Potential of Immobilized Artificial Membranes for Predicting Drug Penetration Across the Blood–Brain Barrier

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Purpose. The present study evaluates immobilized artificial membrane (IAM) chromatography for predicting drug permeability across the blood-brain barrier (BBB) and outlines the potential and limitations of IAMs as a predictive tool by comparison with conventional methods based on octanol/water partitioning and octadecylsilane (ODS)-HPLC.
Methods. IAM- and ODS-HPLC capacity factors were determined in order to derive the hydrophobic indices log k_{IAM} and log k_W for two sets of compounds ranging from very lipid soluble (steroids) to more hydrophilic agents (biogenic amines). The uptake of the compounds across the *in vivo* BBB expressed as brain uptake index (BUI) has been correlated with these HPLC capacity factors as well as octanol/water partition (ClogP) and distribution coefficients (log $D_{7.4}$).
Results. For both test groups log k_{IAM} correlates significantly with the respective log BUI of the drug (r^2 = 0.729 and 0.747, $p < 0.05$), whereas with log k_W, log $D_{7.4}$ and ClogP there is only a correlation for the group of steroids (r^2 = 0.789, 0.659 and 0.809, $p < 0.05$) but not for the group of biogenic amines. There is a good correlation between log k_{IAM} and log k_W, ClogP or log $D_{7.4}$ for the group of steroids (r^2 = 0.945, 0.867 and 0.974, $p < 0.01$) but not for the biogenic amines.
Conclusions. All physico-chemical descriptors examined in this study equally well describe brain uptake of lipophilic compounds, while log k_{IAM} is superior over log $D_{7.4}$, ClogP and log k_W when polar and ionizable compounds are included. The predictive value of IAMs combined with the power of HPLC holds thus great promise for the selection process of drug candidates with high brain penetration.

KEY WORDS: blood-brain barrier (BBB); drug transport; prediction of brain uptake; immobilized artificial membrane (IAM); HPLC.

INTRODUCTION

The continuous layer of the brain endothelium lining brain capillaries forms the main barrier for drug entry from blood into the CNS, the so called blood-brain barrier (BBB). The multi-stranded tight junctional complexes between adjacent endothelial cells in the brain effectively restrict paracellular pathways and result in the individual endothelial cell membranes acting as a continuous lipidic boundary (1). Consequently, the lipid solubility of solutes has long been considered to determine the rate at which they are capable of entering the brain via the lipid-mediated pathway (2). Since success in CNS drug development depends as much on the specific pharmacological activity of the drug candidate at the target site as it depends on the efficacy of its delivery to that site, simple and reliable predictors of drug penetration through the BBB would be very powerful tools in the drug discovery process (3,4). A great deal of effort is currently being made to identify physico-chemical parameters which correlate with the brain uptake *in vivo*, and hence are a satisfactory surrogate for predicting drug entry into CNS.

Although the lipid solubility of a solute as measured by its partition behaviour between octanol and water (or buffer) is still the most commonly used model of its transmembrane permeability (4), the octanol/water partition coefficient (log P_{oct}) is, unfortunately, an unreliable predictor for drug penetration across cellular barriers (5,6), and, therefore, alternative models have been proposed. Among the most prominent models are systems relying on measures of hydrogen bonding (ΔlogP) (7), hydrophobicity estimates by reversed-phase HPLC (8), partitioning into liposomes (9), solvatochromic parameters (10), measures of the surface activity (11) and various topological indices derived from computational approaches (12). As yet, however, none of these models is entirely satisfactory for predicting brain uptake or easily applicable for a large throughput screen of drug candidates.

Recently, immobilized artificial membranes (IAMs) which are solid phase models of fluid membranes have been proposed for assessment of drug permeability through cell membranes (13). IAMs consist of phosphatidyl choline residues covalently bound to silica propylamine and are used as a chromatographic interface in HPLC. The ordered monolayer of immobilized phosphatidylcholine residues in the IAM column is intended to mimic the lipid phase of cell membranes and thereby affect retention of solutes on the basis of solute-membrane interactions (14). Drug partitioning into IAMs has shown promise for predictions of drug permeability across tight epithelial barriers such as human skin, rat and mice intestinal epithelium, and Caco-2 cells (15). The predictive value of IAMs for brain penetration cannot simply be extrapolated from these studies as the cell membranes of the cerebral endothelium express a unique composition which differs from that elsewhere in the body (16) and will thus affect drug permeation (17). The purpose of the present study was to examine the potential of the IAM technology for predicting drug delivery to the brain.

