with ambient illumination with only one subject in the same room (and a research assistant) per session. During the first trial, the NeuroCart test battery was subsequently performed at time points 30, 60, 120, 180, 195, 240, 480, 600 and 1320 minutes and for the second trial at time points 30, 80, 130, 180, 230, 280, 360 and 480 minutes post-dose. Washout periods between occasions were at least one week in both studies.

All subjects were thoroughly trained and familiarized with the psychometric tests within 21 days preceding study start to minimize inter-individual variability at baseline and to make sure subjects were able to understand and perform the tests. Each baseline assessment (pre-dose battery of tests) was performed twice at the beginning of each occasion. The mean of the two pre-dose measurements was used as baseline. A combination of tests evaluating neurophysiological and cognitive variables was analysed. Tests were included in the PK-PD analysis if they showed a statistically significant effect at 30 mg when compared to placebo. The blood pressure was modelled as a secondary measure since it was also used to determine the maximum tolerable dose for the second study, to predict the tolerability of mecamylamine in healthy subjects.

ADAPTIVE TRACKER TEST

The test evaluates attention and executive skills such as visuo-motor coordination (Borland and Nicholson, 1984; van Steveninck *et al*, 1991). Subjects were asked to use a joystick to keep a randomly moving target on the screen inside a circle. The percentage of time that the target was kept in the circle was calculated. Even though attention is a cognitive process involved in numerous functional areas and therefore can be indirectly measured via many cognitive tests, the adaptive tracker is a more specific test for attention (arousal, vigilance) as the complexity of the test resides in sustained attention since it is very simple to perform from a psychomotor performance point of view. We have shown earlier that the adaptive tracker test was very sensitive to subtle disturbances in attention caused by ethanol, sleep deprivation, and benzodiazepines, and also to subtle enhancements by e.g. caffeine and donepezil in healthy subjects (van Steveninck *et al*, 1991, 1999; de Visser *et al*, 2003).

N-BACK TEST

Subjects were instructed to remember and correlate a sequence of letters presented in a random order, thereby allowing evaluation of (short-term) working memory (Lim *et al*, 2008). Performance was expressed as the percentage of correct answers on the 0-back paradigm, and as reaction time of all answers on the 2-back paradigm. The fraction of correct answers was logit-transformed prior to model fitting.

Based on exploratory data analysis, the following NeuroCart tests were not considered in the model, because no significant effect of 30 mg of mecamylamine compared to placebo was observed: Visual Analogue Scales (evaluating alertness mood and calmness), Finger Tapping (evaluating motor fluency), Visual Verbal Learning Test (evaluating verbal working memory), Milner Maze Test (evaluating visuo-spatial working memory) and electroencephalogram (EEG). The electroencephalogram was measured tasks free during one minute with eyes closed.

SOFTWARE

Pharmacokinetics and pharmacodynamics analyses were performed using non-linear mixed-effect (NLME) modelling in NONMEM v7.2 and v7.3 (Beal *et al*, 2009). The database and all graphs were created using R v2.13.1 (R Core Team, 2013). Statistical analysis and calculations were performed using SAS software for windows v9.4 (SAS Institute, Inc., Cary, NC, USA).

MODEL DEVELOPMENT AND EVALUATION

Plasma mecamylamine concentration-time dependent data were analysed using a consecutive NLME modelling approach; once the best pharmacokinetic model was obtained, the individual pharmacokinetic parameter estimates were fixed to develop the pharmacodynamic models. The first order conditional estimation method with interaction (FOCE-I) was used. Several compartment models were explored for the pharmacokinetic model. Weight, height, age, body mass index and body surface area (calculated using DuBois's formula) were tested as potential covariates for parameters on which inter-individual variability (IIV) could be identified and were incorporated in the model as covariates if needed.

For the pharmacodynamic endpoints, several structures including direct and indirect (using an effect compartment) sigmoidal, truncated, linear, exponential and E_{max} model structures were tested. Delay compartments were taken into consideration for the pharmacodynamics models only when an indirect model was chosen.

For all models, once the structural model was defined additive, proportional, exponential or combined error models were tested. IIV was tested in each parameter estimate and correlations between post-hoc Bayesian parameter estimates and between post-hoc Bayesian parameter estimates and potential covariates were explored using coefficient of determination (r^2). Correlations with an $r^2 \geq 0.4$ that were considered clinically relevant were taken forward in formal testing of omega block structures and covariate analysis (weight, age and height). Competing models were compared based on their Goodness of Fit (GOF) plots, decrease of the objective function value (OFV), plausibility of parameter estimates, residual error, parameter precision (in terms of residual standard error; RSE), shrinkage and parameter distribution. The OFV is a goodness of fit statistic defined as minus two times the logarithm of the likelihood and it is provided in each model's output file provided by NONMEM. A decrease in the OFV of at least 3.84 units ($p<0.05$) was considered statistically significant. GOF plots included observations vs. population and individual predictions, conditional weighted residuals with interaction (CWRESI) vs. time and CWRESI vs. observations and IIV frequency distribution, boxplots and QQ graphs. The VPCs were obtained by simulating 1000 subjects, using the population parameter estimates and the full variance-covariance matrix. Covariates were sampled from the observed population distribution.

RESULTS

MODEL DEVELOPMENT – PLASMA MECAMYLAMINE CONCENTRATIONS

Shortly after oral mecamylamine administration, plasma mecamylamine concentrations increased rapidly and, once they reached the equilibration phase, plasma mecamylamine concentrations decreased gradually (Figure 5.1). A one-compartment (consisting of a dose and a central compartment) linear pharmacokinetic model structure best described the plasma mecamylamine pharmacokinetic data. A two-compartment linear model resulted in a negligible inter-compartmental clearance estimate (0.000022) with a gradient that approached zero and therefore the model was abandoned. Non-linear (Michaelis-Menten) kinetics was also tested. This provided no improvement in the fit or OFV and produced an estimated K_m above the measured concentrations (158 $\mu g \cdot L^{-1}$) and was therefore rejected. Inter-individual variability could be identified on the lag time related to the oral administration (ALAG time), absorption rate constant (K_{12}) and clearance (CL).

