Neuropharmacokinetics: a bridging tool between CNS drug development and therapeutic outcome

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WHO classified neurological disorders to be among 6.3% of the global disease burden. Among the most central aspects of CNS drug development is the ability of novel molecules to cross the blood–brain barrier (BBB) to reach the target site over a desired time period for therapeutic action. Based on various aspects, brain pharmacokinetics is considered to be one of the foremost perspectives for the higher attrition rate of CNS biologics. Although drug traits are important, the BBB and blood–cerebrospinal fluid barrier together with transporters become the mechanistic approach behind CNS drug delivery. The present review emphasizes neuropharmacokinetic parameters, their importance, an assessment approach and the vast effect of transporters to brain drug distribution for CNS drug discovery.

Introduction

Neurological ailments constitute ~6.3% of the total disease burden globally, and this figure is estimated to increase by 12% by 2030 [1,2]. This is an alarming sign and the development of new drugs for the therapy of central nervous system (CNS) disorders are therefore much needed. Despite advanced technology in the area of drug discovery, nervous system drug therapy remains challenging for researchers. CNS drug discovery encounters higher attrition rates than other areas of drug development preclinically and clinically. Currently, treatment available for CNS disorders is disappointing and only focuses on the symptoms rather than curing the disease. Research are being implemented for the development of neurotherapeutics with advanced technological approaches (Table 1), including examples like the development of vitamin-incorporated solid lipid nanoparticles (SLNs) of dimethyl fumarate for effective brain delivery; because numerous pharmacokinetic obstacles prevent its penetration across the blood–brain barrier (BBB) [3,4]. Therefore, it could be proposed that the factors responsible for CNS drug failures are physicochemical properties, penetration efficacy across the BBB, protein binding and binding to receptors (i.e., pharmacokinetics; see Glossary).

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Understanding the basic principles of CNS pharmacokinetics would enable prediction of novel drug BBB-penetration and accumulation. It is a well-known fact that drugs developed for neurological disorders must cross major CNS barriers over a desirable time period so the drug can be therapeutically active, driving its pharmacological action [5]. Among numerous perspectives, brain-pharmacokinetics/neuropharmacokinetics (neuroPK) is considered to be the most significant determinant for the failure of CNS drug discovery. It is concerned with the quantitation of neuroactive lead molecules in the brain, brain interstitial fluid (BISF), brain cerebrospinal fluid (BCSF) and plasma using different techniques [6].

Several compounds are screened daily for CNS drug development but only a few of them meet the criteria for treatment of neurological disorders. Therefore, it is important to evaluate a molecule for its prerequisite neuroPK characteristics before it enters the post-marketing surveillance phase for various neurological disorders like Parkinson's disease [7], Alzheimer's disease [8], Huntington's disease [9], epilepsy [10], NeuroAIDS [11,12], among others. Although it is impossible to measure the drug concentration directly in any species, the surrogate biological matrices, for example the CSF and brain homogenate, are considered to be the most reliable surrogate matrix for analyzing the unbound form of

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GLOSSARY

Pharmacokinetics PK – defined as the time-course of drug absorption, distribution, metabolism and excretion and their relationship with therapeutic efficacy and toxic effects of drugs.
Neurological disorders The condition that affects the physiological conditions of neuronal tissues.
Bioavailability Defined as the rate and extent of the drug in systemic circulation.
CSF Cerebrospinal fluid.
Transepithelial/endothelial electrical resistance TEER – an important method for in vitro barrier tissue integrity assay measures for body-on-a-chip barrier tissue devices owing to its usefulness and noninvasive nature [84].
BBB Blood–brain barrier.
HPLC High-performance liquid chromatography.
LC–MS/MS High-throughput liquid chromatography tandem mass spectrometry.