We used a set of very hydrophobic (6 steroids) and a set of more hydrophilic compounds (6 biogenic amines) to determine the respective HPLC capacity factors which are measures of solute-surface interaction. The uptake of the compounds across the *in vivo* BBB expressed as brain uptake index (BUI) has been correlated with the HPLC capacity factors. BUI values rather than permeability-surface area (PS) products or blood-brain steady state distribution coefficients have been taken because uptake as studied by the BUI technique (i) is dominated by solute-membrane interactions due to the very short exposure time of the test compound to the brain endothelium, and (ii) is not affected by processes unrelated to membrane partitioning such as plasma protein binding and metabolism (18). The evaluation of the IAM technology was carried out by comparison of the correlation coefficients derived from correlation of log BUI* (corrected for drug size) with log k_{IAM} and the classical parameters octanol/water partition coefficients (ClogP), octanol/buffer distribution coefficient (log $D_{7.4}$) and ODS-HPLC capacity factors (log k_W).

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METHODS

Chemicals

Steroids (progesterone, testosterone, estradiol, corticosterone, cortisol and aldosterone) and biogenic amines (amphetamine, serotonin, tryptophol, dopamine, epinephrine and norepinephrine) were purchased from SIGMA. Phosphate buffered saline (tablets), HPLC grade water and acetonitrile were obtained from BDH Chemicals, Poole, UK.

IAM and ODS Chromatography

Retention times of the test compounds were determined using either an IAM column (150 × 4.6 mm IAM.PC.MG 12 PAS column, Regis Technologies Inc., Morton Grove, Illinois, USA) or a C18 column (250 × 4.6 mm Spherisorb 5 ODS, HPLC Technology, Macclesfield, UK). For all studies, the injection volume was 20 µl of a solute aqueous solution (~1 mg/ml) and the flow rate was 1 ml/min. Steroids were detected using UV absorption at 250 nm and biogenic amines were detected electrochemically (E = 720 mV). Chromatograms were obtained using a LDC/Milton Roy HPLC pumping system equipped with a LDC/Milton Roy spectromonitor/II or a EC& G EC detector, respectively, and were analysed using Kontron HPLC Analysis software (Data system MT2).

IAM chromatography was carried out isocratically using a 0.01 M phosphate buffered saline solution (PBS) at pH 7.4, while the elution of the test compounds using ODS chromatography required the addition of organic modifier (acetonitrile) to the aqueous buffer to prevent collapse of the octadecyl chains as would occur in a 100% aqueous phase.

HPLC Solute Capacity Factors

IAM Chromatography

Averaged triplicates of the retention times (t_R) of the test compounds were transformed into capacity factors (k_{IAM}) according to the following equation:

$$k = (t_R - t_0)/t_0 \quad (1)$$

where t_R and t_0 are the retention times of the test compound, and a compound which is not retained by the column (e.g. citrate) to indicate the column dead time/void volume. Capacity factors were expressed as logarithm (log k_{IAM}).

ODS Chromatography

The respective log k_W values corresponding to the logarithm of the capacity factors for a 100% aqueous eluent were determined polycratically by measuring the retention times of the test compounds with varying percentages (φ) of organic modifier (increments of 10% from 30% to 80% of acetonitrile) in 0.01 M PBS using the following linear extrapolation:

$$\log k_W = \log k_\varphi + S * \varphi \quad (2)$$

where log k_φ is the capacity factor as calculated from the retention time for the respective volume fraction of acetonitrile in the eluent (φ), S is the slope and log k_W the intercept of the log k_φ – φ plot (11). The log k_φ values were derived from averaged triplicates of retention times according to equation (1). The polycratic determination of log k_W values could only be applied for the set of steroids but not for the biogenic amines.

