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A simple physiologically based pharmacokinetic model evaluating the effect of anti-nicotine antibodies on nicotine disposition in the brains of rats and humans

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Abstract

Physiologically based pharmacokinetic (PBPK) modeling was applied to investigate the effects of anti-nicotine antibodies on nicotine disposition in the brains of rats and humans. Successful construction of both rat and human models was achieved by fitting model outputs to published nicotine concentration time course data in the blood and in the brain. Key parameters presumed to have the most effect on the ability of these antibodies to prevent nicotine from entering the brain were selected for investigation using the human model. These parameters, which included antibody affinity for nicotine, antibody cross-reactivity with cotinine, and antibody concentration, were broken down into different, clinically-derived *in silico* treatment levels and fed into the human PBPK model. Model predictions suggested that all three parameters, in addition to smoking status, have a sizable impact on anti-nicotine antibodies' ability to prevent nicotine from entering the brain and that the antibodies elicited by current human vaccines do not have sufficient binding characteristics to reduce brain nicotine concentrations. If the antibody binding characteristics achieved in animal studies can similarly be achieved in human studies, however, nicotine vaccine efficacy in terms of brain nicotine concentration reduction is predicted to meet threshold values for alleviating nicotine dependence.

Keywords and phrases

Physiologically based pharmacokinetic model; PBPK; Nicotine vaccine; Anti-nicotine antibodies; Nicotine disposition; Nicotine dependence

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Conflict of interest statement

The authors declare that they have no conflicting interests.

Author contributions

Kyle Saylor carried out the data acquisition, model design and construction, and model output analysis, in addition to drafting the manuscript. Dr. Chenming Zhang contributed to project conception, manuscript revision, and final approval of the manuscript contents for submission.

Transparency document

The Transparency document associated with this article can be found in the online version.

1. Background

The use of tobacco products is the leading preventable cause of death in the world today, resulting in nearly six million deaths annually and assisting in the manifestation of many other diseases (World Health Organization, 2013). In addition, the economic damages caused by tobacco smoking in the US alone over the past four decades could exceed \$7 trillion USD (extrapolated from CDC reported data, unpublished). Existing therapeutic treatments have proven to be more and more ineffective at assisting those with the desire to quit (Messer et al., 2008) and, if current trends continue, more than 1 billion people may die from smoking related diseases in the 21st century (World Health Organization, 2013).

Nicotine is the main addictive compound found in tobacco products (Benowitz, 2009). It has been shown to induce rewarding behavior through stimulation of brain mesolimbic dopamine neurons (Corrigall et al., 1992), prompting addiction and directly instigating the withdrawal symptoms experienced by those trying to quit (de Villiers et al., 2010). Intuitively, the addictive properties of tobacco could be avoided if nicotine molecules were prevented from entering the brain. The resulting effect could be a significant reduction in the number of potential quitters that eventually relapse. One possible therapeutic option that applies this concept as a mode of stemming nicotine addiction is the nicotine vaccine.

There are currently a number of studies claiming the successful application of a conjugate nicotine vaccine in animals (Hieda et al., 1997, 1999, 2000; Keyler et al., 1999, 2008; Pentel et al., 2000; Pentel and Malin, 2002; Satoskar et al., 2003; de Villiers et al., 2010, 2013; Moreno et al., 2010; Cornish et al., 2011; Pravetoni et al., 2011, 2012; Lockner et al., 2013, 2015; Pryde et al., 2013; Hu et al., 2014; Miller et al., 2014; Zheng et al., 2015). In the majority of these studies, immunogenic carrier proteins are conjugated to nicotine molecules *via* a linker modification added to nicotine's pyrrolidine ring or pyrodine ring (Keyler et al., 1999). These newly formed protein-hapten conjugates are then injected into patients with an accompanying adjuvant, eliciting an immune response and prompting the generation of antibodies with a high affinity for nicotine (Raupach et al., 2012). After successful vaccination, exposure to tobacco products (either firsthand or secondhand) results in nicotine being sequestered in the blood and extracellular fluid by the anti-nicotine antibodies (Pentel and Malin, 2002). It is important to note that these antibodies do not "eliminate" nicotine from the body, but rather only temporarily impede the mobility and pharmacokinetic activity of nicotine molecules by forming nicotine-antibody complexes (Hieda et al., 1997). Ultimately, the formation of these nicotine-antibody complexes induces a dampening effect that reduces spikes of nicotine concentration in the brain and provides a controlled, incremental release of nicotine in the blood (Keyler et al., 1999). Dampening nicotine kinetics in this way has been proven to aid the dissolution of nicotine addiction in humans who achieve high immunogenic responses to conjugate nicotine vaccines (Hatsukami et al., 2005, 2011; Cornuz et al., 2008).

Appreciable anti-nicotine antibody concentrations have been achieved in human subjects using protein-hapten conjugate vaccines in the past, with some studies even proving successful enough to proceed to clinical trials (Hatsukami et al., 2005, 2011; Maurer et al., 2005; Cornuz et al., 2008; Wagena et al., 2008; Esterlis et al., 2013). However, none of these

studies have proceeded past Phase III clinical trials due to vaccinated subjects' failure to exhibit increased abstinence rates when compared to subjects given a placebo (Raupach et al., 2012). The overall lack of positive results from human studies illuminates a need for new approaches to the nicotine vaccine design process. Therefore, the majority of current nicotine vaccine research focuses on the investigation of the key variables thought to have the most impact on the dampening effect described earlier, such as vaccine immunogenicity and vaccine-elicited antibodies' affinity for nicotine and its major metabolites (Raupach et al., 2012).

A review of factors that are suspected to have a significant impact on nicotine vaccine efficacy prior to additional animal and human experimentation could aid in understanding the success gap between these studies. Additionally, it might prove particularly useful when evaluating human-specific factors that cannot be evaluated in animal studies, such as nicotine elimination rates, cross reactivity of the major nicotine metabolite cotinine with anti-nicotine antibodies, and those factors that have the most impact on the ability of nicotine to enter the brain, such as cerebral volume and blood flow rate. Now that sufficient public data exists on the subject of nicotine vaccination and nicotine metabolism, it may be prudent to employ an *in silico* method outside of *in vivo* experimentation to evaluate all of these variables.

As computational capacities continue to improve, *in silico* methods are becoming an increasingly relevant approach to drug evaluation outside of living systems. When paired with a pharmacokinetic model, they can effectively save time, money, and resources by predicting a drug's disposition and pharmacokinetics prior to animal and human trials. Currently, the most widely used form of pharmacokinetic model for *in silico* research is the physiologically based pharmacokinetic model (PBPK) (Yang et al., 2004). These models are usually multi-compartmental constructs that utilize species-specific, tissue-to-blood partition coefficients coupled with tissue volumes or weights, average tissue blood flow rates, and mathematical relationships to make predictions about xenobiotic kinetics and disposition. Complexity of PBPK models varies greatly between individual studies, as their design is based upon the complexity of output variables and the desired scale and accuracy of model predictions. Many pharmacokinetic and toxicokinetic models of nicotine and cotinine disposition kinetics have been proposed in literature (Gabrielsson and Bondesson, 1987; Plowchalk et al., 1992; Robinson et al., 1992; Schwartz et al., 1993; Yamazaki et al., 2010; Teeguarden et al., 2013; Gajewska et al., 2014). However, to date there have been no models constructed that evaluate the effect of anti-nicotine antibodies on nicotine disposition in the blood and in the brain.

