Brain drug targeting: a computational approach for overcoming blood–brain barrier

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The treatment of brain disorders is limited by the insufficiency in delivering therapeutic drugs into brain relating to highly limited transport of compounds through blood–brain barrier (BBB). Therefore, a lot of attempts have been made to rise above this problem using a variety of approaches. In this way, *in silico* techniques try to predict the brain permeability based on a range of physicochemical descriptors resulting from structures of the corresponding compounds. Most of the models have some disadvantages, which preclude making conclusive decision. The major defect is ignoring the main parts of process of permeability using only total concentrations for modeling. Moreover, the role of transporters is underestimated in addition to neglecting the complex nature of BBB, which, collectively, leads to uncertain results.

Introduction

The process of rational drug design using different computational approaches has resulted in great advances in the discovery of new drug entities. However, it seems that there is an exceptional part: central nervous system (CNS) drugs which have greatly underdeveloped market and although many promising drug candidates have been discovered, most of these candidates have the lowest chance of success [1]. In this context, the most important factor limiting the chance of new entities is blood–brain barrier (BBB) [2,3]. In fact, BBB is the defensive tool to maintain the homeostasis of brain. This fact puts a great obstacle for drug targeting to CNS [4].

Therefore, a variety of strategies have been tried to overcome this problem using various approaches such as combinatorial sciences, computational methods and novel drug delivery systems. However, owing to the complex nature of BBB in addition to the potential interference by several concurrent physio/pathological factors, most of these efforts have been failed to suggest a global solution.

Blood–brain barrier (BBB): structure and physiology

BBB makes the most important part of the natural mechanism active in protection of the brain from exposure to potential hazardous xenobiotics. It has some distinguishing features causing highly effective impediment for the entry of chemical compounds into CNS [5]. The most important physical structure of BBB is the brain capillary endothelial cells (BCECs) having some unique characteristics such as the presence of tight junctions between the neighboring cells in addition to the lack of fenestrations precluding paracellular transport of the solute molecules. Furthermore, very limited vesicular transport (endocytosis) and high metabolic activity of BBB-forming cells are additional factors preventing different molecules from being entered into the brain parenchyma [6].

Although a well-established relationship exists between lipophilicity of a penetrant and the efficiency of brain penetration, there is a common misconception that small lipophilic molecules easily diffuse the BBB. In fact, some of these small solutes do not penetrate the brain as their lipid solubility may suggest. This phenomenon is due to the presence of some active transporters in BBB, more importantly the members of ATP-binding cassette (ABC) superfamily of transporters, which play crucial roles in active influx/efflux of the drugs regardless the concentration gradient across BBB [7]. It was proved that the BBB contains several ABC transporters, which expel a multiplicity of drugs from the CNS, like P-glycoprotein (P-gp), multidrug resistance protein (MRP) and breast cancer resistance protein (BCRP) [7]. Some natural transport systems are presented in the surface of BBB which are intended inherently for the transport of some especial large polar compounds into brain. In this manner,

