

A Review of Methods for Computational Prediction of Blood-Brain Partitioning

Yiannis N. Kaznessis*

Department of Chemical Engineering and Materials Science, and Digital Technology Center, University of Minnesota, Minneapolis, MN 55112, USA

Abstract: Advances in combinatorial synthesis and high throughput screening have resulted in libraries containing hundreds of thousands of drug candidate compounds. Computational prediction of properties that will determine the utility of a drug molecule has become a *sine qua non* in the pharmaceutical industry, because of the appreciation that ADMET properties must be considered early in the discovery process and the higher cost of experimental alternatives. In this paper we are reviewing the models developed recently to predict the permeation of organic molecules through the blood-brain barrier.

Keywords: Blood-brain partition coefficient, computer simulations, QSAR.

INTRODUCTION

The blood-brain barrier is in essence a mechanism for preventing the entry of unnecessary or toxic blood molecules into the central nervous system, while allowing the circulation of adequate amounts of arterial blood through brain tissue. Since the brain consumes more than 20% of available oxygen in the human body, it is important to maintain sufficient amounts of blood while excluding potentially harmful molecules. [1-3].

Already in the late 19th century the presence of the blood-brain barrier became evident, when scientists observed that dyes readily penetrated in other organs from the blood circulation, but not in the brain.

The blood-brain barrier can be identified with the capillary wall of neural vessels. Cerebral endothelial cells are distinguished from those in other tissues by the tight junctions between them. Passage of small molecules via the intercellular space is prevented by these tight junctions. There are different mechanisms of active transport through the BBB, such as carrier mediated efflux/influx transport, receptor-mediated transcytosis and adsorptive-mediated transcytosis, and these are the subject of intensive research efforts. In this paper we are concerned with passive diffusion through the BBB, and in particular the computational tools that have been developed to predict the passive permeation of small organic molecules.

At the molecular level, the principal barrier component for passive diffusion is the lipid bilayer of the capillary endothelial cell membrane, through which compounds have to diffuse to reach the brain. There is thus a hydrophilic interface and a hydrophobic core that permeating molecules interact favorably with.

The relative affinity for the blood or brain tissue can be expressed in terms of the blood-brain partition coefficient, $\log BB = \log(C_{brain}/C_{blood})$, where C_{brain} and C_{blood} are the equilibrium concentrations of the drug in the brain and the blood respectively.

Researchers in the pharmaceutical industry are interested in designing central nervous system (CNS) drugs that penetrate the BBB. Equally important is that peripherally acting drugs do not penetrate in the cerebrospinal fluid. With the advent of combinatorial chemistry and high throughput screening, very large sets of compounds can potentially become drug candidates. As the cost of drug discovery increases progressively in later stages of the process, methods are necessary that can quickly filter these candidates for poor absorption, distribution, metabolism, excretion and toxicity (ADMET) properties, including the blood-brain barrier permeation, much before deciding to examine molecules in the clinic. Indeed, recent studies suggested that up to half of all candidates actually fail as drugs because of poor pharmacokinetics and animal toxicity. Furthermore, knowledge of ADMET properties can be beneficial even before the synthesis of new compounds, not available in libraries. [4]

Computational methods have shown promise in predicting ADMET properties and are attractive means because of their inherent speed and low cost. They also offer the only solution when the compounds have not been synthesized yet. Based on the premise that the structure of molecules contains all the information needed to predict the partition between blood and brain fluids, models have been developed that identify the important characteristics resulting in BBB permeation.

Quantitative structure–activity relationships (QSAR) are typically developed that correlate $\log BB$ and a set of molecular descriptors of molecules with known $\log BB$ values. QSARs are ‘trained’ using a dataset of molecules to develop a typically linear relationship between $\log BB$ and the descriptors, and then the model is validated using a different test dataset of molecules. The descriptors that participate in

*Address correspondence to this author at the Department of Chemical Engineering and Materials Science, and Digital Technology Center, University of Minnesota, Minneapolis, MN 55112, USA;
E-mail: yiannis@cems.umn.edu

the QSAR with the highest statistical significance, in terms of their predictive ability, are thought to be the molecular properties important for BBB permeation. Ideally, design principles can be developed for molecules that penetrate the BBB using the information of important molecular properties.

In what follows we describe the available datasets of compounds with known *logBB*, the accuracy of experimental results and its impact on models, and we discuss recent advances in predictive QSAR models for BBB permeation. The physical insight gained in terms of molecular characteristics for high *logBB* and directions for possible future research are also discussed.

DATASETS

The paucity of available data has been the most significant hurdle for the development of *logBB* prediction QSAR models. The number of organic compounds with publicly available *logBB* values has grown to over 100 only recently, with the majority of studies limited to around 60-80 compounds.

Both *in vivo* [2,5] and *in vitro* [6-9] experiments have been conducted for measuring *logBB* of organic compounds, and both are costly and difficult. In *in vivo* experiments, peripheral application of radiolabeled compounds to rats is followed by brain concentration level measurements. In *in vitro* experiments, the partition of the compound between an aqueous and an organic phase, or its penetration in specific cell types is measured and the results are used for relative *logBB* ranking of compounds. For the majority of the *logBB* QSAR models developed, *in vivo* experimental results have been employed, directly measuring *logBB*.