TABLE 1

List of recently FDA-approved formulations for neurological disorders

Drug	Year	Neurological disorder	Remarks
Aimovig	2018	Migraine	
Austedo	2017	Chorea associated with Huntington's disease and tardive dyskinesia	
Brineura		Late infantile neuronal ceroid lipofuscinosis type 2	
Amantadine		Parkinson's disease dyskinesia	
Valbenazine		Tardive dyskinesia	
Edaravone		Amyotrophic lateral sclerosis	
Safinamide		Parkinson's disease	
Brivaracetam	2016	Partial onset seizures related to epilepsy	
Carbamazepine		Seizures	Replacement therapy when oral administration is not feasible
Sumatriptan nasal powder		Migraine	
Nusinersen		Spinal muscular atrophy	
Oxycodone + naltrexone		Severe pain	
Daclizumab		Relapsing multiple sclerosis	
Aripiprazole lauroxil	2015	Schizophrenia	Extended-release injectable
Buprenorphine		Severe pain	
Carbidopa and levodopa		Motor fluctuations in patients with advanced Parkinson's disease	Enteral suspension
Carbidopa and levodopa		Parkinson's disease	Extended-release tablets
Meloxicam		Osteoarthritis pain	
Cariprazine		Schizophrenia and bipolar disorder	
Suvorexant	2013–2014	Insomnia	
Diclofenac sodium injection		Mild, moderate or severe pain	
Tasimelteon		Non-24-hour sleep-wake disorder	In totally blind people
Alemtuzumab		Relapsing multiple sclerosis	
Naloxegol		Opiod-induced constipation in adults	Chronic noncancer pain
Memantine hydrochloride + donepezil hydrochloride		Moderate to severe dementia of the Alzheimer's type	Memantine hydrochloride is extended release
Droxidopa		Neurogenic orthostatic hypotension	
Peginterferon β-1a		Relapsing multiple sclerosis	
Topiramate		Partial onset and primary generalized tonic-clonic seizures and Lennox–Gastaut syndrome	
Targiniq ER extended-release tablets		Severe chronic pain	(Oxycodone hydrochloride + naloxone hydrochloride)
Indomethacin		Acute pain	
Xartemis XR extended release		Acute pain	(Oxycodone hydrochloride and acetaminophen)
Eslicarbazepine acetate		Partial-onset seizures	
Levomilnacipran		Major depressive disorder	
Nimodipine		Reduction of incidence and severity of ischemic deficits following subarachnoid hemorrhage	
Lopiramate		Partial onset, tonic-clonic and Lennox–Gastaut syndrome seizures	
Hydrocodone bitartrate Extended-release capsules		Severe pain	
Buprenorphine and naloxone		Opioid dependence	

drug accessible in the human brain. Investigations have been performed to ratify the predictability of CSF drug assessment for unbound drug concentration in the brain, plasma and CSF [13]. Furthermore, today, many *in vitro*, *in vivo* or *in situ* methods are available to measure CNS drug exposure but a translational approach like physiological-based pharmacokinetic modeling has also been the predominant technique for CNS drug development.

Apart from the above brief introduction, the present review will discuss the major obstacles during neuropharmaceutical discovery, important parameters to be measured after drug development during preclinical trials and their quantification using recent technological advances. In reviewing the literature on neuroPK, the authors searched PUBMED MEDLINE for articles using the keywords 'brain pharmacokinetics'; 'BBB penetration'; 'PBPK' and 'physicochemical properties'; from the past 5 years. The authors would also like to emphasize the interference caused owing to the presence of transporters on the BBB membrane regarding bioavailability of therapeutically active neuroactive lead molecules.

Facets of the neuroactive lead molecule

The decrease in product output for CNS therapeutics needs an innovative drug discovery approach. For designing and development of a centrally acting lead molecule, the physicochemical properties of the optimized novel candidate become one of the crucial factors for crossing the BBB (Box 1). Various researchers reported that, for a productive output, a lead compound for neuro drug should have: (i) molecular weight less than or equal to 400 Da; (ii) logP value close to 2; (iii) cumulative number of H-bonds less than ten; and (iv) the compound should be in a non-ionized form or basic in nature [14]. The neuroactive compounds should also accompany the ionizable functional group on its structure enabling facile BBB permeation to reach the target site at an effective concentration. Further, topological surface area (TPSA) is another important aspect to be considered during CNS drug development [15]. A molecule with TPSA of <90 Å is indicated for development in CNS therapeutics.

Furthermore, considering recent advances, the concentration of unbound drug molecule in plasma is the most important feature for CNS drug development. Depending upon its physicochemical properties, the molecule binds to the acidic/basic proteins present in plasma leaving the remaining amount of drug to be available for entering the brain [16]. Neuroactive molecule stability within the brain, brain-tissue binding and brain drug-metabolism are the main factors affecting the bioavailability of the molecule for therapeutic efficacy. After reaching the target site, the physicochemical properties of the drug affect the drug distribution within the cerebral regions. Drugs that are basic in nature (pK_a 8.9) (e.g., thioridazine) accumulate in the intracellular acidic compartment of the cytosol resulting in a many-fold increase of the drug after detachment of the lysosomal compartment from the cytosolic compartment that ameliorates its distribution phenomenon, eventually improving bioavailability to the target site [17]. Only 19–20% of the unbound form of a drug that is acidic in nature (e.g., salicylic acids) crosses the BBB [16]. Hence, it is beneficial to determine the unbound concentration of a drug in plasma which is further distributed at the cytosolic and lysosomic level within the brain; and its penetration efficacy across the BBB during CNS drug development [14,16]. Despite the characteristics of a molecule, the barriers present in the brain limit the distribution of neuroactive compound and thus hinder the bioavailability and therapeutic outcome. These barriers will be the next target for discussion in the present review.

