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A mathematical model of a recombinant humanized anti-cocaine monoclonal antibody's effects on cocaine pharmacokinetics in mice

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Abstract

Aims—A recombinant humanized anti-cocaine monoclonal antibody (mAb), h2E2, is at an advanced stage of pre-clinical development as an immunotherapy for cocaine abuse. It is hypothesized that h2E2 binds to and sequesters cocaine in the blood.

Main Methods—A three-compartment model of the effects of h2E2 on cocaine's distribution was constructed. The model assumes that h2E2 binds to cocaine and that the h2E2-cocaine complex does not enter the brain but distributes between the central and peripheral compartments. Free cocaine is eliminated from both the central and peripheral compartments, and h2E2 and the h2E2-cocaine complex are eliminated from the central compartment only. This model was tested against a new dataset measuring cocaine concentrations in the brain and plasma over one hour in the presence and absence of h2E2.

Key findings—The mAb significantly increased plasma cocaine concentrations with a concomitant significant decrease in brain concentration. Plasma concentrations declined over the 1-hour sampling period in both groups. With a set of parameters within reasonable physiological ranges, the three-compartment model was able to qualitatively and quantitatively simulate the increased plasma concentration in the presence of the antibody and the decreased peak brain concentration in the presence of antibody. Importantly, the model explained the decline in plasma concentrations over time as distribution of the cocaine-h2E2 complex into a peripheral compartment.

Significance—This model will facilitate the targeting of ideal mAb PK/PD properties thus accelerating the identification of lead candidate anti-drug mAbs.

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Conflict of Interest statement: Dr. Norman is named as a co-inventor on a patent application for the matter and use of the humanized anti-cocaine monoclonal antibody, h2E2.

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Keywords

Mathematical modeling; pharmacokinetics and pharmacodynamics; humanized monoclonal antibody; immunotherapy; cocaine; addiction

Introduction

Immunotherapy represents a promising potential treatment for substance use disorders [1]. This includes anti-drug monoclonal antibodies (mAbs), which are hypothesized to bind to these drugs of abuse and prevent their entry into the brain, thus nullifying the central nervous system (CNS) effects of these drugs. This approach has shown success in preclinical animal studies of an anti-methamphetamine mAb [2], which has advanced into clinical trials [3]. A hybridoma-derived anti-cocaine mAb with a human heavy chain and a murine light chain (designated 2E2) and the reengineered recombinant version of this anti-cocaine mAb, designated h2E2, have also been developed. The mAb h2E2 has high affinity and selectivity for cocaine over its inactive metabolites [4,5] and has a long plasma half-life in both rats and mice. In rats, the recombinant humanized mAb has been shown to decrease brain concentrations of cocaine [4] and prevent cocaine-induced priming of self-administration behavior [6]. In mice, h2E2 can antagonize the distribution of cocaine's active metabolite, cocaethylene, into the brain [7]. However, the effects of h2E2 on cocaine's distribution to the brain in mice has never been reported. Since mice are a common model species, it is important to determine these effects in this species.

Considerable effort has been dedicated to developing anti-drug mAbs with ideal binding and pharmacokinetic profiles. Generally, it has been assumed that mAbs with high binding affinity for their target drug, selectivity over inactive metabolites and with long biological pharmacokinetic half-lives are superior. The mAb should also be able to rapidly alter the distribution and pharmacokinetics of the target drug. However, many of these assumptions have been called into question. For example, it is speculated that high selectivity of the humanized anti-cocaine mAb h2E2 over the major inactive metabolite benzoylecgonine may not be ideal, and may exacerbate the increase in cocaine consumption that is observed after the mAb has been surmounted [6]. Extensive study of anti-methamphetamine mAbs with varying pharmacological profiles has also revealed that *in vitro* affinities and *in vivo* pharmacokinetic half-life are not always predictive of the magnitude and duration of action of these antibodies [8]. This underscores the need for *in vivo* animal efficacy testing of anti-drug mAbs. However, these studies require large quantities of purified mAb and are time consuming and costly to conduct. Alternatively, a mathematical model of the effects of an anti-drug antibody on its ability to alter the distribution and pharmacokinetics of the target drug could be used to efficiently provide valuable information in determining the ideal characteristics of an anti-drug mAb.