Researchers have been careful in using measurements obtained with similar experimental protocols, but experimental measurements for compounds used in modeling have been conducted by multiple groups. An updated set of organic compounds is described recently in ref. [10] containing 115 compounds with *logBB* values measured by multiple different groups. There is thus an inevitable element of variability that will impact the accuracy of models. This will in turn limit the accuracy of computational models. Since QSAR models are trained on experimental blood-brain permeation data, their accuracy cannot exceed the biological measurements one.

The narrow range of observed *logBB* values can also present an obstacle to accurate predictive modeling of BBB permeation. Most published values of *logBB* cover only a range between -2.00 and $+1.00$, in contrast to values of octanol/water partition coefficients *logP* that span a range between -4 and $+8$. Typically, molecules with *logBB* >0.3 permeate readily the BBB, while values less than -1.0 indicate very small permeation. There is however scant information about the experimental variability and the expected error bars in measurements are in the order of 0.3-0.4 log units. [11]

For example, some of the molecules used in *logBB* QSAR development were consistently identified as outliers by multiple, different research groups and this deviation has

been explained in terms of the difficulty of experimental measurements or attributed to metabolism or possible active transport mechanisms.

It follows that a most pressing need is the generation of larger datasets with accurate measurements of *logBB* coefficients. Since *in vivo* experiments are particularly difficult to conduct in a reproducible manner, *in vitro* models of BBB permeation appear to present an attractive alternative. [12-16] Conditionally immortalized cell lines have been discovered that show promise in preserving the *in vivo* permeation character. For example, bovine microvessel endothelial cell (BMEC) lines have been used successfully as a model for BBB permeation. [12]

It appears that the physiological methods can now be complemented with molecular biology techniques that allow measurements for very large datasets in a controlled, reproducible manner. Certainly, more *in vivo* experiments are required to ascertain the relevance of *in vitro* models, but low cost, high-throughput techniques are becoming available for measuring actual or relative *logBB* values.

Furthermore, recent government efforts in the United States and elsewhere are resulting in publicly available information for hundreds of thousands of organic compounds. Typically, information on this size scale molecular datasets was the privilege of pharmaceutical companies. A repository is being established by the National Institutes of Health to acquire, maintain and distribute a collection of up to 1 million chemical compounds and a central database, called PubChem is already freely available to the entire scientific community. [17] Partnering with biotechnology companies with high-throughput expertise and resources, scientists will be able to measure a variety of biochemical and cellular assays for hundreds of thousands of molecules. One can thus safely predict that computational researchers should soon be able to use a wealth of *logBB* data for developing predictive models.

COMPUTATIONAL METHODS

Substantial progress has been made in the last few years in the development of accurate QSARs for organic compound *logBB* values, even in the absence of very large datasets. There are many ways to categorize and present these methods and we will focus on the type of molecular information used to build the QSAR. Structural fragments and molecular fields, entire molecule static properties (not dependent on time) and entire molecule dynamic properties have been correlated to experimental *logBB* measurements with different degrees of success. Researchers have also developed correlations between *logBB* and other physico-chemical properties of molecules, such as the octanol/water partition coefficient and the solvation energy.

Structural Fragments and Molecular Fields

Ideally, libraries of structural fragments, such as hydroxyl, benzyl and guanidinium groups can be created and linear relationships developed between the number of these fragments in molecules of training sets and the physiological measurement of BBB permeation. However, because of the absence of polyfunctional molecules in the datasets, drug-

like molecules cannot be treated appropriately with this conceptually straightforward approach.

In early efforts to model BBB permeation, physico-chemical properties were calculated for molecular fragments and these were in turn correlated to BBB permeation. For example, Abraham and co-workers [18-20] constructed the following model equations using a fragment-based scheme for a set of 57 compounds:

$$\log BB = -0.038(\pm 0.064) + 0.198(\pm 0.100)R_2 - 0.687(\pm 0.125) \frac{H}{2} - 0.715(\pm 0.334) \frac{H}{2} - 0.698(\pm 0.107) \frac{H}{2} + 0.995(\pm 0.096)V_x, \\ n = 57, r = 0.952, s = 0.197, F = 99.2 \quad (1a)$$

$$\log BB = 0.023 \log P_{ow} - 0.507 \frac{H}{2} - 0.500 \frac{H}{2} + 0.055, \\ n = 49, r = 0.949, s = 0.201, F = 136.1 \quad (1b)$$

where R_2 is an excess molar refraction, $\frac{H}{2}$ is the dipolarity/polarizability parameter, $\frac{H}{2}$ and $\frac{H}{2}$ are the solute hydrogen-bond acidity and basicity, respectively, and V_x is the characteristic volume of McGowan. [18] In equation 1, n is the number of compounds used in the model, r is the correlation coefficient, s is the standard error, and F is the Fischer value that gives a measure for the statistical significance of the relationship.

Equations 1a and 1b illustrate the significance of hydrogen-bonding potential in the partition of compounds between the blood and brain fluids. The main shortcoming of fragment-based methods is that they require the calculation of many parameters for fragments. Moreover, the additivity of those parameters to molecular level properties is problematic, since this scheme assumes no intramolecular interactions between these fragments.

