Challenging the cholinergic system : ageing, cognition & inflammation

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MODEL-BASED EXPOSURE-RESPONSE ANALYSIS TO QUANTIFY AGE RELATED DIFFERENCES IN THE RESPONSE TO SCOPOLAMINE IN HEALTHY SUBJECTS

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ABSTRACT

Subjects with increasing age are more sensitive to the effects of the anti-muscarinic agent scopolamine, which is used (among other indications) to induce temporary cognitive dysfunction in early phase drug studies with cognition enhancing compounds. The enhanced sensitivity has always been attributed to incipient cholinergic neuronal dysfunction, as a part of the normal aging process. The aim of the study was to correlate age-dependent pharmacodynamic neuro-physiologic effects of scopolamine after correcting for differences in individual exposure. We applied a pharmacokinetic and pharmacodynamic modelling approach to describe individual exposure and neuro-cognitive effects of intravenous scopolamine administration in healthy subjects. A two-compartment linear kinetics model best described the plasma concentrations of scopolamine. The estimated scopolamine population mean apparent central and peripheral volume of distribution was 2.66 ± 1.050 L and 62.10 ± 10.100 L, respectively and the clearance was 1.09 ± 0.096 $L \cdot min^{-1}$. Age was not related to a decrease of performance in the tests following scopolamine administration in older subjects. Only the saccadic peak velocity showed a positive correlation between age and sensitivity to scopolamine. Age was however correlated at baseline with an estimated slower reaction time while performing the cognitive tests and to higher global δ and frontal ϑ frequency bands measured with the surface EEG. Most of the differences in response to scopolamine administration between young and older subjects could be explained by pharmacokinetic differences (lower clearance) and not to an enhanced sensitivity when corrected for exposure levels.

INTRODUCTION

The involvement of the cholinergic system in the etiology of geriatric cognitive dysfunction was already suggested in the early eighties (Bartus *et al*, 1982). At that time, evidence from different sources using mostly semi-parametric statistical methods, suggested that cholinergic neuronal dysfunction played a major role in the brains of healthy older subjects, subjects with Mild Cognitive Impairment (MCI) and to a greater extent in those of patients with dementia (Coyle *et al*, 1983). Research in older subjects show that cognitive abilities such as encoding new memories of episodes or facts, working memory (the simultaneous short-term maintenance and manipulation of information involving executive processes) and processing speed seem to be the most affected by ageing (Hedden and Gabrieli, 2004). Recently, the age-related decrease in specific areas in the brain of healthy subjects was quantified prospectively with MRI imaging. Acetylcholine-rich regions in the brain including the hippocampus, the entorhinal cortex, the inferior temporal cortex and the prefrontal white matter, decreased with age. The hippocampus was the only structure that showed a non-linear increased rate of shrinkage with increasing age, providing evidence of an age-related decline in hippocampal volume in subjects with increasing age and indirectly suggesting a decline in hippocampal neurons, an area rich in cholinergic neurons (Raz *et al*, 2005).

The cholinergic hypothesis was further strengthened by evidence that the severity of cognitive deficits appeared to be related to the extent of cholinergic impairment (Mufson *et al*, 2000), and to the observation that anti-cholinergic compounds induce cognitive dysfunction (Broks *et al*, 1988; Liem-Moolenaar *et al*, 2011; Newhouse *et al*, 1994). The most commonly used cholinergic challenge utilizes scopolamine as a selective competitive muscarinic antagonist. Scopolamine has a high affinity for all five muscarinic receptor subtypes (M_1– M_5) and a negligible affinity for histaminergic and dopaminergic receptors (Ali-Melkkilä *et al*, 1993). Intravenous administration of scopolamine leads to a reduction in attention, arousal, verbal and non-verbal working memory and episodic memory. The reduction in cognitive functioning was observed in both healthy young and healthy older subjects,

but appeared to be more severe in healthy older subjects. A significant difference between young adults compared to older subjects was observed in several cognitive tests evaluating short-term verbal and numeric working memory (Molchan *et al*, 1992; Ray *et al*, 1992; Zemishlany and Thorne, 1991), attention (Zemishlany and Thorne, 1991), acquisition (Zemishlany and Thorne, 1991), visuo-spatial praxis (Flicker *et al*, 1992) and episodic memory (Molchan *et al*, 1992). Additionally, some authors have suggested that scopolamine use in clinical practice in older patients might induce a higher risk of cognitive impairment when compared to younger subjects (Flicker *et al*, 1992; Seo *et al*, 2009). It is hypothesized that this difference between young adults and older subjects receiving scopolamine is due to incipient cholinergic neuronal dysfunction in the healthy older subjects and hence an enhanced sensitivity to anti-cholinergic drug effects (Dumas and Newhouse, 2011; Terry and Buccafusco, 2003). Consequently, a lower scopolamine dose in the older subjects will result in similar effects compared to younger subjects. However, none of these studies, measured age-related difference in plasma scopolamine concentrations.

In the current paper, a non-linear mixed effects (NLME) approach was used to quantitatively correlate the pharmacodynamic neuro-physiologic effects of scopolamine as an indirect measure of muscarinic brain activity after correcting for differences in exposure (pharmacokinetics). This allows the identification of pharmacokinetic (PK) and pharmacodynamic (PD) differences at population and individual level, and their relationships with covariates like age. NLME methods are a useful tool when differences in exposure explain a different measurable effect, while biological differences such as inter-subject and intra-subject random variability, are also taken into consideration and may be explained by covariates (e.g.: body weight, height, BMI, age, etc.).