The goal of the current study was to develop a pharmacokinetic model of the interaction between the anti-cocaine mAb h2E2 and cocaine. This model is validated against a new data set determining the effects of h2E2 on cocaine's distribution to the brain in mice. This model will provide valuable information on how the pharmacological properties of the mAb will

likely effect *in vivo* efficacy as measured by cocaine distribution to the brain, leading to more targeted design of future anti-drug mAbs for the treatment of overdose and substance use disorders.

Methods

Effects of h2E2 on cocaine distribution in mice

All studies were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* [9] and under a protocol approved by the Institutional Animal Care and Use Committee at the University of Cincinnati. Adult male jugular vein catheterized Swiss-Webster mice (19-22 g) were purchased from Taconic Farms. Mice were housed individually on a 14/10- hour light/dark schedule with unrestricted access to food and water. Mice were injected with h2E2 (120 mg/kg) or vehicle (PBS) i.v. One hour later, mice were injected with an equimolar dose of cocaine HCl (0.56 mg/kg) i.v. At 45 s, 1.5, 3, 5, 10, 20, 40 and 60 minutes following cocaine injection, 3 mice per time point were anesthetized and decapitated and trunk blood was collected and spun to separate plasma. Brains were removed and immediately placed on dry ice. At 3 min prior to decapitation mice were anesthetized with sodium pentobarbital (45 mg/kg i.p.). All samples were stored at -20 °C. Cocaine was quantified using gas-chromatography/mass-spectrometry using methods reported previously [10].

Materials

Cocaine HCl was provided by the Research Triangle Institute under the National Institute on Drug Abuse Drug Supply Program. Deuterated cocaine-D3 (0.1 mg/mL in acetonitrile), was used as an internal standard, unlabeled cocaine (1 mg/mL in acetonitrile), used to generate a standard curve, and the derivatizing reagent N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane were purchased from Cerilliant. Recombinant h2E2 was produced from stably transfected CHO-cell lines by Catalent Pharmsolutions using their proprietary GPEx technology [11].

Model Construction

A diagrammatic depiction of the model is shown in Fig. 1. In summary, a three-compartment model of the effects of h2E2 on cocaine's distribution was constructed. The three compartments represent the central compartment, or blood and well-perfused organs, the peripheral compartment, and the brain compartment. The model assumes that h2E2 binds to cocaine and that the h2E2-cocaine complex does not enter the brain but is able to distribute between the central and the peripheral compartments. This assumption is based on the knowledge that antibodies are unable to cross the blood-brain-barrier, whereas lipophilic small molecular weight drugs such as cocaine can [1]. It was assumed that h2E2 can enter peripheral compartments based on the distribution phase observed in pharmacokinetic studies in mice [12]. Since cocaine is much smaller than h2E2 and presumably interacts only with the binding site, h2E2 is assumed to be eliminated and distributed at the same rates whether bound or unbound, h2E2 is eliminated only from the central compartment, while cocaine is eliminated from both compartments. Initial values for central cocaine and antibody were set equimolar (since the experimental doses were equimolar) at the

experimental Cmax in the presence of h2E2. This value was also used in the control simulations, as the Cmax at time zero should be equal for both groups since the same dose was injected i.v in both groups. Terms for the relative volumes of the compartments (C/B and C/P in the equations) were used to convert data from amounts to concentrations when the molecules travel between different compartments. Brain tissue was assumed to have density of 1 g/mL for analysis of the experimental data. The system of ordinary differential equations (ODEs) describing this system is shown below.