Recently, more involved methods of modeling and predicting pharmacokinetic properties were based on the calculation of molecular interaction fields. Norinder and co-workers [21] used MolSurf [22] parametrization to calculate various properties related to the molecular valence region, and combined it with the Partial Least Squares to Latent Structures (PLS) method [23] to develop a QSAR with three statistically significant components (the components were obtained by means of the Principal Component Analysis, PCA, method [24]) and the following statistics: $n=56$, $r=0.913$, $s=0.312$, $F=86.95$.

Luco [25] employed the PLS technique to develop a QSAR based on several topological descriptors. This analysis also resulted in a significant three-component model with the following statistics: $n=58$, $r=0.922$, $s=0.318$ and $F=102$.

Crivori and coworkers applied a new technique, Volsurf, which generates molecular descriptors for developing QSARs for ADME properties. [26] VolSurf calculates three-dimensional molecular interaction fields and converts them into molecular descriptors that describe molecular size, shape, polarity, and hydrophobicity. [27] Based on more than 70 Volsurf descriptors, Crivori and coworkers developed a binary decision model suitable for organic compounds, assigning a score of 1 to compounds that cross the BBB and a score of -1 for non-permeating compounds.

One shortcoming of molecular field and PLS based methods is that they generally do not provide a clear physical insight, with a very large number of descriptors. Nonetheless, they are computationally fast and reach high levels of predictive accuracy.

Static Properties of Entire Molecules

Descriptor properties such as the molecular volume, dipolar moment and solvent accessible surface are calculated for the entire molecule. If M descriptors are calculated for N molecules a linear relationship is formed between the blood-brain partition coefficient $\log BB$ and the values D_{mn} of the m_{th} property of the n_{th} molecule:

$$\log BB = C_o + \sum_{m=1}^M \sum_{n=1}^N C_m D_{mn} \quad (2)$$

The coefficients c are determined by regression analysis, so that the pairwise correlation coefficient between the calculated $\log BB$ using equation 2 and the experimentally measured $\log BB$ is maximum.

For example, using this method Van de Waterbeemd and Kansy [28] established the following QSAR for 20 molecules:

$$\log BB = -0.021(\pm 0.003)PSA - 0.003(\pm 0.001)V_{mol} + 1.643(\pm 0.465), \\ r = 0.835, s = 0.448, F = 19.5 \quad (3)$$

where PSA is the molecular polar surface area, and V_{mol} is the molecular volume. PSA can be considered as a hydrogen bonding descriptor. These results directly indicate the importance of the polar surface area and molecular size for permeation through the BBB.

More involved methods have been recently developed that employ quantum mechanical calculations to compute descriptors used in QSAR building. Hutter used semi-empirical AM1 calculations to compute molecular electrostatic potentials and fundamental electronic properties such as the ionization potential and use those to compute properties such as the polar surface area of compounds. [29]

In addition to simple multiple linear regression methods, a number of comprehensive computational approaches based on neural networks and genetic algorithms resulted in the development of $\log BB$ QSARs. [30-34]

For example, Teixido and coworkers successfully developed a genetic algorithm to identify and design peptides that permeate through the BBB. [33]

Recently, Fu and coworkers used computed values of the molecular volume, the sum of the absolute values of the net atomic charges of oxygen and nitrogen atoms which are hydrogen-bond acceptors, and the sum of the net atomic charges of hydrogen atoms attached to oxygen or nitrogen atoms to train and artificial neural network for $\log BB$. They used 56 compounds and a test set of 5 compounds and their neural network resulted in root mean squared error of 0.258 between experimental $\log BB$ values and predicted $\log BB$ values. [32]

The main advantage of these methods over PLS methods is the inclusion of nonlinear terms in the models developed. On the other hand, neural networks are not transparent and they do not provide physical insight that can be used for drug design.

Ensemble Averages

All of the approaches described so far are based on mechanistically chosen topological descriptors or the calculation of properties of stand-alone molecules in the lowest gas-phase energy conformation.

The solvation of compounds in water and a lipid phase might be accompanied by significant conformational changes that in turn lead to changes in the molecular properties. These changes will be more pronounced for large, flexible molecules like fexofenadine in Fig. (1).

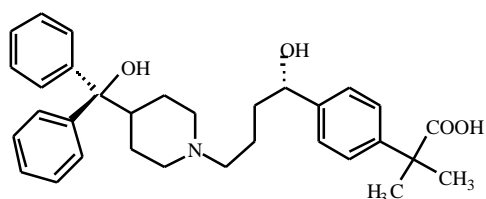


Fig. 1. Structure of fexofenadine

The simulation of diffusion of molecules through a lipid bilayer would in principle be an ideal solution. Molecular dynamics simulations of various molecules in bilayers embedded in an aqueous environment have been reported, and these atomistic simulations provide a detailed molecular level understanding of the partition of molecules in heterogeneous environments [35,36].

Iyer and coworkers used such simulations to build a *logBB* QSAR. [37] Organic compounds were simulated in a dimyristoylphosphatidylcholine (DMPC) bilayer. DMPC is one of a class of PC lipids which are major components of mammalian cell walls. The simulations generated a very large number of molecular conformations and ensemble averaged properties were calculated for organic molecules in the lipid bilayer. Based on these properties, a membrane-interaction QSAR (MI-QSAR) was developed that predicted the blood/brain coefficient fairly accurately ($R^2 = 0.845$), demonstrating that atomistic simulations of molecules in lipid-rich heterogeneous environments can help predict *logBB* values.