METHODS

STUDY POPULATION

A total of 135 healthy subjects participated in four separate clinical studies performed at the Centre of Human Drug Research (Leiden, the Netherlands). Subjects' demographics can be found in Table 2.1. A medical ethics committee approved the four different 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 CNS performance (including smoking and drug or alcohol abuse), consuming more than five cups of caffeine-containing drinks per day and evidence of relevant medical abnormalities. Older subjects were required to score 28 points (or higher) on the Mini-Mental State Examination (Folstein *et al*, 1975) (MMSE) at screening.

STUDY DESIGN

Data for this analysis were obtained from all four studies. In the first two trials the effects of two different glycinergic compounds were studied using a scopolamine challenge model (Liem-Moolenaar *et al*, 2010a, 2010b), the third one was a study in which the effects of scopolamine on cognitive functions were compared with those of mecamylamine (a nicotinic acetylcholine receptor antagonist). In the fourth study, the effects of an a_7 nicotinic acetylcholine receptor agonist were examined in older subjects using a scopolamine challenge without a placebo-control. Only the data from the occasions where scopolamine, scopolamine in combination with placebo or only placebo (comparison placebo group) was administered were included for analysis.

An intravenous dose of 0.5 mg of scopolamine hydrobromide was administered to subjects below 65 years and an intravenous dose of 0.3 mg to the older subjects group (≥ 65 years), according to previous recommendations (Flicker *et al*, 1992; Snyder *et al*, 2005; Thomas *et al*, 2008). In all four studies, scopolamine was administered as an intravenous infusion over 15 minutes. Plasma scopolamine concentrations were determined using a validated, selective and sensitive liquid LC-MS/MS method with a Lower Limit of Quantification of 10 pg · mL^{-1}. Across the study sample analytical runs, the accuracy (expressed as the percentage of bias) of scopolamine QC samples ranged from 2.0 to 3.3% and assay precision (expressed as the percentage of CV) ranged from 2.5 to 4.0%.

A battery of CNS tests (neurophysiological, psychomotor and cognitive tests, subjective drug effects) was performed to quantify the pharmacodynamics effects of scopolamine, which provided information on the effects of scopolamine on different functional CNS domains. All pharmacodynamic tests were performed in a quiet room with ambient illumination with only one subject in the same room with a research assistant per session. All subjects were thoroughly trained and familiarized with the psychometric tests within 14 days preceding study start to minimize learning effects during the study. Each baseline or pre-dose assessment was performed twice at the beginning of each occasion. The lack of placebo arm in the older subjects group was handled by using the PD baseline measurements to estimate the scopolamine effects. A combination of tests evaluating both neurophysiological and cognitive variables on which an effect was previously reported (Liem-Moolenaar *et al*, 2011) were analysed:

N-BACK TEST ✳ Subjects were asked to remember and correlate a sequence of letters presented in a random order, thereby allowing evaluation of (short-term) working memory. This test evaluates working memory (Lim *et al*, 2008). Subjects were asked to remember and correlate a sequence of letters presented in a random order. Performance was expressed as the percentage of correct answers on the 0-, 1- and 2-back paradigms, and as reaction time on the 0-back paradigm. N-back ratio data was analysed as count data and therefore the data was logit-transformed.

ADAPTIVE TRACKER TEST ✳ The test evaluates attention and executive skills 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 accuracy was recorded.

SACCADIC EYE MOVEMENTS TEST ✳ This is one of the most sensitive tests for sedation (van Steveninck *et al*, 1991). Subjects were requested to look at a randomly moving target in the computer screen. With help of two electrodes, the horizontal peak velocity eye movements and inaccuracy were measured.

ELECTRO-ENCEPHALOGRAM ✳ Four cranial superficial electrodes (Fz, Cz, Pz, Oz) were placed following the 10-20 system and subjects were asked to close their eyes. Fast Fourier transformed absolute power (μV) was calculated from the raw measurements in the α [7.5 – 11.5 Hz], β [11.5 – 30 Hz], δ [0.5–3.5 Hz] and ϑ [3.5 – 7.5 Hz] frequency ranges in two bipolar leads: Fz-Cz and Pz-Oz).

SOFTWARE ✳ Pharmacokinetics and pharmacodynamics modelling analyses were performed using non-linear mixed-effect (NLME) modelling in NONMEM v7.2 and v7.3 (Beal *et al*, 2009) (ICON Development Solution, Hanover, MD). The database and all graphs were created using R v2.13.1 (R Core Team, 2013) (R Foundation for Statistical Computing, Vienna, Austria).

MODEL DEVELOPMENT AND EVALUATION

Plasma scopolamine concentration-time dependent data was analysed using a consecutive NLME modelling approach; once the best pharmacokinetic model was obtained, the individual PK parameter estimates were fixed to develop the pharmacodynamic models. The first order conditional estimation method with interaction (FOCE-I) was used for all data except for the N-back ratio data where a Laplacian method for count data was also compared to FOCE-I. Several compartment models were explored for the pharmacokinetic model, before inter-individual variability (IIV) was tested for PK parameters, as were additive, proportional or combined error models. Body weight, height and age were tested as potential covariates for parameters on which IIV could be identified.