In this study, data relevant to nicotine and cotinine metabolism, nicotine and cotinine affinity to anti-nicotine antibodies, maximal concentrations of antibody achieved in humans using clinically approved materials and procedures, and various other parameters necessary for the generation of a compartmental model were gathered for both rats and humans. All of this information was applied to two PBPK models (one rat and one human) in order to investigate key issues that are thought to negatively affect nicotine vaccine efficacy in terms of percent decrease in brain nicotine concentration. The ultimate goal of this project was to

provide a novel approach to evaluating the efficacy of potential nicotine vaccines prior to human trials and extended animal trials.

2. Methods

2.1. Approach to model construction

Human and rat pharmacokinetic data were obtained from literature and applied to two mechanistic, multi-compartmental nicotine and cotinine PBPB models constructed in Matlab's SimBiology application. Similar to existing studies, it was assumed that simple mass action kinetics and flow-limited tissue distributions could accurately predict nicotine and cotinine systemic distribution (Plowchalk et al., 1992; Teeguarden et al., 2013; Gajewska et al., 2014). Model design was inspired by previous works used to predict nicotine and cotinine pharmacokinetics and pharmacodynamics in both rats and humans, with the most notable works being those of Plowchalk et al. (1992) and Teeguarden et al. (2013). It was initially determined that a simple model consisting of only a blood, brain, and liver compartment would be sufficient to simulate nicotine metabolism and evaluate the effect of anti-nicotine antibodies on nicotine systemic distribution, similar to the model proposed by Yamazaki et al. (2010). This approach was abandoned, however, due to evidence presented by Satoskar et al. (2003) that suggests reduced nicotine concentrations in the brain not only result from anti-nicotine antibodies sequestering nicotine in the blood, but also from the distribution of nicotine to the tissues being disproportionately redirected away from the brain. This effect could not be accounted for in the three compartment model.

Parameters acquired from literature were calibrated using existing nicotine and cotinine blood and tissue concentration time course data in rats (Hieda et al., 1999; Keyler et al., 1999) and in humans (Benowitz et al., 1982; Benowitz et al., 1991; Hukkanen et al., 2005). Model accuracy simulations were subsequently performed. Calibration was achieved *via* SimBiology's data fit task followed by manual adjustment of parameters to provide an optimal visual fit. Less emphasis was placed on the accuracy of tissue concentrations while more emphasis was placed on the accuracy of both the venous and arterial blood concentrations due to the large variability in both calculated (Poulin and Theil, 2000; Schmitt, 2008; Peyret et al., 2010) and experimentally determined (Gabrielsson and Bondesson, 1987; Benowitz et al., 1990; Plowchalk et al., 1992; Thompson et al., 2012) partition coefficients. However, a single exception to this approach was made for the nicotine brain-to-blood partition coefficient, since the evaluation of nicotine concentration in the brain was one of the main foci of this study.

2.2. Major model assumptions

Many key assumptions were made that allowed for the construction of the PBPB models described in this study. In order to include the effect of anti-nicotine antibodies on nicotine distribution, it was assumed that peak antibody concentrations had already been achieved *via* vaccination. These antibody concentration values were based off of previously published data and did not represent immunogenic capacity of nicotine vaccines as a whole. Making this assumption led to model predictions overestimating vaccine efficacy at all time points other than when peak anti-nicotine antibodies had been achieved. This result was intentional,

as these predictions could be viewed as good indicators of vaccine potential. Alternatively, different antibody concentrations could be applied to these models in order to predict vaccine efficacy at different time points during the course of the vaccination process. It was assumed that the anti-nicotine antibodies' affinity for cotinine would be an important factor in the efficacy of any nicotine vaccine in humans, an assumption that was based on the large proportion of serum nicotine that is metabolized into cotinine, the long residence time of serum cotinine, and the structural similarities between the two molecules (de Villiers et al., 2010). Blood composition was assumed to be 55 parts serum to 45 parts red blood cells (Ballard, 1997), values that allowed for the conversion of the serum antibody concentrations reported in literature to the whole blood antibody concentrations that were defined in the model. Antibody binding capacities were calculated using whole blood antibody concentrations and the assumptions that each antibody can only bind a maximum of two nicotine molecules at any given time and that anti-nicotine antibodies have an average molecular weight of 150 kDa (Hieda et al., 1999; Keyler et al., 1999). Additionally, many more important assumptions were included in the model (following the conventions set by previous PBPK models), such as assuming instantaneous mixing within compartments (Plowchalk et al., 1992; Teeguarden et al., 2013), partition coefficients independent of time and concentration parameters (Plowchalk et al., 1992), minimal cellular uptake and protein binding of chemical species (Hukkanen et al., 2005), complete access of entire blood volume to circulatory flow (Plowchalk et al., 1992; Teeguarden et al., 2013), and even distribution of nicotine and cotinine between red blood cells and serum (Yamazaki et al., 2010).

2.3. Compartmental design

The majority of tissues included in the model were chosen based on their perceived relevance to overall nicotine and cotinine distribution and the availability of necessary data. Partitioning between compartments was achieved by using published tissue-to-blood partition coefficients (defined in this study as steady-state tissue to venous blood concentration ratios), tissue volumes, and tissue blood flow rates. The eleven compartments selected for use in this model are shown in Fig. 1. Blood flow was modeled in a cyclic manner, with the lung compartment accounting for all blood flow between the venous and arterial compartments. Returning blood flow was distributed between the remaining interior compartments and was based on published and calibrated tissue blood flow rates. Partition coefficients were used to account for the flow-limited (Hukkanen et al., 2005) distribution of nicotine and cotinine between blood and tissue.

The antibody sub-compartments illustrated in Fig. 1 represent compartment specific anti-nicotine antibody species and the arrows represent antibody association and dissociation with nicotine (rat and human models) and cotinine (human model). Tissue retention kinetics (aggregation and dispersion) were conceptualized for the brain compartment in order to account for tissue concentration changes that could not be accounted for by using a partition coefficient alone. Antibody mobility between compartments was not accounted for in either the rat or the human model due to lack of necessary parameters, such as the tissue-to-blood partition coefficients associated with antibody systemic distribution. Blood volume was divided between arterial and venous blood, with arterial blood accounting for approximately three quarters of the total blood volume (Dill and Costill, 1974). Dosing was applied directly

to the lung compartment when published, experimental data was obtained from cigarette smoking or nicotine inhalation. Following similar logic, when published, experimental data were obtained from intravenous infusion, the simulation dose was applied to the venous blood compartment.

2.3.1. Clearance organ compartments—The final model design applied hepatic and renal elimination directly to the arterial blood compartment, which is similar to the approach taken by Teeguarden et al. (2013). This method can effectively account for these processes because the hepatic and renal elimination rates of both nicotine and cotinine are measured as a function of changes in blood concentration, as opposed to changes in liver and kidney concentrations (Hukkanen et al., 2005). The metabolism of nicotine to cotinine in the human model was directed to the liver compartment to avoid immediate metabolism of cotinine. Being stripped of their primary physiological function, both the liver and the kidney compartments only served as partitions between the venous and arterial blood compartments. It was assumed that this function would still provide a significant contribution to model predictive accuracy, however, due to the large portion of cardiac output supplied to these tissues.

