Brain Availability Is the Key Parameter for Optimising the Permeability of Central Nervous System Drugs

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Abstract

Drug disposition across the blood–brain barrier is frequently determined by measuring the distribution coefficient of drug between brain and plasma *in vivo* or by predicting the distribution coefficient from *in vitro* experiments. To render the distribution coefficient between brain and plasma (B/P ratio) a useful parameter for lead optimisation, it needs to be complemented with a measure of brain tissue binding, because it has been shown that only the free concentration of a drug in the brain relates to its pharmacodynamics. Methods for assessing drug disposition and brain tissue binding were reviewed and a new concept of brain availability has been introduced. Drug availability in the brain is defined as the product of the B/P ratio and the unbound fraction of drug in the brain. An easily accessible brain efficacy scale is introduced based on the proposed brain availability. Brain efficacy is defined as the ratio of brain availability over the drug's inhibition constant. This relates the drug's ability to penetrate the brain to its potency. Hence, this efficacy scale provides a new means of ranking drug candidates according to their dose-independent effect in the brain.

Keywords

Blood–brain barrier (BBB), brain free fraction, passive transport, brain disposition, brain tissue binding, brain membrane affinity, central nervous system (CNS) drugs, CNS side effects, logBB, screening, pharmacokinetics, *in vitro* assays, permeability-limiting glycoprotein (Pgp)

Disclosure: The author is CEO of Sovicell GmbH.
Received: 26 April 2010 **Accepted:** 20 May 2010 **Citation:** *Drug Discovery*, 2010;7:57–60
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Early assessment of drug candidate availability in the central nervous system (CNS) is essential for CNS drug development and useful for optimising the toxicity profile of non-CNS drugs.[1,2] Designing pharmaceutical agents so that they pass the blood–brain barrier and are freely available to interact with receptors is one of the great challenges in CNS drug development,[3] because more than 98% of all new candidates do not cross the blood–brain barrier efficiently.[4] Hence, one of the significant challenges in treating CNS conditions is drug passage across the blood–brain barrier.[5,6] However, at least equally important is the extent of brain tissue binding.[7,8] The stronger the binding of the drug candidate to the brain tissue, the lower the unbound fraction of the drug that can freely interact with the target receptors. The unbound fraction greatly influences the extent of the free drug concentration in the brain that ultimately interacts with the target receptors. Therefore, investigation of drug disposition into the brain as well as brain tissue binding will improve efforts in drug discovery.

Rate and Extent of Brain Penetration

Rate and extent of drug absorption into the brain are considered key parameters for the evaluation of CNS drug likeness. Several end-points have been used to estimate brain disposition in terms of rate and extent. *Table 1* compares the existing variety of nomenclatures used in the field. The rate of drug absorption from blood to brain can be of direct relevance, in particular for indications such as antiepileptic agents where rapid action is desired. However, the rate is not directly related to the time it takes a drug to reach its equilibrium distribution between blood and brain. It has been noted that drugs with a low permeability rate can reach equilibrium very rapidly *in vivo*.[9,10] Notwithstanding these difficulties in the direct interpretation of rate measurements, the rate of drug uptake into the brain is often considered proportional to the extent of brain penetration. Thus, rate measurements frequently replace extent measurements based on the distribution coefficient between brain and plasma (B/P ratio or, on the log scale, logBB; see *Table 1*), because it became apparent that the distribution coefficient varies over a wide range even for highly efficacious CNS drugs (see *Figure 1*).