BOX 1

General physicochemical properties of neuroactive molecule

Typical physicochemical properties of drugs crossing the blood–brain barrier

Compound should not be ionized.
log*P* value should be close to 2.
Molecular weight <400 Da.
Cumulative number of H-bonds should not be >8–10.
Drug nature (acidic or basic) should be known.
Unbound drug reaches the brain.

Blood–brain barrier

The BBB is an active, dynamic and selective interface between the blood and brain made up of endothelial cells, astrocytes, pericytes and neuronal terminations (Fig. 1). It regulates the transport of exogenous and endogenous substances into the CNS, predominantly through two main channels: the paracellular and transcellular pathways [18]. Hence, the BBB is one of the major barriers to the successful development of active CNS compounds. Therefore, development of neuroactive molecules should be done under consideration of the properties of the BBB. In the past, the pharmaceutical industry believed that the best chance of success for a potential CNS drug was by optimizing the rate and extent of drug delivery [19]. However, in the present century, research has been more focused on an integrated approach estimating drug concentrations at the active site within the brain compartment. Numerous drugs with neurotherapeutic activity have been quantified in the brain to assess BBB permeability and the brain:plasma ratio. Furthermore, drug quantification in any specific region of any tissue also depends upon the technique employed for assessment [20–22]. Singh *et al.* quantified the nobiletin (neuroprotective agent) concentration in brain and plasma, concluding that drug exposure was three-times higher in the brain compared with the plasma [23]. They determined that higher penetration of nobiletin in the brain signalled the drug could be a lead molecule for any neuropharamacological disease. Similarly, Patil *et al.* analyzed the pharmacokinetics of flupirtine in different brain regions, concluding that the drug reaches the brain through the systemic circulation where it is enzymatically converted into its metabolite drug (D-13223), which might be accountable for its therapeutic activity against neonatal seizures [24]. Hence, neuroPK is the foremost parameter for identification of pharmacotherapeutic efficacy of a drug within the brain. Furthermore, drug quantification in a specific region of a tissue also depends on the technique employed for assessment [20–22].

Owing to the problem of delivering molecules across the BBB, neuroPK in CNS drug discovery and development is a valuable tool to be appraised. Transportation of a substance such as low molecular weight, lipid-soluble molecules, nutrients and a few peptides across the BBB is either by passive diffusion or specific transport mechanisms [25,26]. To overcome the restriction of transportation of a potential lead molecule into the BBB offers extensive research