$$\begin{aligned}\frac{dA}{dt} &= ak31 \cdot PrA - (ak10 + ak13)A - CCF \\ \frac{dC}{dt} &= (ck21 \cdot BC + ck31 \cdot PrC) - (ck10 + ck12 + ck13) \cdot C - CCF \\ \frac{dAC}{dt} &= ak31 \cdot PrAC - (ak13 + ak10) \cdot AC + CCF \\ \frac{dBC}{dt} &= CB \cdot (ck12 \cdot C - ck21 \cdot BC) \\ \frac{dPrC}{dt} &= CP \cdot (ck13 \cdot C - ck31 \cdot PrC - ck30 \cdot PrC) - PrCF \\ \frac{dPrA}{dt} &= CP \cdot (ak13 \cdot A - ak31 \cdot PrA) - PrCF \\ \frac{dPrAC}{dt} &= CP \cdot (ak13 \cdot AC - ak31 \cdot PrAC) + PrCF\end{aligned}$$

Species

A = central antibody concentration

C = central cocaine concentration

AC = central antibody cocaine complex concentration

BC = brain cocaine concentration

PrC = peripheral cocaine concentration

PrA = peripheral antibody concentration

PrAC = peripheral antibody cocaine complex concentration

Binding constants:

koff = antibody-cocaine disassociation constant

kon = antibody-cocaine association constant

Pharmacokinetic constants for the antibody h2E2

ak10 = rate of elimination of the antibody h2E2 from the central compartment

ak31 = antibody or antibody-cocaine complex rate of distribution from the peripheral to central compartment

ak13 = antibody or antibody cocaine complex rate of distribution from the central to the peripheral compartment

Pharmacokinetic constants for cocaine

$ck10$ = rate of elimination of cocaine from the central compartment

$ck30$ = rate of elimination of cocaine from the peripheral compartment

$ck21$ = rate of cocaine distribution from the brain to the central compartment

$ck12$ = rate of cocaine distribution from the central compartment to the brain

$ck13$ = rate of cocaine distribution from the central to the peripheral compartment

$ck31$ = rate of cocaine distribution from the peripheral to the central compartment

Relative volumes of the compartments:

$$\begin{aligned} C/B &= \frac{\text{central volume}}{\text{brain volume}} \\ C/P &= \frac{\text{central volume}}{\text{peripheral volume}} \end{aligned}$$

Antibody-cocaine complex formations

$PrCF$ = peripheral complex formation = $kon \cdot PrA \cdot PrC - koff \cdot PrAC$

CCF = central complex formation = $kon \cdot A \cdot C - koff \cdot AC$

Determination of parameter values and parameter sweep

An original set of parameters was determined by visually fitting the simulations to the data. In order to sample the parameter space, a set of 120,000 random values within a 10-fold range on either side of the original values were generated. 12,000 simulations were then executed, where each simulation selected a random value for each parameter. The total difference between the simulation values and the experimental values in all four data sets (plasma and brain concentrations in vehicle and h2E2 treated animals) was used to estimate goodness of fit.

Phase plane analysis

In order to carry out phase plane analysis, the model is simplified to two differential equations:

$$\begin{aligned} \frac{dx}{dt} &= ck21 \cdot y + ck31 \cdot PrC - (ck13 + ck12) \cdot x \\ \frac{dy}{dt} &= (-ck21 \cdot y + ck12 \cdot x) \cdot C/B \end{aligned}$$

with:

x = free cocaine in central compartment

y = free cocaine in brain

$$\text{PrC} = \text{TC} - (x + y) - \text{TA}$$

Where TC = Total cocaine; TA = total antibody

by assuming the following:

1. Degradations are much slower than antibody distribution as well as cocaine-h2E2 complex association and dissociation;
2. High affinity between antibody and cocaine;
3. The concentration of total cocaine is higher than the concentration of total antibody, therefore there is free cocaine in the system.

These two equations were used to compute the phase plane.

Sensitivity analysis

To determine the relative impact of each of the parameters on the output of the model, a sensitivity analysis was performed. Peak brain concentrations were chosen as a single value output of the model, as this has been shown to be a key determinant of the probability of reinstatement of self-administration behavior in animals, and therefore may be a predictor of relapse [13]. Each parameter value was both increased and decreased by 10-fold, and the peak brain concentrations yielded in both vehicle and h2E2 simulations were recorded.