For the PD endpoints, indirect E_{max} model structures were preferred to assess the age-related sensitivity to scopolamine, in terms of EC_{50} and E_{max}. Delay compartments were taken into consideration for the pharmacodynamics models. Once the structural model was defined, IIV was tested in each parameter estimate, as were additive, proportional or combined error models. Correlations between post-hoc Bayesian estimates and between post-hoc Bayesian estimates and potential covariates were explored using Pearson's correlation coefficients (r^2). A coefficient of

determination ≥ 0.4 was considered relevant and taken forward in testing of the omega block structures and covariate analysis (body weight, age and height). Age was tested as a covariate in all models regardless of correlation. Competing models were compared based on their Goodness of Fit (GOF) plots, improvement in OFV, plausibility of parameter estimates, residual error, parameter precision (in terms of residual standard error, RSE), shrinkage and parameter distribution. A decrease in the OFV of 3.84 units ($p<0.05$) was considered statistically significant. GOF plots included population and individual predictions vs. observations, time- and observations-dependent conditional weighted residuals (with interaction) and IIV distribution graphs. Visual Predictive Checks (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 (assuming a normal distribution, with resampling).

RESULTS

MODEL DEVELOPMENT – PLASMA SCOPOLAMINE CONCENTRATIONS

Pharmacokinetic model graphical representation and parameter estimates can be found in Figure 2.1 and Table 2.2, respectively. A two-compartment linear model structure proved superior compared to a one-compartment linear plasma scopolamine pharmacokinetic model (ΔOFV = -1474 points). Inter-individual variability could be identified on the central (V_c) and peripheral (V_p) volumes of distribution and clearance. As displayed in Equation 2.1, age (in years) and body weight (in kilograms) were identified as covariates on clearance (ΔOFV = -29 and -38 points, respectively). Body weight was also identified as a covariate on peripheral volume of distribution (ΔOFV = -13 points; Equation 2.2).

$$CL = \alpha \cdot e^{\{[CAC \cdot \log(\frac{AGE}{28})] + [CWC \cdot \log(\frac{WGT}{78.5})]\}} \quad (2.1)$$

$$Vp = \beta \cdot e^{\{CWV \cdot \log(\frac{WGT}{78.5})\}} \quad (2.2)$$

From the estimated clearance and volumes of distribution we computed the second order rate elimination and inter-compartmental distribution, which are shown in Equations 2.3.

$$k_{10} = \frac{CL}{V_c}, k_{12} = \frac{Q}{V_c} \text{and } k_{21} = \frac{Q}{V_p} \quad (2.3)$$

Finally, the best model's differential equations (2.4 and 2.5) are shown below for the central A_c and peripheral A_p compartments.

$$\frac{dA_c}{dt} = -k_{10} \cdot A_1 - k_{12} \cdot A_1 + k_{21} \cdot A_2 \quad (2.4)$$

$$\frac{dAp}{dt} = k_{12} \cdot A_1 - k_{21} \cdot A_2 \quad (2.5)$$

MODEL DEVELOPMENT – SCOPOLAMINE EFFECTS

The scopolamine effect on the β frequency in the EEG (in both Fz-Cz and Pz-Oz leads) was negligible and therefore the parameter was excluded from the analysis. An indirect E_{max} model accurately described scopolamine's effect on the other pharmacodynamics tests in the CNS test battery.

REACTION TIME IN THE 0-BACK PARADIGM OF THE N-BACK TEST

✳ Older subjects had a slower (prolonged) reaction time at baseline when compared to young subjects while performing the 0-back paradigm of the N-back test (average per group 402 ms for young vs. 476 ms for older subjects). Following scopolamine administration, the Reaction Time of the 0-Back paradigm increased significantly from baseline. Adding IIV to E_{max} provided a non-significant decrease in the OFV (3 points) and therefore was abandoned. Age was identified as covariate for the baseline (presented in Equation 2.6; K_{in}) estimated value (Figure 2.4), which provided a significant decrease in the OFV (24 points) and a better fit.

$$k_{IN} = \lambda \cdot e^{\{\frac{AGE}{CAB}\}} \quad (2.6)$$

SACCADIC EYE MOVEMENTS (SEM) TEST – INACCURACY ✳ Scopolamine increased the saccadic inaccuracy in the SEM test. IIV was identified for K_{in} (baseline), EC_{50} and E_{max}. An omega block structure between EC_{50} and E_{max} was tested. This did not reduce the OFV but provided a reasonable shrinkage reduction from 90.3 to 36.9 % and improved the GOF of the model, and was therefore kept in the model.

SACCADIC EYE MOVEMENTS (SEM) TEST ✳ Saccadic Peak Velocity decreased the saccadic peak velocity in the SEM test. IIV was identified for baseline (K_{in}) and EC_{50}. IIV on E_{max} did not significantly improve the model fit. Age was identified as covariate for EC_{50}, which decreased the OFV (25 points), improved the GOF, and the IIV on the parameter decreased (CV = 3.5 vs. 2.8). Figure 2.3 provides a visual representation of the correlation between age and estimated EC_{50} values. This was the only model from the battery of tests were age was correlated with the estimated EC_{50} (Equation 2.7), indicating a higher sensitivity to scopolamine with increasing age where ε and CAE are estimated coefficients.

$$EC_{50} = \varepsilon \cdot e^{\left\{-\frac{AGE}{CAB}\right\}} \quad (2.7)$$

ADAPTIVE TRACKER TEST (PERCENTAGE OF ACCURACY) ✳ Shortly after scopolamine was administered, an abrupt decrease on the performance in the Adaptive Tracker test was observed. In previous models where E_{max} was estimated, the result was consistently 1 (100% decrease in performance). Fixing E_{max} at 1 resulted in a reduction of the parameter uncertainty, improved the model's stability and resulted in no change in the OFV or GOF. Therefore E_{max} was fixed at 1. The addition of an exponent in the E_{max} function (γ=1.1 ± 0.063), even though this value is near to 1 and has a low variability, improved the model fit and decreased the OFV by approximately 7 points and therefore was accepted. IIV was identified for k_{in} and EC_{50}.