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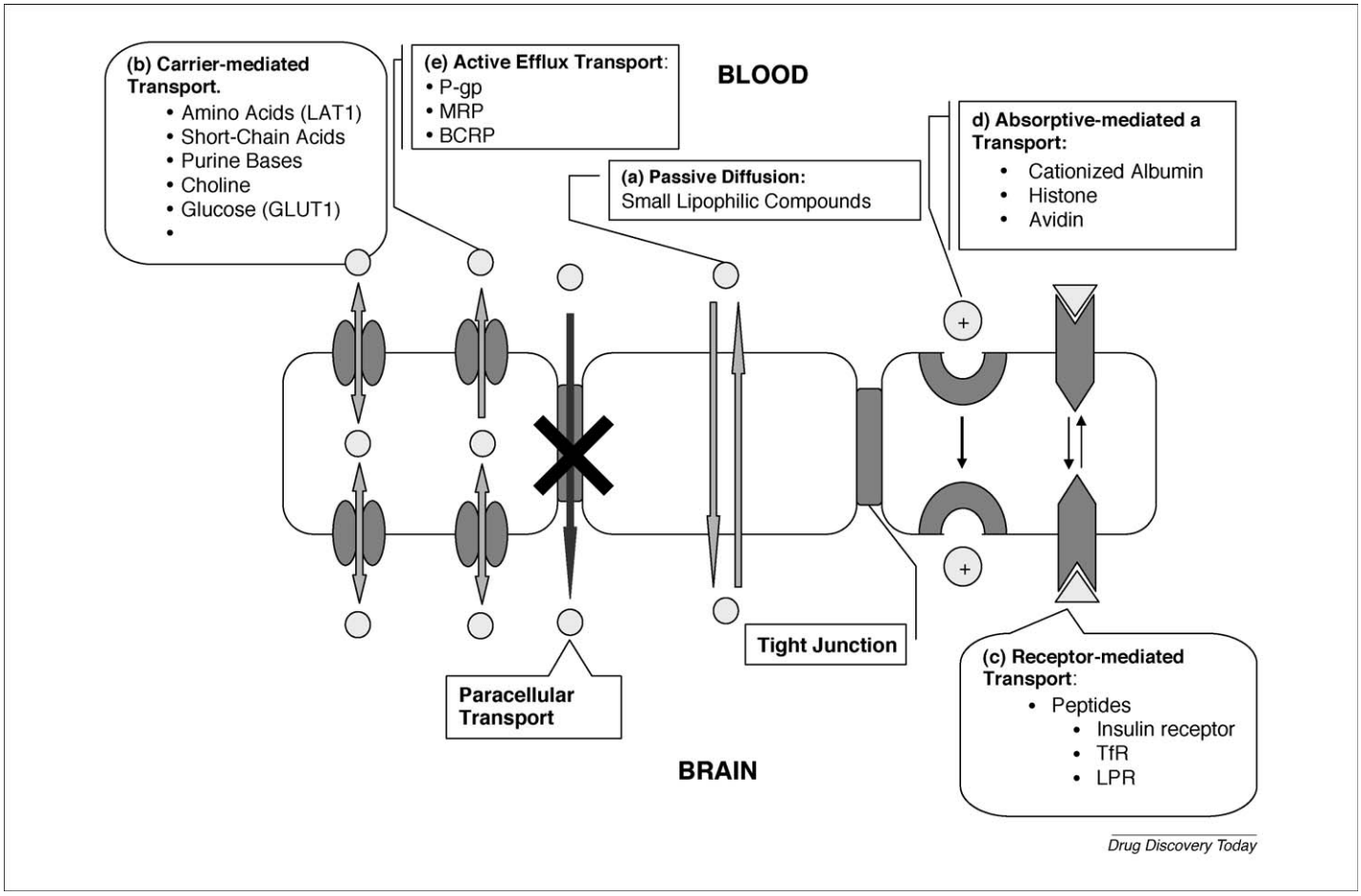

FIGURE 1

A schematic diagram of BBB: there are several mechanisms which are interfering in the permeability of compounds through BBB. **(a)** Passive diffusion is the most common role by which most small lipophilic compounds penetrate through BBB. **(b)** Carrier-mediated transport which usually happened for small hydrophilic compounds (LAT1: Large neutral Amino acids Transporter 1; GLUT1: Glucose transproter1). **(c)** Receptor-mediated transport which is generally taken place for large hydrophilic compounds like peptides and protein and has considerable role in the transport of biopharmaceuticals (TfR: Transferrin Receptor; LPR: LDL-related protein receptor). **(d)** Absorptive-mediated transcytosis is a relatively nonspecific mechanism for positively charged peptides. **(e)** Active efflux transport is an antiport system which attenuates the CNS concentration of many drugs by active efflux of them against concentration gradient from brain into blood (P-gp: P-glycoprotein; MRP: Multidrug Resistance Protein; BCRP: Breast Cancer Resistance Protein).

they can also work as pseudotransporters via carrier-mediated transcytosis for small molecules and receptor-mediated transcytosis for large biopharmaceuticals [1,7]. For instance, some transporters of nutrient such as glucose transporter (GLUT1) or large neutral amino acids (LAT1) have been used as transporter of some drugs and therefore enhance their uptake into brain. Moreover, definite large-molecule peptides or proteins undergo transport from brain to blood via receptor-mediated transcytosis across the BBB. In this way, the most widely used transporters for the delivery of large therapeutics compounds are transferrin receptor (TfR), LDL-related protein receptor (LPR) and insulin receptor [7,8]. A schematic diagram of BBB including the main transporters is shown in Fig. 1.