Software

Wolfram Mathematica V.10 was used to develop the model and the Manipulate function was used as a graphical user interface to determine parameter values with a good fit to the data. Random iterations and sensitivity analysis were executed using Python's SciPy library. Phase-plane analysis was carried out with Xppaut (<http://www.math.pitt.edu/~bard/xpp/xpp.html>) and plotted with MatLab (The MathWorks Inc., Natick, MA, 2015).

Results

Experimental

Mice were treated with the mAb h2E2 or vehicle, then injected with an equimolar dose of cocaine. Blood and brain samples were taken at varying time-points from 45 s to 1 h. Mean $\pm$ SEM peak plasma concentrations in controls were significantly increased ($p=0.003$, t-test) from 593 ± 71.2 nmol/L in control mice to $14,026 \pm 3,734$ nmol/L in treated animals. The area under the time-concentration curve (AUC) was increased 61-fold from $218,557$ nmol·s·L⁻¹ in controls to $13,329,523$ nmol·s·L⁻¹ in treated animals (Fig. 2A). Plasma concentrations in both groups declined over the 1-hour sampling period. In contrast, peak brain concentrations were significantly lower ($p=0.01$, t-test) in the presence of the antibody ($4,146 \pm 448$ nmol/L in vehicle treated mice compared to 650 ± 344 in h2E2-treated mice). The AUC was also decreased 85% from $3,402,451$ nmol·s·L⁻¹ to $495,205$ nmol·s·L⁻¹ in the presence of h2E2 (Fig. 2B).

Computational

A three-compartment model was constructed (Fig.1). The model was able to reasonably capture all data (Fig 2A and B). Parameter values that produced these simulations are shown in Table 1. 12,000 random simulations with parameter values varying from 10% to 10-fold of the values in Table 1 were run. Out of these 12,000, only two yielded smaller total distances between the experimental data and simulated data (0.6% and 7% overall improvements). However, all improvement was in the fit to the brain data, at the cost of poorer fits to the plasma data, including systematic deviation from the data at the lower concentrations. Since the plasma data is more trustworthy given the experimental setting, these “*improved*” simulations were not accepted and the original parameter values were maintained.

A phase plane analysis where the system was simplified to two variables (cocaine in central compartment and cocaine in the brain) showed a linear relationship between cocaine in the central compartment and cocaine in the brain, where (assuming all positive constants) increasing antibody always decreased brain concentrations of cocaine. This highlights the robustness of the model. For example, note that the central/brain volume ratio (C/B) has an effect on the quantitative features of the system, however, it does not change the shape of the balance curves, and hence does not affect the qualitative features of the system (Fig 3).

The model predicts that almost all of the cocaine seen in the plasma in the presence of h2E2 is bound (antibody-cocaine complex); AUC= 579,481 nmol·s·L⁻¹ for free and 12,754,905 nmol·s·L⁻¹ for bound (Fig. 4A). Simulated results for the peripheral compartments showed an accumulation of cocaine in both the treated and control sets. The AUC for peripheral cocaine in control simulations was 10,952,351 nmol·s·L⁻¹. In the presence of the antibody, most of the peripheral cocaine was bound (AUC= 15,714,775 nmol·s·L⁻¹ for bound, and 579,481 nmol·s·L⁻¹ for free) (Fig. 4B). As an assumption of the model, all cocaine in the brain was free in both data sets.

A sensitivity analysis was performed, where parameter values were both increased and decreased 10-fold and the effect on peak brain concentration was observed. In rank order, the system was sensitive to (largest to smallest change) $ck_{12} > k_{on} = k_{off} > ck_{21} > C/B > ck_{13} > ck_{31} > C/P > ak_{13} > ak_{10} > ck_{10} > ak_{31} > ck_{30}$ (Fig. 5).