ELECTRO-ENCEPHALOGRAM ✳ Scopolamine induced a decrease of the absolute power in the *α* frequency band in both Fz-Cz and Pz-Oz leads. On the other hand, scopolamine increased the absolute power in both δ and ϑ frequency bands (with a greater effect on the δ frequency band) in the EEG of the analysed bipolar leads (Fz-Cz and Pz-Oz). Addition of E_{max} IIV resulted in a significant decrease of the OFV of 196 points in the *α* Fz-Cz EEG and 22 points in the *α* Pz-Oz models and was therefore accepted. Addition of age as covariate in any of the parameters of the *α* models (both Fz-Cz and Pz-Oz) resulted in an increase in OFV and a worse fit and therefore was abandoned. Addition of E_{max} IIV resulted in a significant decrease of the OFV of 29 points in the δ Fz-Cz EEG and 38 points in the δ Pz-Oz models and therefore was accepted. Age could be identified as covariate for the baseline (K_{in}) estimates in the δ frequency band in Fz-Cz (average of the older 1.360 μV and the younger adults group 1.745 μV) and Pz-Oz (older 1.468 μV vs. younger adults 1.776 μV). Once incorporated, the OFV decreased 68 points in the Fz-Cz and 12 points in the Pz-Oz model. In the δ Fz-Cz model, IIV could be identified for EC_{50} and E_{max} and K_{in}. An omega block structure between EC_{50} and E_{max} and between EC_{50} and K_{in} was tested. This resulted in a model improvement (ΔOFV = -36 and -29 points respectively). In the ϑ frequency band in the Fz-Cz lead (but not in the Pz-Oz lead) IIV tested for E_{max} did not significantly improve any of the models. Age was used as covariate for the baseline measurements (older group average 1.535 μV vs. younger adults 2.024 μV).

PERCENTAGE OF CORRECT ANSWERS IN THE 0-, 1- AND 2-BACK PARADIGMS OF THE N-BACK TEST ✳ Subjects scored at baseline (pre-dose measurements) on average 97.75% for the 0-back, 96.54% for the 1-back and 94.48% for the 2-back test. Following scopolamine administration, the number of correct answers decreased significantly compared to placebo. In all models (0-, 1- and 2-back) IIV was identified on E_{max}, however IIV at EC_{50} could not be identified. For the 0- and 2-back models, IIV was identified at baseline (E_0) and an omega block (variance –covariance structure) between E_{max} and E_0 was required. In the 1-back model IIV was identified at K_{in} and E_{max}, but could not be identified in E_0. Only for the 0-back test, the addition of transit compartments to create a delay for the effect was required (ΔOFV = -80 points). E_{max} was fixed to 1 as this reduced the parameter uncertainty and improved the model's stability while providing only a marginal increase

of the OFV (a maximum of +10 points). No covariates could be identified in any of the estimated model parameters.

MODEL EVALUATION

In general, for the pharmacokinetic, Adaptive Tracker, EEG (α, ϑ and δ frequencies of the Fz-Cz and Pz-Oz leads) and Saccadic Eye Movement (Peak Velocity and Inaccuracy) tests, the GOF plots indicate that the central and individual trend of the data is well described, and that no bias occurs over time or observations. The RSE's are relatively small, indicating acceptable accuracy of the parameter estimates, with acceptable shrinkage (Table 2.2). The VPCs indicate that the variability for these parameters is well described as 95% of the data lie within the 95% prediction interval (Figure 2.1). However, for N-back ratios, even though the VPC graphs indicate that the observations are contained within the 95% prediction interval and the GOF plots show a good description of the central and individual trends of the data, the estimated variability (appreciated by the 95% prediction interval in the VPC graphs shown in Figure 2.1) is considerable, probably related to the great inter- and intra-individual variability observed in the individual observations during the test where a substantial IIV is estimated to describe outliers; this was also reflected in the high estimated shrinkage in the E_{max} (42.1%) of the 0-back and the K_{in} (47.3%) of the 1-back ratio models. Similarly, the shrinkage values for the α frequency EC_{50} (48.4%) in the Fz-Cz lead and δ frequency E_{max} (48.0%) in the Pz-Oz lead were considerable; probably related to the high inter-individual variability of the test and the modest effect of scopolamine on the EEG. Finally, the central volume of distribution V_c in the PK model showed a high shrinkage (49.3%).

DISCUSSION

Our results show that age-related increase in scopolamine exposure (pharmacokinetics), rather than previously hypothesized 'higher sensitivity' of older subjects, explains enhanced age-related anti-cholinergic drug effects. The developed structural model is in agreement with the two-exponential equation describing bi-phasic pharmacokinetics (central and peripheral volume of distribution) as reported in previous literature using non-linear regression (non-compartmental analysis) and NLME methods (Ebert *et al*, 2001; Liem-Moolenaar *et al*, 2011; Putcha *et al*, 1996; Renner *et al*, 2005). Also, the relatively large apparent volume of distribution, mainly of the peripheral compartment, is in agreement with the weak basic non-polar alkaloid high lipophilicity physicochemical properties of scopolamine. Body weight and age were not earlier reported as covariates associated with plasma scopolamine pharmacokinetics. Body weight was directly related and age was inversely related to the total clearance. Scopolamine is hardly detected unchanged in urine (~2.6%), and based on *in vitro* and interaction studies in humans, scopolamine seems to be mainly eliminated hepatically, with CYP3A4 as the responsible elimination pathway (Renner *et al*, 2005). Increasing age has been correlated with a diminished blood flow to the liver and a reduced liver volume and functional capacity, explaining reduced clearance with increase in age (Woodhouse and James, 1990; Wynne *et al*, 1989). Medication was controlled in the current studies, however use of concomitant CYP3A inhibitors, thereby further decreasing clearance, could explain the higher incidence of side effects in other studies in older subjects after scopolamine administration, especially if scopolamine exposure was not taken into consideration.