Brain drug targeting: computational background

In silico methods for the evaluation of blood–brain partitioning of drugs endeavor to predict the brain permeability based on a variety of physiochemical descriptors resulting from the chemical structures of corresponding compounds [9]. Earlier studies were concentrated on small molecules and the linear relationships between BBB permeability and lipid-to-water partition coefficient in various solvent systems. Conversely, during the past decade, progresses occurred in computational tools along with the development of efficient modeling algorithms have associated with the routine development of more complicated models. Furthermore, in recent times, it has become possible to calculate the permeability of large molecules.

One of the most recent debates in this field is regarding which parameter(s) can be used as to classify compounds as having 'good' or 'poor' CNS distribution. In this way, from the very early time of *in silico* attempts, log *BB* was calculated by the following equation [9]:

$$\log BB = \log \frac{C_{\text{brain}}}{C_{\text{blood}}} \quad (1)$$

In which it is a measure of the extent of partitioning into brain but not necessarily at steady state. This parameter is similar to $K_{\text{p,brain}}$, being defined as [10]:

$$K_{\text{p,brain}} = \frac{AUC_{\text{tot,brain}}}{AUC_{\text{tot,blood}}} \quad (2)$$

However, some experts believe that log *BB* or $K_{\text{p,brain}}$ does not actually describe BBB permeability and in fact '*Whole brain/blood*

TABLE 1

Summary of models using conventional *in silico* methods

		Data Size	Experimental parameter	*In silico* method	Used descriptors	Statistical performance	Ref
1	Levin	27	Log P_c	LR	Log *P*	$r = 0.91$	[16]
2	Young	20	Log *BB*	MLR	Δlog *P*	$r = 0.831$	[17]
3	Abraham *et al.*	57	Log *BB*	MLR	Solvation parameters	$r = 0.952$	[18]
		148	Log *BB*	MLR	Solvation parameters	$r = 0.843$	[19]
		30	Log *PS*	MLR	Solvation parameters	$r = 0.87$	[24]
4	Luco	58	Log *BB*	PLS	Topological indices	$r = 0.922$ $q = 0.867$	[20]
5	Norinder *et al.*	45	Log *BB*	PLS	Polar surface area	$R^2 = 0.720$ $Q^2 = 0.707$	[23]
		70	Log *BB*	PLS	Polar surface area	$R^2 = 0.756$ $Q^2 = 0.746$	
6	Liu *et al.*	23	Log *PS*	MLR	TPSA, log *D*	$R^2 = 0.74$	[25]
		11	Log *PS*	MLR	Solvation parameters	$R^2 = 0.61$	
7	Bendels *et al.*	77	Log *BB*	PLS	Different physiochemical parameters	$R^2 = 0.78$	[26]
		37	Log CSFPR	PLS	Different physiochemical parameters	$R^2 = 0.75$	
8	Wan *et al.*	108	f_u	LR	Log *P*	$R^2_P = 0.756$	[12]
				MLR	A variety of structural descriptors	$R^2_P = 0.744$	
				PLS	A variety of structural descriptors	$R^2_P = 0.794$	

partitioning reflects nothing but an inert partitioning process of drug into lipid material' [11] because these parameters are highly influenced by the relative binding affinity of compounds for protein and lipid contents of both sides [10]. Therefore, in recent years, some other parameters are introduced as indicator of BBB permeability. On the basis of the 'unbound drug hypothesis', it is speculated that it is the unbound drug that exerts the physiological effect [10,12]. So, it is believed that parameters that were calculated based on unbound fraction of drugs in CNS should be used. In this way, Pardridge and Martin suggested replacing log *BB* with the BBB permeability–surface area (PS) product; because it predicts the level of free drug in brain [13,14]. However, some others believed that the PS product by itself cannot predict the unbound fraction of drug in CNS because PS product is an estimate of net influx clearance and influenced by the possible association of drug with active influx or efflux [10].