Discussion

The ability of the anti-cocaine mAb h2E2 to prevent cocaine's entry into the brain in mice is consistent with all previous reports [4,7,10]. Our model was able to adequately simulate these results, including cocaine concentrations in the brain and plasma of mice in the presence and absence of the anti-cocaine mAb h2E2 (Fig 2). Though this model was validated against a dataset using mice as a model species, parameter values could be readily adjusted to allow for it to apply to other species, such as rats and eventually humans. This indicates that the model proposed has physiological relevance, and that the parameter values used to generate the simulations are reasonable. The difference between the data and simulated values observed in the brain in the presence of the antibody could be due to blood in the brain. The data is generated using whole homogenized brain, so some blood will be

included in the sample. Since, in the presence of h2E2, plasma levels of cocaine are highly elevated, it is possible that the measured values are artificially high. This could account for the observed differences between the experimental observations and simulations. Both the distribution and elimination constants were much smaller for h2E2 than for cocaine, which is consistent with h2E2's much larger molecular weight (303 Da for cocaine, about 150,000 Da for h2E2), its slower distribution (0.5 min for cocaine, 13.5 hours for h2E2) and slower elimination (16 min for cocaine, 7.8 days for h2E2) half-lives reported in rats [4].

Overall, this model predicts that increases in antibody concentration will always result in decreased brain concentrations of cocaine. Though, in cases when the cocaine to mAb ratio is very high, these differences may be very small and of little physiological relevance. The magnitude of this effect will be determined the concentration of free cocaine in the central compartment, which is a function of the inter-compartmental rate constants and the affinity of the antibody for the drug. Consequently, the model should be able to predict how much drug is able to enter the brain under all circumstances. This prediction is robust despite the fact that many parameter values are unknown or imprecise. For example, the C/B volume ratio will not change the shapes of the balance curves, and hence will not change the conclusions of the model (Fig 3). This is because cocaine concentration in the brain is a direct function of free cocaine in the plasma, which is drastically lowered by h2E2. This is consistent with the finding that increasing the dose of the murine 2E2 decreased brain concentrations of cocaine following a single dose of cocaine [10]. This predicts that increasing antibody concentrations would be a robust strategy to decrease drug penetration into the brain for target drugs that have a large range of PK/PD properties. However, in clinical trials of a cocaine vaccine, high antibody titers were not associated with decreased urine samples positive for cocaine, where positive urine samples were used as a measure of efficacy [14]. More work is needed to explain the apparent discrepancies between the pre-clinical and modeling results and the clinical trials of a cocaine vaccine. Modeling of cocaine self-administration behavior in the presence of an antibody may be informative.

It should be noted that it is technically very difficult to measure how much cocaine is free or bound *in vivo*. According to this model, the large observed increase in cocaine concentrations in the plasma is mainly due to an increase in bound cocaine, not free (Fig 4A). Therefore, one would expect that the enzymatic degradation of cocaine would be slowed since h2E2 would restrict access of cocaine to enzymes. However, cocaine appears to leave the plasma at approximately the same rate in the presence and absence of h2E2 in rats [4]. The same was observed for cocaethylene disappearance in mice [7] and the present study also observed the same effect on cocaine disappearance in the presence of h2E2 in mice. It has been previously speculated that this disappearance of cocaine from plasma actually represented a delayed distribution into the peripheral compartments, instead of an unchanged rate of elimination of the drug [7]. The current model supports this hypothesis. This model predicts a relatively large accumulation of cocaine in the peripheral compartment in the presence of h2E2. Throughout the hour, peripheral concentrations of cocaine continue to accumulate, while in control simulations, the peak was reached almost immediately (Fig 4B). Therefore, the observed disappearance of cocaine out of the plasma could be accounted for by distribution of h2E2-bound cocaine into peripheral compartments. Additionally, since most of the cocaine is bound, h2E2 might be expected to ameliorate any

peripheral effects of cocaine in addition to the central effects of cocaine. If so, anti-cocaine mAbs may be useful to treat all toxic effects of cocaine at any site of action within the body.