2.3.2. Calibration compartment—Parameters associated with the ‘other’ compartment served as a means of model calibration for venous nicotine concentrations in both the human and rat model and for venous cotinine concentration in the human model when antibodies were absent. This seemed to be the most logical approach to calibration when considering that the ‘other’ compartment represents organs and tissues that are absent from both models due to lack of published parameters. Calibration of the blood flow rate in the rat model resulted in blood flow fractions that added to a value greater than one, indicating a physiologically impossible situation in which blood flow to the tissues was greater than cardiac output. This issue could be corrected by providing adjusted blood flow fractions, but no adjustment was made due to priority being placed on providing references for all physiological parameters. Blood flow rate through the ‘other’ compartment in the human model was determined by calculating the cardiac output and then balancing that value *versus* all of the blood flow rates published for the remaining tissue compartments. Partition coefficients for the ‘other’ compartment were also calibrated in both the rat and the human model.

2.3.3. Brain compartment—Brain partition coefficient, blood flow rate, and retention parameters in the rat PBPK model were calibrated using data from Hieda et al. (1999). This calibration step resulted in brain partition coefficients that do not physiologically represent a true steady state tissue to blood concentration ratio but rather represent overall nicotine transport to the brain when paired with the tissue retention parameters. Published brain nicotine concentrations were converted from units of mass nicotine per mass tissue to units of moles nicotine per volume tissue using a mean brain tissue density value of 1.04 g/L (Brown et al., 1997) so that they could be compared with brain nicotine concentrations predicted by the models. Identical calibration steps to those performed in the rat model involving the brain could not be completed in the human model due to the lack of available

data. As such, the brain partition coefficient and retention parameters calibrated in the rat model were also applied to the human model.

2.4. Model parameters

Parameters necessary for the construction of both the rat and human PBPK models were collected from various sources, with the majority consisting of averaged values (non-specific for sex, ethnicity, breed, *etc.*). Mean tissue volume fractions were published as a function of subject mass and were obtained from literature for both the rat and human compartmental models (Brown et al., 1997). Nicotine partition coefficients associated with each tissue were obtained from Teeguarden et al. (2013) for both the rat and human compartmental models, with the exception of the calibrated values in the brain and ‘other’ compartments. Cotinine partition coefficients for all tissue compartments were calibrated visually using published starting values (Thompson et al., 2012) and were only used in the human model where cotinine is a major metabolite. Cardiac output for the rat and human models were calculated based on subject mass (Brown et al., 1997; Gajewska et al., 2014). Tissue blood flow rates were obtained from literature and based on a percentage of overall cardiac output (Brown et al., 1997), with the only exception being the calibrated ‘other’ compartment blood flow rate. Rat brain nicotine retention kinetics were accounted for by using dispersion and aggregation parameters. These values were calibrated by fitting model predictions for rat brain concentration with Hieda et al. (1999) brain nicotine concentration time course data *via* the data fit task in SimBiology. Human metabolism rates were obtained from literature (Hukkanen et al., 2005) and rat metabolism rates were calibrated using Keyler et al. (1999) blood nicotine concentration time course data. Parameters used by the human and rat PBPK models can be found in Tables 1–3.

2.5. Vaccine efficacy factors

The key variables that were used to evaluate vaccine efficacy in the human model were antibody concentration, antibody affinity for nicotine, and antibody cross-reactivity with cotinine. These variables have been identified in previous studies as being the most important factors influencing nicotine access to the brain (Raupach et al., 2012). The effect of resting cotinine levels (which are correlated with smoking status) on vaccine efficacy was also investigated. Increasing systemic antibody concentration allows more nicotine to be bound in the blood, decreasing the likelihood that free nicotine will be able to traverse the blood-brain barrier (Keyler et al., 2008). Antibody affinity for nicotine characterizes nicotine-antibody interactions, which determine how quickly nicotine associates with and dissociates from anti-nicotine antibodies. Antibody cross-reactivity with cotinine may have a profound effect on the number of free antibodies able to bind nicotine when cotinine levels are elevated in the blood (Hukkanen et al., 2005; de Villiers et al., 2010). Finally, high resting cotinine levels could have a detrimental effect on vaccine efficacy, especially when anti-nicotine antibodies have a high affinity for cotinine. Different treatment levels of these key variables were selected using published values in human clinical trials as references and then applied to the human PBPK model in order to determine their effect on vaccine efficacy. Due to programming limitations in SimBiology, antibody affinity had to be divided into associative (on) and dissociative (off) terms. This was achieved by estimating a reasonable antibody-nicotine dissociation rate using limitations described by Poulsen et al.

(2011) and then using this value along with a published antibody affinity value to calculate the association rate. The cotinine dissociation rate was estimated to be double that of the nicotine dissociation rate. Anti-nicotine antibody affinity for cotinine had to be estimated using cotinine and nicotine IC₅₀ (half maximal inhibitory concentration) ratios paired with nicotine antibody affinity values reported in literature. Since the ratio between off and on rates is what determines the extent of antibody binding, setting an antibody dissociation rate that slightly deviates from the true pharmacokinetic value should have little influence on model predictions.

2.6. Calibration

Nicotine concentration time course data were obtained from published sources along with dosing, antibody concentration range, and mean antibody affinity data. The calibration of the rat PBPK model parameters was then performed in three subsequent steps. The first step involved calibrating the nicotine metabolism rate, the 'other' blood flow rate, and the 'other' partition coefficient using data published by Keyler et al. (1999). The second step involved taking these newly calibrated values and applying them to a second calibration of brain blood flow, partition coefficient, and retention parameters using data published by Hieda et al. (1999). The values obtained in the first two steps were then applied to a third and final calibration step in which antibody concentration was adjusted within the published range for each study. Calibrated values were set as primary parameters in the model and then simulations were run using dosing and mean antibody affinity data published alongside the nicotine time course concentration data in each respective study.

The human PBPK model was constructed by substituting physiological parameters in the rat model with human values obtained from literature. There was only one calibration step and it involved adjusting nicotine and cotinine partition coefficients for the 'other' compartment so that simulation predictions would fit nicotine and cotinine venous concentration time course data published by Benowitz et al. (1991). As was done with the rat model, calibrated values were set as primary parameters in the human model and then simulations were run using dosing data published alongside nicotine and cotinine time course data in order to test model accuracy in unvaccinated subjects. Accuracy of venous nicotine concentration predictions were tested using time course nicotine concentrations reported by Benowitz et al. (1982, 1983) and accuracy of venous cotinine concentrations were tested using time course cotinine concentrations also reported by Benowitz et al. (1983). Nicotine concentration time course data that included anti-nicotine antibody effect was unavailable, so model accuracy could not be verified for vaccinated subjects.

