

Predicting the kinetics of drugs in the brain

RICHARD J. DIMELow, PAUL D. METCALFE, SIMON THOMAS*

*Corresponding author

Scientific Computing Group, Cyprotex Discovery Ltd, 15 Beech Lane, Macclesfield, SK10 2DR, United Kingdom

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ABSTRACT: Predicting the detailed kinetics of drugs in the brain from molecular properties and *in vitro* data alone could potentially be a cost-effective screening tool in drug discovery. Whilst this currently appears to be beyond the state of the art, we propose that a physiologically-based model of the brain offers the best way forward in this endeavour, although several problems are outstanding. Such a model will require compound specific parameters, such as the diffusive and active transport components of the blood-brain barrier permeability and the degree of binding in the brain cells. These parameters may come directly from an *in silico* approach (such as QSAR) or alternatively may be derived from *in vitro* assays, such as drug binding in a brain homogenate or drug transport across an MDR1-MDCK or caco-2 cell monolayer. The challenge here is translating the *in vitro* measurements into the *in vivo* parameters for use in the physiologically-based model. Furthermore, having built the model it must be validated against actual *in vivo* drug concentrations in the brain. For rat, microdialysis data is available, but the problem of validating a model of the human brain remains.

INTRODUCTION

Predicting drug concentration in the brain is an important step towards understanding a drug's pharmacological action and toxicity *in vivo*. But the kinetics of drugs in the brain can be very different to the kinetics in blood: the brain is protected from compounds in the bloodstream by the blood-brain barrier, a tightly-jointed layer of endothelial cells separating the bloodstream from the brain interstitial fluid (1, 2). Additional protection is provided by an array of active transporters that pump a broad range of substrates from the endothelial layer back into the bloodstream. In this review, we restrict our attention to the MDR1 transporter, thought to be the most significant drug transporter in the blood-brain barrier (3, 4). Similarly, the cerebrospinal fluid of the brain is separated from the capillaries by the blood-cerebrospinal fluid barrier at the choroid plexus (5). Unlike the blood-brain barrier, the capillaries at the blood-cerebrospinal fluid barrier are fenestrated with microscopic pores (6); the barrier here is provided by the tight junctions of the epithelial cells of the choroid plexus surrounding these capillaries. The MDR1 transporter protein also

contributes to the blood-cerebrospinal fluid barrier (7). The cerebrospinal fluid flows through the brain and then into the dural venous sinuses (8) which provides a mechanism for the clearance of drugs from the brain. There is also a smaller flow of interstitial fluid, some of which flows into the cerebrospinal fluid (9). A schematic example of the brain is shown in Figure 1.

Drug molecules in the interstitial fluid can bind to receptors located on the surface of or within the neurons to mediate a cellular response. G-protein coupled receptors, for example, activate a wide range of cellular responses (10, 11), and hence are the targets of many drugs (12). Once we know the drug kinetics in the brain, and if we have some idea of receptor affinity, we can begin to predict receptor binding and hence pharmacodynamics (13, 14).

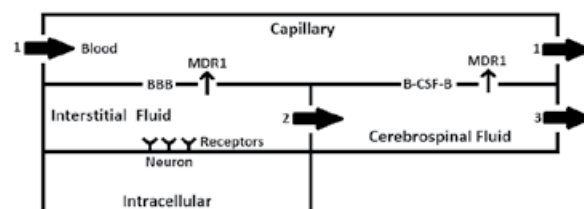


Figure 1. A cartoon brain. The different compartments of the brain: capillary, interstitial fluid, cerebrospinal fluid and intracellular space. Also shown are the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (B-CSF-B), with the action of the MDR1 transporter proteins given by the vertical arrows. The blood flow into and out of the capillaries is represented by the arrow 1, the flow of interstitial fluid into the cerebrospinal fluid by arrow 2 and the flow of cerebrospinal fluid out of the brain by arrow 3. The receptors are potential drug targets in the neuronal membrane.