By contrast, it was suggested that some parameters, related to unbound drug concentration, are used. In this manner, $K_{p,uu}$ was recommended assessing the unbound concentration gradient across the BBB and calculated by the following equation:

$$K_{p,uu} = \frac{AUC_{u,brain,ISF}}{AUC_{u,blood}} \quad (3)$$

$K_{p,uu}$ is related to BBB equilibration effects such as passive diffusion and active influx/efflux. Therefore, it can show whether drug is transported by passive diffusion ($K_{p,uu}$ would be near unity) or actively influxed ($K_{p,uu}$ would be higher than unity) or actively effluxed ($K_{p,uu}$ would be lesser than unity). Furthermore, it is independent of protein binding in blood or binding to tissue component of brain which is one of the main problems of using log *BB* [10,15]. Alternatively, fraction of unbound drug in brain is also considered as measure unbound drug concentration and it has been used directly in modeling process [12]. Finally, the best answer is that just one factor cannot explain all aspects of drug permeation into brain. Thus, it can be best enlightened by three factors: permeability clearance, unbound drug in brain and intra-brain distribution of drug [10].

Small molecules

It can be assumed that the first exact BBB permeability model was obtained by Levin in 1980 (Table 1). Using a set of 27 molecules of divers structures, he found that the best fit between BBB permeability coefficient (log *Pc*) and log *P* was attained for compounds with molecular weights below 400 Da [16]. In 1988, Young *et al.*, synthesized 20 compounds active on H_2 histamine receptors and measured their blood/brain partitioning (log *BB*). They obtained a reasonable relation between log *BB* and Δlog *P*, with the latter being defined as [log *P* octanol/water (O/W) – log *P* cyclohexane/water (C/W)], using the following equation:

$$\log BB = -0.485(\Delta \log P) + 0.889$$
$$n = 20, r = 0.831, F = 40.23 \quad (4)$$

According to this equation, they concluded that log *P* (C/W) might play a role in partitioning process in non-polar regions of brain, while log *P* (O/W) might reflect protein binding which limits the free drug from crossing BBB [17].

Abraham *et al.* performed a series of analyses using solvation parameters including dipolarity/polarizability (*S*), H-bond acidity (*A*), H-bond basicity (*B*), McGowan characteristic molar volume (*V*), and excess molar refraction (*E*), at first using 57 compounds [18], then increased to 148 chemical entities [19]:

$$\log BB = 0.044 + 0.511E - 0.886S - 0.724A - 0.666B + 0.861V$$
$$n = 148, r = 0.843, F = 71.0 \quad (5)$$

Nevertheless, Abraham model constructed by 57 compounds was validated by Norinder and Haeberlein finding that a more predictive model could be achieved by excluding *E* and *A* variables [9].

Luco used partial least square (PLS) and topological indices approach. This model showed promising performance, as proved by an external test set for its predictive ability. However, topological indices and large number of descriptors make its interpretation difficult [20].

Polar surface area (PSA) is a parameter which seems to have a distinct relationship with *BB* partitioning, because it has been proved that low polar surface area is consistent with the ability

of compounds to cross biological membranes [21]. Some studies have proved model ability of this parameter [22]. In a remarkable study, Osterberg and Norinder first confirmed that PSA had suitable correlation with some straightforward structural parameters (the number of hydrogen bond accepting oxygen and nitrogen atoms and the number of hydrogen atoms bonded to them). Then, these parameters along with log *P* were used to construct a model by PLS. The result was a simple predictive equation, while interpretation of the findings of this model would be under debate [23].

As mentioned in previous section, most of recent reviews criticized that reliability of log *BB* as indicator of CNS permeation of compounds mainly because log *BB* reflects the total drug concentration (both free and bound) in brain, while drug action is directly dependent on free drug concentration in brain. Therefore, some efforts were done to use other parameters which are thought to be more relevant to free drug concentration in the brain. In this way, two models have been reported for log *PS* on the basis of reasons made by Pardridge and Martin. Abraham constructed an equation using 30 neutral compounds. He found that log *PS* has good relationship with his descriptors (solvation parameters) [24]. Simultaneously, Liu *et al.* have reported some equations on different data set using a variety of parameters such as TPSA, log *D*, and also Abraham solvation parameters [25]. Again, their results showed no obvious new trend other than those found for log *BB*.

Alternatively, a recent study has used log[CSF-to-plasma concentration ratio (CSFPR)] and log *BB* to model brain permeability. The attractive point was that log *P* had a negative effect on log CSFPR whereas positively affected log *BB*. It can be concluded that lipophilicity is a positive factor in favor of tissue distribution, while partitioning to aqueous media, such as CSF, relates reversely to lipophilicity [26].