2.7. Human vaccine efficacy predictions

One of the main goals of this project was to predict human vaccine efficacy *via in silico* methods without needing to commit to costly clinical trials. In order to do this, pre-existing data from human studies that quantified achievable antibody binding characteristics were paired with the human PBPK model described earlier and multiple simulations were run. A crude sensitivity analysis was designed in an attempt to identify antibody binding characteristics that would have the most impact on preventing nicotine from reaching the brain, and this analysis was followed by several simulations aimed at predicting the

maximum effect antibodies could have on the distribution of nicotine to the brain in humans under plausible physiologic conditions.

2.7.1. Sensitivity analysis—Simulations using the human model were conducted using all possible combinations of the following treatments and treatment levels: high and low antibody binding capacity, high and low antibody affinity for nicotine, and high and low cross reactivity with cotinine. High and low antibody affinity for nicotine was defined as the maximum and minimum values of the range reported by Maurer et al. (2005), respectively. In order to quantify antibody affinity for cotinine, two cross reactivity factors were defined using the ratio of cotinine and nicotine IC₅₀ values for the worst performing hapten (GK56KLH) and the best performing hapten (IP31KLH) described by de Villiers et al. (2010). These cross reactivity factors were then multiplied by the antibody affinity for nicotine treatment levels, resulting in four test cotinine affinities for anti-nicotine antibodies. High and low antibody binding capacities were concluded using the mean and standard deviation for antibody concentrations reported in multiple human trials (see Table 9) (Hatsukami et al., 2005, 2011; Wagena et al., 2008). The low antibody binding capacity was defined as the mean value from these studies while the high antibody binding capacity was defined as this same mean value plus three standard deviations. Eight simulations were run using all possible variable combinations for smokers (who maintain baseline cotinine concentrations) and non-smokers (who do not maintain baseline cotinine concentrations). These simulations were also paired with control simulations in which anti-nicotine antibodies were not present. Reduction in brain nicotine concentration with respect to control concentrations were calculated and plotted *versus* cigarette number.

2.7.2. Predicting maximal antibody effect in humans—The initial vaccine efficacy predictions made by the human model seemed less promising than expected when compared to results published from rat studies in which brain nicotine concentration reductions of up to 90% were reported (Raupach et al., 2012). As a result, additional tests were designed in order to predict the maximum reduction in human brain nicotine concentration that could theoretically be achieved using first generation nicotine vaccination technologies and approaches. Geometric mean antibody concentration time course data paired with vaccine and booster shot application times published by Hatsukami et al. (2011) were used to predict maximal antibody concentrations when utilizing the most beneficial time intervals between booster applications (measured as the average time between each booster application and the resulting maximum serum antibody concentration). The total number of booster applications was varied using this prediction method in order to generate plausible serum antibody concentration test values. These data can be found in Table 6 (*6B16W* refers to administration of vaccine and six subsequent booster shots over the course of sixteen weeks, with *10B24W* and *14B32W* following identical nomenclature). The total dose per cigarette was not changed from the assigned value in earlier tests, but the dose time, dose rate, and recorded total dose were modified to make predictions better represent smokers who are trying to quit (the most likely candidate for clinical use of a nicotine vaccine). Other alterations to the model included changing the affinity for nicotine value to that of the average value in one set of simulations and the maximal value for a second set of simulations (values were derived from the range reported by Maurer et al. (2005) and using

only the smallest IC50 ratio reported by de Villiers et al. (2010) as a cotinine cross-reactivity factor. Simulations were designed around three variables (antibody concentration, antibody affinity for nicotine, and smoking status) and were run in addition to a control simulation for each consecutive dose. Reductions in brain nicotine concentration with respect to control concentration were calculated and plotted *versus* cigarette number.

3. Results

3.1. Rat model calibration

The results from the rat model calibration simulations can be seen in Figs. 2–4. Fig. 2 shows the successful calibration of the rat model using venous nicotine concentration time course data and antibody binding characteristics (see Table 4) reported by Keyler et al. (1999) in response to both the CMUNic vaccine as well as the negative control. The model simulation output was able to remain well within the standard deviations defined for each data point, indicating proximity of predictions to reality over the range of time described in the data set. The success of this calibration step was confirmed in Fig. 3 by the close proximity of model predictions to the venous nicotine time course data reported by Hieda et al. (1999) when using the newly calibrated values and the antibody binding characteristics defined for the new data set (see Table 5). Fig. 4 shows the successful calibration of the rat model in terms of brain nicotine concentration using data again described by Hieda et al. (1999). The close proximity of the model output to the above-mentioned time course data (values are within the standard deviation defined for each data point) again indicates proximity of predictions to reality.

3.2. Human model calibration and testing

The results from human model calibration and accuracy testing simulations can be seen in Figs. 5–8. Fig. 5 shows the successful calibration of human model venous nicotine and cotinine concentration predictions using time-course data reported by Benowitz et al. (1991) (dosing information can be found in Table 6). Predictions remain within one standard deviation of each data point with the exception of two cotinine concentration values at 2 and 3 h that appear to be outliers within the published data set. Accuracy of these newly calibrated values was confirmed using controlled-dose smoking data (see Table 7) reported by Benowitz et al. (1982) and unrestricted-dose smoking data (see Table 8) reported by Benowitz et al. (1983). Fig. 6 shows that the calibrated human model can approximate nicotine venous concentrations in smokers when applying lower and average nicotine doses (0.4 mg and 1.2 mg of nicotine per cigarette), but there appears to be sizeable discrepancy between model predictions and published time-course data when applying higher nicotine doses (2.5 mg of nicotine per cigarette). This is most likely due to self-regulation of nicotine uptake during the administering of higher doses (Benowitz et al., 1982). Figs. 7 and 8 show that the calibrated human model can accurately approximate nicotine and cotinine venous concentrations, respectively, at dosing rates comparable to those that smokers would typically experience. Additionally, the simulation results in these figures have not been adjusted to averaged peak-trough values, so they may serve as better representatives of nicotine and cotinine concentrations in venous blood upon nicotine inhalation.

3.3. Sensitivity analysis results

The sensitivity analysis results can be seen in Fig. 9 (active smokers with high resting cotinine systemic concentrations) and Fig. 10 (nonsmokers with negligible resting cotinine systemic concentrations). Together, Figs. 9 and 10 show that antibody binding capacity, affinity for nicotine, and cross reactivity with cotinine have a sizeable impact on the ability of potential nicotine vaccines to prevent nicotine from entering the brain. Additionally, the ability of vaccine-elicited antibodies to impede nicotine's passage into the brain was reduced upon sequential, *in silico* dosing with nicotine (for dosing information, see Table 10). The percent decrease in brain nicotine concentration never reaches zero, however, but rather approaches a constant value that appears to depend on the initial antibody binding characteristic treatment combination and the nicotine dosing rate. When comparing Figs. 9 and 10, the sensitivity analysis results showed that resting cotinine levels and smoking status have a detrimental effect on nicotine vaccine performance, especially when the elicited antibodies have a high affinity for cotinine. The results obtained from simulations using low cotinine cross-reactivity factors were similar in models describing both smokers and non-smokers. Also, the largest antibody effect predicted in both smokers and non-smokers was obtained when a high antibody concentration, a high antibody affinity for nicotine, and a low antibody affinity for cotinine were used in the simulation. In contrast, the smallest antibody effect was predicted in simulations that included a low antibody concentration, a low antibody affinity for nicotine, and a high antibody affinity for cotinine. Legend nomenclature found in each figure refers to the combination of various levels for each test parameter, with *NhChAbh* representing the simulation run using high antibody affinity for nicotine (*Nh*), high cotinine cross-reactivity factor value (*Ch*), and high blood antibody concentration (*Abh*). Similarly, *NIcIAbl* represents the simulation run using low antibody affinity for nicotine (*NI*), low cotinine cross-reactivity factor value (*CI*), and low blood antibody concentration (*AbI*).