The estimation of the elimination rate (K_{20}) was dependent on the clearance and the central apparent volume of distribution as showed in Equation 5.1.Body weight was identified as covariate on the central volume of distribution (V) (r^2=0.66, $p<0.01$) and incorporated as mean body weight-

normalised covariate (ΔOFV = -27 points; Equation 5.2), which completely explained the inter-individual variability (IIV) on this parameter. Equation 5.3 and 5.4 show the one-compartment model differential equations and the way the lag time (ALAG) was incorporated. The rate of absorption (K_{12}) was negatively correlated with the lag time or ALAG (r^2 = 0.53, p<0.01) and an omega block structure (variance-covariance structure) was used, reducing the IIV of the ALAG (from 0.276 to 0.099) without influencing the OFV. Pharmacokinetic model graphical result estimates can be found in Table 5.2.

$$k_{20} = \frac{CL}{V_C} \quad (5.1)$$

$$\frac{V_C}{F} = e^{\left\{\left[\log(\vartheta_{V_C})\right] + \left[CWC \cdot \log\left(\frac{WGT}{77.698}\right)\right]\right\}} \quad (5.2)$$

$$\frac{dA_A}{dt} = -k_{12} \cdot A_A \cdot (t > ALAG1) \quad (5.3)$$

$$dA_C = k_{12} \cdot A_A \cdot (t > ALAG1) - k_{20} \cdot A_C \quad (5.4)$$

MODEL DEVELOPMENT – MECAMYLAMINE EFFECTS

PERCENTAGE OF ACCURACY OF THE ADAPTIVE TRACKER TEST ✳ Figure 5.2 shows the effect over time of mecamylamine administration on adaptive tracker performance (%-point accuracy). At baseline, subjects consistently scored a mean of 29 ± 0.82 %. Mecamylamine, compared to placebo, produced a decrease in performance of -1.89 %-point (confidence interval: -3.90 – 0.12; p=0.0647) after administration of 10 mg of mecamylamine, -2.06 % (-3.97 – -0.15; p=0.0355) after 20 mg of mecamylamine and -3.27 (-4.58 – -1.97; p<0.0001) after 30 mg of mecamylamine. The effect was observed promptly at the first time point after mecamylamine administration. In accordance, during PK model development, a direct E_{max} model proved similar when compared to an indirect model structure (ΔOFV = 0.6 points) and the direct model structure was therefore chosen. Equation 5.5 depicts the equation used to relate plasma mecamylamine concentrations (C) with the effect. The right side of the equation has as a consequence a reduction in the baseline (BL) or pre-dose value. Addition of a learning or practice effect linear and exponential function to describe the placebo data was unsuccessful since estimated OFV decreased by 12 points. Moreover it gave negligible improvement in the fit and caused difficulties estimating the learning function (parameter with the highest gradient and covariate step aborted) and was therefore abandoned.

Adding an exponent (γ) to the E_{max} model function provided a non-significant decrease in the OFV (1 point), however it improved the shrinkage, the uncertainty of the parameters and the fit of the model and therefore was accepted. In the best model, IIV was identified for BL (ΔOFV = -809 points) and EC_{50} (ΔOFV = -152 points). An omega block was required between BL and EC_{50}.

$$Tracker = BL \cdot \left\{1 - \left[\frac{E_{MAX} \cdot C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}}\right]\right\} \quad (5.5)$$

PERCENTAGE OF CORRECT ANSWERS IN THE 0-BACK PARADIGM OF THE N-BACK TEST ✳ Following mecamylamine administration, the number of correct answers decreased significantly with the highest dose when compared to placebo (Figure 5.3). Administration of mecamylamine 10 mg produced an average decrease in the 0-back ratio of correct answers of -0.03 % of correct answers (-0.08 – 0.01; p=0.1348), 20 mg -0.02 (-0.06 – 0.03; p=0.4714) and 30 mg produced a significant reduction of -0.023 (-.044 – -.003; p=0.0270). Compared to an indirect model, a direct model performed best (Equation 5.6). An E_{max} model proved superior compared to linear, truncated and exponential model structures (ΔOFV = 5157). A sensitivity analysis was performed to investigate the impact of one extreme outlier (Subject 6) on parameter estimation and uncertainty. Excluding this subject resulted in near identical parameter estimates and the SEM of the EC_{50} decreased from 17.1 to 4.8 $\mu g \cdot L^{-1}$, indicating that this data point has no substantial influence of model performance. Subject 6 was included in the final model run.

$$0\ back\ correct\ answers = BL \cdot \left\{\frac{E_{MAX} \cdot C}{EC_{50} + C}\right\} \quad (5.6)$$

REACTION TIME IN THE 2-BACK PARADIGM OF THE N-BACK TEST ✳ Figure 5.4 presents how mecamylamine administration increased the reaction time of the majority of the subjects during the 2-back test. Administration of mecamylamine 10 mg produced a non-significant increase of 7 milliseconds (-37–51; p=0.7503), 20 mg -1 milliseconds (-43 – 41; p=0.9677) and 30 mg produced a significant increase of 28.3 milliseconds (2.0 – 54.6; p=0.0356) in the 2-back reaction time when compared to placebo. Addition of intra-occasion variability at baseline occurred at an early stage of model development since it was observed when fitting the data and, once implemented, resulted in a significant drop in OFV of 165 points and improved the fit of the data. The best model structure proved to be a direct model. An exponential model provided a better fit and results when compared to an E_{max} model (exponential model decreased the OFV by 22 points). The parameter estimates provided by the E_{max} model were also above the measured mecamylamine concentrations and therefore this model was rejected. Variability (inter-occasion variability) was identified only at baseline and this was sufficient to describe the data correctly. One equation was needed to correctly describe the learning or practice effect without the influence of mecamylamine (E_0), where a time-dependent function described an ascending trend seen in all subjects (Equation 5.7). Afterwards, this function was used in Equation 5.8 to characterize the effect mecamylamine exerted in the *reaction time of the 2-bask test*. Again, the concentrations (C) in the exponent multiplied by a constant (λ) related the concentrations with the effect on the test.