Unfortunately, the time scales of diffusion of most small molecules can span scales of microseconds and molecular dynamics can simulate atomistic systems for up to 100 nanoseconds with typically available computational resources. Hence, it is not clear that simulations of organic molecules in heterogeneous atomistic systems reach an equilibrium state. In particular, it is not a straightforward task to demonstrate that the simulation results are independent of the initial configuration of the molecules in the system, i.e., the position and orientation of the simulated molecule with respect to the lipid interface.

Therefore, one has to turn to semi-empirical approaches that both address the issue of the solvent's influence on the

molecular conformations and can reach equilibrium in attainable simulated times.

Jorgensen and co-workers [38,39] demonstrated that Monte Carlo simulations of molecules in water can be successfully employed to predict the gas to liquid free energies of solvation in hexadecane, octanol and water, the $\log P_{ow}$ and the water solubility $\log S$.

This methodology was employed for 76 molecules and a QSAR was developed for the prediction of the blood-brain partition coefficient, demonstrating the utility of molecular mechanics simulations in QSAR building. [40]

Specifically, each molecule was solvated in 500 water molecules (Fig. 3). The TIP4P model [41] was used for the waters and the OPLS force field was used with AM1 partial charges. [42-44] The systems were simulated in the NPT ensemble at 25 °C and 1 atm generating 3×10^5 equilibration configurations.

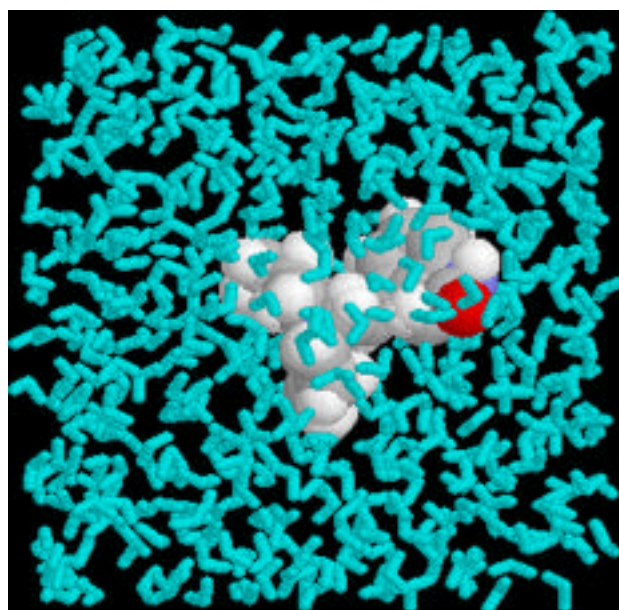


Fig. 2. Schematic of one of the simulated molecules (SKF 89124) inside a cubic box with 500 water molecules.

Ensemble averages were calculated during the simulations for properties like Coulomb energy between solute and solvent (ESXC), solvent accessible surface area (SASA), aromatic component of SASA (ARSA), dipole moment of solute (DIPO), number of solute hydrogen-bond donors (HBDN), number of solute hydrogen-bond acceptors (HBAC), and molecular volume (MVOL). In Fig. (3), the Coulomb interaction energy between one of the simulated organic molecules (SKF 89124) and the water solvent is shown. During the simulation data are stored in regular intervals of 6000 Monte Carlo trials. It is clear that thermal fluctuations influence significantly the descriptors used for QSAR building.

In the statistical analysis, in addition to the descriptors described previously, the term $HBACXHBDN^{1/2}/SASA$ was used. This cohesive index represents an electrostatic surface tension term, and was introduced by Jorgensen and co-workers [38,39]. The fractional power in *HBDN* reflects pos-

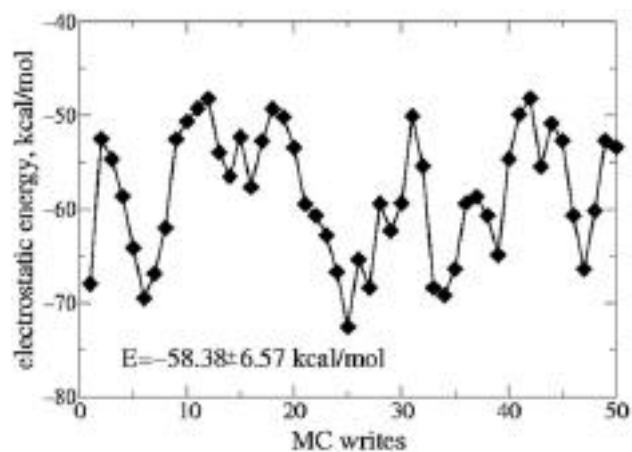


Fig. 3. Simulated electrostatic interaction energy between a simulated organic molecule (SKF 89124) and water.

sible saturation effects expected for molecules with a large number of acceptor and donors, in which case it is not likely that all of them will be simultaneously satisfied.

Using the ensemble averaged values of simulated properties a multiple linear regression analysis produced equation 4

$$\log BB = -0.2339(\pm 0.013)HBAC + 0.00147(\pm 0.00011)MVOL - 31.6099(\pm 4.0837)HBAC \times HBDN^{1/2} / SASA - 0.04579(\pm 0.05808) \\ n = 76, r = 0.97, s = 0.173, F = 311.307 \quad (4)$$

With only three terms $\log BB$ is predicted very accurately, with the highest correlation coefficient reported in the literature, because of averaging over many molecular conformations. Furthermore, a clear physical picture emerges of the molecular mechanisms involved in cerebrovascular transport, with the size and the numbers of hydrogen bond acceptors and donors playing a significant role. This QSAR clearly indicates that hydrophilicity negatively impacts the blood-brain permeation.