Finally, on the basis of the recent literatures, the most appropriate parameters are those that include the unbound fraction of drugs in the brains. Summerfield *et al.* showed that fraction of unbound drug in the brain has inverse relation with *c* log *P* indicating that although CNS diffusion may be enhanced by growing lipophilicity, great amount of compounds will be nonspecifically bound to brain tissue. Therefore, increasing lipophilicity will not necessarily result in increasing efficacy of CNS compounds and it is essential to be a balance between sufficient penetration and satisfactory free drug in brain [27]. Similar observation was reported by Wan *et al.* finding a strong inverse relationship between unbound drug fraction (f_u) and lipophilicity ($r = -0.78$). Furthermore, they highlighted a cut-off requirement ($C \log P < 4$) for sufficient unbound drug fraction ($f_u > 1\%$). Interestingly, they found that several aromatic atoms have negative influence on f_u while solvent accessible polar surface area correlated positively [12] while it was assumed that low polar surface area was an essential factor for penetrating biological barriers [21].

New modeling methods

In recent years, the increasing computational possibilities and development of sophisticated modeling algorithms have resulted in more robust and especially accurate predictions [28]. Neural network (NN) is one of the novel approaches that have shown its promising ability in different modeling processes. A variety of NN methods are used in the prediction of BBB permeability. Winkler and Burden used Bayesian regularized neural network (BRNN) to model BB partitioning of 106 diverse compounds. Their results showed that BRNN is a robust approach, with resistance to overfitting and poor data, although the used model did not give high performance ($R^2 = 0.81$ and $Q^2 = 0.65$) (Table 2). In this procedure, automatic relevance determinant process was applied to find the important variables of models. As expected, log *P* and PSA were among the most important ones [29]. By contrast, Hemmateenejad tried to apply the hybrid approaches. In this way, first, principal component analysis was run to reduce the dimensions of data and, then, different variables of selection methods such as correlation-ranking and genetic algorithms in association with back-propagation neural network were used to model log *BB* [30]. In an additional trial, quantum chemical descriptors in combination with topological indices and log *P* were modeled by genetic neural network. Final results showed very high-quality statistics (R^2 of prediction for three best models were 0.904, 0.943 and 0.979). Furthermore, models noticeably showed the importance of mentioned descriptors [31]. However, such complex hybrid models make the interpretations very difficult.

A less complex novel method was introduced by Zhang: nonlinear regression analysis which was applied for partitioning to many tissues [21,32]. In this method, a linear model was obtained by stepwise selection and, then, obtained parameters were used in an exponential equation [33]. The predictive capacity of such

TABLE 2

Summary of models using novel *in silico* methods

		Data size	Experimental parameter	*In silico* method	Used descriptors	Statistical performance	Ref
1	Winkler and Burden	106	Log *BB*	Bayesian regularized neural network	Property-based descriptors Topological indices CIMI/bc descriptors	$R^2 = 0.74$ $Q^2 = 0.65$ $R^2 = 0.61$ $Q^2 = 0.65$ $R^2 = 0.54$ $Q^2 = 0.64$	[29]
2	Hemmateenejad *et al.*	115	Log *BB*	Principal component-neural network	Different physiochemical parameters	$R^2_P = 0.988$	[30]
		123	Log *BB*	Genetic neural network	Different physiochemical parameters + quantum chemical descriptors	$R^2_P = 0.979$	[31]
3	Zhang *et al.*	35	Log *BB*	Nonlinear regression analysis	Different physiochemical parameters	$R^2 = 0.920$ $Q^2 = 0.891$	[33]
		160	Log *BB*	Nonlinear regression analysis	Different physiochemical parameters	$r = 0.906$	[21]
4	Wan *et al.*	108	f_u	Neural network	A variety of structural descriptors	$R^2_P = 0.819$	[12]
		108	f_u	Support vector machine	A variety of structural descriptors	$R^2_P = 0.871$	

nonlinear equations was usually better than corresponding linear ones.

In an interesting study, Mente and Lombardo performed series of modeling using bagged recursive decision trees. Then, a set of 289 compounds was used as test set for which ratio of log *BB* between P-gp-knocked out and wild-type mice was observed in Pfizer laboratories. The average ratio was 8.14 and nearly 60% of these compounds had substantial P-gp-mediated efflux issues (ratio > 3). It was shown that test set gave very poor results when was applied to obtained models. However, when compounds with ratio > 10 were removed, the model constructed by PSA showed better performance [34].