$$E_0(t) = BL - t^{\gamma} \quad (5.7)$$

$$2\ back\ RT = E_0 \cdot \{e^{(C \cdot \lambda)}\} \quad (5.8)$$

SYSTOLIC AND DIASTOLIC BLOOD PRESSURE ✳ The blood pressure decrease effect of mecamylamine was the limiting factor for the dose increase in the studies and therefore was also modelled. Figure 5.7 presents the time dependent graphs per mecamylamine dose. The systolic and diastolic blood pressure (SBP and DBP, respectively) were modelled simultaneously since they are intimately correlated. Rhythmic oscillations around an identity (base) line were observed in the data from the placebo group. In order to describe the baseline circadian variability, a one-cosine function was used (Van Rijn-Bikker *et al*, 2013). Shortly after mecamylamine was administered, both the SBP and DBP decreased in a dose-dependent manner. A direct truncated effect model performed better than both an E_{max} (ΔOFV = -22 points) and a linear model (ΔOFV = -12 points). A direct model structure was chosen. IIV best described the data when placed at baseline. SBP and DBP baselines were highly correlated (r^2=0.37) and physiologically plausible, therefore an omega block was placed, reducing the IIV of both parameters. The Body Mass Index (BMI) was also highly correlated to the baseline SBP (r^2 = 0.49, p<0.01), adding it as a covariate produced a OFV decrease of 13 points and provided a better fit to the data. *Systolic and Diastolic Blood Pressure* were calculated with a cosine function of time (Equation 5.9), which was correlated to the plasma mecamylamine concentrations with an exponent (Equation 5.10). The amplitude (AMP) of the oscillations, the frequency (FREQ) and the point in the daytime that it starts (PHS) were estimated parameters. Only for the systolic blood pressure, the body mass index (BMI) was divided by the population BMI value and a constant (CBMIB) was used as correction factor to calculate the baseline as shown in Equation 5.11.

$$E_0(t) = BL + AMP \cdot \cos\left\{2 \cdot \Pi \cdot \left[\frac{t\text{-}PHS}{FREQ}\right]\right\} \quad (5.9)$$

$$BP = E_0 - (BASE \cdot e^{C}) \quad (5.10)$$

$$BLS = e^{\left\{\left[log(\vartheta_{BLS})\right] + \left[CBMIB \cdot \log\left(\frac{BMI}{23.25}\right)\right]\right\}} \quad (5.11)$$

MODEL EVALUATION

The GOF plots for all models indicate that the central and individual trend of the data is well described, and that no bias occurs over time or observations. The shrinkage was acceptable in all models except for the E_{max} estimated

in the 0-back percentage of correct answers (41.3 %) and the baseline of the 2-back reaction time (31.2 %). The VPCS indicate that the variability for these parameters is well described as 95% of the data appears lie within the 95% prediction interval.

DISCUSSION

This is the first time that neurocognitive and neurophysiological effects of mecamylamine have been quantified using an exposure-effect (pharmacokinetic and pharmacodynamic) relationship approach.

Mecamylamine is a highly lipophilic secondary amine that acts by binding non-competitively and non-selectively to the nicotinic acetylcholine receptor as an antagonist to the voltage gated function of the ion channel (Varanda *et al*, 1985). Due to its chemical properties, mecamylamine distributes profusely in the body including the Blood Brain Barrier and therefore exerts its effect in the Central Nervous System without delay or use of an effect compartment in the model. Bioavailability of mecamylamine is unknown. Even though mecamylamine has been administered intravenously in the past, plasma concentrations have not been determined; probably due to the fact that the drug was developed more than 70 years ago when plasma concentration methods were not available (Allanby and Trounce, 1957). It has been previously reported in literature that mecamylamine bioavailability is complete, however it was determined after comparing the reduction of the systolic and diastolic blood pressure after oral and intramuscular administration in healthy subjects, without measuring plasma mecamylamine concentrations (Ford *et al*, 1956). The reported one-compartment linear-kinetic model and the estimates obtained for mecamylamine are comparable to a model developed for dexmecamylamine. Dexmecamylamine (TC-5214) is a compound with similar chemical structure, when compared to mecamylamine. The compound is currently in clinical development to treat hyperactive bladder symptoms (Xu *et al*, 2014). The authors also reported that the corrected body weight was an important covariate directly correlated to the apparent central volume of distribution, as corroborated in our model. Non-linear kinetics proved not better than zero-order clearance of plasma mecamylamine in our model. While in our model Michaelis-Menten kinetics were tested, the value estimated for the K_M, concentration at which the reaction rate is half of v_{max}, was above the measured plasma concentrations, not excluding that at higher concentrations saturation of the system may be present.

Based on previously reported work (Ellis *et al*, 2006; Little *et al*, 1998; Voss *et al*, 2010), effects induced by mecamylamine doses below 20 mg have been previously difficult to quantify in healthy subjects. In this study, we performed PK-PD modelling on statistically significant effects of mecamylamine in a dose range of 10-30 mg. This demonstrated consistent effects on all evaluated neuro-physiologic tests even at dose levels as low as 10 mg. We were able to characterize the effects of administration of mecamylamine in a set of tests that were not earlier reported such as attention, vigilance and visuo-motor coordination (Adaptive Tracker) and confirm the effects previously reported in literature: impairment of learning and retrieval or working memory (N-back percentage of correct answers) and increase in reaction time (N-back reaction time).

Subjects receiving mecamylamine were more prone to commit mistakes during the 0-back paradigm compared to those in the placebo group. The estimated EC_{50} and E_{max} were low (8.7 $\mu g \cdot L^{-1}$ and 30%, respectively) resulting in long-lasting effects (higher possibilities of making mistakes) even at low plasma concentrations. Previously reported cognitive effects after administration of 20 mg of mecamylamine (and even 10 mg of mecamylamine in elderly) include an increase in working memory errors and reaction time, compared to the placebo group (Newhouse *et al*, 1992), consistent with our findings. Nicotinic blockade in humans produces impairment in the recall and integrative brain pathways (both needed to respond correctly in the N-back paradigms), probably secondary to nAChR inactivation in the basal forebrain structures where the receptor density is high (Zoli *et al*, 2015). Despite the fact that only 18 of the total 491 (4%) plasma mecamylamine concentrations were above the EC_{50} in the Adaptive Tracker model, the E_{max} model structure described the data substantially better when compared to more simple models and was therefore accepted as most appropriate model structure. As a result, the predictive value of E_{max} for higher doses should be careful considered.