Table 1. Brain Uptake[a] and Various Physico-Chemical Parameters of the Group of Steroids

	log BUI*	log k_{IAM}	log k_W	log $D_{7.4}$	ClogP
progesterone	0.110	2.28	2.47	3.25	3.775
testosterone	0.116	1.80	2.04	2.72	3.219
estradiol	0.119	1.68	1.85	2.29	3.784
corticosterone	0.0864	1.34	1.42	1.82	1.163
aldosterone	0.0314	0.89	0.829	1.08	−0.139
cortisol	0.00987	1.00	0.947	1.54	0.537

[a] Brain uptake indices taken from (20), corrected for molecular weight and expressed as log BUI*.

Log $D_{7.4}$, CLogP and BUI Values

Octanol/buffer distribution coefficients (log $D_{7.4}$) of the two sets of test compounds determined at pH 7.4 were taken from Hansch & Leo (19) and the MEDCHEM™ data base, respectively. Octanol/water partition coefficients were calculated using ClogP software. Data for brain uptake indices (BUI) for steroids were taken from Pardridge and Mietus (20) and for biogenic amines from Cornford et al. (21) and corrected for the size of the molecule by dividing log BUI by the square root of the molecular weight of the test compounds (log BUI*).

Statistics

Correlation coefficients were obtained using linear regressions analysis of log/log plots of the data. We denote the number of data points in a correlation n, the correlation coefficient r, the standard deviation s, and the F-statistic F. The quality of the curve fit was assessed by r^2, s and F values and a correlation was judged significant when $p < 0.05$.

RESULTS AND DISCUSSION

The present study examines the potential of IAM chromatography for predicting drug permeability across the BBB on the basis of comparisons with conventional lipophilicity measures based on octanol/water partitioning and hydrophobicity indices measured by ODS-HPLC (Table 1 and 2). Table 3 summarises the correlations between brain uptake (log BUI*) of the group

Table 2. Brain Uptake[a] and Various Physico-Chemical Parameters of Biogenic Amines

	log BUI*	log k_{IAM}	log $D_{7.4}$	ClogP
amphetamine	0.213	0.972	−0.84	1.592
tryptophol	0.184	0.581	−1.47	1.173
serotonin	0.0369	−0.117	−1.75	0.606
dopamine	0.0582	0.393	−2.36	0.019
epinephrine	0.0384	0.302	−2.59	−0.685
norepinephrine	0.0615	0.335	−2.97	−0.989

[a] Brain uptake indices taken from (21), corrected for molecular weight and expressed as log BUI*.

Table 3. Linear Regression of the Brain Uptake of Steroids and Biogenic Amines with Different Physico-Chemical Descriptors

Log BUI* vs	Steroids	Biogenic amines
Log $D_{7.4}$	log BUI = (0.0479 ± 0.0172)log $D_{7.4}$ − (0.0225 ± 0.0385) n = 6, r^2 = 0.659, s = 0.0307, F = 7.72, not significant	log BUI = (0.0800 ± 0.0296) log $D_{7.4}$ + (0.258 ± 0.0629) n = 6, r^2 = 0.646, s = 0.0522, F = 7.3, not significant
ClogP	log BUI = (0.0242 ± 0.0059) ClogP + (0.0289 ± 0.0153) n = 6, r^2 = 0.809, s = 0.0229, F = 17.0, $p < 0.05$	log BUI = (0.0615 ± 0.0172) ClogP + (0.0810 ± 0.0224) n = 6, r^2 = 0.643, s = 0.0524, F = 7.19, not significant
Log k_W	log BUI = (0.0849 ± 0.0168) log k_W − (0.0246 ± 0.0285) n = 6, r^2 = 0.789, s = 0.0241, F = 14.9, $p < 0.05$	n/a
Log k_{IAM}	log BUI = (0.0765 ± 0.0234) log k_{IAM} − (0.0357 ± 0.0369) n = 6, r^2 = 0.729, s = 0.0275, F = 10.6, $p < 0.05$	log BUI = (0.190 ± 0.0551) log k_{IAM} + (0.0207 ± 0.0289) n = 6, r^2 = 0.747, s = 0.0441, F = 11.8, $p < 0.05$