Another approach is using consensus modeling in which some different models are constructed and final predictions are obtained by averaging the predictions of each compound made by individual models for continuous QSAR and by majority votes from classifications. For the first time, Subramanian and Kitchen employed the consensus approach to find better prediction in both regression and classification trials; however, their results showed that consensus models did not significantly improve the final predictions [35]. Very recently, Zhang *et al.* obtained various models by nearest neighborhood and support vector machine and, then, final prediction achieved by consensus modeling. Their results revealed that although consensus prediction always provides the most stable and robust solutions, it does not necessarily give the best results [21]. Furthermore, Kortagere *et al.* used a consensus model based on support vector machine and generalized regression and aimed to classify 389 compounds based on a majority vote. Like previous studies, combined predictions did not improve the final results compared to each modeling methods alone [36].

The only report on modeling of unbound drug fraction using new computational approaches was the work of Wan *et al.* which used neural network and support vector machine (SVM). The results confirmed that SVM model was a robust and truthful predictor of f_u [12] (Table 2).

Targeting by prodrugs

The prodrug approach involves the administration of a drug in an inactive form but readily able to transverse biological barriers and is converted into active form solely in target site and this lipophilic form will no longer be able to exit [7]. On the basis of this approach, brain targeted chemical delivery systems (CDS) represent rational drug design considering both delivery and targeting simultaneously [37]. In recent years, a lot of efforts have been devoted to the preparation of lipophilic compounds targeting CNS by structure–activity relationship (SAR) modifications. Perioli *et al.* designed a prodrug backbone for non-steroidal anti-inflammatory agents to cure Alzheimer disease. In this respect, they attached compounds to 1,4-dihydropyridines [38]. Then, they used BBB VolSurf model which was developed by Crivori *et al.* [39] and transformed 3D fields of molecules into descriptors and correlated them to BBB permeability by a discriminant partial least square process. Their results showed that all 17 prodrugs are BBB+ and among them, 7 compounds were better candidates. Furthermore, log *P* of prodrugs had a good correlation with BBB penetration [38]. Recently, in another study, the same results were obtained for *C* log *P* and log *k′* (retention in reverse-phase HPLC) ($R^2 = 0.71$) [40].

Peptides

Peptides have the great potential as potent pharmaceuticals for efficient specific cure of many CNS maladies. On the basis of rules governing small molecules, peptides larger than tripeptide do not seem to cross BBB. However, various larger peptides are capable of penetrating BBB using various routes even passive diffusion [41].

First trials were carried out to relate BBB penetration of peptides to different types of partition coefficients [42]. Similar to small molecules, it was observed that CNS permeability has a poor correlation with log $P_{Oct/Wat}$ ($R = 0.6$) [43], whereas it showed good connection with $\Delta \log P$ or $\log P_{hexane/ethylene\ gylcol}$ ($R > 0.94$) [43,44]. Furthermore, general permeability of peptides through biological membrane showed a distinct relationship with several potential hydrogen bonds ($R > 0.8$) [43–45]. However, some observations revealed that brain penetration of peptides cannot be simply predicted through individual molecular properties or net charges [46]. This indicated the emergence of more complex and comprehensive models to evaluate the possible BBB permeability of peptides. In a successful attempt, Giralt group constructed a genetic algorithm (GA)-based model in which nine physiochemical parameters, such as log *P*, conformation and aromaticity considered to model the BBB penetration of randomly generated peptides. In the first generation, some of these random peptides, randomized on the basis of predicted permeability, were produced and experimentally evaluated using four different techniques and, then, peptides were ranked based on *in vitro* results, which was, in turn, a basis for the development of second generation in a manner that the second generation contained the best results of previous generation. Such a process confirmed that BBB permeability could hardly been anticipated by simple rules of small molecules and needs such combined approaches [41].

In very recent years, a family of short peptides known as cell penetration peptides (CPPs) has attracted attention as potential peptide-based delivery vectors [47]. Therefore, some efforts were made to predict sequences capable of penetrating biological barriers, in particular BBB. Although most of these attempts were done by trial and error, lately it was tried to model the permeability by a set of descriptors called *Z*-scaled, derived from a lot of physiochemical parameters for each amino acid. Results showed that bulk property values ($z\sum/n$) had good predictive ability [48]. However, the main drawback of these descriptors is ignoring the sequence of peptides because values of $z\sum/n$ are similar for a set of scrambles analogues.