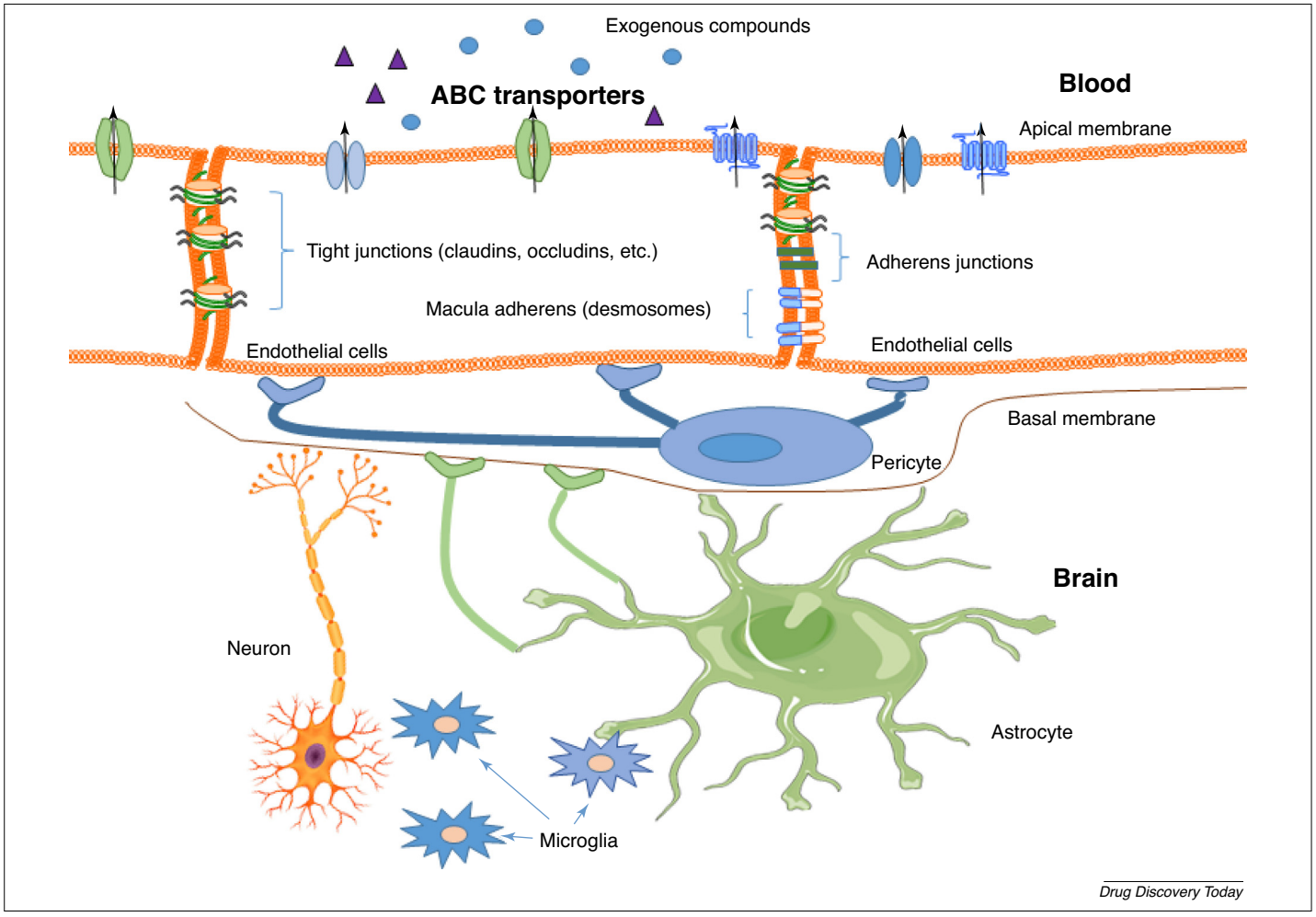

FIGURE 1

Graphical representation of the blood–brain barrier (BBB) structure and transport across the membrane.

on the development of new drug delivery strategies to deliver drug molecules more effectively at the CNS or target site. Moreover, modeling analysis has also been focused to optimize the relationship between drug concentration and therapeutic efficacy that belongs to pharmacokinetic and pharmacodynamic (PK/PD) modeling [19,27]. Further, in combination with PBPK, PK/PD analysis would also be used to estimate the effect and dose of novel molecules. Although these models need to be validated but *in vivo* exposure could be best predicted from the newly developed translational approach.

Brain–cerebrospinal-fluid barrier (BCSFB)

The CSF resides in the subarachnoid space and the ventricular system around or inside the brain parenchyma, and its mechanical cushioning or buffering nature protects the brain from mechanical stress. Approximately 20% of CSF in the human brain originates from brain ISF [28]. The free drug is the only drug that is present for pharmacological activity; therefore, CSF could be used as the direct biomarker for brain drug distribution analysis because there is no or minimal protein binding under standard physiological milieu [16,29]. For example, selumetinib (for plexiform neurofibromas and gliomas in children) was quantified by Gross *et al.* for CNS penetration using CSF of non-human primates as a surrogate marker. They found that the pharmacokinetic aspects, the area under the curve and half-lives of humans and the CSF surrogate model, were similar. Hence, the surrogate CSF could be the useful model for analyzing drug distribution and pharmacokinetic parameters within the brain after applying the correction factor of plasma protein binding [30].

Parameters for measuring brain pharmacokinetics

Anatomical and physiological parameters of the BBB are important characteristics that should be considered during drug discovery when neuroPK is being evaluated [31].

Cerebral blood flow (CBF)

CBF is defined as the extent of blood that flows per unit mass per unit time in brain tissue through the brain capillaries and arteries and expressed in terms of $ml_{blood}/(100\ g_{tissue}\ min)$ [32]. The CBF represents the upper limit of the rate of brain penetration as it exposes the brain to the maximum amount of drug delivered and, hence, limits the entry of the drug into the brain *in vivo*.

Drug permeability to the brain (P)

Depending on the BBB membrane properties and drug physicochemical characteristics, the speed of drug permeability to the

brain varies accordingly. Furthermore, the permeability is not an easy quest to acquire *in vivo*; therefore, the product of P and surface area (SA) is often implemented, which measures the rate at which drug crosses the BBB; and the distribution of drug into the brain is calculated simultaneously. The drug concentration at a particular timepoint could also be calculated using the volume of distribution and systemic drug clearance after i.v. administration. PSA and brain:plasma ratio can also be applied for the determination of the extent of drug exposure at any time after systemic exposure [6].