EQUILIBRIUM DISTRIBUTION

A first step in predicting drug kinetics in the brain is the prediction of the blood-brain partition coefficient, defined as the equilibrium ratio of drug concentration in the brain to the drug concentration in blood. In general, greater partitioning into the brain can be achieved by increasing the octanol-water partition coefficient or decreasing the polar surface area (15-17). *In silico* models based on this principle are useful virtual screening tools for drug discovery, but are often confined to the compound space spanned by the model training set. It is also becoming clear that the blood-brain partition coefficient is of limited use in assessing the pharmacodynamic potential of drugs, and therefore *in silico* models which predict this parameter are of limited value also (18). Alternatively, the concentration ratio of unbound drug in brain

interstitial fluid to that in the plasma has been estimated from the blood-brain partition coefficient (19). An *in silico* model was then used to predict this interstitial fluid to plasma ratio from sixteen molecular descriptors, the two most significant being the polar surface area and the number of hydrogen bond acceptors. *In vitro* assays measuring binding in a brain homogenate, on the other hand, are more expensive and time consuming to carry out, and of course require compound synthesis, but do have the advantage that the result is real and not extrapolated from molecular properties (20, 21).

BLOOD-BRAIN BARRIER PERMEABILITY

In silico

QSAR methods can reliably predict the blood-brain barrier permeability of a compound provided that it is passively transported across the blood-brain barrier (22, 23). Like the blood-brain partition coefficient, the blood-brain barrier permeability tends to increase by increasing the octanol-water partition coefficient or by decreasing the polar surface area (22). Active efflux at the blood-brain barrier is mediated by the MDR1 transporter protein which has a wide substrate specificity. If a drug is an MDR1 transporter substrate then the blood-brain barrier permeability cannot be predicted from the octanol-water partition coefficient and the polar surface area alone. Properties associated with the molecular surface, polarizability and hydrogen bonding appear to be strong predictors of MDR1 activity (24), although models incorporating this information to predict the blood-brain barrier permeability for MDR1 substrates have not been published.

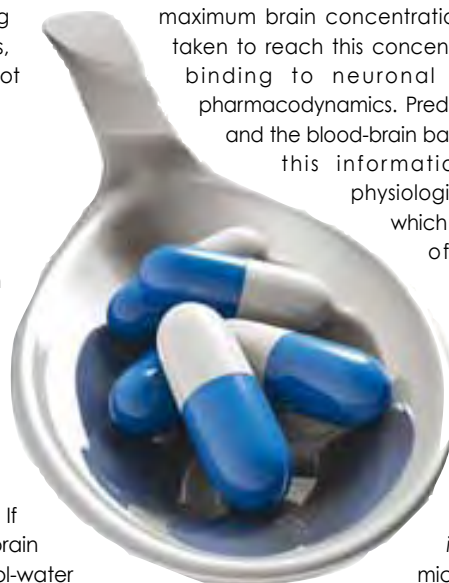
In Vitro

Transport of drug across an MDR1-MDCK or Caco-2 cell monolayer can provide information about the passive and active components of permeability. The MDR1-MDCK cells over-express the MDR1 transporter protein and can therefore be used to measure MDR1-mediated transport (25, 26). Caco-2 cells express MDR1 as well, but also express a variety of other transporter proteins (26) which can potentially mask the effect of MDR1. We therefore expect the MDR1-MDCK cell line to be more useful for quantifying MDR1-mediated transport. Furthermore, measuring the transport of drugs across a bovine brain capillary endothelial cell monolayer has been shown to be superior to Caco-2 (human intestinal carcinoma) cells at predicting the *in vivo* blood-brain barrier permeability (28). The authors argue this is to be expected since the blood-brain and the intestinal barriers are fundamentally different systems. In the same vein transport across isolated rat brain capillaries (29) should, at least in theory, give more accurate and reliable blood-brain barrier permeability predictions.