Molecular modeling of BBB transporters

Transporters are polytopic membrane proteins involved in uptake or removal of different compounds into/from different living cells. It has been proven that many drugs are substrates of such transporters especially in BBB. However, most conventional 2D-QSAR studies ignore the role of transporters, both influx and efflux ones, resulting in significant movement away in modeling from proper prediction of substrates of transporters. This idea is generally anchored in a misconception that most compounds cross through biological barriers via passive diffusion and carrier-mediated transport is just an exception in this way. However, latest studies challenge this inspiration and support this idea that such a transport is more likely to be a common trend rather than an exception [49]. Therefore, complete understanding of transporter structures

and functions would be of considerable value in CNS drug design. However, this context is currently limited because of limited availability of high-resolution 3D structures of BBB transporters. In this way, *in silico* methods are good tools to circumvent such problems. Actually, there are two major approaches to model transporters: transporter-based design, when suitable template of transporters is available and substrate-based design, when appropriate knowledge of substrates against the transporter is available [50,51].

Until now, a series of BBB transporters have been subjects of various transporter-based methods like homology (comparative) modeling and molecular dynamic simulations. In this case, ABC transporters have been extensively studied because of their remarkable role in drug efflux. However, up to now, models have been based on bacteria origin. For example, P-gp has been modeled using structure of an *E. coli*-based protein, MsbA, sharing 30% homology with P-gp. Although using such proteins in modeling transporters have provided some valuable insights about transporters mechanisms and structures, unfortunately, these prokaryote proteins are not drug transporters. Therefore, a human ABC transporter structure is crucial for further studies [52].

By contrast, indirect methods like comparative molecular field analysis (CoMFA) and comparative molecular similarity index analysis (CoMSIA) have been used to gather information about transporter structures through common pharmacophores of their substrates. By these methods, it would be possible to derive general pharmacophoric pattern of substrates/inhibitors. Recent efforts have resulted in identifying pharmacophore for some ABC transporters such as P-gp or MRPs [53]. Also, recently, models have been developed for choline transporter used as a vector for CNS drug delivery [54]. On the basis of such modeling, a theoretical rendering of active binding site of choline transporters has been achieved [55,56].

Concluding remarks

Despite several years of efforts, CNS drug design remains the bottleneck of drug development process mainly because of the very efficient limiting factor of BBB. This challenge prompted a wide variety of studies to overcome the problem of brain penetration. In this way, computational approaches have shown a promising trend toward transversing BBB. In this context, different *in silico* methods have been developed. However, there is a never-ending challenge between interpretability and predictive ability of models in view of the fact that simple models, generally derived by linear regression, provide good vision about the process of permeation, while not being usually high-quality predictors. By contrast, novel modeling approaches like neural networks afford very accurate predictions while their elucidation would be near to impossible task. Consequently, complex models cannot be used in lead optimization because the main question of designer for the next step is '*which compound should I make next?*' [28].

Besides, the main problem seems to be completely ignored '*which parameter should be used in modeling as surrogate of brain permeability of drugs?*' although, in recent years some efforts were performed to make clear answer. In this way, most of literatures based on experimental analysis come to this conclusion that parameters derived from total concentration like log *BB* are very imperfect measure of BBB penetration [10,15]. Despite this conclusion, it seems that computational divisions close the eyes to this fact and up to now nearly all papers still use log *BB*. Conversely, although based on 'unbound drug hypothesis', switching from log *BB* into parameters related to unbound drug concentrations might give better idea, again relying on just on these parameters would be the next misleading trend because only one parameter cannot give complete details about BBB permeability and it consists of at least three parts of permeability clearance, brain unbound fraction and also intra-brain distribution [10]. Finally, rapid changes of CND drug discovery in recent years call attention to this reality that just stick into modeling of concentrations and related pharmacokinetic data cannot create a good perception of CNS activity of drugs which is highly dependent on biology and pharmacology basis of both compounds and brain [15]. Thus, it would be inevitable to combine the pharmacodynamic and pharmacokinetic data including all information about molecular/cellular processes of BBB permeability of drugs parallel to their CNS actions.

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