In order to use mecamylamine as a cognitive challenge model drug to explore the nicotinic central activity, and manipulate the system with drugs that exert their mechanism of action through the same nAChR receptor, it is useful to first analyse the concentration-effect relationships. The choice of the optimal mecamylamine dose should depend on the balance between desired and unwanted effects, including central and peripheral effects. Mecamylamine exerts its action in a dose-dependent manner in different brain areas, translated in an individual dose-effect relationship per cognitive area. The different evaluated effect-concentration relationships per test are shown in Figure 5.5. Compared with other functions, accuracy and reaction time in N-back test of working memory are relatively sensitive to mecamylamine. The decrease of the accuracy and increase in the reaction time observed in the N-back test occurs at low concentrations and reaches a steady maximum around 100 $\mu g \cdot L^{-1}$. Above this concentration other less sensitive but potentially undesirable effects will be observed without a further clinically significant decrease in the performance on working memory. On the other hand, performance in the Adaptive Tracker (a tests evaluating attention and executive skills as visuo-motor coordination) may still decrease with higher doses of mecamylamine, since the estimated EC_{50} concentration (97.2 $\mu g \cdot L^{-1}$) was barely surpassed with the administration of mecamylamine 30 mg. Mecamylamine has been used in the past as a drug to treat moderately severe- to severe-hypertension due to its parasympathetic ganglionic effect. Mecamylamine effects only caused an average decrease in systolic blood pressure of 5 mmHg in our healthy subject population. This was less pronounced than the reduction of approximately 20 mmHg in hypertensive patients with hypertension, after oral administration of 20 mg of mecamylamine (Ford *et al*, 1956). Our findings further suggest, as has previously been assumed(Shytle *et al*, 2002), that one third or even less of the usual dose used to treat hypertension is enough to produce measurable central effects and higher doses than 30 mg of mecamylamine would not provide a greater decrease in tests evaluating working memory but would further decrease the blood pressure in an exponential way. Higher doses should only be considered after a careful hemodynamic risk assessment has been performed and if other cognitive areas rather than working memory are the main outcome.

Using the currently developed model could help simulate new scenarios with different mecamylamine doses based on the cognitive area of interest. A dose of 20 mg seems reasonable to induce disturbance in memory with minimum changes in the SBP as shown in Figure 5.5. On the other hand, the previous dose would seem insufficient to induce a decrease in visuo-spatial coordination where as shown in Figure 5.6, however higher doses should provide a greater decrease in performance with as consequence a more sensitive inflection point with small dose changes or co-administration of nicotinergic agonists, showed a significant effect.

It has been proposed that elderly subjects and patients with mild cognitive impairment are more sensitive to mecamylamine effects (Newhouse *et al*, 1994a, 1994b). The developed models may be helpful to further quantitate these differences by using age as covariate in the different estimates, e.g.: the estimated EC_{50}, Hill exponent, E_{max}, depending on the structural model used. Other applications of the PK-PD-models could be the translational integration of pre-clinical and clinical study results, to further understand the implications of manipulation of the nicotinic cholinergic neuronal system.

A learning effect secondary to consecutive testing, measured as a slight improvement in performance after several repetitions during the course of the occasion, was identified for the reaction time of the N-back as has been previously described (Bartels *et al*, 2010; Collie *et al*, 2003; Goldberg *et al*, 2015). This learning effect was successfully incorporated in both models using time-dependent functions (Ito *et al*, 2010; Samtani *et al*, 2015). Mecamylamine also induced a reduction of the practice or learning curve resulting of repetition during the reaction time of the 2-back test. Even though mecamylamine 10 mg administration by itself did not produced a statistically significant effect in this test, modelling showed that corresponding low levels did decrease the ability of subjects to learn (or perform better after practicing) in a quantifiable way.

Scopolamine induces more sedative effects (Robbins *et al*, 1997) when compared to mecamylamine, and it is possible that scopolamine-induced cognitive deficits are at least partly related to sedation rather than direct disturbances of muscarinic brain cortical and basal areas involved in cognitive processing. The most sensitive tests to measure sedation (induced

by sleep deprivation or pharmacological agents), namely the adaptive tracker and saccadic eyes movement tests (de Haas *et al*, 2008; van Steveninck *et al*, 1991, 1999) were less affected by mecamylamine when compared to scopolamine. The fact that scopolamine produced more sedative effects than mecamylamine is in accordance with the fact that muscarinic receptors populate more densely the brain stem (including the ascending reticular ascending system), which regulates arousal (Flynn *et al*, 1997).

The mecamylamine pharmacological challenge model is useful to investigate the role of nAChR in neuro-physiological functions and to support clinical research. The better understanding of the relationship between the plasma concentrations of mecamylamine and its pharmacodynamic effects that this model has yielded, will aid to quantify the more subtle differences in performance that with other statistical methods are not discovered. This is of particular importance when trying to show cognitive improvement due to drugs that are being developed, as detrimental effects of psychoactive compounds on cognition are already difficult to demonstrate, but reversal or improvement of cognitive functions has rarely been reported (Buccafusco, 2009). Using a pharmacokinetic and pharmacodynamic model we provide a better insight into the complexity of the mechanism of action of central nicotine receptor blockade in healthy subjects. Antagonism of the nicotinic cholinergic system using mecamylamine resulted mainly in impairment of cognitive functions such as acquisition, processing and execution. The mecamylamine model in humans could be useful as a proof of pharmacology tool in drug development of novel nicotinic agents.

REFERENCES

Allanby KD, Trounce JR (1957). Excretion of mecamylamine after intravenous and oral administration. *Br Med J* **2**: 1219–21.

Alvarez-Jimenez R, Groeneveld GJ, Gerven JMA van, Goulooze SC, Baakman AC, Hay JL, *et al* (2016). Model-based exposure-response analysis to quantify age related differences in the response to scopolamine in healthy subjects. *Br J Clin Pharmacol* **82**: 1011–21.

Bajo R, Pusil S, López ME, Canuet L, Pereda E, Osipova D, *et al* (2015). Scopolamine effects on functional brain connectivity: a pharmacological model of Alzheimer's disease. *Sci Rep* **5**: 9748.

Bartels C, Wegrzyn M, Wiedl A, Ackermann V, Ehrenreich H (2010). Practice effects in healthy adults: A longitudinal study on frequent repetitive cognitive testing. *BMC Neurosci* **11**: 118.

Beal S, Sheiner LB, Boeckmann A, Bauer RJ (2009). NONMEM User's Guides. 210.

Borland RG, Nicholson a N (1984). Visual motor co-ordination and dynamic visual acuity. *Br J Clin Pharmacol* **18 Suppl 1**: 69S–72S.

Buccafusco J (2009). The Revival of Scopolamine Reversal for the Assessment of Cognition-Enhancing Drugs. *Methods Behav Anal Neurosci* at <http://www.ncbi.nlm.nih.gov/books/NBK5233/>.

Collie A, Maruff P, Darby DG, McStephen M (2003). The effects of practice on the cognitive test performance of neurologically normal individuals assessed at brief test-retest intervals. *J Int Neuropsychol Soc* **9**: 419–428.