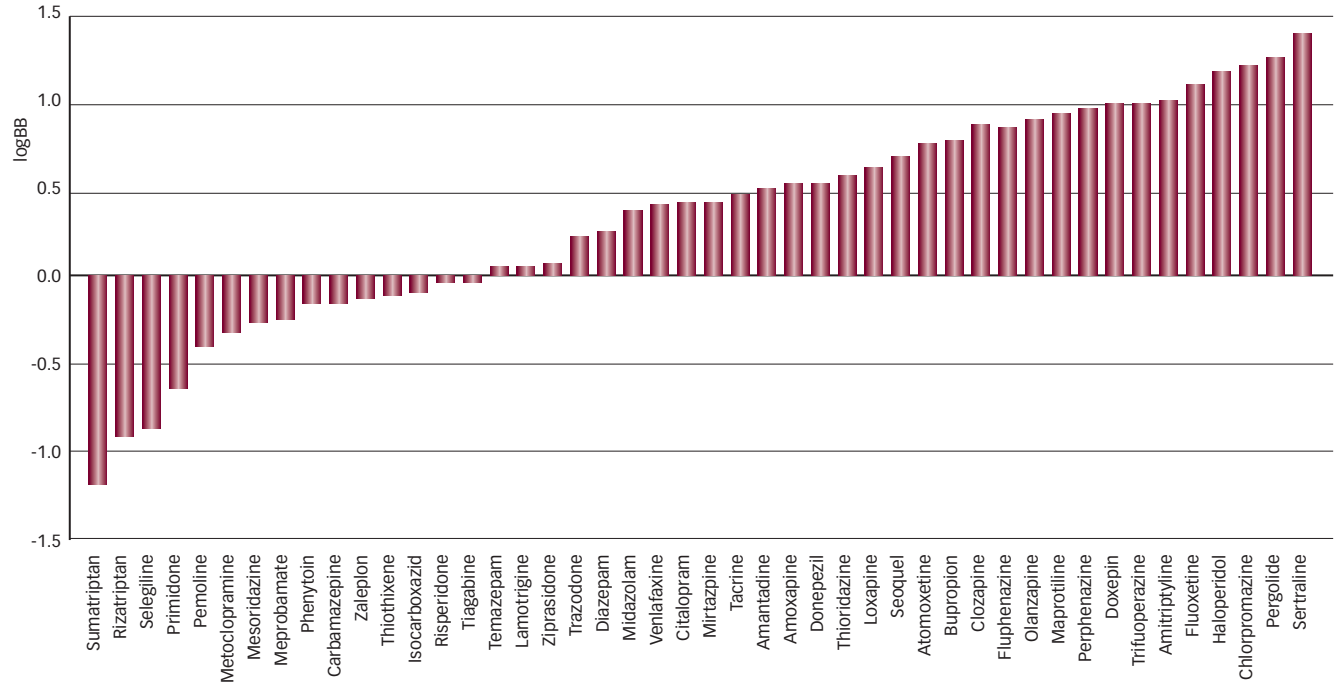
However, estimating the extent of brain penetration from the rate of brain penetration requires several assumptions about influx, efflux, clearance rates, capillary surface areas and transporter density that render such estimates somewhat inaccurate. Moreover, it has been shown that the key problem with the extent estimate based on the brain-to-plasma distribution coefficient is its deficiency in predicting the unbound drug concentration that is freely available to interact with receptors in the brain's interstitial fluid.[7,11] Extent estimates based on permeability rates cannot solve this problem. They are also not suitable for assessing the amount of drug bound to brain tissue. Moreover, permeability-rate-based extent estimates tend to worsen the problem because of their inherently lower accuracy compared with direct brain-to-plasma distribution estimates. Hence, it is more promising to combine a high-quality estimate of the extent of drug absorption with an estimate of brain tissue binding, because tissue binding affects the free concentration in the brain more effectively

Table 1: Comparison of End-points Used to Characterise Brain Disposition and Brain Tissue Binding

End-point	Definition	Description
B/P ratio	$B/P = \frac{[D]_{brain}}{[D]_{plasma}}$	Distribution coefficient of total drug between brain and plasma
logBB	$\log BB = \log\left(\frac{[D]_{brain}}{[D]_{plasma}}\right)$	Distribution coefficient of total drug between brain and plasma on logarithmic scale
K_p	$K_p = B/P = \frac{[D]_{brain}}{[D]_{plasma}}$	Distribution coefficient of total drug between brain and plasma
$K_{p,free}$	$K_{p,free} = \frac{f_{u,\,brain} \cdot [D]_{brain}}{f_{u,plasma} \cdot [D]_{plasma}}$	Distribution coefficient of free drug between brain and plasma
$P_{app,brain}$	$P_{app} = \frac{V_D \cdot V_R}{(V_D + V)\,S \cdot t} \ln\left[\frac{V_D}{V_D - f_t\,(V_D + V)}\right]$	Permeability rate of drug across a cell monolayer or organic solvent as in PAMPA determined on the basis of the fraction of drug transported, f_t, the surface area, S, the incubation time, t, and the donor and receiver volumes, V_D and V_R, respectively
PS	$K_{in} = v_f = F \cdot (1 - e^{PS/v_f F})$	Product of the permeability, P, and the capillary surface area, S, (PS product) determined by solving the equation for the unidirectional influx constant, K_{in}, using estimates of the plasma flow, $v_f F$

The total concentration of drug is denoted by [D], total concentration of drug in brain is denoted by $[D]_{brain}$ and total concentration of drug in plasma is denoted by $[D]_{plasma}$.

Figure 1: Distribution Coefficient logBB Between Brain and Plasma of 46 Marketed Central Nervous System Drugs



Sertraline exhibits the highest brain penetration (logBB 1.6), while sumatriptan exhibits the lowest brain penetration (logBB -1). However, both drugs are highly efficacious central nervous system (CNS) drugs. This indicates that the distribution coefficient, which varies 400-fold between these two drugs, carries only little information about a drug or drug candidate's efficacy in the brain.

than the total concentration in the brain.[10] In fact, the better a drug is able to penetrate the brain, the greater the likelihood that it will be tightly bound to the brain tissue (see *Figure 2*). Drugs entering the brain more easily tend to be more lipophilic, and since the brain's dry mass is dominated by lipids they will bind to the lipid fraction of the brain to a greater extent than less lipophilic compounds.