A major shortcoming of atomistic simulations is the required computational resources. An average of 0.75 h of CPU is required for each compound on a 1.2 GHz Intel-PentiumIV LINUX-based PC. Although the overall cost for simulated compounds in currently available datasets is negligible, this method is not readily amenable for high-throughput computational screening of hundreds of thousands of drug-like molecules.

Correlation between $\log BB$ and other Physicochemical Properties

The permeation through the BBB can be thought as being controlled by the ratio and topological distribution of polar and hydrophobic molecular groups. These also influence other physicochemical properties such as the octanol/water partition coefficient or the energy of solvation. Since these properties are more readily measured than $\log BB$, researchers have attempted to correlate $\log BB$ with them.

Young and co-workers [5] proposed the correlation between $\log BB$ and $\log P$, shown in equation 5. $\log P$ is known as the Seiler parameter [45], and is defined as $\log P = \log P_{ow} - \log P_{cycbw}$, where P_{ow} and P_{cycbw} are the octanol/water and cyclohexane/water partition coefficients, respectively. $\log P$ is considered to be a measure of hydrogen-bonding potential [46].

$$\log BB = -0.485(\pm 0.160) \log P + 0.889(\pm 0.500), \\ n = 20, r = 0.831, s = 0.439, F = 40.23 \quad (5)$$

Lombardo and co-workers [47] correlated $\log BB$ with the free energy of solvation G_w with the following equation

$$\log BB = 0.054(\pm 0.005) G_w + 0.43(\pm 0.07), \\ n = 55, r = 0.82, s = 0.41, F = 108.3 \quad (6)$$

This correlation provides an elegant means for successful $\log BB$ prediction. It, however, relies on calculating the energy of solvation from *ab initio* calculations in the gas phase. The solvent might play an important role in the conformations of the solute, which will in turn lead to different values for a number of parameters, such as the solvent-accessible surface area (SASA), the molecular volume and the molecular dipole moment, all of which influence the energy of solvation.

Kaliszan and Markuszewski [48] re-established the correlation of $\log BB$ with $\log P_{ow}$ and refined it, employing the molecular mass as an additional descriptor of molecular bulkiness:

$$\log BB = -0.088(\pm 0.051) + 0.272(\pm 0.017) \log P - 0.001116(\pm 0.00049)M_m, \\ n = 33, r = 0.947, s = 0.126, F = 131.1 \quad (7)$$

These authors indicated that a molecular bulkiness descriptor should be used to better account for non-specific dispersive properties of molecules.

Feher and co-workers [49] built the following model

$$\log BB = -0.1092 \log P_{ow} - 0.3873n_{acc,solv} - 0.0017PSA + 0.4275, \\ n = 61, r = 0.854, s = 0.424, F = 51 \quad (8)$$

where $n_{acc,solv}$ is the number of hydrogen-bond acceptors.

Recently Hou and coworkers [10] revisited the $\log BB / \log P_{ow}$ correlation building the following regression model

$$\log BB = 0.00845 + 0.197 \log P - 0.0135HCPSA - 0.0140(MW - 360) \quad (9)$$

with $n=78, r=0.88, q=0.86, s=0.36$, and $F=81.5$.

In equation 9, HCPSA is the high-charged polar surface areas computed with the Gasteiger partial charges and MW is the molecular weight. [10] The n-octanol/water partition coefficient was calculated using the SLOGP approach, which estimates $\log P$ by summing the contribution of atom-weighted solvent accessible surface areas (SASA) and correction factors. The excess molecular weight above 360 improves the accuracy of the model and indicates that smaller molecules with $MW < 360$ permeate the BBB, whereas for larger molecules their size diminishes their BBB penetration.

PHYSICAL INSIGHT

A fairly clear consensus has emerged concerning the molecular properties that influence the permeation of organic compounds through the blood-brain barrier. [50-55] Most all studies conclude that hydrophilicity and size negatively impact *logBB* values. Hydrophilicity has been expressed in terms of polar surface areas, number of hydrogen bond acceptors and donors and dipolarity/polarizability values. More refined correlations suggest that molecular weight plays a detrimental role in BBB permeation only for larger molecules, with MW=360 being a reasonable cutoff.

Kelder and co-workers [56] examined the distribution of the polar surface area of for 776 CNS and 1590 non-CNS drugs and deduced that penetration of molecules is possible only if their polar surface area is less than 120 \AA^2 and suggested that drugs can be tailored for brain penetration by decreasing the polar surface to less than 60 \AA^2 .

Seelig and co-workers [57,58] identified the molecular parameters governing the passive diffusion of the molecules through lipid membranes, using theoretical arguments. They suggested that the optimal characteristics for a molecule to penetrate the BBB are (i) amphiphilicity $G_{am} > -3 \text{ kJ/mol}$ (they defined amphiphilicity as $G_{am} = G_{aw} - G_{mic}$ where G_{aw} and G_{mic} are the free energies of partitioning into the air-water interface and of micelle formation respectively), (ii) a value for the air/water partition coefficient K_{aw} in the range of 10^5 - 10^3 M^{-1} , and (iii) a molecular cross-sectional area $A_D < 80 \text{ \AA}^2$.