Court J., Piggott M., Lloyd S, Cookson N, Ballard C., McKeith I., *et al* (2000). Nicotine binding in human striatum: elevation in schizophrenia and reductions in dementia with Lewy bodies, Parkinson's disease and Alzheimer's disease and in relation to neuroleptic medication. *Neuroscience* **98**: 79–87.

Coyle J, Kershaw P (2001). Galantamine, a cholinesterase inhibitor that allosterically modulates nicotinic receptors: effects on the course of Alzheimer's disease. *Biol Psychiatry* **49**: 289–99.

Danhof M, Jongh J de, Lange ECM De, Pasqua O Della, Ploeger B a, Voskuyl R a (2007). Mechanism-based pharmacokinetic-pharmacodynamic modeling: biophase distribution, receptor theory, and dynamical systems analysis. *Annu Rev Pharmacol Toxicol* **47**: 357–400.

Ebert U, Kirch W (1998). Scopolamine model of dementia: electroencephalogram findings and cognitive performance. *Eur J Clin Invest* **28**: 944–9.

Ellis JR, Ellis K a, Bartholomeusz CF, Harrison BJ, Wesnes K a, Erskine FF, *et al* (2006). Muscarinic and nicotinic receptors synergistically modulate working memory and attention in humans. *Int J Neuropsychopharmacol* **9**: 175–89.

Ellis JR, Nathan PJ, Villemagne VL, Mulligan RS, Ellis K a, Tochon-Danguy HJ, *et al* (2009). The relationship between nicotinic receptors and cognitive functioning in healthy aging: An in vivo positron emission TOMOGRAPHY (PET) study with 2-[(18)F]Fluoro-A-85380. *Synapse* **63**: 752–63.

Flicker C, Ferris SH, Serby M (1992). Hypersensitivity to scopolamine in the elderly. *Psychopharmacology (Berl)* **107**: 437–41.

Flynn DD, Reever CM, Ferrari-DiLeo G (1997). Pharmacological strategies to selectively label and localize muscarinic receptor subtypes. *Drug Dev Res* **40**: 104–116.

Ford R V, Madison JC, Moyer JH (1956). Pharmacology of mecamylamine. *Am J Med Sci* **232**: 129–43.

Goldberg TE, Harvey PD, Wesnes KA, Snyder PJ, Schneider LS (2015). Practice effects due to serial cognitive assessment: Implications for preclinical Alzheimer's disease randomized controlled trials. *Alzheimer's Dement Diagnosis, Assess Dis Monit* **1**: 103–111.

Haas SL de, Visser SJ de, Post JP van der, Schoemaker RC, Dyck K van, Murphy MG, *et al* (2008). Pharmacodynamic and pharmacokinetic effects of MK-0343, a GABA(A) alpha2,3 subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers. *J Psychopharmacol* **22**: 24–32.

Hurst R, Rollema H, Bertrand D (2013). Nicotinic acetylcholine receptors: from basic science to therapeutics. *Pharmacol Ther* **137**: 22–54.

Ito K, Ahadieh S, Corrigan B, French J, Fullerton T, Tensfeldt T (2010). Disease progression meta-analysis model in Alzheimer's disease. *Alzheimer's Dement* **6**: 39–53.

Levin ED (2002). Nicotinic receptor subtypes and cognitive function. *J Neurobiol* **53**: 633–40.

Liem-Moolenaar M, Boer P de, Timmers M, Schoemaker RC, Hasselt JGC van, Schmidt S, *et al* (2011). Pharmacokinetic-pharmacodynamic relationships of central nervous system effects of scopolamine in healthy subjects. *Br J Clin Pharmacol* **71**: 886–98.

Liem-Moolenaar M, Zoethout RWM, Boer P de, Schmidt M, Kam ML de, Cohen AF, *et al* (2010a). The effects of the glycine reuptake inhibitor R213129 on the central nervous system and on scopolamine-induced impairments in psychomotor and cognitive function in healthy subjects. *J Psychopharmacol* **24**: 1671–9.

Liem-Moolenaar M, Zoethout RWM, Boer P de, Schmidt M, Kam ML de, Cohen AF, *et al* (2010b). The effects of a glycine reuptake inhibitor R231857 on the central nervous system and on scopolamine-induced impairments in cognitive and psychomotor function in healthy subjects. *J Psychopharmacol* **24**: 1681–7.

Lim H-K, Juh R, Pae C-U, Lee B-T, Yoo S-S, Ryu S-H, *et al* (2008). Altered verbal working memory process in patients with Alzheimer's disease: an fMRI investigation. *Neuropsychobiology* **57**: 181–187.