Methods for determining BBB permeability

Earlier, studies were executed using *in silico* techniques for determination of the passive permeability of a molecule that correlates the physicochemical properties of a drug or CNS distribution and efficacy (Table 2). Lipophilicity was one of the primary parameters considered for assessing the permeability through computational modeling (i.e., logP value of a CNS drug candidate). logP >0 was contemplated as the most lipophilic molecule that would enter the brain easily, whereas a molecule with logP <−1 would have limited entrance to the brain. Moreover, brain uptake and logP ratio present in the neuroactive agent also become important factors for assessing drug permeability [14].

The *in silico* technique is restricted to the passive diffusion that relies on physicochemical properties of a drug within the limited experimental dataset provided by these models; therefore, in association with *in silico* tools, *in vitro* cell culture methods have been developed for estimation of CNS drug permeability during the early stages of drug discovery (Table 3). Newly developed *in vitro* models include primary cell co-culture models with mouse endothelial cells and astrocytes, and an immortalized mouse cerebral and cerebellar endothelial cell line (cEND, cereBEND) with TEER value ranging from 300 to 800 $\Omega\,cm^2$ [33]. When reviewing for rat origin cell lines, the models like bovine brain capillary endothelial cells (BMEC), porcine brain capillary endothelial cells (PMEC) yield approximately 1–2 million cells per brain, which is not sufficient for permeability studies [34]. Moreover, the transporter expression in the brain capillary cell lines is not well evidenced and the TEER is also less than the *in vivo* TEER value (8000 $\Omega\,cm^2$) because the endothelial tight junction is reported to be greatly reduced. Hence, the porcine and bovine cells are not highly applicable to BBB permeability studies. Further, validation of an immortalized human (hBMEC) *in vitro* BBB model has been recently done using some reference drugs that are BBB-permeable, whereas other drugs are impermeable [35]. An immortalized endothelial clonal (hTERT/SV40-immortalized hCMEC/D3) cell line derived from human epileptic stem cells represents the most used, optimized and well-developed culture model for BBB permeation. Moreover, human pluripotent cells (hPSCs) originating from human blastocysts and induced pluripotent stem cells (iPSCs) by reprogramming somatic cells to a pluripotent state have also been given consideration for assessment of drug BBB permeation. When co-cultured with astrocytes, hPSCs reach TEER value 1450 $\Omega\,cm^2$. Other cord-blood-derived human stem cells have been developed utilizing co-culture with pericyte/astrocyte with TEER value of 180 $\Omega\,cm^2$ for pericyte and 60 $\Omega\,cm^2$ with astrocyte. This lacks validation and optimization of different receptors, transporters, proteins and influx and efflux pumps expressed in the culture.

Alternatively, cells of non-cerebral origin can be used for brain permeability estimation among which Madin Darby canine kidney (MDCK) cells of renal epithelial origin (TEER 2000 $\Omega\,cm^2$) have been widely used for investigating the BBB permeability of compounds. For transporter-related drug–receptor and drug–drug interaction, the MDCK cell line was transfected to develop a recombinant MDCK cell line with overexpressed P-gp transporter [36]. The test compounds were screened at different concentrations, incubated with an integrity marker and the efflux ratio of bidirectional component (apical to basolateral or vice versa) was assessed using high-throughput analyzing techniques (LC-MS/MS) through Eqs. (1) and (2).

$$\text{Efflux ratio} = P_{app\ (B\text{-}A)}/P_{app\ (A\text{-}B)} \quad (1)$$

TABLE 2

CNS drugs under development using *in silico* modeling

Drug category	Target	Property	Software	Refs
Series of aroylhydrazones of 2H-chromene and Coumarincarbaldehydes	Human GABAA receptor (PDB ID 4COF)	Log*P*, PSA, H-bond acceptors and donors count, molecular weight, logBB	Molecular operating environment (MOE, version 2016.08) and Marvin 16.2.8.0 software	[48]
Acridone-based 1,2,4-oxadiazoles as anticonvulsants	BZD receptors		Autodock tools (1.5.6)	[49,50]
Acetylcholine esterase inhibitors (AchE), butylcholine esterase inhibitors	Neurotransmitter acetylcholine	pK_a, log*P*, PSA, number of H-bond donors and acceptors	GOLD v. 5.1., 3D QSAR modeling	[51,52]
Dual target AchE and phosphodiesterase 5 (PDE5) inhibitors	Alzheimer's disease		TriposSybyl 2.0 software	[53]
BACE-1 ligand complexes	Alzheimer's disease	H-bond frequency	Protein–ligand interaction profiler	[54]
Nipecotic acid and acetonapthone hybrids	Epilepsy	Lipophilicity	Molecular docking	[55]
Kynurenic acid, natural compounds	Alzheimer's disease (AchE, NMDA receptor binding), BACE-1 inhibition		Molecular docking, 3D QSAR Pharmacophore modeling	[56,57]
2-Amino-*N*-[2-(3,4-dihydroxy-phenyl)-ethyl]-3-phenyl-propionamide	Dopamine		Glide software	[58]