The neuro-physiologic eye movement parameter saccadic peak velocity was the only test where an age related augmented sensitivity to scopolamine was found, a finding not earlier reported. Age was correlated with a decline in the EC_{50} in the Peak Velocity of the horizontal Saccadic Eye Movements test with a decrease mainly observed above ~45 years. Pons and midbrain, both located in the brainstem, are particularly rich in muscarinic acetylcholine receptors (Kobayashi *et al*, 1978) which are an important binding site for anti-muscarinic compounds as demonstrated *in vivo* with imaging techniques that measured enrichment of radiolabeled scopolamine in these brain areas (Frey *et al*, 1992). The horizontal Saccadic Movements test evaluates the coordinated action of brain areas that involve the occipital lobe for acquisition and brain stem structures such as the pons and midbrain for execution, explaining the high sensitivity of this test in detecting scopolamine effects.

Previous studies have reported significant differences in sensitivity of older subjects compared to younger subjects after scopolamine administration, only while carrying out cognitive tests involved in short term acquisition or verbal and numeric working memory (Molchan *et al*, 1992; Ray *et al*, 1992; Zemishlany and Thorne, 1991) or visuo-spatial praxis(Flicker *et al*, 1992). The N-back test was the most sensitive experiment performed in our battery of memory tests specifically evaluating verbal acquisition, processing and execution. Even though in all three paradigms (0-, 1- and 2-back) a small percentage of the subjects in the older subjects group scored lower even when receiving a lower dose compared to the younger group, the great majority did not have a larger decrease in the performance. The above-mentioned outliers in the older subjects group scored consistently poorer in the three tests as reflected in the individual E_{max} estimates graphed per age (Figure 2.2). A larger number of older subjects scored lower compared to the younger group where subjects with an estimated E_{max} above the sample's upper 95% CI were above 65 years, even when receiving a lower dose, at the population level there was no significant difference between the higher decrease in the score or baseline and age. This might provide evidence that, while in general older subjects perform similarly to younger adults after scopolamine administration in tests evaluating verbal working memory and execution, the number of subjects who perform worse during the test increases with a higher age; suggesting that although age as such is not correlated with a worse performance during scopolamine administration, the number of subjects with a higher sensitivity to the anti-cholinergic compound increases with age. Future research should be directed to longitudinally study whether these subjects are prone to develop Mild Cognitive Impairment.

Contrary to previous literature, we suggest that scopolamine administration does not lead to an increase in sensitivity of cognitive function tests (e.g.: decrease in performance) in healthy older subjects compared to healthy young. This finding suggests that increased sensitivity to scopolamine in healthy older subjects may not be due to incipient cholinergic neuronal degeneration in the majority of older subjects. Healthy subjects of advanced age perform similar to younger subjects after scopolamine administration in tests evaluating verbal working memory and execution. This finding seems to be

in line with previous experiments that could not demonstrate a decline in the availability of nicotinic acetylcholine receptors with increasing age in healthy human older subjects with a MMSE score above 27 points, a similar population in the current study (Ellis *et al*, 2009). Prescription of scopolamine in healthy older subjects should be in general safe and well tolerated, however caution is advised when co-administered with CYP3A4 inhibitors or in subjects with a low weight since the apparent peripheral volume of distribution was influenced by total body weight. Further studies should be performed in subjects above 78 years and in subjects with cognitive impairment.

Regarding the reaction time measured during the N-back test at baseline, older subjects were slower in responding than younger subjects. Nevertheless once scopolamine was administered, the increase in the reaction time was similar in both groups. According to the estimations in our model, on the average population estimate, a subject would increase his/her reaction time with a rate of 4.46% every 10 years (Figure 2.4), similar to a decrease previously reported (Ratcliff *et al*, 2001).

Scopolamine effects on the resting state EEG performed with eyes closed included a decrease in α and an increase in δ and ϑ frequencies of both Fz-Cz and Pz-Oz leads. In addition, no difference in sensitivity was found between older and younger subjects after scopolamine administration. These findings are consistent with previous literature; intramuscular administration of 0.25 mg of scopolamine in subjects between 24 and 31 years produced a significant increase in absolute δ power and in relative δ and ϑ power in subjects with eyes closed, mainly over central and parieto-occipital regions, and a decrease in absolute and relative α power, widespreaded but mainly over frontal regions (Kikuchi *et al*, 1999). Higher intramuscular doses of scopolamine (0.5 and 0.75 mg) induced a dose-related increase of relative power in low- and high-frequencies and a decrease in the α range in the posterior leads (Sannita *et al*, 1987). A previously published PK-PD model that described the EEG effects of an intravenous and intramuscular administration of 0.5 mg of scopolamine reported a decrease in the α power over the frontal, central, and occipital brain areas compared to placebo, explained by a sigmoidal E_{max} model. Even though non-significant, the appreciable changes in the δ, ϑ and β frequencies were previously reported (Ebert *et al*, 2001).