3.4. Maximal antibody effect prediction results

When maximal antibody effect predictions were made using hypothetical antibody concentration thresholds within the human model, the largest reductions in brain nicotine concentration were ~22% in both smokers and non-smokers when using an averaged antibody-nicotine affinity value (Fig. 11) and ~26% and ~27% in smokers and nonsmokers, respectively, when using a maximized antibody affinity for nicotine value (Fig. 12). The ability of vaccine elicited antibodies to impede nicotine's passage into the brain was reduced after each successive nicotine dose, a similar effect to that seen in the sensitivity analysis results. When comparing Figs. 11 and 12, it appears that there are fewer discrepancies between model predictions in smokers and non-smokers when a low cotinine cross-reactivity factor is supplied to the model. This result was also observed when performing the sensitivity analysis. Additionally, increasing the affinity of *anti*-nicotine antibodies for nicotine from $44.05 \mu\text{M}^{-1}$ to $71.43 \mu\text{M}^{-1}$ resulted in an additional decrease in brain nicotine concentration of ~3, ~2.5, and ~2% for the first, second, and third nicotine doses, respectively.

4. Discussion

Predictions provided by the rat PBPK model fit well with published, experimental data, confirming that this model could be a good predictor of whole blood and brain nicotine concentrations in rats with and without anti-nicotine antibodies present. It is worth noting that the model predicted the steady-state brain nicotine half-life in rats without antibody action to be approximately 40 min, which is comparable to the 52 min described by Ghosheh et al. (1999). The data presented herein indicates a successful conceptualization and construction of a rat PBPK model that can evaluate nicotine vaccine efficacy. However, additional confirmation of model prediction accuracy should be performed in the future when more data becomes available.

Agreement between simulations and experimental data confirmed the validity of the human PBPK model in terms of predicting venous nicotine and cotinine concentrations without the presence of anti-nicotine antibodies. Given the accuracy of the predictions made by the rat model and the similarity between rat and human nicotine binding kinetics in the brain reported by Court et al. (1994), it was assumed that a similar level of accuracy would be achieved in the human model. However, assuming that the brain partition coefficient and retention parameters found in rats are comparable to those found in humans may lead to some inaccuracies in model predictions due to variable interspecies brain pharmacokinetics (Syvanen et al., 2009). While it is unfortunate that the physiological accuracy of key human model predictions cannot be confirmed, applying the brain pharmacokinetic parameters calibrated in the rat model to the human model was necessary in order to draw conclusions about nicotine vaccine efficacy in humans. Various pieces of published data support the relative accuracy of the brain nicotine concentration predictions described in this study. First, the nicotine concentration half-life predicted in human brains when antibody effect is not applied (~43 min) is comparable to that illustrated in Nyback et al. (1994) for the active stereochemical form of nicotine in nonsmokers (~39 min). Second, the range of brain nicotine concentration reductions predicted during the maximal antibody effect study (17–26%) encompasses the value of 23.6% reported by Esterlis et al. (2013). Therefore, percent reduction in nicotine brain concentrations using this model could remain accurate even in the event that predictions produced by the human model vary from their true physiological values. While these results certainly do not confirm the accuracy of the human PBPK model predictions, it can be argued that they do present evidence of model proximity to reality.

The investigation of antibody specific factors that contribute to nicotine vaccine efficacy in humans provided results indicating that smoking status appears to have a sizable impact on nicotine access to the brain. This is especially true when a high cotinine cross-reactivity factor is defined for the simulation, indicating that increased antibody affinity for cotinine is detrimental to the effectiveness of any nicotine vaccine that is intended for use in humans. Since it is assumed that active smokers will occasionally “cheat” during quitting attempts, controlling this factor could prove to be vital to vaccine success in humans. Consequently, results from these simulations strongly support the hypothesis that choice of nicotine hapten could play a significant role in vaccine efficacy due to its profound effect on the affinity of anti-nicotine antibodies for both nicotine and cotinine. The human PBPK model predicts that increasing antibody affinity for nicotine and antibody concentration leads to decreased

nicotine access to the brain, a result that is intuitive in nature. The greatest reduction in brain nicotine concentration that could be achieved using the most optimal antibody pharmacokinetic parameters published from human trials was ~15% in smokers and ~16% in non-smokers after the first *in silico* dose, which is much lower than many of the reductions that have been described in animal studies. A steady drop off in the amount of nicotine prevented from entering the brain occurred over the next doses, indicating antibody saturation and competition with newly formed cotinine for antibody binding sites. This drop off did not approach zero when additional doses were applied, however, but rather converged on a constant value, indicating that antibody saturation does not completely nullify the impact anti-nicotine antibodies have on brain nicotine concentrations. The results from these simulations could explain the issues that have been encountered in past clinical trials. Additionally, these results seem to indicate that if future nicotine vaccines cannot elicit the production of antibodies with more beneficial pharmacokinetic properties, achieving desired clinical effectiveness might be difficult.

The brain nicotine concentration reductions achieved after the initial *in silico* dose in the maximal antibody effect prediction simulations are above the 23.6% described by Esterlis et al. (2013) that coincides with a 50% reduction in cigarette use. However, the model also predicted brain nicotine levels to decline by ~20% in non-smokers and ~16% in smokers when compared with controls after applying the third and final nicotine dose, values that were below the 50% effectiveness threshold previously described. This result indicates that the effectiveness of nicotine vaccines may be reduced upon the application of additional nicotine doses. It is worth noting that the antibody binding capacities applied within these simulations were much higher than those that have been achieved in humans to date. Serum binding capacities of greater than 1 μM (~75 $\mu\text{g/mL}$) have been achieved in many rat studies (Keyler et al., 1999; Pentel et al., 2000; Lockner et al., 2013), however, so it is not a stretch to assume that the same can be accomplished in humans after extensive optimization of the nicotine vaccine concept. Until this binding capacity is achieved, however, the reduction in brain nicotine concentration elicited by nicotine vaccines in humans is predicted to remain below the threshold described by Esterlis et al. (2013).

Most variables used in this study were obtained from literature and in many cases they were not checked for physiological accuracy. Additionally, parameters that were not obtained from literature were either assumed or calculated/estimated using available data. Great effort was made to create two PBPK models that could predict the concentration of nicotine in the blood and brain of rats and humans, but the accuracy of concentration predictions in other tissues included in these models was largely ignored. Consequently, the accuracies of all nicotine concentration predictions in the rat model compartments other than those of the brain and mixed venous/arterial blood are questionable and should not be used to make conclusions about physiological processes involving nicotine. This is equally true for the cotinine and nicotine concentration predictions outside of the venous blood compartment in the human model.