The blood-brain partition coefficient *logBB* also correlates positively with the octanol/water partition coefficient suggesting that lipophilic molecules traverse the BBB more readily, in agreement with studies based only on molecular properties.

CONCLUDING REMARKS

From the described methods it becomes evident that there has been considerable progress in the development of semi-empirical models for the relative affinity of compounds for blood and brain compartments, with considerable predictive ability.

Quantitative structure-property relationships from properties calculated in molecular mechanics simulations appear to most accurately predict the blood/brain partition coefficient, albeit with the highest computational cost. Of course, constant improvement of available computer power and the development of more accurate force-fields for lipids and organic molecules render increasingly attractive the employment of more complex models of organic compounds in lipid layers, which do provide a clear atomistic level picture of phenomena relevant to BBB permeation.

One can envision a hierarchical scheme and various stages of virtual screening from binary decision models to QSARs from gas phase molecular structures to simulations in heterogeneous lipid environments. With the molecular structure as the sole input, *logBB* values can be calculated

and used in decision-making without the need to resort to expensive experiments.

With the advent of high-throughput techniques, *in vitro* models for *logBB* measurements, and publicly available structures and properties for hundreds of thousands of compounds an automated decision-making engine (ADME) [59] is not considered out of reach.

ACKNOWLEDGEMENTS

The author expresses his deep gratitude to the scientists of Pfizer Global Research and Development, Ann Arbor Laboratories for introducing him to the exciting world of computer-aided drug discovery and bioinformatics

REFERENCES

- [1] De Vries, H. E.; Kuiper, J.; De Boer, A. G.; Van Berkel, T. J. C.; Breimer, D. D. The Blood-Brain Barrier in Neuroinflammatory Diseases. *Pharmacol. Rev.* **1997**, *49*, 143-155.
- [2] Pardridge, W.M. Blood-Brain Barrier Biology and Methodology. *Neurovirol.* **1999**, *5*, 556-69.
- [3] Tamai, I.; Tsuji, A. Drug Delivery Through the Blood-Brain Barrier. *Adv. Drug Delivery Rev.* **1996**, *19*, 401-424.
- [4] van de Waterbeemd, H. High-Throughput and In Silico Techniques in Drug Metabolism and Pharmacokinetics *Curr. Opin. Drug Disc. & Develop.* **2002**, *5*, 33-43.
- [5] Young, R. C.; Mitchell, R. C.; Brown, T. H.; Ganellin, C. R.; Griffiths, R.; Jones, M.; Rana, K. K.; Saunders, D.; Smith, I. R.; Sore, N. E.; Wilks, T. J. Development of a New Physicochemical Model for Brain Penetration and Application to the Design of Centrally Acting H2 Receptor Histamine Antagonists. *J. Med. Chem.* **1988**, *31*, 656-671.
- [6] Eddy, E. P.; Maleef, B. E.; Hart, T. K.; Smith, P. L. *In Vitro* Models to Predict Blood-Brain Barrier Permeability. *Adv. Drug Deliv. Rev.* **1997**, *23*, 185-198
- [7] Reichel, A.; Begley, D. J. Potential of Immobilized Artificial Membranes for Predicting Drug Penetration Across the Blood-Brain Barrier. *Pharm. Res.* **1998**, *15*, 1270-1274.
- [8] Feng, M.R. Assessment of Blood-Brain Barrier Penetration: In Silico, *In Vitro* and *In Vivo*. *Curr Drug Metab.* **2002**, *3*, 647-57.
- [9] Lohmann, C.; Huwel, S.; Galla, H.J. Predicting blood-brain barrier permeability of drugs: evaluation of different *in vitro* assays. *J. Drug Target.* **2002**, *10*, 263-76.
- [10] Hou, T.J.; Xu, X.J., ADME Evaluation in Drug Discovery. 3. Modeling Blood-Brain Barrier Partitioning Using Simple Molecular Descriptors. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 2137-2152.
- [11] Clark, D.E., In Silico Prediction of Blood-Brain Barrier Permeation *Drug Disc. Today*, **2003**, *8*, 927-933.
- [12] Terasaki, T.; Ohtsukia, S.; Horia, S.; Takanagaa, H.; Nakashimab E.; Hosoyac, K. New Approaches to *in vitro* Models of Blood-Brain Barrier Drug Transport. *Drug Discov Today*. **2003**, *8*, 944-54.
- [13] Lundquist, S.; Renftel, M.; Brillault, J.; Fenart, L.; Cecchelli, R.; Dehouck, M.P. Prediction of Drug Transport Through the Blood-Brain Barrier *In Vivo*: a Comparison Between Two *In Vitro* Cell Models. *Pharm Res.* **2002**, *19*, 976-81.
- [14] van Bree, J.B.; de Boer, A.G.; Danhof, M.; Ginsel, L.A.; Breimer, D.D. Characterization of an "In Vitro" Blood-Brain Barrier: Effects of Molecular Size and Lipophilicity on Cerebrovascular Endothelial Transport Rates of Drugs. *J Pharmacol Exp Ther.* **1988**, *247*, 1233-9.
- [15] Gumbleton, M.; Audus, K.L. Progress and Limitations in the Use of *In Vitro* Cell Cultures to Serve as a Permeability Screen for the Blood-Brain Barrier. *J Pharm Sci.* **2001**, *90*, 1681-98.
- [16] Franke, H.; Galla, H.J.; Beuckmann, C.T. An improved low-permeability *in vitro*-model of the blood-brain barrier: transport studies on retinoids, sucrose, haloperidol, caffeine and mannitol. *Brain Res.* **1999**, *818*, 65-71.
- [17] NIH website: <http://pubchem.ncbi.nlm.nih.gov/>

