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Review

Assessment of the blood–brain barrier in CNS drug discovery

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ABSTRACT

A wide variety of models have been developed over the years to predict blood–brain barrier (BBB) penetration, most of them have focussed on predicting total concentrations of drug and then expressing this as a brain:blood (or plasma) ratio. This approach is somewhat flawed and fails to address the critical issue of understanding the relationship between access of free drug to the requisite site of action. In this short review, we highlight the need for an integrated approach and whilst blood–brain barrier permeability is an important determinant in achieving efficacious CNS drug concentrations it should not be viewed or measured in isolation. Optimal CNS penetration is achieved through the correct balance of permeability, a low potential for active efflux and the appropriate physicochemical properties that allow for drug partitioning and distribution into brain tissue. Such an approach should enhance and accelerate our understanding and ability to predict CNS efficacy in terms of free drug concentrations and the rate at which they are achieved.

Contents

Introduction

Despite much progress by the pharmaceutical industry over the last few years, the number of products launched to treat diseases of the central nervous system (CNS) has not met with expectation nor demand. Increases in the costs associated with drug development, the risk and subsequent cost of failure in clinical trials, a basic shortfall in the understanding of both the underlying biology behind the disease and the myriad of factors that contribute to optimised CNS drug delivery have severely limited successful drug development in this area (Pardridge, 2002).

The CNS is highly protected by the blood–brain barrier (BBB) and the blood–cerebrospinal–fluid barrier (BCSFB). The BBB is a unique barrier that regulates and controls the selective and specific transport of both exogenous and endogenous materials to the brain (Begley and Brightman, 2003; Abbott et al., 2006). The BBB is made up of three cell types; endothelial cells, astrocytes and pericytes. The endothelial cells that are present in the BBB surround the brain capillaries and are relatively impermeable, possessing extensive tight junctions with no fenestrations and reduced pinocytic vesicular transport. These cells also contain both uptake and efflux transporters (Ohtsuki and Terasaki, 2007) and are metabolically competent (Meyer et al., 2007), a combination that restricts drug permeation. Historically the pharmaceutical industry has believed that the best chance of success for a potential CNS drug has been by optimising the rate and extent of drug delivery and simply ranking compounds on the basis of the faster the delivery and the greater the amount delivered the better (Hitchcock 2008; Hitchcock and Pennington, 2006). However, current research is now focussed on a more integrated approach that is aimed at understanding the drug concentrations at the active site within the brain compartment, the factors that need to be considered in order to optimise this and the relationship between drug concentration and pharmacological activity i.e. pharmacokinetics and pharmacodynamics (Ploeger et al., 2009).

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Basic concepts: An historical perspective

The general assumption in drug discovery departments has been that for those lead compounds which are intended for CNS indications "good CNS penetration" is required and that the two key parameters have generally been used to define "CNS penetration" are; *Kp* which describes the ratio between brain and blood (or plasma) concentration, usually measured under steady-state conditions and *PS* which describes BBB permeability and is quantified as the permeability × surface area product. There are numerous reports in the literature that describe such approaches (see Cecchelli et al., 2007; Feng, 2002 and Reichel, 2006 for historical reviews) but more recently this type of approach has been called into question (Hammarlund-Udenaes et al., 2008; Jeffrey and Summerfield, 2007; Liu et al., 2008). The determination of *Kp* alone has somewhat limited value in drug discovery; in measuring the brain–plasma ratio of 32 structurally diverse marketed CNS drugs in the mouse, values between 0.06 to 24 were obtained (Doran et al., 2005) refuting the claim that all CNS drugs require "good CNS penetration" and emphasising the point that *Kp* represents nothing more than "an inert partitioning process of drug into lipid material" (Van de Waterbeemd et al., 2001). The determination of *PS* provides quantitative information on the rate at which a compound will cross the BBB but used in isolation can provide no assessment on the extent of drug permeation in the brain.

The central tenet in pharmacokinetics is that it is the unbound or free drug concentrations that are responsible for drug action and that under steady-state equilibrium conditions these concentrations will be equal across the intracellular space and intracellular and extravascular extracellular spaces. However, this is not the case with the BBB. Compounds with a high *Kp* value may not necessarily provide a solid platform for efficacy, since high levels on non-specific binding within the brain tissue compartment may reduce the unbound drug concentrations required for efficacy. Drug transporters, both uptake and efflux, at the level of the BBB (Ohtsuki and Terasaki, 2007) and the complex interactions of bulk flow of brain interstitial fluid (Abbott, 2004) and cerebrospinal fluid (Johanson et al., 2008) all have implications for drug delivery and clearance within the various compartments of the CNS.

Current approaches to understanding and quantifying CNS penetration have moved on from expressing a single value representing rate and/or extent and are now focussed on a greater understanding of the three main factors that modulate and control drug disposition in the brain; 1) passive membrane permeability, 2) the role of facilitated transport at the BBB (primarily focussed on the efflux transporter P-gp) and 3) the relative degree of tissue binding between the brain and plasma (or blood) compartments. This concept is illustrated in Fig. 1 and highlights the fact that these processes do not act in isolation but are all linked together and require an integrated approach to understand which parameter(s) dictate the overall rate and extent of brain penetration for a single compound or any series of compounds with similar physicochemical properties.