To the best of our knowledge, this has been the first attempt to quantify anti-nicotine antibody effect on nicotine systemic distribution in both rats and in humans by using PBPK modeling. The work outlined in this study is preliminary in nature and will require

refinement in the future. The lack of published, comparative nicotine concentration data in humans vaccinated against nicotine makes it impossible to determine prediction accuracy, and it is possible that there will never be sufficient data available to completely validate the human model in terms of the effect anti-nicotine antibodies have on nicotine distribution to the brain. Also, there may be additional biological mechanisms that affect the distribution of nicotine in the presence of anti-nicotine antibodies that have not been considered in either of the models described in this study. Until the accuracy of the human PBPK model outputs can be validated, predictions should only be viewed as guidelines that can direct the development of future human nicotine vaccines and not as true representations of physiological concentrations. However, this model could potentially be used to predict brain nicotine concentration reductions in rats and humans without requiring brain removal based on measurements in antibody titer and affinity. A possible benefit of the *in silico* modeling in combination with animal trials would be the reduction in study costs and the number of experiments necessary to quantify antibody effect. The model may also be used to assess brain nicotine levels in humans upon nicotine challenge using indirectly measured parameters. Applying the model to different species for further vaccine analysis is also possible through slight modification, as has already been demonstrated with the construction of the human PBPK model within the scope of this study.

5. Conclusions

In this study, two physiologically based pharmacokinetic models were successfully constructed in order to investigate the effects of anti-nicotine antibodies on nicotine disposition in the brains of rats and humans. The good fit between predicted and published blood and brain nicotine concentrations in the rat model suggests that this approach can effectively quantify the complex, biological processes associated with nicotine pharmacokinetics and nicotine-antibody interactions. Investigation of the key parameters presumed to have the most effect on the ability of anti-nicotine antibodies to prevent nicotine from entering the brain in the human model, such as antibody affinity for nicotine, antibody cross-reactivity with cotinine, and overall antibody concentration, confirmed the conclusions made in previous studies about antibody binding characteristics necessary for a successful vaccine. Predictions made by the human model also suggest that the antibodies elicited by current vaccines do not have sufficient binding characteristics to reduce brain nicotine concentrations to clinically significant levels. However, the human model also predicts that if antibody binding characteristics similar to those achieved in animal studies can be achieved in human studies, human vaccine efficacy in terms of brain nicotine concentration reduction should meet the threshold values for smoking cessation that have been recently described in literature. Ultimately, both of the models presented in this study can be used to predict nicotine vaccine efficacy in terms of brain nicotine concentration reduction prior to human trials and extended animal studies, a feature that can save both time and money in future endeavors for nicotine vaccine development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Material balance equations

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.taap.2016.07.017>.

Abbreviations

PBPK	physiologically based pharmacokinetic model
USD	United States dollar
CDC	Centers for Disease Control and Prevention
IC50	half maximal inhibitory concentration
CMUNic	6-(carboxymethylureido)-(+)-nicotine

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Fig. 1. Overall schematic for the rat and human PBPK models. Grey boxes represent antibody binding nicotine (and cotinine in the human model), blue boxes represent tissue retention of nicotine, and arrows represent nicotine and cotinine mass action kinetics or metabolism. All associated equations are shown in Supplementary Material. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 2.

Rat whole blood nicotine concentration prediction using parameters listed in Tables 1–3 along with serum nicotine concentration time course data reported by Keyler et al. (1999). Whole blood refers to the pooling of both the venous and arterial blood compartments and then re-evaluation of nicotine blood concentration. Serum was acquired from trunk blood (mixed venous and arterial blood). Simulation predictions were compared to experimental data collected with (CMUNic) and without (control) the presence of anti-nicotine antibodies. Additional simulation-specific parameters can be viewed in Table 4. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3.

Rat whole blood nicotine concentration prediction using parameters listed in Tables 1–3 along with serum nicotine concentration time course data reported by Hieda et al. (1999). Whole blood refers to the pooling of both the venous and arterial blood compartments and then re-evaluation of nicotine blood concentration. Serum was acquired from trunk blood (mixed venous and arterial blood). Simulation predictions were compared to experimental data collected with (CMUNic) and without (control) the presence of anti-nicotine antibodies. Additional simulation-specific parameters can be viewed in Table 5. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 4.

Rat brain nicotine concentration prediction using parameters listed in Tables 1–3 along with brain nicotine concentration time course data reported by Hieda et al. (1999). Simulation predictions were compared to experimental data collected with (CMUNic) and without (control) the presence of anti-nicotine antibodies. Additional simulation-specific parameters can be viewed in Table 5.

Fig. 5.

Human model simulation of venous nicotine and cotinine concentrations was calibrated using published time course data reported by Benowitz et al. (1991) for a 24 h intravenous nicotine infusion. Hour zero represents the start of infusion. Additional simulation-specific parameters can be viewed in Table 6.

Fig. 6.

Venous nicotine time course data were reported by Benowitz et al. (1982). Simulation data points are presented as the average of trough (prior to dose) and peak (post dose) nicotine concentrations. Hour zero represents the start of smoking. Additional simulation-specific parameters can be viewed in Table 7.

Fig. 7.

Venous nicotine time course data that were originally reported by Benowitz et al. (1983) were obtained from Hukkanen et al. (2005). Nicotine concentrations were achieved during ‘unrestricted smoking’ and no dosing regimen was described. As such, a reasonable dosing regimen was estimated. Simulation data are presented with trough (prior to dose) and peak (post dose) nicotine concentrations. The simulation was conducted for a week *in silico* and results were recorded during the final day. Hour zero represents the start of smoking. Additional simulation-specific parameters can be viewed in Table 8.

Fig. 8.

Venous cotinine time course data that were originally reported by Benowitz et al. (1983) were obtained from Hukkanen et al. (2005). Cotinine concentrations were achieved during ‘unrestricted smoking’ and no dosing regimen was described. As such, a reasonable dose regimen was estimated. Simulation data is presented with trough (prior to dose) and peak (post dose) cotinine concentrations. The simulation was conducted for a week *in silico* and results were recorded during the final day. Hour zero represents the start of smoking. Additional simulation-specific parameters can be viewed in Table 8.

Fig. 9. Vaccine efficacy predictions for active smokers presented as the percent decrease in peak brain nicotine concentration when compared with control values. Cigarette number represents sequential dosing of nicotine to the lung compartment over time (refer to Table 10). Additional simulation-specific parameters can be viewed in Table 11.

Fig. 10. Vaccine efficacy predictions for non-smokers presented as the percent decrease in peak brain nicotine concentration when compared with control values. Cigarette number represents sequential dosing of nicotine to the lung compartment over time (refer to Table 10). Additional simulation-specific parameters can be viewed in Table 11.

Fig. 11.

Vaccine efficacy predictions for smokers (S) and non-smokers (NS) using various antibody concentration values. Level one (L1), level two (L2), and level three (L3) antibody concentrations coincide with the 6B16W, 10B24W, and 14B32W values presented in Table 6, respectively. Data are presented as the percent decrease in peak brain nicotine concentration when compared with control values. The average antibody affinity for nicotine value was used in each simulation. Cigarette number represents sequential dosing of nicotine to the lung compartment over time (refer to Table 12). Additional simulation-specific parameters can be viewed in Table 13.