Little JT, Johnson DN, Minichiello M, Weingartner H, Sunderland T (1998). Combined nicotinic and muscarinic blockade in elderly normal volunteers: Cognitive, behavioral, and physiologic responses. *Neuropsychopharmacology* **19**: 60–69.
Maelicke A, Albuquerque EX (2000). Allosteric modulation of nicotinic acetylcholine receptors as a treatment strategy for Alzheimer's disease. *Eur J Pharmacol* **393**: 165–70.
Maelicke A, Samochocki M, Jostock R, Fehrenbacher A, Ludwig J, Albuquerque EX, *et al* (2001). Allosteric sensitization of nicotinic receptors by galantamine, a new treatment strategy for Alzheimer's disease. *Biol Psychiatry* **49**: 279–88.
Molchan SE, Martinez RA, Hill JL, Weingartner HJ, Thompson K, Vitiello B, *et al* (1992). Increased cognitive sensitivity to scopolamine with age and a perspective on the scopolamine model. *Brain Res Brain Res Rev* **17**: 215–26.
Newhouse PA, Potter A, Corwin J, Lenox R (1992). Acute nicotinic blockade produces cognitive impairment in normal humans. *Psychopharmacology (Berl)* **108**: 480–484.
Newhouse PA, Potter A, Corwin J, Lenox R (1994a). Age-Related Effects of the Nicotinic Antagonist Mecamylamine on Cognition and Behavior. *Neuropsychopharmacology* **10**: 93–107.
Newhouse PA, Potter A, Corwin J, Lenox R (1994b). Modeling the Nicotinic Receptor Loss in Dementia Using the Nicotinic Antagonist Mecamylamine : Effects on Human Cognitive Functioning. *Drug Dev Res* **31**: 71–79.
R Core Team (2013). R: A language and environment for statistical computing. 99doi:10.1111/j.1541-0420.2010.01426.x.
Ray PG, Meador KJ, Loring DW, Zamrini EW, Yang XH, Buccafusco JJ (1992). Central anticholinergic hypersensitivity in aging. *J Geriatr Psychiatry Neurol* **5**: 72–77.
Rijn-Bikker PC Van, Snelder N, Ackaert O, Hest RM Van, Ploeger BA, Montfrans GA Van, *et al* (2013). Nonlinear mixed effects modeling of the diurnal blood pressure profile in a multiracial population. *Am J Hypertens* **26**: 1103–1113.
Robbins TW, Semple J, Kumar R, Truman MI, Shorter J, Ferraro A, *et al* (1997). Effects of scopolamine on delayed-matching-to-sample and paired associates tests of visual memory and learning in human subjects: comparison with diazepam and implications for dementia. *Psychopharmacology (Berl)* **134**: 95–106.
Samtani MN, Xu SX, Russu A, Adedokun OJ, Lu M, Ito K, *et al* (2015). Alzheimer's disease assessment scale-cognitive 11-item progression model in mild-to-moderate Alzheimer's disease trials of bapineuzumab. *Alzheimer's Dement Transl Res Clin Interv* **1**: 157–169.
Shytle RD, Penny E, Silver AA, Goldman J, Sanberg PR (2002). Mecamylamine (Inversine®): an old antihypertensive with new research directions. *J Hum Hypertens* **16**: 453–457.
Snyder PJ, Bednar MM, Cromer JR, Maruff P (2005). Reversal of scopolamine-induced deficits with a single dose of donepezil, an acetylcholinesterase inhibitor. *Alzheimers Dement* **1**: 126–35.
Sperling R, Greve D, Dale A, Killiany R, Holmes J, Rosas HD, *et al* (2002). Functional MRI detection of pharmacologically induced memory impairment. *Proc Natl Acad Sci U S A* **99**: 455–60.
Steveninck AL van, Berckel BN van, Schoemaker RC, Breimer DD, Gerven JM van, Cohen AF (1999). The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. *J Psychopharmacol* **13**: 10–7.
Steveninck AL van, Schoemaker HC, Pieters MSM, Kroon R, Breimer DD, Cohen AF (1991). A comparison of the sensitivities of adaptive tracking, eye movement analysis, and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. *Clin Pharmacol Ther* **50**: 172–180.
Strougo a, Zuurman L, Roy C, Pinquier JL, Gerven JM a van, Cohen a F, *et al* (2008). Modelling of the concentration--effect relationship of THC on central nervous system parameters and heart rate -- insight into its mechanisms of action and a tool for clinical research and development of cannabinoids. *J Psychopharmacol* **22**: 717–726.
Thompson JC, Stough C, Ames D, Ritchie C, Nathan PJ (2000). Effects of the nicotinic antagonist mecamylamine on inspection time. *Psychopharmacology (Berl)* **150**: 117–9.
Varanda W a, Aracava Y, Sherby SM, VanMeter WG, Eldefrawi ME, Albuquerque EX (1985). The acetylcholine receptor of the neuromuscular junction recognizes mecamylamine as a noncompetitive antagonist. *Mol Pharmacol* **28**: 128–37.
Visser SJ de, Post JP van der, Waal PP de, Cornet F, Cohen AF, Gerven JMA van (2003). Biomarkers for the effects of benzodiazepines in healthy volunteers. *Br J Clin Pharmacol* **55**: 39–50.
Voss B, Thienel R, Reske M, Habel U, Kircher T (2010). Cognitive performance and cholinergic transmission: influence of muscarinic and nicotinic receptor blockade. *Eur Arch Psychiatry Clin Neurosci* **260 Suppl**: S106-10.
Xu H, Henningsson A, Alverlind S, Tummala R, Toler S, Beaver JS, *et al* (2014). Population pharmacokinetics of TC-5214, a nicotinic channel modulator, in phase I and II clinical studies. *J Clin Pharmacol* **54**: 707–18.
Zemishlany Z, Thorne a. E (1991). Anticholinergic challenge and cognitive functions: A comparison between young and elderly normal subjects. *Isr J Psychiatry Relat Sci* **28**: 32–41.
Zoli M, Pistillo F, Gotti C (2015). Diversity of native nicotinic receptor subtypes in mammalian brain. *Neuropharmacology* **96**: 302–311.

TABLE 5.1 Subject demographics.

Mean ± Standard Deviation. ξ Mean (minimum-maximum).

	Exploratory (first) trial	Confirmatory (second) trial	All
Subjects (n)	14	30	44
Age ξ (years)	25.4 (19 - 36)	23.5 (19 – 35)	24.3 (19 - 36)
Weight (kg)	80.3 ± 9.14	75.2 ± 8.47	77.3 ± 9.06
Height (cm)	182.5 ± 6.22	181.6 ± 6.16	182.0 ± 6.17
BMI ($kg \cdot m^{-2}$)	24.0 ± 2.18	22.8 ± 2.44	23.3 ± 2.41
Mecamylamine doses (mg)	Placebo, 10 and 20 mg	Placebo and 30 mg	NA

NA: not applicable.

TABLE 5.2 Population estimates for pharmacokinetic and pharmacodynamic models for mecamylamine.

Parameters are reported as population estimate.

	Parameter	Units	Parameter estimate	SEM	IIV	Shrinkage	Observations
Mecamylamine pharmacokinetics	$ALAG_1$	min	26.8	1.04	0.096*	19.10	Correlation -0.294
	K_{12}	min^{-1}	0.0335	0.0088	2.115 *	4.59	
	CL	$L \cdot min^{-1}$	0.279	0.0154	0.326	7.63	
	$\frac{V_C}{F}$	L	291 †	5.15	-	-	
	CWV	-	0.794	0.132	-	-	
	σ^2	-	0.0328	0.00446	-	-	Proportional
Adaptive Tracker (percentage of accuracy)	EC_{50}	$\mu g \cdot L^{-1}$	97.2	28.1	1.0399 *	26.7	Correlation 0.0451
	BL	-	29.0	0.82	0.182 *	0.66	
	E_{max}	%	0.27	0.053	-	-	
	γ	-	1.58	0.548	-	-	
	σ^2	-	11.5	1.2	-	-	Additive
Correct Answers of the o-back § (percentage of correct answers)	EC_{50}	$\mu g \cdot L^{-1}$	8.74	17.1	-	-	
	E_{max}	%	0.377	0.163	0.885	41.3	
	BL	% correct answers	3.66	0.125	0.150	22.9	
	σ^2	-	0.00174	0.000322	-	-	Additive
Reaction Time of the 2-back paradigm (milliseconds)	BL	ms	561	12.5	0.0446¶	31.2	
	γ_{bl}	-	0.568	0.0345	-	-	
	γ_{eff}	-	0.000763	0.000219	-	-	
	σ^2	-	0.0132	0.00119	-	-	Proportional
Systolic and Diastolic Blood Pressure (mmHg)	BL_D	mmHg	70.2	0.931	0.080 *	6.91	Correlation 0.055
	BL_S ‡	mmHg	121	1.26	0.064 *	6.24	
	AMP_D	mmHg	1.81	0.438	-	-	
	AMP_S	mmHg	1.12	0.422	-	-	
	PHS_D	min	8e-04	-	-	-	
	PHS_S	min	802	74.5	-	-	
	$FREQ_D$	min-1	676	-	-	-	
	$FREQ_S$	min^{-1}	833	-	-	-	
	$BASE_D$	-	0.0227	0.00329	-	-	
	$BASE_S$	-	0.0284	0.00318	-	-	
	CBMIB	-	0.229	0.107	-	-	
	σ2D	-	0.0109	0.001	-	-	Proportional
	σ^2_S	-	0.00615	0.000598	-	-	Proportional