TABLE 3

***In vitro* blood–brain barrier permeability models used for drug distribution to brain**

BBB model used	Purpose	Techniques employed	Refs
MDCK-MDR1 monolayers	To elucidate the transport mechanism, interplay of OATP1A2-mediated transcellular transport with P-gp across brain	LC-MS/MS	[59,60]
MDCKcmdr1-KO, MDCK cells overexpressing human MDR1 transporter (hmdr1) with cmdr1 knockout (MDCK-hmdr1cmdr1-KO), brain sectioning technique	To estimate drug–brain permeability alteration owing to drug–drug interaction	Mass spectrometry imaging	[61,62]
MDCK-pHaMDR cell monolayer model	BBB permeability of Lignans and Malabaricones from the seeds of *Myristicafragrans*	HPLC	[63]
MDCK-pHaMDR cell monolayer	Blood–brain barrier Transport of twelve coumarins	HPLC	[64]
MDCKII cell line, stem-cell-derived human brain microvascular endothelial cells (BC1-hBMECs)	To evaluate the drug is P-gp substrate and acetyl choline esterase reactivation	HPLC, fluorescence microscopy	[65]
Parallel artificial membrane permeability assay (PAMPA)	Prediction of BBB permeability	Quantitative structure–permeability relationship (QSPR) methodology	[66]
PAMPA	Predicting PAMPA BBB power for permeation assay	HPLC-UV	[67]
PAMPA and porcine brain endothelial cell models	Assessment of the blood–brain barrier permeability	HPLC-UV, LC-MS/MS	[39,68]
ECV304 and bEnd3 cell layers	Characterization of suitable cell lines that mimics the *in vivo* BBB permeability conditions	LC-MS/MS, immunofluorescence microscopy	[69,70]

Where, $P_{app} = ((dQ/dT)/(C_0*A))$ (2)

And, dQ/dt: rate of penetration of the drug across the cells, C_0: donor compartment concentration at time zero and A: area of the cell monolayer.

Other *in vitro* assays that are extensively applied are parallel artificial membrane assays (PAMPAs) and Caco2 cells for permeability assessment between donor and acceptor compartments using a lipid-impregnated membrane established on a solid filter support [37]. Most of the research uses an *in vivo* method to measure the rate of CNS drug permeation rather than performing *in situ* brain perfusion techniques that require high-level technical skills and are labor-intensive, which is almost not suitable in routine drug discovery analysis.

Total brain:plasma ratio (Kp)

In an *in vivo* method, the compound concentration is measured at predetermined time intervals in plasma and brain samples after oral administration to the animal model [38]. Eq. (3) is used for the estimation of BBB permeability

$$K_{in} = AUC_{(brain,t)}/AUC_{(plasma,0-t)} \quad (3)$$

Where, K_{in} is the rate of CNS penetration, $AUC_{(brain,t)}$ is quantity of drug in the brain at time t and $AUC_{(plasma,0\text{-}t)}$ is the amount of drug plasma exposure to the time t. From Eq. (3), it was postulated that, for high-permeability compounds, the upper limit of K_{in} is CBF and for low permeability compounds it is PS, but this does not include the factor protein binding of a compound.

Unbound brain:plasma ratio (Kp, uu)

Defined as the mean ratio of the amount of free drug in ISF and amount of free drug in plasma at an equilibrium state and the parameter that directly proportionates the pharmacological efficacy of neuroactive agents [39,40], calculated using Eq. (4).

$$K_{p,uu(brain)} = C_{u(brainISF)}/C_{u(plasma)} \quad (4)$$

Where $C_{u(brainISF)}$ is the extent of free drug in brain ISF and $C_{u(plasma)}$ is the extent of free drug plasma. The *in vitro* and *in vivo* approach has been considered to be more usable in which $K_{p,uu(brain)}$ is determined by Eq. (5).

$$K_{p,uu(brain)} = ((K_{p(brain)})/(V_{u(brain)}*F_{u(plasma)})) \quad (5)$$

Where $K_{p(brain)}$ is total brain:plasma ratio, $V_{u(brain)}$ is unbound volume of distribution in brain determined by brain slice technique and $F_{u(plasma)}$ is the unbound fraction of drug in plasma determined by the brain homogenate technique. The drugs with $K_{p,uu(brain)} < 1$ are under the category of efflux transporter substrates and $K_{p,uu(brain)} > 1$ are considered to be the substrates of influx transporters, whereas a the value near 1 shows passive diffusion. Hence, the compounds with an unbound brain:plasma ratio >1 are known to be highly permeable through the BBB and therapeutically active for CNS neurodegenerative disorders during the drug discovery process.