Fig. 12.

Vaccine efficacy predictions for smokers (S) and non-smokers (NS) using various antibody concentration values. Level one (L1), level two (L2), and level three (L3) antibody concentrations coincide with the 6B16W, 10B24W, and 14B32W values presented in Table 6, respectively. Data are presented as the percent decrease in peak brain nicotine concentration when compared with control values. The maximal antibody affinity for nicotine value (K_a) was used in each simulation. Cigarette number represents sequential dosing of nicotine to the lung compartment over time (refer to Table 12). Additional simulation-specific parameters can be viewed in Table 14.

Table 1

Nicotine–cotinine pharmacokinetic model parameters for human and rat model.

Model parameter	Human value	Source	Rat value	Source
Tissue volume (fraction of body weight)				
Lung	0.0076	Brown et al. (1997)	0.0048	Brown et al. (1997)
Heart	0.0050	Brown et al. (1997)	0.0029	Brown et al. (1997)
Brain	0.0193	Brown et al. (1997)	0.0058	Brown et al. (1997)
Adipose	0.2326	Brown et al. (1997)	0.0761	Brown et al. (1997)
Liver	0.0248	Brown et al. (1997)	0.0324	Brown et al. (1997)
Kidney	0.0038	Brown et al. (1997)	0.0067	Brown et al. (1997)
Skin	0.0339	Brown et al. (1997)	0.1743	Brown et al. (1997)
Muscle	0.3883	Brown et al. (1997)	0.3922	Brown et al. (1997)
Other	0.1940	Brown et al. (1997)	0.1965	Brown et al. (1997)
Arterial blood	0.0190	Brown et al. (1997)	0.0178	Brown et al. (1997)
Venous blood	0.0570	Brown et al. (1997)	0.0534	Brown et al. (1997)
Nicotine partition coefficients				
Lung	0.870	Teeguarden et al. (2013)	0.870	Teeguarden et al. (2013)
Heart	0.490	Teeguarden et al. (2013)	0.490	Teeguarden et al. (2013)
Brain	2.650	Rat Data	2.650	Hieda calibration
Adipose	0.190	Teeguarden et al. (2013)	0.190	Teeguarden et al. (2013)
Liver	7.600	Teeguarden et al. (2013)	7.600	Teeguarden et al. (2013)
Kidney	7.600	Teeguarden et al. (2013)	7.600	Teeguarden et al. (2013)
Skin	0.970	Teeguarden et al. (2013)	0.970	Teeguarden et al. (2013)
Muscle	1.000	Teeguarden et al. (2013)	1.000	Teeguarden et al. (2013)
Other	13.721	Benowitz infusion calibration	7.100	Keyler calibration
Cotinine partition coefficients				
Lung	0.901	Optimized, $K_{apriori}$ starting values from Thompson et al. (2012)		
Heart	0.832	Optimized, $K_{apriori}$ starting values from Thompson et al. (2012)		
Brain	0.892	Optimized, $K_{apriori}$ starting values from Thompson et al. (2012)		
Adipose	0.828	Optimized, $K_{apriori}$ starting values from Thompson et al. (2012)		
Liver	0.770	Optimized, $K_{apriori}$ starting values from Thompson et al. (2012)		
Kidney	0.822	Optimized, $K_{apriori}$ starting values from Thompson et al. (2012)		
Skin	1.019	Optimized, $K_{apriori}$ starting values from Thompson et al. (2012)		
Muscle	1.178	Optimized, $K_{apriori}$ starting values from Thompson et al. (2012)		
Other	0.658	Benowitz infusion calibration		

Table 2

Nicotine–cotinine pharmacokinetic model parameters for human and rat model, cont.

Model parameter	Human value	Source	Rat value	Source
Mean body weight, BW (kg)	70	–	0.35	–
Cardiac output (L/hr)	$15 * BW_{(kg)}^{0.74}$	Gajewska et al. (2014)	$14.2 * BW_{(kg)}^{0.75}$	Brown et al. (1997)
Blood flow (fraction of cardiac output)				
Lung	1.000	Brown et al. (1997)	1.000	Brown et al. (1997)
Heart	0.040	Brown et al. (1997)	0.051	Brown et al. (1997)
Brain	0.114	Brown et al. (1997)	0.046	Hieda Calibration
Adipose	0.052	Brown et al. (1997)	0.070	Brown et al. (1997)
Liver	0.227	Brown et al. (1997)	0.183	Brown et al. (1997)
Kidney	0.175	Brown et al. (1997)	0.141	Brown et al. (1997)
Skin	0.058	Brown et al. (1997)	0.058	Brown et al. (1997)
Muscle	0.191	Brown et al. (1997)	0.278	Brown et al. (1997)
Other	0.143	Flow balance	0.178	Keyler Calibration
Tissue Ab conc. (fraction of serum Ab conc.)				
Lung	0.029	Satoskar et al. (2003)	0.029	Satoskar et al. (2003)
Heart	0.042	Satoskar et al. (2003)	0.042	Satoskar et al. (2003)
Brain	0.002	Satoskar et al. (2003)	0.002	Satoskar et al. (2003)
Adipose	0.125	Satoskar et al. (2003)	0.125	Satoskar et al. (2003)
Liver	0.029	Satoskar et al. (2003)	0.029	Satoskar et al. (2003)
Kidney	0.03	Satoskar et al. (2003)	0.03	Satoskar et al. (2003)
Skin	0.01	Satoskar et al. (2003)	0.01	Satoskar et al. (2003)
Muscle	0.05	Satoskar et al. (2003)	0.05	Satoskar et al. (2003)
Other	0.047	Satoskar et al. (2003)	0.047	Satoskar et al. (2003)
Nicotine tissue retention (s^{-1})				
Brain nicotine dispersion	0.00343	Rat value	0.00343	Hieda calibration
Brain nicotine aggregation	0.00201	Rat value	0.00201	Hieda calibration

Table 3

Nicotine–cotinine pharmacokinetic model parameters for human and rat model, cont.

Model parameter	Value	Source
Human clearances (L/h)		
Nicotine total clearance	79.0720	Hukkanen et al. (2005)
Nicotine renal clearance	3.7520	Hukkanen et al. (2005)
Nicotine hepatic clearance	75.3200	Hukkanen et al. (2005)
Fraction nicotine hepatic clearance to cotinine	0.8000	Hukkanen et al. (2005)
Cotinine total clearance	3.2720	Hukkanen et al. (2005)
Cotinine hepatic clearance	2.9120	Hukkanen et al. (2005)
Cotinine renal clearance	0.3600	Hukkanen et al. (2005)
Rat clearances (L/h)		
Nicotine total clearance	0.756	Keyler calibration
Molecular weights (g/mol)		
MW of nicotine	162.23	
MW of cotinine	172.22	
MW of antibody	150,000	
Other parameters		
Fraction plasma in whole blood	0.55	Dill and Costill (1974)
Fraction nicotine protein binding	0.05	Hukkanen et al. (2005)
Fraction cotinine protein binding	0.05	Hukkanen et al. (2005)
Nicotine concentration ratio of blood to plasma	1	Yamazaki et al. (2010)
Cotinine concentration ratio of blood to plasma	1	Yamazaki et al. (2010)
Antibody concentration ratio of blood to plasma	0.55	
Percent total blood volume in arterial compartment	0.25	
Percent total blood volume in venous compartment	0.75	
Baseline systemic cotinine in smokers (μM)	1.2000	Simulation prediction
Antibody decomposition rate (day ⁻¹)	0.01155	Maurer et al. (2005)

Table 4

Keyler et al. (1999) Published Dose and Antibody Parameters.