IIV: Inter-individual Variability expressed as Coefficient of Variation. ‡ BMI used as a covariate.
*†Weight used as covariate. *Omega block structure. γ Exponent. 4 buffer compartments.*
§ Parameters reported as natural log odds. ¶Highest inter-occasion variability. F: oral bioavailability.

FIGURE 5.1 Mecamylamine pharmacokinetics.

Plasma mecamylamine concentrations visual predictive check graphs versus time after mecamylamine administration (time point zero) per dose. The solid line represents the model population prediction and the **grey** area the 95% predicted interval. Circles represent the observations. **Red** lines represent the 95% confidence interval and the dotted line in between the median of the observations.

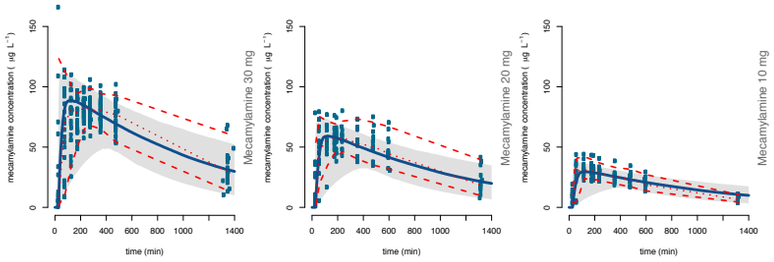

FIGURE 5.2 Mecamylamine effects on the Adaptive Tracker.

Performance during the Adaptive Tracker test versus time after oral mecamylamine administration (time point zero) per dose. The solid line represents the model population prediction and the **grey** area the 95% predicted interval. The **purple** lines represent the individual predictions. Circles represent the observations.

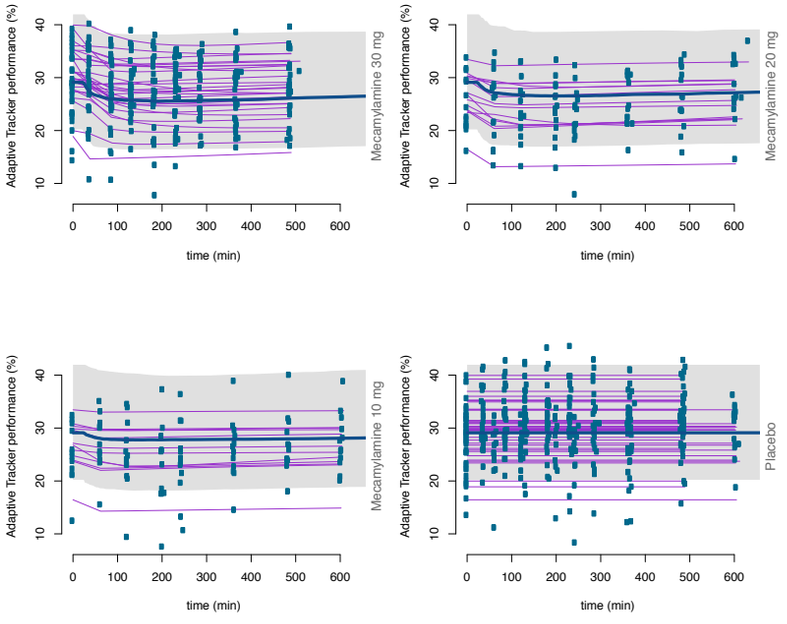

FIGURE 5.3 Mecamylamine effects on the ratio of correct answers of the 0-back paradigm.

Ratio of correct answers during the 0-back paradigm versus time after oral mecamylamine administration (time point zero) per dose. The solid line represents the model population prediction and the **grey** area the 95% predicted interval. The **purple** lines represent the individual predictions. Circles represent the observations.

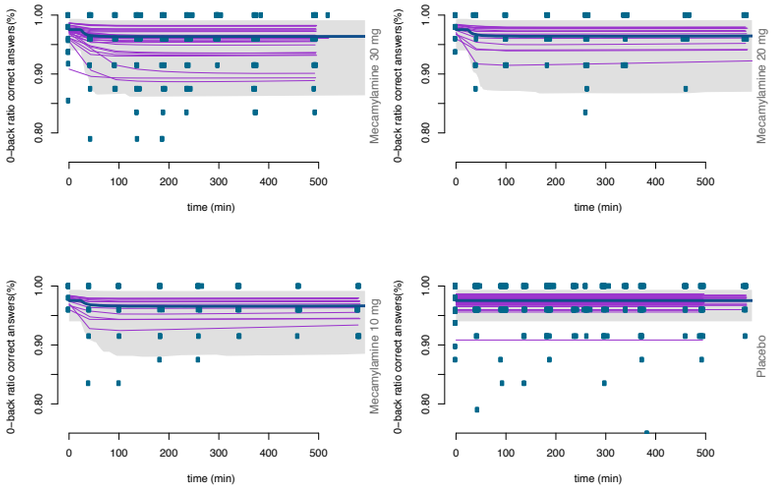

FIGURE 5.4 Mecamylamine effects on the reaction time of the 2-back paradigm.

Reaction time during the 2-back paradigm versus time after oral mecamylamine administration (time point zero) per dose. The solid line represents the model population prediction and the **grey** area the 95% predicted interval. The **purple** lines represent the individual predictions. Circles represent the observations.