But herein lies the problem. A mathematical model of the *in vitro* system is needed to extract an *in vitro* permeability from the experimental measurements. Whether this *in vitro* permeability is in any way predictive of the *in vivo* blood-brain barrier permeability not only depends on the choice of *in vitro* system, but also on the mathematical model used to interpret the experimental results. For cell monolayers the simplest mathematical model defines the permeability as the ratio between the initial flux per unit area across the monolayer and the initial concentration on the dosing side. The MDR1 transporter protein actively transports its substrates to the apical side of the monolayer, so for MDR1 substrates the apical to basolateral permeability will be less than the basolateral to apical permeability. These two measurements can then be combined to deduce the diffusive and active components of the monolayer permeability (28). More detailed and realistic mathematical models have also been investigated (29-31).

PHYSIOLOGICALLY-BASED MODELS

In drug discovery it would be useful to know how a drug distributes between the interstitial fluid and brain cells. It would also be beneficial to have an idea about the kinetics of the drug during the time taken to reach distributional equilibrium. For instance, the maximum brain concentration reached, as well as the time taken to reach this concentration, will have implications for binding to neuronal receptors and hence the pharmacodynamics. Predicting the blood-brain partitioning and the blood-brain barrier permeability does not provide this information. On the other hand, a physiologically-based model of the brain, which is a mathematical representation of the brain's anatomy and physiology, can overcome these shortcomings. Such a model could simulate the kinetics of drugs in the brain and therefore could have a major impact in the screening of potential drug candidates. To date such models have been developed to reproduce actual *in vivo* data taken from rat microdialysis experiments (32-34). The microdialysis technique simultaneously measures the unbound concentration of drug in



both the plasma and brain interstitial fluid (35). A physiologically-based model can help us fully interpret this microdialysis data by predicting the passive and active transport components of the blood brain barrier permeability, and showing how the intracellular drug concentration must vary in time. A few examples are worth mentioning. Xie et al. (32) used a model to quantify the effect of probenecid on the transport of morphine-3-glucuronide across the blood-brain barrier. Bouw et al. (33), on the other hand, used the model to show how the blood-brain barrier affects the delay in the antinociceptive effect of morphine-6-glucuronide. Kielbasa et al. (34) used a model to determine that brain penetration of atomoxetine was high and transport across the blood-brain and blood-cerebrospinal fluid barrier is mainly passive. However, if a physiologically-based model of the brain is to be useful in early drug discovery then it must be capable of making sufficiently accurate predictions from molecular properties and *in vitro* data alone. We are only aware of Fenneteau et al. (28) who have attempted to do this for a small range of drugs; the general utility of such an approach has not been tested. The challenge is in translating the molecular properties and *in vitro* data into the model parameters. There is no prescribed structure for a physiologically-based model of the brain and the formula for obtaining these model parameters will depend in part on the choice of model being used.

CONCLUSION

Relatively simple properties such as the blood-brain barrier permeability and blood-brain partition coefficient can be predicted, with some degree of reliability, by QSAR methods. More complex properties, such as MDR1 transport, are harder to predict by QSAR, but cannot be ignored as the MDR1 transporter protein has broad substrate specificity. To further understand drug kinetics in the brain, data from more expensive and time consuming *in vitro* and *in vivo* assays of varying complexity are available. Brain partitioning can be measured *in vitro* or *in vivo*, and permeabilities and active transport can be measured in cell monolayers. However, the significance of any of these measurements is unclear. It may be useful to rank compounds according to permeability or

MDR1 transport, but the quantitative interpretation of these results is essentially unknown. Physiologically-based pharmacokinetic models of brain distribution offer an attractive means of combining experimental data to gain understanding of brain kinetics. However, *a priori* predictions are currently beyond the state of the art, requiring the correct scaling of the *in vitro* or *in silico* parameters discussed herein. *In silico* models of the rat brain can be validated using microdialysis data, but the far more important human brain models cannot be validated in any straight forward way. Finally, it is unclear just how good predictions need to be: the ultimate test of a model is how well the therapeutic window of a novel compound can be predicted, which requires insight into on-target effects, off-target effects, and toxicology. Despite these challenges, physiologically based models in drug discovery have the potential to be an important and useful tool to support decision making about novel compounds.

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