Several key studies highlight recent progress in this area. In evaluating a set of CNS drugs ($n=48$) with a set of non-CNS drugs ($n=45$) Mahar Doan et al. (2002) investigated the relationship between physicochemical descriptors and *in vitro* passive membrane permeability with P-gp substrate liability using the MDR1-MDCKII cell line. The majority of CNS drugs (46 out of 48) had passive permeabilities (*Papp*) greater than 150 nm/s whilst 13 out of the 45 non-CNS drugs had permeabilities less than 150 nm/s. A similar cut-off value of 200 nm/s was advocated by Lin (2004) using the LLC-PK1 cell line whilst Wang et al. (2005) reported a much reduced *Papp* value of 30 nm/s based on a comparison of CNS and non-CNS drugs evaluated *in vitro* in an MDR-MDCK cell based assay. Interestingly, a comparison of the Papp values obtained for the same 14 compounds that were used in two separate studies (Mahar Doan et al., 2002 and Wang et al., 2005) showed very little correlation between the data

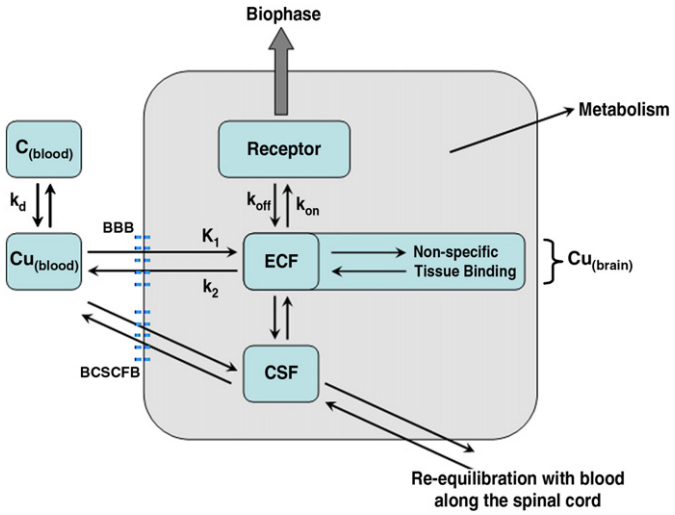

Fig. 1. Schematic of the major brain compartments and rate constants associated with brain penetration and efficacy. Unbound drug is assumed to be the fraction available to cross the BSCFB or BBB, the latter of which is associated with the influx clearance constant K_1 (or Kin) and k_2 denoting back exchange to the peripheral arterial blood by means of passive diffusion or active transport. Association and dissociation constants between blood and receptor are denoted by k_d, k_{on} and k_{off}. C (blood) and C (brain) represent total tissue concentrations while Cu (blood) and ECF (extracellular fluid) describe the free drug available in the peripheral and central compartments, respectively (Jeffrey and Summerfield, 2007).

sets (Jeffrey and Summerfield, 2007). Although there are a number of well characterised *in vitro* cell based models for investigating BBB permeability, metabolism and transporters, they cannot reproduce all aspects of the *in vivo* system (Abbott et al., 2008). Problems associated with cell-lines are quite varied; cultured brain endothelial cell-lines preserve many features of the BBB phenotype but suffer from down regulation or altered expression levels of tight junctions, transporters, enzymes and receptors to varying degrees (Abbott et al., 2008 and Di et al., 2008). Immortalised cell-lines are generally leakier that primary cultures and have greater batch-to-batch variability; thus the ability of any cell monolayer to resolve and rank drug permeabilities will be influenced by the original source and culture conditions. Whilst the determination of drug permeability across the BBB and understanding the role of transporters is important, the lack of a single, well characterised, fully validated and uniformly acceptable *in vitro* cell line highlights a major challenge for researchers in this field; that the results obtained and conclusions drawn will be very much dependent on the experimental conditions and cell-lines employed by the respective groups. Therefore, it is very difficult to combine data from different groups to create a large single date set with which to analyse, model and predict.

An early study that highlighted the importance and impact of obtaining a unified data set that could then be applied in a drug discovery setting was described by Kalvass and Maurer (2002). Using 18 proprietary compounds a number of evaluations were performed; relative plasma, brain and cerebrospinal fluid (CSF) concentrations and unbound fractions in plasma and brain data were collated for the rat, *Papp* was performed using Caco-2 cells assessing both permeability and the potential for active efflux mediated by P-gp and then correlated with *in vivo* studies in wild-type (FVB) vs MDR 1a/1b knockout mice. Measurement of free fractions in both rat plasma and brain was determined using high-throughput equilibrium dialysis and correlated with total plasma and brain concentrations and CSF (obtained via the *cisterna magna*) concentrations. Although no chemical structures were disclosed the results demonstrated that brain:plasma ratios could be predicted within 2-fold for 89% of the compounds evaluated and that passive permeability was not influenced by active transport. For those compounds that were