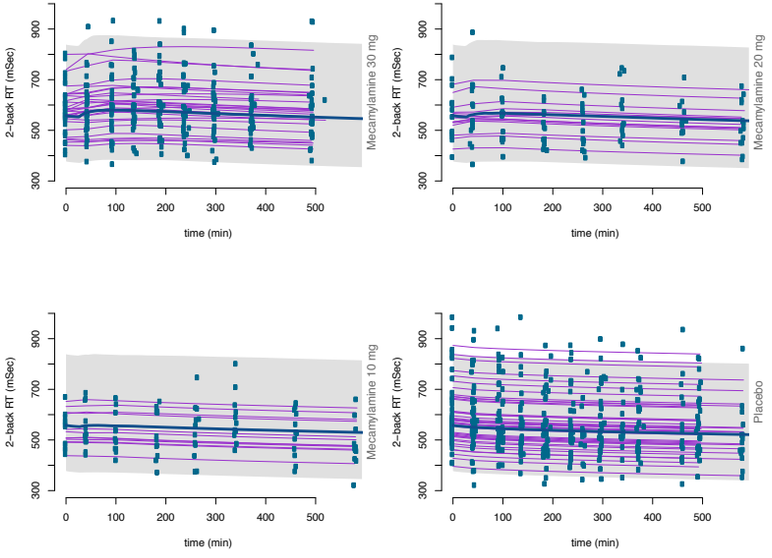

FIGURE 5.5 Mecamylamine concentration-effect relationships.

Plasma mecamylamine concentrations versus the effect per (neuro-) physiological test (N-back percentage of correct answers and reaction time, Adaptive Tracker and Systolic and Diastolic Blood Pressure). The solid line represents the model population prediction. The dots represent the individual predictions.

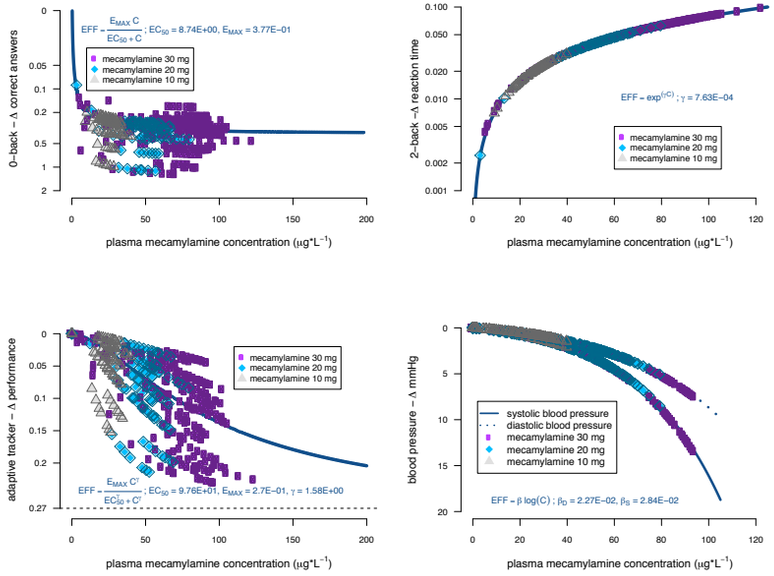

FIGURE 5.6 Mecamylamine simulation of different doses.

Plasma mecamylamine concentrations and resulting effects in the different physiologic and neurologic tests versus time. The simulations were performed using a normalized weight of 70 kg.

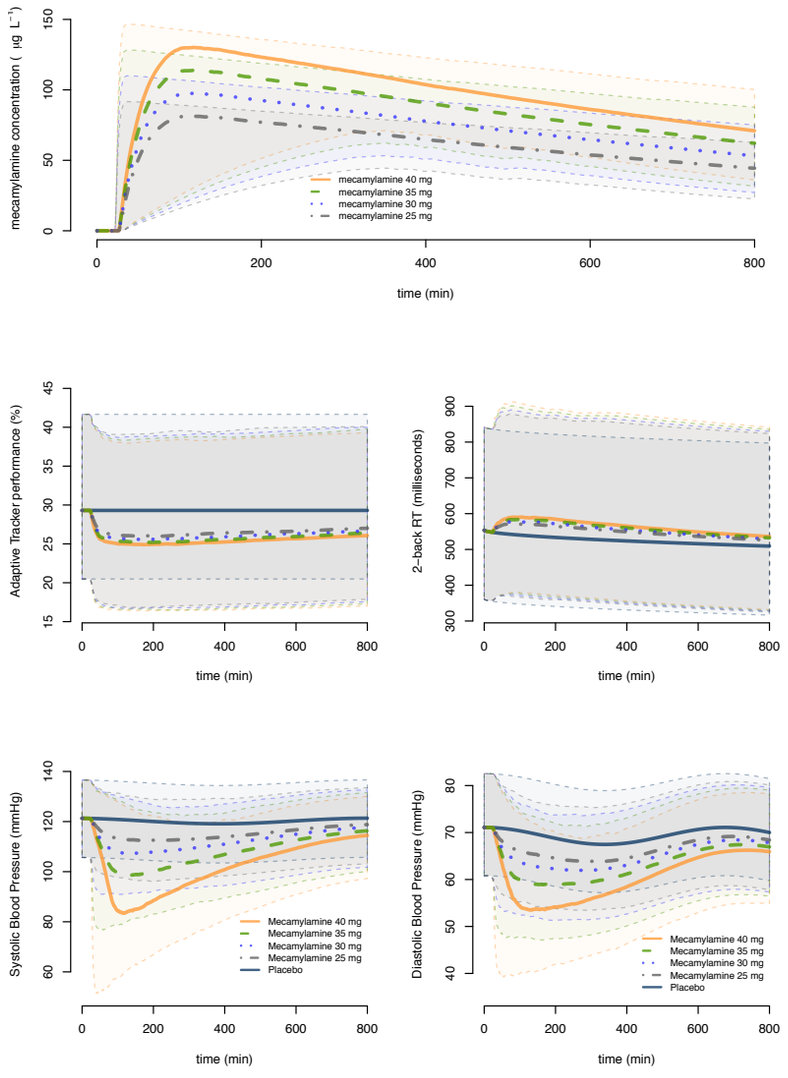

FIGURE 5.7 Mecamylamine effects on the blood pressure.

Blood pressure versus time after oral mecamylamine administration (time point zero) per dose. The solid line represents the model population prediction and the **grey** area the 95% predicted interval. The **purple** lines represent the individual predictions. Circles represent the observations.