Note: Slope and intercept are given with standard errors.

of steroids and different physico-chemical descriptors as measured by IAM-HPLC (log k_{IAM}), octanol/water partition (ClogP), octanol/buffer distribution (log $D_{7.4}$) and ODS-HPLC (log k_W). A comparison shows that apart from log $D_{7.4}$ all other parameters correlate with the brain uptake of steroids with log k_W and ClogP giving the best curve fits though not significantly better than log k_{IAM}. The results suggest that the lipophilicity parameter ClogP, and the hydrophobicity indices log k_W and log k_{IAM} predict the brain uptake of very lipid soluble compounds such as steroids equally well.

For the group of biogenic amines, however, only log k_{IAM} but neither the lipophilicity parameter ClogP nor the distribution coefficient log $D_{7.4}$ correlate with log BUI* (Fig. 1). The polycratic method required for the determination of log k_W using ODS-HPLC was unsuited for biogenic amines, hence this hydrophobicity parameter could not be measured. The correlation of log BUI* vs. log k_{IAM} is significantly better ($p < 0.05$), than log BUI* vs. log $D_{7.4}$ or log BUI* vs. ClogP which lack a common relationship (Table 3). Indeed, brain uptake of serotonin, dopamine, epinephrine and norepinephrine does not depend on octanol/buffer partition and distribution coefficients, although there appears to be some relationship for the more lipid soluble compounds tryptophol and amphetamine. However, the brain uptake of both the hydrophilic and the more hydrophobic biogenic amines shows a common relationship with partitioning into IAMs.

Although the present set of compounds is limited, the data is sufficient to reveal important trends and to draw first conclusions about the utility of IAM in predicting drug entry into brain. Taking the results for both groups of test compounds together it becomes evident that the predictive potential of IAMs for drug brain penetration is equivalent to that of ODS-HPLC, octanol/water partitioning and octanol/buffer distribution as long as very or moderately lipid soluble compounds are concerned. The IAM parameter however appears superior to octanol/water partitioning for predicting BBB penetration of test sets which consist of or include rather hydrophilic compounds. This conclusion is supported by the significant correlation between log k_{IAM} and log $D_{7.4}$ (r^2 = 0.974, $p < 0.001$), ClogP (r^2 = 0.867, $p < 0.01$) and log k_W (r^2 = 0.945, $p < 0.005$) for the hydrophobic set of compounds (steroids), and the lack of any such relationship for the hydrophilic biogenic amines (log k_{IAM} with log $D_{7.4}$: r^2 = 0.285, and with ClogP: r^2 = 0.253). Consequently, the behaviour of lipid soluble compounds is governed by similar forces in all these systems, while only the forces determining the partition behaviour in IAMs