- [18] Abraham, M. H.; Chadha, H. S.; Mitchell, R. C. Hydrogen Bonding. 33. Factors that Influence the Distribution of Solutes Between Blood and Brain. *J. Pharm. Sci.* **1994**, *83*, 1257-1268
- [19] Abraham, M. H.; McGowan, J. C. The Use of Characteristic Volumes to Measure Cavity Terms in Reversed Phase Liquid Chromatography. *Chromatographia* **1987**, *23*, 243-246.
- [20] Abraham, M. H.; Chadha, H. S.; Mitchell, R. C. Hydrogen Bonding. Part 36. Determination of Blood Brain Distribution Using Octanol-Water Partition Coefficients. *Drug Des. Discov.* **1995**, *13*, 123-131
- [21] Norinder, U.; Sjöberg, P.; Osterberg, T. Theoretical Calculation and Prediction of Brain-Blood Partitioning of Organic Solutes Using MolSurf Parametrization and PLS Statistics. *J. Pharm. Sci.* **1998**, *87*, 952-959.
- [22] Sjöberg, P. MolSurf - a Generator of Chemical Descriptors for QSAR. In: 'Computer-Assisted Lead Finding and Optimization', Eds. Han van de Waterbeemd, Bernard Testa, Gerd Folkers, Verlag Helvetica Chimica Acta, CH - 4010 Basel, Switzerland, 1997, pp. 83-92.
- [23] Wold, S.; Johansson, E.; Cocchi, M. PLS – Partial Least Squares Projections to Latent Structures. In *3D QSAR in Drug Design*; Kubinyi, H., Ed.; ESCOM: Leiden 1993; pp 523-550.
- [24] Jolliffe, I. T. *Principal Component Analysis in Chemistry*; Springer-Verlag: New York, 1986.
- [25] Luco, J. M. Prediction of the Brain-Blood Distribution of a Large Set of Drugs from Structurally Derived Descriptors Using Partial Least-Squares (PLS) Modeling. *J. Chem. Inf. Comput. Sci.*, **1999**, *39*, 396-404.
- [26] Cruciani, G.; Pastor, M.; Mannhold, R. Suitability of Molecular Descriptors for Database Mining. A Comparative Analysis. *J. Med. Chem.* **2002**, *45*, 2685-94
- [27] Cruciani, G.; Pastor, M.; Guba, W. VolSurf: a New Tool for the Pharmacokinetic Optimization of Lead Compounds. *Eur. J. Pharm. Sci.* **2000**, Suppl 2, S29-39.
- [28] van de Waterbeemd, H.; Kansy, D. Hydrogen-Bonding Capacity and Brain Penetration. *Chimia* **1992**, *46*, 299-303.
- [29] Hutter, M.C. Prediction of blood-brain barrier permeation using quantum chemically derived information, *J. Comp-Aid. Mol. Des.* **2003**, *17*, 415-433.
- [30] Winkler, D.A.; Burden, F.R. Modelling Blood-Brain Barrier Partitioning Using Bayesian Neural Nets. *J. of Mol. Graph. & Mod.* **2004**, *22*, 499-505.
- [31] Hou, T.J.; Xu, X.J. ADME Evaluation in Drug Discovery - I. Applications of Genetic Algorithms to the Prediction of Blood-Brain Partitioning of a Large Set of Drugs. *J. Mol. Mod.* **2002**, *8*, 337-349.
- [32] Fu, X. C.; Wang, G. P.; Liang, W. Q.; Yu, Q. S. Predicting blood-brain barrier penetration of drugs using an artificial neural network. *Pharmazie*. **2004**, *59*(2), 126-130.
- [33] Teixidó, M.; Belda, I.; Roselló, X.; González, S.; Fabre, M.; Llorá, X.; Bacardit, J.; Garrell, J.M.; Vilaró, S.; Albericio, F.; Giralt, E. Development of a Genetic Algorithm to Design and Identify Peptides that can Cross the Blood-Brain Barrier: 1. Design and validation in silico. *QSAR & Combinatorial Science*, **2003**, *22*, 745-753.
- [34] Doninger, S.; Hofmann, T.; Yeh, J. Predicting CNS Permeability of Drug Molecules: Comparison of Neural Network and Support Vector Machine Algorithms. *J. Comp. Biol.* **2002**, *9*, 849-864.
- [35] Bassolino-Klimas, D.; Alper, H. E.; Stouch, T. R. Solute Diffusion in Lipid Bilayer Membranes: an Atomic Level Study by Molecular Dynamics Simulation. *Biochemistry* **1993**, *32*, 12624-12637.
- [36] Jin, B.; Hopfinger, A. J. Characterization of Lipid Membrane Dynamics by Simulation: 3. Probing Molecular Transport Across the Phospholipid Bilayer. *Pharm. Res.* **1996**, *13*, 1786-1794.
- [37] Iyer, M.; Mishra, R.; Han, Y.; Hopfinger, A. J. Predicting Blood-Brain Barrier Partitioning of Organic Molecules Using Membrane-Interaction QSAR Analysis. *Pharm. Res.* **2002**, *19*, 1611-1621.
- [38] Jorgensen W.L.; Duffy E. M. Prediction of Drug Solubility from Monte Carlo Simulations. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 1155-4755.