Brain Availability

Relative differences in the free concentrations of the drug candidates can be predicted by the drug's brain-to-plasma distribution (B/P ratio) and its unbound fraction in the brain. In fact, brain availability, A, defined as the product of the B/P ratio and the free fraction, f_u of the drug in the brain, is proportional to the free concentration in the brain:

$$A = \frac{[D]_{brain}}{[D]_{plasma}} \cdot f_u(D)_{brain} \sim c_u(D)_{brain}$$

where $[D]_{brain}$ denotes the total concentration of the drug in the brain and $[D]_{plasma}$ denotes the total concentration of the drug in plasma, while $c_u(D)_{brain}$ denotes the free concentration of the drug in the brain. Hence, the availability of a drug in the brain represents the fraction of drug entering the brain that is unbound and thus freely available to interact with receptors. This availability scale can be used to rank compounds by their potential effect at the receptor site.

The free concentration, $c_u(D)_{brain}$, further depends on the drug's dosage, which is related to its pharmacodynamic properties. Consequently, the availability scale can be further improved by relating the fraction of drug entering the brain that is unbound to the drug's potency, i.e. its inhibition constant. This yields the efficacy, *E*, of a drug in the brain and can be obtained by dividing the availability of the candidates by their inhibition constant, *Ki*:

$$E = \frac{A}{K_i} .$$

This efficacy scale, *E*, expresses the dosage-independent effect of a drug. A low *E* value requires a high drug dosage, while a large *E* value requires only a small dosage. Therefore, this scale is well suited for ranking compounds based on their efficacy and provides a means of relating data from toxicological studies to the drug's potency.

Estimating Brain Tissue Binding

Brain tissue binding is a key parameter for determining brain availability of drugs. Several methods are available for estimating tissue binding (see *Table 2*). Brain microdialysis evaluates tissue binding *in situ*. It is the most realistic and accurate measurement; however, it requires extensive resources, cannot be easily applied to lipophilic compounds and has a very low throughput.

The brain slice method is the second most realistic approach as it maintains the tissue integrity and thus minimises binding to structures that may become exposed when preparing crude extracts, as is the case for dialysis with brain extract.[12,13] However, this approach is also somewhat resource-demanding and cannot be performed in high-throughput formats.

A more rapid alternative is dialysis with rat brain homogenate.[7] This approach allows fast assessment of brain tissue binding and requires far fewer resources than the brain slice method. The main caveats with this method are the preparation of rat brains and the downstream analytical process requiring quantification of compound concentrations in the lipid phase. The latter may lead to matrix effects such as ion suppression in mass spectrometry and consequently to unreliable results.

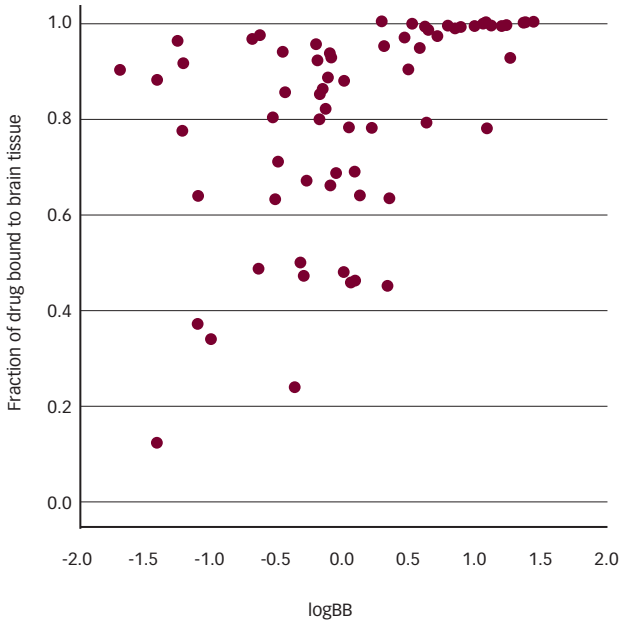
Another rapid technique to determine brain tissue binding is to measure the affinity to brain membranes via solid supported TRANSIL brain absorption beads. This matrix-free method yields comparable results to dialysis with brain homogenate, while it is amenable to high-throughput screening, requires fewer resources and saves animals.