Dose (mg nicotine/kg rat mass)	0.1
Dose time (sec)	60
Mean antibody serum concentration (mg/ml)	0.13 ± 0.10
Calibrated antibody serum concentration (mg/ml)	0.149
Mean antibody binding affinity (M^{-1})	1.83×10^8
Dose compartment	Venous blood

Table 5

Hieda et al. (1999) published dose and antibody parameters.

Dose (mg nicotine/kg rat mass)	0.03
Dose time (sec)	10
Mean antibody serum concentration (mg/ml)	0.38 ± 0.32
Calibrated antibody serum concentration (mg/ml)	0.423
Mean antibody binding affinity (M^{-1})	1.4×10^7
Dose compartment	Venous blood

Table 6

Benowitz et al. (1991) published dosing parameters.

Dose (mg nicotine/kg human mass)	0.288
Dose time (hours)	24
Dose compartment	Venous blood

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Table 7

Benowitz et al. (1982) published dosing parameters.

High dose (mg/cigarette)	2.5
Usual brand dose (mg/cigarette)	1.2
Low dose (mg/cigarette)	0.6
Dose time (min)	4
Dose rate (cigarette/h)	2
Total dose (cigarette)	30
Dose compartment	Lungs

Table 8

Benowitz et al. (1983) estimated dosing parameters.

Dose (mg/cigarette)	1.2
Dose time (min)	4
Dose rate (cigarette/h)	1.5
Total dose (cigarette)	24
Dose compartment	Lungs

Table 9

Human serum nicotine-specific antibody concentrations from clinical trials.

Source	Max serum concentration (µg/mL)
Hatsukami et al. (2005)	27
Hatsukami et al. (2011)	44
Wagena et al. (2008)	10.8
Parameter	Value
Mean (µg/mL)	27.27
Standard deviation (µg/mL)	16.60

Table 10

Human vaccine efficacy dosing parameters for Fig. 9 & Fig. 10.

Dose (mg/cigarette)	1.2
Dose time (min)	5
Dose rate (cigarette/h)	1.5
Total Dose (cigarette)	20
Dose compartment	Lungs

Table 11

Human vaccine efficacy prediction parameters for Fig. 9 & Fig. 10.

Model parameter	Value	Source
Serum concentration ($\mu\text{g/mL}$)		
High	77.07	Mean + 3 * SD
Low	27.27	Mean
Binding capacity (μM)		
High	0.57	Calculation
Low	0.2	Calculation
Affinity for nicotine, $K_{A,\text{Nic}}$ (μM^{-1})		
High	71.43	Maurer et al. (2005)
Low	16.67	Maurer et al. (2005)
Cotinine cross-reactivity factor, CRF_{Cot}		
High	1.183E-01	de Villiers et al. (2010)
Low	7.321E-03	de Villiers et al. (2010)
Affinity for cotinine, $K_{A,\text{Cot}}$ (μM^{-1})		
High	$K_{A,\text{Nic}} * \text{CRF}_{\text{Cot}}$	Estimate
Low	$K_{A,\text{Nic}} * \text{CRF}_{\text{Cot}}$	Estimate
Estimated antibody off rates (sec^{-1})		
Nicotine, $k_{\text{off},\text{Nic}}$	5.50E-04	Estimate
Cotinine, $k_{\text{off},\text{Cot}}$	1.10E-03	Estimate
Antibody on rates ($\mu\text{M}^{-1} \text{ s}^{-1}$)		
Nicotine, $k_{\text{on},\text{Nic}}$	$k_{\text{off},\text{Nic}} * K_{A,\text{Nic}}$	Calculation
Cotinine, $k_{\text{on},\text{Cot}}$	$k_{\text{off},\text{Cot}} * K_{A,\text{Cot}}$	Calculation

Table 12

Human vaccine efficacy dosing parameters for Fig. 11 & Fig. 12.

Dose (mg/cigarette)	1.2
Dose time (min)	5
Dose rate (cigarette/h)	1
Total Dose (cigarette)	3
Dose compartment	Lungs

Table 13

Human vaccine concentration effect parameters for Fig. 11.

Model parameter	Value	Source
Serum antibody concentration ($\mu\text{g/mL}$)		
6B16W	94.3	Estimate
10B24W	128.6	Estimate
14B32W	162.9	Estimate
Blood antibody binding capacity (μM)		
6B16W	0.6915	Calculation
10B24W	0.9431	Calculation
14B32W	1.195	Calculation
Affinity for nicotine (μM^{-1})		
$K_{A,\text{Nic}}$	44.05	Maurer et al. (2005)
Cotinine cross-reactivity factor		
CRF_{Cot}	7.321E-03	de Villiers et al. (2010)
Affinity for cotinine (μM^{-1})		
$K_{A,\text{Cot}}$	0.3225	Estimate
Estimated antibody off rates (sec^{-1})		
Nicotine, $k_{\text{off},\text{Nic}}$	5.50E-04	Estimate
Cotinine, $k_{\text{off},\text{Cot}}$	1.10E-03	Estimate
Antibody on rates ($\mu\text{M}^{-1} \text{ s}^{-1}$)		
Nicotine, $k_{\text{on},\text{Nic}}$	2.42E-02	Calculation
Cotinine, $k_{\text{on},\text{Cot}}$	3.55E-04	Calculation

Table 14

Human vaccine concentration effect parameters for Fig. 12.

Model parameter	Value	Source
Serum antibody concentration ($\mu\text{g/mL}$)		
6B16W	94.3	Estimate
10B24W	128.6	Estimate
14B32W	162.9	Estimate
Blood antibody binding capacity (μM)		
6B16W	0.6915	Calculation
10B24W	0.9431	Calculation
14B32W	1.195	Calculation
Affinity for nicotine (μM^{-1})		
$K_{A,\text{Nic}}$	71.43	Maurer et al. (2005)
Cotinine cross-reactivity factor		
CRF_{Cot}	7.321E-03	de Villiers et al. (2010)
Affinity for cotinine (μM^{-1})		
$K_{A,\text{Cot}}$	0.5229	Estimate
Estimated antibody off rates (sec^{-1})		
Nicotine, $k_{\text{off},\text{Nic}}$	5.50E-04	Estimate
Cotinine, $k_{\text{off},\text{Cot}}$	1.10E-03	Estimate
Antibody on rates ($\mu\text{M}^{-1} \text{ s}^{-1}$)		
Nicotine, $k_{\text{on},\text{Nic}}$	3.93E-02	Calculation
Cotinine, $k_{\text{on},\text{Cot}}$	5.75E-04	Calculation