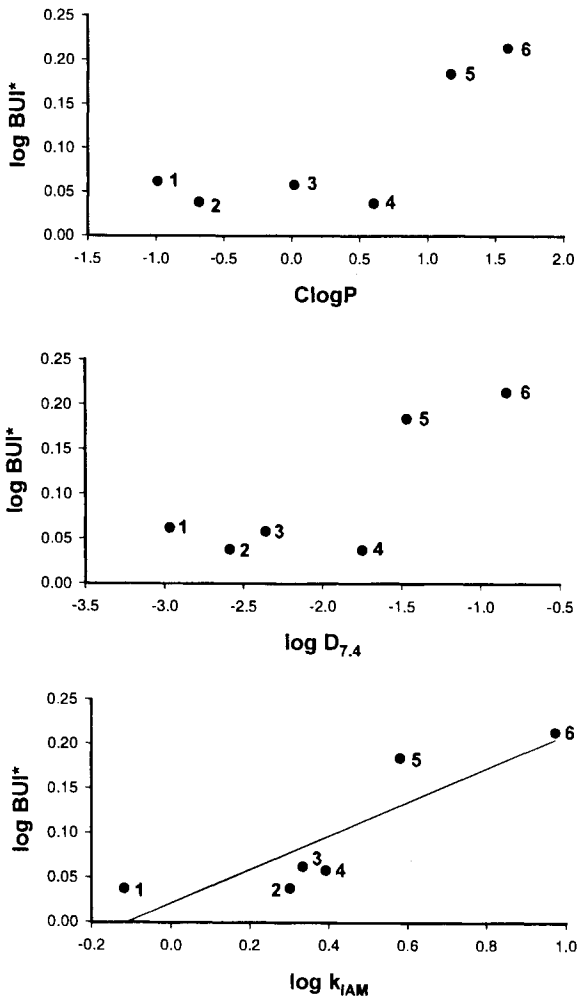

Fig. 1. Relation of the brain uptake of biogenic amines (log BUI*, corrected for molecular weight) with the lipophilicity parameter ClogP (top), the octanol/buffer distribution coefficient log $D_{7.4}$ (middle) and the IAM parameter log k_{IAM} (bottom). A regression line was drawn only when the correlation was statistically significant ($p < 0.05$). 1 norepinephrine, 2 epinephrine, 3 dopamine, 4 serotonin, 5 tryptophol, 6 amphetamine.

but not in the octanol/water system reflect the brain uptake of polar and ionizable solutes. These findings also suggest that

the mechanisms of solute retention in IAMs give some insight into the factors determining the BBB permeation of drugs.

Pidgeon and colleagues have studied extensively the major interactions between solutes and IAMs and the respective chromatographic retention mechanisms (15). Their work has shown that partitioning of a compound into IAMs comprises both hydrophobic and hydrophilic interactions as well as electrostatic forces, all of which are thought to be involved in the process of solute partitioning into cell membranes (22). IAMs therefore appear to model more diverse types of solute-membrane interactions than octanol/water partitioning which reflects mainly hydrophobic solute-surface interactions (4). Indeed, good correlations between log P_{oct} and log k_{IAM} are usually found for homologous sets of hydrophobic compounds which interact predominantly with the non-polar hydrocarbon region of membrane lipids, while solutes with more functional groups or ionised species also interact with the zwitterionic head groups of phospholipids (23), and hence their relationship between these two parameters is poor (9,24).

Moreover, partitioning of a solute into chromatographic surfaces is thermodynamically a more appropriate reflection of the dynamics of the interactions between a flowing solute (bulk phase) and the cell membrane (interfacial phase) than the shake-flask method (partitioning between to bulk phases) generally applied for the determination of partition coefficients (8). Further increasing the potential of IAM chromatography, there are also some practical advantages such as ease of automation, high speed and reproducibility. In contrast to ODS-HPLC which generally requires the polycratic determination of log k_W, the log k_{IAM} of most compounds can be determined isocratically and IAM-HPLC is therefore less cumbersome and requires less sample volume.

The IAM technology has thus both theoretical and a practical value in that it aids our understanding of the forces involved in membrane permeation as well as providing a promising tool for ranking BBB drug penetration. As IAMs are solid phase models of fluid membranes they do however not mimic lipid molecular dynamics such as lateral diffusion, membrane flip-flop and axial displacement (14). Other limitations are that although they model partitioning of solutes into membranes they do not mimic diffusion across a membrane bilayer. As the use of the IAM technology relies on the assumption that the rate limiting step for permeation across the BBB is partitioning of the drug into the brain endothelial cell membrane, it may fail when other processes limit brain uptake such as plasma protein binding, carrier-mediated transport or metabolism, which would have to be examined *in vitro* using cell cultures (25,26) or *in vivo* using whole animals (18).

CONCLUSIONS

Though based on a limited set of compounds the present study suggests that the IAM technology is suitable for ranking of compounds with respect to entry into brain. The predictive potential of the parameter log k_{IAM} is comparable to the conventional physico-chemical descriptors log $D_{7.4}$, ClogP, log k_W as long as lipid soluble compounds are concerned, but is superior when more polar compounds are considered. The higher predictability of log k_{IAM} for brain uptake of heterogeneous groups of compounds is thought to be due to a better reflection of the complexity of the forces governing drug permeation across the blood-brain barrier. The combination of the predictive value of IAMs with the power of HPLC may provide a promising tool supporting the selection process of drug candidates with high brain penetration.

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