Estimating Brain-to-plasma Distribution

The B/P ratio measures how much drug crosses the blood–brain barrier. Taken alone, this measure is difficult to interpret. As mentioned above, compounds that cross the blood–brain barrier more readily than others have a high tendency to bind to brain tissue. As illustrated in *Figure 1*, marketed CNS drugs span a broad range of B/P ratios from 0.1 to 40. Thus, these marketed CNS drugs span a 400-fold range of B/P ratios while they span only a five-fold range in free concentration in the brain.[10] This indicates that while the B/P ratio alone is less helpful for predicting CNS druggability, the free fraction as well as the availability is a good indicator for CNS drug likeness.

As an alternative to measuring the B/P ratio *in vivo*, it can be predicted by the ratio of the free fraction of the drug in plasma over the free fraction of the drug in the brain[14] or by a prediction model

Figure 2: Brain Tissue Binding of 72 Marketed Drugs in Relation to the Distribution Coefficient Between Brain and Plasma (logBB)



The greater the fraction of the drug entering the brain, the greater the likelihood that the drug binds to brain tissue.

Table 2: Comparison of Methods for Assessing the ADME Properties of Drug Candidates Relevant for Central Nervous System Penetration

Assay	Rate	Extent	
		Concentration	Free Fraction
Brain-to-plasma Distribution (B/P ratio, logBB)			
In vivo	NA	+	NA
IAM	NA	+	NA
Uptake Rates			
PAMPA	0	–	NA
Caco-2	0	–	NA
MDCK II	+	–	NA
BCEC/astrocyte co-culture	++	0	NA
PS product (*in situ* perfusion)	++	+	NA
Brain Free Fraction			
In situ dialysis	NA	NA	++
Brain slices	NA	NA	++
Microdialysis with brain extract	NA	NA	+
Solid supported brain membranes (TRANSIL)	+	+	+

The methods are ranked based on relative suitablility on a scale from – (very poor) to ++ (very good). The abbreviation NA denotes that the method is not designed to assess that particular parameter.

based on the drug's polar surface area, plasma protein binding and affinity to brain membrane using TRANSIL brain absorption. Both predictions assume absence of active transport processes. Hence, a significant influence of efflux transporters on brain-to-plasma distribution will result in overestimation of total brain concentration using either approach. For practical purposes this overestimation may not be critical, since successful CNS drugs on the market are not substrates for efflux transporters and because *in vitro* evidence for efflux transport does not always manifest *in vivo*.[11]

Active Transport

The pharmacokinetics of brain delivery is further complicated by active influx and efflux transport at the blood–brain barrier. The mechanisms by which efflux transporters act will influence brain pharmacokinetics in different ways.[15] It has been suggested that the permeability-limiting glycoprotein (Pgp), the best known and, according to current understanding, most important efflux transporter for exogenous substances, lowers brain drug concentrations through two mechanisms: a gate-keeper function that prevents molecules from entering the brain and an extrusion mechanism via which molecules already present in the cytoplasm of the blood–brain barrier endothelial cells are transported back to the blood.[16] The effect on the brain concentration time profiles will depend on which of these two functions is predominant. It has also been speculated that some compounds are transported from the brain interstitial fluid into the endothelial cells by abluminal transporters and thereafter by luminal transporters from the blood–brain barrier to the blood. Hence, transporters located at the two membranes of the BBB may work together to reduce or increase brain concentrations of certain compounds.

Lead Compound Prioritisation

Several competing and complementary methods exist to assess active and passive brain disposition as well as brain tissue binding of drug candidates (see *Table 2*). Recent developments have provided new rationales and methods to assess crucial absorption, distribution, metabolism and excretion (ADME) properties of CNS drugs. Methods assessing drug disposition into the brain based on passive diffusion are generally much less resource-demanding than methods investigating the active transport components. If the active transport component is an efflux process, its relevance depends on the degree of passive influx and brain tissue binding. Only if the drug's availability is reasonably high will the efflux process significantly reduce the net disposition into the brain. Hence, for lead candidate selection of CNS drugs it will be most economical to estimate the availability or efficacy of drugs in the brain based on passive permeability processes first, and to focus further resource-demanding investigations of active transport processes only on those drug candidates that have reasonably high CNS efficacies. This approach will save resources in the ADME screening of lead optimisation.

However, screening for CNS availability will not only be instrumental for CNS drug development; drug discovery and development projects for other indication areas may also benefit from the inclusion of CNS availability in the lead optimisation assay portfolio, as this parameter is tightly linked to CNS side effects. Non-CNS drugs that are highly available in the brain will have high free concentrations in the brain. As a result, the likelihood that these drugs will unspecifically interact with receptors and cause typical CNS side effects such as nausea, headache, insomnia, etc. is substantially increased. Screening for brain availability of next-generation antihistamines, antiarrhythmics or antihypertension drugs bears the chance of reducing their side effects and thus increasing their market potential. n

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