In the present study, an age related difference in the EEG baseline was detected in the low frequency ranges (δ and ϑ) but not in the *α* frequency. Older subjects had a decreased absolute power in the δ frequency and ϑ frequency. These findings are consistent with reported literature where an age dependent decrease in EEG low frequency activity was identified (Leirer *et al*, 2011).

Due to teratogenicity risks related to other studied drugs rather than scopolamine, no women were allowed to participate in the first three studies, however no sex differences are to be expected based on neuroimaging morphology (Raz *et al*, 2005) and longitudinal assessments in cognitive function (Aartsen *et al*, 2004).

This study provides evidence that plasma scopolamine concentrations in the healthy older subjects are influenced not only by body weight but also by age and once this difference in exposure is corrected for, most age-related increases in anticholinergic sensitivity disappear. The peak velocity of the saccadic eye movements was the only test where sensitivity increased with age.

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TABLE 2.1 Subject demographics.

Mean ± Standard Deviation. Age is presented as Mean (minimum – maximum).

	Trial 1	Trial 2	Trial 3	Trial 4	All
Subjects (n)	43	43	13	36*	135
Age (years)	29.3 (18 - 55)	27.7 (18 – 55)	25.4 (19 – 36)	69.0 (65 - 78)	39.0 (18 - 78)
Weight (kg)	78.6 ± 7.54	78.6 ± 7.74	81.1 ± 9.76	77.8 ± 11.82	78.6 ± 9.08
BMI ($kg \cdot m^{-2}$)	23.6 ± 2.30	23.3 ± 2.69	24.6 ± 2.82	25.9 ± 2.51	24.2 ± 2.73
Scopolamine dose (mg)	0.5	0.5	0.5	0.3	NA

** 13 female subjects. NA: not applicable.*

TABLE 2.2 Population estimates for pharmacokinetic and pharmacodynamic models for scopolamine.

Parameters are reported as population estimate ± standard error. IIV: Inter-individual Variability expressed as Coefficient of Variation and shrinkage between parenthesis.

	CL [α] (L · min^{-1})	IIV (shrinkage)	VC (L)	IIV (shrinkage)	V_p [β] (L)	IIV (shrinkage)	Q (min^{-1})	CAC	CWC	CWV	Error (σ^2)
Scopolamine pharmacokinetics	1.09 ± 0.096[AB]	10.3% (9.7)	2.66 ± 1.050	74.1% (49.3)	62.10 ± 10.100 [B]	9.5% (25.6)	1.01 ± 0.247	-0.12 ± 0.019	0.56 ± 0.097	0.38 ± 0.120	0.045 (prop)
	EC50 [ε] (pg·ml^{-1})	IIV (shrinkage)	E_{max} (%)	IIV (shrinkage)	K_{in} [λ]	IIV (shrinkage)	K_{out}	CAB or CAE	γ [c]	OC	Error (σ_2)
0-back RT (msec)	1300 ± 363	99.8% (33.2)	0.837 ± 0.118	NA	3.42 ± 0.361[A]	10.5% (8.13)	0.00974 ± 0.00099	229 ± 40.9	NA	NA	0.00999(prop)
Saccadic Inaccuracy (%)	55.1 ± 29.8	13.7% (36.9)[e]	0.388 ± 0.079	49.9% (36.2)[e]	22.8 ± 0.731	13.2% (21.86)	3.33 ± 0.0398	NA	NA	-0.06 ± 0.157	0.0493 (prop)
Saccadic Peak Velocity (deg · sec^{-1})	2530 ± 213[A]	277.0% (20.9)	0.232 ± 0.00113	NA	1280 ± 21	9.9% (3.1)	2.73 ± 0.0468	34.0 ± 1.39	NA	NA	768.0 (prop)
Adaptive Tracker (%)	386 ± 22.6	85.5% (10.50)	1	NA	0.636 ± 0.0413	22.4% (4.1)	0.0294 ± 0.0018	NA	1.10 ± 0.063	NA	8.64 (add)
EEG alpha Fz-Cz (μV)	2.55 ± 1.06	1530.0% (48.4)	0.232 ± 0.0258	95.4% (14.9)	0.206 ± 0.0344	46.6% (1.1)	0.0699 ± 0.0113	NA	NA	NA	0.0256 (prop)
EEG alpha Pz-Oz (μV)	14.7 ± 0.168	1309.3% (27.9)	0.443 ± 0.00389	37.6% (18.0)	3.05 ± 0.135	57.9% (2.1)	0.553 ± 0.0055	NA	NA	NA	0.0429 (prop)
EEG delta Fz-Cz (μV)	469 ± 70.8	154.5% (35.5)[e]	0.419 ± 0.0515	325.4% (19.2)[e]	0.0354 ± 0.00369[A]	28.9% (2.1)[e]	0.0166 ± 0.00161	161 ± 30	NA	1.410 ± 0.722 (EC50 vs. E_{max}); -0.153 ± 0.0494 (E_{max} vs. k_{in})	0.0338 (prop)
EEG delta Pz-Oz (μV)	1230 ± 368	1166.2% (29.6)	0.537 ± 0.0601	55.8% (48.0)	0.726 ± 0.186 [A]	30.3% (1.7)	0.348 ± 0.084	210 ± 63.7	NA	NA	0.0304 (prop)
EEG theta Fz-Cz (μV)	4110 ± 1410	2162.7% (31.1)	0.594 ± 0.106	NA	0.863 ± 0.0422[A]	28.4% (1.8)	0.594 ± 0.106	142 ± 24.7	NA	NA	0.0297 (prop)
EEG theta Pz-Oz (μV)	8240 ± 6270	1778.6% (27.9)	1.15 ± 0.179	NA	13 ± 8.79	33.4% (0.5)	6.08 ± 4.23	NA	NA	NA	0.0379 (prop)
	EC50 (pg·ml^{-1})	K_{in}	IIV (shrinkage)	K_{out}	E_{max} (%)	IIV (shrinkage)	E_0	IIV (shrinkage)	NA	OC	Error (σ^2)
0-back ratio (% correct answers)[f]	2140 ± 1720	0.0354 ± 0.00244[d]	NA	0.036 ± 0.0061	1	631.3% (42.1)[e]	3.77 ± 0.274	37.8% (20.1)[e]	NA	0.40 ± 0.141	0.0039 (add)
1-back ratio (% correct answers)[f]	0.624 ± 0.0053	1.10 ± 0.0175	578.5% (47.3)	221 ± 4.35	0.961 ± 0.00511	121.6% (30.0)	3.33 ± 0.0293	NA	NA	NA	0.0059 (add)
2-back ratio (% correct answers)[f]	212 ± 72.4	0.54 ± 0.103	NA	0.317 ± 0.0704	1.09 ± 0.248	109.8% (31.9)[e]	2.84 ± 0.164	35.9% (21.5)[e]	NA	0.23 ± 0.107	0.0085 (add)