ATP-binding cassette (ABC) transporters

Although the BBB is the major interface for the development of neuropharmaceuticals, ABC transporters constitute one of the leading known superfamilies of proteins that hinders the process of CNS drug discovery [41]. Drug transporter proteins are highly conserved and translocate a broad spectrum of molecules across the cell membrane using energy from the hydrolysis of ATP. These modules could be one of the obstacles and the best alternative to enhance the distribution of the drug in the brain. Overexpression and downregulation of transporters is one of the major parameters that modulates the drug bioavailability in brain compartments.

TABLE 4

Recently developed CNS formulations and their transporter-dependent modulation in bioavailability

Name of drug (target)	*in-vitro*/*in-vivo* model illustrated	Samples used for analysis	Alteration in Transporters	Technique employed for estimation	Concluding remark	Refs
Breviscapine (neuroprotective)	Rat	Brain tissue	P-gp inhibition	Ultra-performance liquid chromatography fitted with tuneable ultraviolet absorbance detection	PEG-coated breviscapine-loaded solid lipid nanoparticles were used to increase the bioavailability of drug in brain that inhibits the P-gp transporter	[71,72]
Ketamine	Wild-type and P-gp and BCRP knockout mice	CSF and plasma	P-gp and BCRP inhibition	High-profile liquid chromatography (HPLC-UV)	Drug exposure in CSF was increased in knockout mice	[73]
Emodepside (antiparasitic)	mdr1-deficient and and mdr1-intact CF1 mice	Brain tissue	MDR1 transporter inhibition	LC-MS/MS	The drug concentration across BBB increases that could cause toxicity	[74]
Brain antitumor drugs	Tumor cells	Brain tissue	P-gp is inhibited by Elacridar		Drug penetration in tumor cells increases	[75]
BMS-275,183 (paclitaxel analog)	Mdr1a/1b$^{-/-}$ and wild-type mice	Plasma and brain tissue	Pgp was genetically deleted	HPLC	Oral and intravenous bioavailability in brain was increased in knockout mice	[76]
Naringenin (nanocarriers)	Male Sprague–Dawley rats	Brain tissue	P-gp inhibition	Enzyme-linked immunosorbent assay	Nanoparticles augmented the blood–brain barrier penetration of naringenin	[77]
Lorlatinib (novel anaplastic lymphoma kinase and reactive oxygen species inhibitor)	MDCKII cells transduced with human ABCG2, ABCB1 and mouse Abcg2 and wild-type Abcb1a/1b$^{-/-}$, Abcg2$^{-/-}$, Abcb1a/1b;Abcg2$^{-/-}$ mice Cyp3a$^{-/-}$ mice	Brain tissue and plasma	P-gp and ABCG2 knockout	LC-MS/MS	Bioavailability of lorlatinib was increased four times in Abcb1a/1b$^{-/-}$ mice and Abcb1a/1b;Abcg2$^{-/-}$ mice No alteration in plasma levels of drug	[78]
Encorafenib (LGX818, a BRAF^{V600E} inhibitor)	MDCKII cells transduced with human ABCG2, ABCB1 and mouse Abcg2 and wild-type Abcb1a/1b$^{-/-}$, Abcg2$^{-/-}$, Abcb1a/1b; Abcg2$^{-/-}$ mice Cyp3a$^{-/-}$ mice	Brain tissue and plasma	ABCG2 and ABCB1 modulation	LC-MS/MS	Oral and intravenous administration increases the brain accumulation of drug in Abcb1a/1b;Abcg2$^{-/-}$ mice, poor brain:plasma ratio in all models; low brain penetration limits its efficacy against malignancies	[79]
YZG-331 (adenosine analog, acts as sedative and hypnotic)	Caco-2 and MDCK cell line and *in vivo*	Brain tissue	P-gp was inhibited by verapamil	Transporter ATPase assay kit and human P-gp membranes	The drug levels were increased when co-administered with P-gp inhibitor. *K*p value raises from 0.03 to 0.05 after combination	[80]
Loperamide (P-gp substrate), dantrolene and proprietary compound X (BCRP substrate), imatinib and proprietary compound Y (dual substrate)	Mdr1a$^{(-/-)}$, Mdr1a$^{(-/-)}$/Abcg2$^{(-/-)}$	Brain tissue, plasma and CSF	MDR1 and ABCG2 alteration	LC-MS/MS	Loperamide concentration in brain was increased in Mdr1a$^{(-/-)}$ mice and similar results for all the drugs in their particular strains. CSF to unbound brain concentration ratio was >3 in knockout mice	[81]
Palbociclib (cyclin-dependent kinase 4/6 inhibitor for orthotopic brain tumor)	Orthotopic xenograft model, transgenic mice	Brain	Elacridar was used as an efflux transporter inhibitor thus increasing drug exposure to brain	LC-MS/MS	115-fold increase in bioavailability of drug in brain of transporter-deficient mice as compared to wild-type mice. Drug was intracellularly accumulated when examined through *in vitro* studies concluding the drug to be P-gp substrate and hence unable to effectively treat glioblastoma	[82]
Etamicastat (dopamine beta hydroxylase inhibitor)	Mice, MDCK-II and Caco-2 cells	Brain tissue, plasma	P-gp-mediated efflux of drug limits its penetration to brain	LC-MS/MS	Brain exposure upon intravenous injection were increased after coadministration of transporter inhibitor	[83]
Mitragynine and 7–hydroxymitragynine	Porcine cerebral primary porcine brain endothelialcell lines, Sprague–Dawley rats	Brain tissue, plasma	P-gp inhibition	Brain slice method, LC-MS/MS	Mitragynine showed higher tissue uptake (18-fold) and penetration form apical to basolateral than 7-hydroxymitragynine. Both were found to be inhibitors of P-gp	[39]