This model also predicts that peak brain concentrations were most affected by the rate of distribution into the brain (ck_{12}). This implies that drugs that less rapidly enter the brain will be more amenable to antibody therapy. However, the properties of the target drug are usually out of the control of the investigator. More useful, the system was also extremely sensitive to changes in both the k_{on} and k_{off} binding constants. Affinity for a drug can be experimentally modified using point mutations, such as what was done for an anti-methamphetamine antibody [15]. Importantly, peak brain concentrations were equally sensitive to both values, meaning that alterations to either value (k_{on} or k_{off}) will have equal impact on effectiveness because the ratio between the two parameters (K_d) is an important determining factor for effectiveness. Interestingly, in order to provide an improved fit of the model to the experimental data, both the k_{on} and k_{off} values yielded by the model are much more rapid than is generally assumed. Though the k_{on} used is outside of the range that would be expected from a diffusion-based value, it is not unreasonable if electrostatic interactions are occurring. For example, it has been shown that k_{on} rates for antibody-antigen interactions can be increased by the introduction of charged residues into the framework region of the antibody [16]. Because h2E2 likely has lysine residues at the binding region [17], it is possible that these charged residues are causing electrostatic interactions, leading to an unusually high k_{on} rate. If so, this is overall favorable, since it means that the antibody would be able to more rapidly sequester cocaine in the plasma. The resulting K_d of h2E2 for cocaine from the k_{on} and k_{off} values generated by this model is similar to what was previously reported (2.2 nM) [12].

Conclusions

In conclusion, we have presented a mathematical model that accurately simulates the effects of an anti-cocaine antibody on cocaine's pharmacokinetics in both the plasma and the brain. This model has already generated testable hypotheses about antibody-drug interactions both *in vivo* and *in vitro*. This modeling approach will be a useful tool for probing drug-antibody interactions and accelerating the development of future anti-drug antibodies.

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Fig. 1.

A wire diagram of a three-compartment model of h2E2 binding to cocaine. Kon and koff represent binding constants. ck21, ck12, ck13, ck31, ck30, and ck10 represent pharmacokinetic rate constants for cocaine, ak13, ak31 and ak10 represent pharmacokinetic rate constants for the antibody h2E2. The 10 terms represent the species leaving the system.

Fig. 2.

Experimental data (symbols) and simulations (curves) for h2E2 treatment (blue triangles) and vehicle (green circles) group in plasma (A) and brain (B). Data represent the mean $\pm$ SEM mice at each time point. The simulations display total cocaine.

Fig. 3. Phase-Plane analysis for the system that was simplified into two equations, cocaine in the central compartment and cocaine in the brain. The cocaine brain balance curve (black) as well as the cocaine central balance curves at instances where total antibody (TA) were equal to 0 (blue) and 1000 (dotted red) are shown. The cocaine brain balance curve records the steady state of cocaine in the brain as a function of the concentration of cocaine in the central, and *vice versa*.

Fig. 4. Simulated cocaine concentrations comparing bound (solid lines) and free (dotted lines) in the presence (blue) and absence (green) of h2E2 in the central and peripheral compartments.

Fig. 5. Sensitivity analysis demonstrating the effects of multiplying and dividing each parameter value by 10 on peak brain concentrations. The blue line represents the value obtained from the parameter values in Table 1.

Parameter estimates used to generate the simulations. Kon and koff represent binding constants. ck21, ck12, ck13, ck31 and ck10 represent pharmacokinetic rate constants for cocaine, ak13, ak31 and ak10 represent pharmacokinetic rate constants for the antibody h2E2. Half-life values were calculated as $\ln 2/k$

Table 1

	Parameter	Value	Units	Half-life
Binding	kon	0.21	$\text{nM}^{-1}\text{s}^{-1}$	
	koff	0.54	s^{-1}	1.28 s
	Kd (koff/kon)	2.5 (reported 2.2 nM)	nM	
Cocaine PK	ck10	0.0006	s^{-1}	19 min
	ck12	0.002	s^{-1}	5 min
	ck21	0.0004	s^{-1}	29 min
	ck13	0.045	s^{-1}	0.3 min
	ck31	0.0007	s^{-1}	17 min
	ck30	.00045	s^{-1}	26 min
Antibody PK	ak10	0.000001	s^{-1}	8 days
	ak13	0.001	s^{-1}	12 min
	ak31	.0001	s^{-1}	1.9 days
Volume Ratios	Central/brain	10		
	Central/peripheral	0.45		