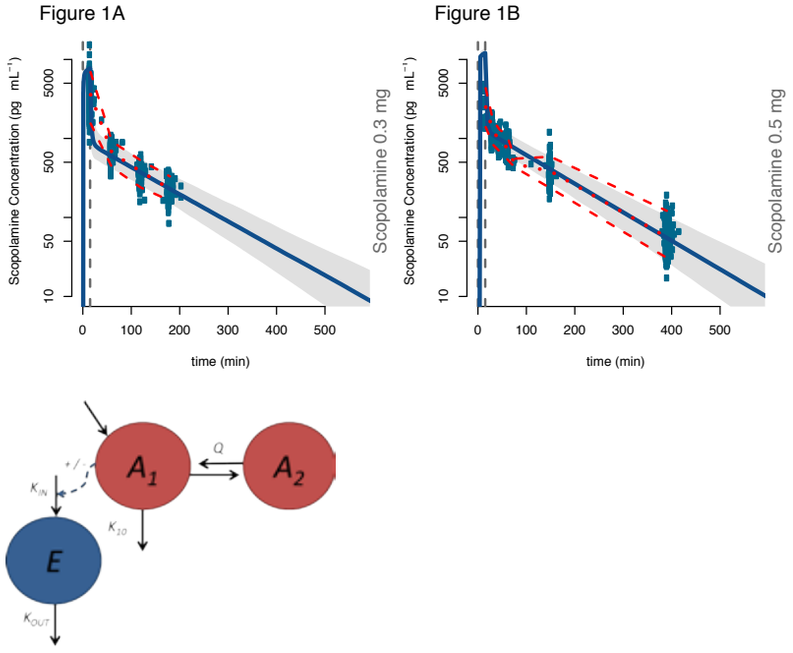
Prop: proportional error. CAC: coefficient relating age and clearance. CWC: coefficient relating age and weight. CWV: coefficient relating apparent peripheral volume of distribution and weight.

Baseline is calculated as the ratio of K_{in} and K_{out}. **A**. Age used as a covariate (ε and λ in Equation 2.1). **B**. Weight used as covariate. **C**. Exponent in E_{max} (γ). **D**. 4 buffer compartments. **E**. Omega block structure. **F**. Parameters reported as natural log odds.

CAB: coefficient relating age and K_{in}. CAE: coefficient relating age and EC50. OC: correlation between Omegas. Eo: baseline. Add: additive error. NA: not applicable.

FIGURE 2.1 Scopolamine pharmacokinetics and pharmacodynamic effects.

The column on the left represents the scopolamine 0.3 mg group, the middle column the scopolamine 0.5 mg group and the column on the right the placebo group. Figure 2.1A and 2.1B represent the plasma scopolamine concentrations. Figure 2.1C is the schematic representation of the model where the **red** circles represent the central (A_1) and peripheral (A_2) PK compartments and the **blue** circle (E) the effect (PD) compartment. Figure 2.1D, 2.1E and 2.1F display the Adaptive Tracker test, Figure 2.1G, 2.1H and 2.1I the Peak Velocity of the Saccadic Eye Movements test, 2.1J, 2.1K, 2.1L the Reaction Time of the 0-Back test. The continuous **blue** line represents the model population predicted values per group and the grey area represents the 95% prediction interval. Circles represent the observations. The middle **red** discontinuous line represents the median of the observations with the upper and lower 95% confidence interval. Dotted, vertical **grey** lines represent the start and stop time of the scopolamine infusion. The differences in baseline-estimated values are indicated by a **red** line and the population value (when applicable). Simulations were performed with 1000 subjects.