Currently, P-gp is considered the most noticeable transporter protein that limits xenobiotics from crossing the BBB and entering the brain. Hence, offering the focus of the researcher to illustrate the role of P-gp with BBB transporter and drug delivery research. Numerous animal experiments and clinical trials proved the modulation of P-gp transporters owing to neurological disorders [42,43].

Breast cancer resistance protein (BCRP)/ABCG2 is another class of ABC transporter in the BBB affecting the bioavailability of CNS drugs limiting their therapeutic efficacy [44,45]. In this context, BCRP and P-gp have shown an overlapping substrate range for distribution of drugs in the brain. Deletion of transporters, proteins and genes affects the probability of neurological disorders, thereby altering required CNS drug concentration for therapeutic activity. Besides P-gp and BCRP, the multidrug resistance protein isoforms (MRP1–5/ABCC1–5) were also discovered at the BBB. MRPs mainly transport organic anions, glucuronide- or sulfate-conjugated compounds, and various nucleoside analogs. MRP1, 4 and 5 were found to be present at the luminal side of brain capillary endothelial cells and confirmed by laser microscopy, whereas the MRP2 and 3 lack reactivity in the human brain. MRP1, 3 and 5 are highly expressed in brain tumors such as glioblastoma, in the tumor-supplying vasculature and in the parenchymal tissue surrounding the tumor where they confer resistance to chemotherapeutics and the epileptic brain [46,47]. Lastly, studies demonstrate that lack of MRP2 at the BBB results in increased drug levels in the brain. This observation offers a challenge for delivering drugs into the brain and limits successful therapeutic treatment of CNS disorders. Alteration in bioavailability of various drugs in the brain as a result of the inhibition or activation of transporters has been listed in Table 4.

Concluding remarks

Unbound drug in cerebral tissues is the main pharmacokinetic factor for neurotherapeutics. Additionally, brief knowledge of kinetics, tissue distribution in peripheral tissue and the target should direct the principle treatment or understanding of any secondary effect, assisting the clinician with the treatment choice and better treatment planning. Particularly for molecules crossing the BBB, the absolute quantitation of drug concentration could be done using different methods such as micro-dialysis, brain slice and homogenate techniques. Furthermore, the presence of membrane transporters constitutes another pathway to modulate the bioavailability of neuroactive drugs in the brain. Thus, the early knowledge of pharmacokinetic parameters including metabolism, phenotyping and inhibition study of a newly discovered drug can reduce side-effects, toxicity and withdrawal of the drug from the market. Potential lead molecules have justified the indispensable criteria of selectivity, oral bioavailability, effectiveness, side-effect profile and therapeutic efficacy. Therefore, the evaluation of the neuropharmacokinetic parameters of CNS drugs will aid in the governance of their pharmacodynamic responses.

Conflicts of interest

The authors have no conflicts of interest to declare.

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