- [39] Duffy, E. M.; Jorgensen, W. L. Prediction of Properties from Simulations: Free Energies of Solvation in Hexadecane, Octanol, and Water. *J. Am. Chem. Soc.* **2000**, *122*, 2878-2888.
- [40] Kaznessis, Y.N.; Snow, M.E.; Blankley, C.J. Prediction of Blood-Brain Partitioning Using Monte Carlo Simulations. *J. Comput. Aided Mol. Des.* **2001**, *15*, 697-708
- [41] Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of Simple Potential Functions for Simulating Liquid Water. *J. Chem. Phys.* **1983**, *79*, 926-935.
- [42] Kaminski, G. A.; Jorgensen, W. L. A Quantum Mechanical and Molecular Mechanical Method Based on CM1A Charges: Applications to Solvent Effects on Organic Equilibria and Reactions. *J. Phys. Chem. B*, **1998**, *102*, 1787-1796.
- [43] Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. Development and Testing of the OPLS All-Atom Force Field on Conformational Energetics and Properties of Organic Liquids, *Journal of the American Chemical Society*, **1996**, *118*, 11225-11236.
- [44] Jorgensen, W. L.; Nguyen, T. B. Monte Carlo Simulations of the Hydration of Substituted Benzenes with OPLS Potential Functions. *J. of Comp. Chem.* **1993**, *14*, 195-205.
- [45] Seiler, P. Interconversion of Lipophilicities from Hydrocarbon/Water systems into the Octanol/Water Systems. *Eur. J. Med. Chem.* **1974**, *9*, 473-479.
- [46] el Tayar, N.; Tsai, R. S.; Testa, B.; Carrupt, P. A.; Leo, A. Partitioning of Solutes in Different Solvent Systems: the Contribution of Hydrogen-Bonding Capacity and Polarity. *J. Pharm. Sci.* **1991**, *80*, 590-598.
- [47] Lombardo, F.; Blake, J. F.; Curatolo, W. J. Computation of Brain-Blood Partitioning of Organic Solutes via Free Energy Calculations. *J. Med. Chem.* **1996**, *39*, 4750-4755
- [48] Kaliszan, R.; Markuszewski, M. Brain/Blood Distribution Described by a Combination of Partition Coefficient and Molecular Mass. *Int. J. of Pharmaceutics*, **1996**, *145*, 9-16.
- [49] Feher, M.; Sourial, S.; Schmidt, J. M.; A Simple Model for the Prediction of Blood-Brain Partitioning. *Int. J. of Pharmaceutics*, **2000**, *201*, 239-247.
- [50] Clark, D.E. Rapid Calculation of Polar Molecular Surface Area and its Application to the Prediction of Transport Phenomena. 2. Prediction of Blood-Brain Barrier Penetration. *J. Pharm. Sci.* **1999**, *88*, 815-21
- [51] Ecker, G.; Noe, C.R. In silico prediction models for blood-brain barrier permeation. *Current Medicinal Chemistry* **2004**, *11*, 1617-1628.
- [52] Terasaki, T.; Ohtsuki, S.; Hori, S.; Takanaga, H.; Nakashima, E.; Hosoya, K. New approaches to *in vitro* models of blood-brain barrier drug transport. *Drug Discovery Today*, **2003**, *8*, 944-954.
- [53] Fu, X.-C.; Chen, C.-X.; Liang, W.-Q.; Yu, Q.-S. Predicting blood-brain barrier penetration of drugs by polar molecular surface area and molecular volume. *Acta Pharmacologica Sinica*, **2001**, *22*, 663-668.
- [54] Sippl, W. Computational approaches for the prediction of blood-brain barrier permeation. *Cur. Med. Chem.: Central Nervous System Agents*, **2002**, *2*, 211-227.
- [55] Gratton, J.A.; Abraham, M.H.; Bradbury, M.W.; Chadha, H.S. Molecular factors influencing drug transfer across the blood-brain barrier. *J. Pharmacy and Pharmacology* **1997**, *49*, 1211-1216.
- [56] Kelder, J.; Grootenhuis, P. D.; Bayada, D. M.; Delbressine, L. P.; Ploemen, J. P.; Polar Molecular Surface as a Dominating Determinant for Oral Absorption and Brain Penetration of Drugs. *Pharm. Res.* **1999**, *16*, 1514-1519.
- [57] Fischer, H.; Gottschlich, R.; Seelig, A. Blood-Brain Barrier Permeation: Molecular Parameters Governing Passive Diffusion. *J. Membr. Biol.* **1998**, *165*, 201-211.
- [58] Seelig, A.; Gottschlich, R.; Devant, R. M. A Method to Determine the Ability of Drugs to Diffuse Through the Blood-Brain Barrier. *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 68-72.
- [59] van de Waterbeemd, H.; Gifford, E. ADMET in Silico Modelling: Towards Prediction Paradise? *Nature Rev. Drug. Discov.* **2003**, *2*, 192-204.