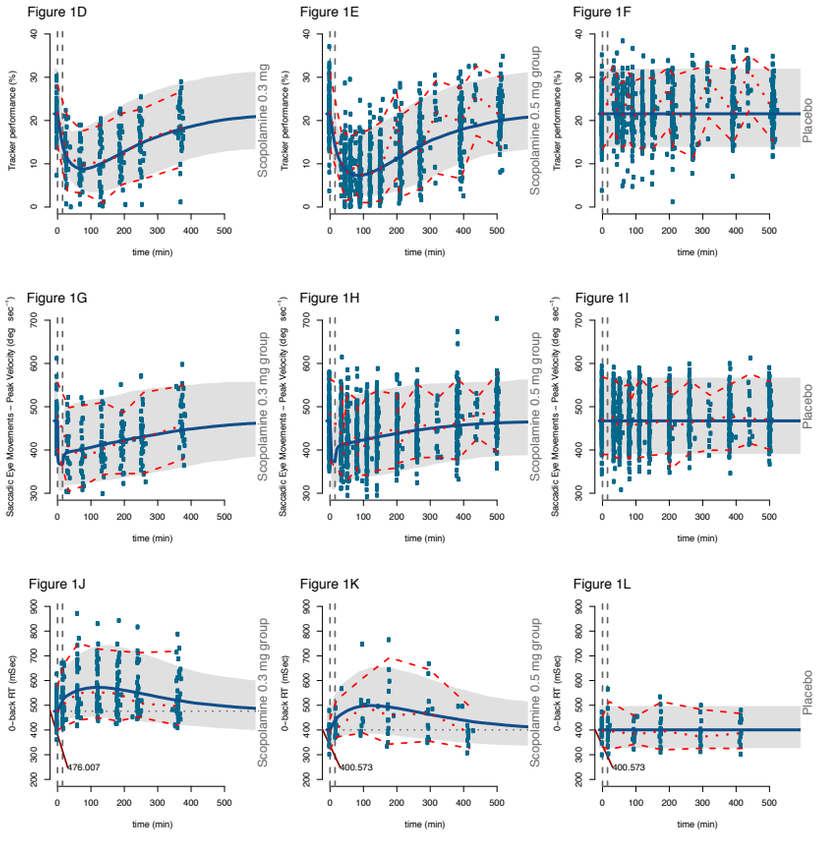


FIGURE 2.2 Scopolamine pharmacodynamic effects in the N-back (number of correct answers).

The row on the left represents the scopolamine 0.3 mg group (Figures 2.2A, 2.2D and 2.2G), the middle row the scopolamine 0.5 mg group (Figures 2.2B, 2.2E and 2.2H) and the row on the right the estimated E_{max} (Figure 2.2C, 2.2F and 2.2I). The first row represents the 0-back, the second row the 1-back and the last row the 2-back percentage of correct answers in the paradigm. The continuous **blue** line represents the model population predicted values per group and the **grey** area represents the 95% prediction interval. Circles represent the actual observations. Dotted, vertical **gray** lines represent the start and stop time of the scopolamine infusion. Simulations were performed with 1000 subjects.

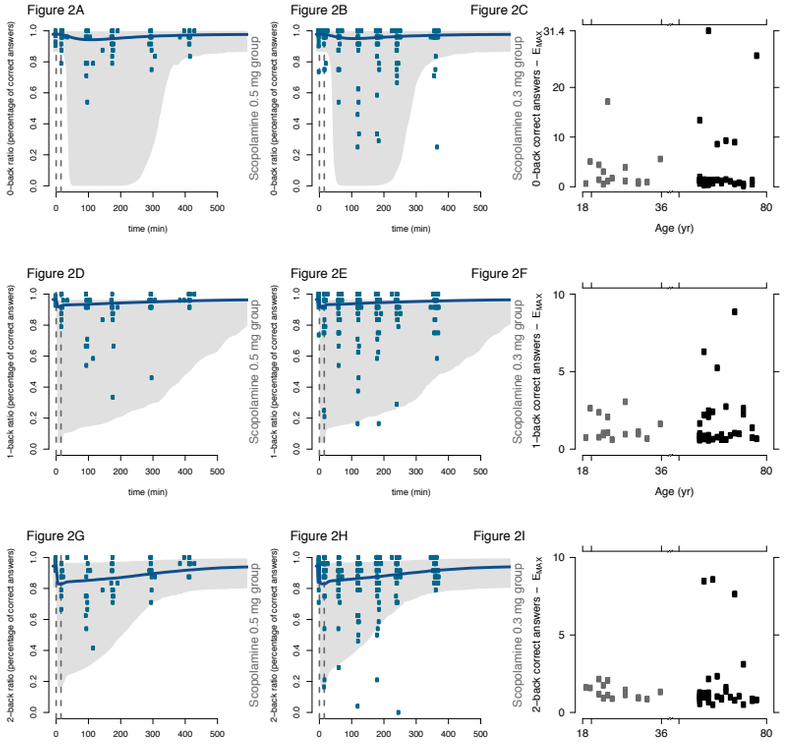


FIGURE 2.3 Relationship between EC_{50} and age in the Saccadic Eyes Movement Peak Velocity.

The estimated EC_{50} value is represented by the **black** (younger adults) and **grey** (older adults) circles plotted against age. The continuous line represents the function with the population values for EC_{50}.

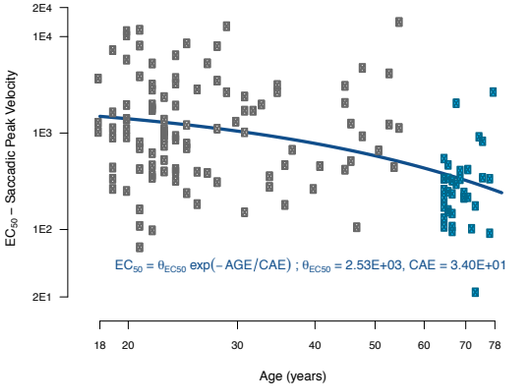


FIGURE 2.4 Relationship between κ_{in}, baseline and age in the Reaction Time of the 0-back test.

The estimated κ_{in} value is represented by the **black** (younger adults) and **grey** (older adults) circles plotted against age. The right column represents the baseline value in milliseconds. The continuous line represents the function with the population values for κ_{in}.