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Optimization and Validation of an *In Vitro* Blood Brain Barrier Permeability Assay Using Artificial Lipid Membrane

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Abstract

Blood-Brain Barrier (BBB) is one of the key issues in the pharmaceutical industry since the Central Nervous System (CNS) drugs need to penetrate the barrier, while the peripherally acting drugs should be impaired in the passage. Most of the CNS drugs enter the brain by transcellular passive diffusion mechanism due to the presence of zonula occludens and limited transport pathways. In the present study two different *in-vitro* methods to predict BBB permeability of drugs were compared and evaluated. We focused our attention on the effect of time on the permeability in PAMPA model to maximize the high through put nature by decreasing the incubation time. Moreover, we have compared the permeability of 16 structurally diverse, commercially available drugs assessed in two different PAMPA models: (1) a PAMPA-PBL (Porcine brain lipid) (2) a PAMPA- Phosphatidylcholine lipid. Both the models successfully identify CNS+ (High brain penetration) and CNS- (Low brain penetration) drugs. A comparison of the permeability by plotting P_{app} values from both methods allows forecasting capacity of the assays. The correlation of the P_{app} value of the both assays with the literature reports showed good correlation of r^2 of 0.9487 and 0.930. The robustness of the established models was further evaluated by establishing correlation of *in silico* generated logBB values and the experimental logBB values (r^2 0.915). Thus, the developed models have the ability to identify the CNS penetration with reduced incubation times, which in turn will shorten the assay time especially when high throughput screening is employed.

Keywords: Artificial membrane; Blood Brain Barrier (BBB); Central Nervous System (CNS); Parallel Artificial Membrane Permeability Assay (PAMPA); Porcine Brain Lipid (PBL); Phosphatidylcholine; High Throughput Screening (HTS)

Introduction

The blood-brain barrier is composed of non-fenestrated capillary endothelial cells and astrocytes which limit the brain penetration of most of the CNS drug candidates [1]. Large number of compounds enters the brain by transcellular passive diffusion, which is driven by concentration gradient between blood and the brain [2]. There are also two active processes involved in the BBB that influence penetration: active influx transporters (e.g. amino acid, peptides) and active efflux transporters (e.g. P-glycoprotein's, multi-drug resistant proteins) [3,4]. In addition, plasma protein binding which reduces the free drug concentration available for BBB penetration and partial metabolism as well as renal excretion will also limit its penetration into the brain. Overall the passive diffusion is the major driving force moving most molecules into the brain.

Several *in vitro* methods and *in silico* models have been employed for the prediction of blood-brain barrier permeability. The *in vitro* methods include cell cultures derived from cerebral (brain capillaries as well as primary, low passage or immortalized brain endothelial cells) and non-cerebral sources (MDCK cell lines) as well as non-cell based *in vitro* systems. Although, the prediction with brain endothelial cells gives the best scoring to the *In vivo* system [5] but difficulties in establishing and maintaining primary culture, as well as high time consuming methods, make the assay unfeasible as a high throughput screening assay.

Parallel artificial membrane permeability assay was originally reported with 10% (w/v) egg lecithin in dodecane and further its variants has been studied [6-8]. A comparison of the three most used PAMPA models viz., Hexadecane Membrane (HDM), Dioleyoylphosphatidylcholine (DOPC) and Double Sink (DS-PAMPA) were recently carried out by Avdeef and Tsinman [9] and explaining permeability differences. Because of the specific nature of the permeability, it was used mainly for the prediction of the gastrointestinal absorption [10]. Attempts to modify the monolayer to improve the prediction of BBB penetration were done using porcine polar brain lipids [11].

We have developed a modified approach for measuring steady-state drug permeability using a permeation barrier made of a tight layer of phospholipids (Porcine brain lipid and Phosphatidylcholine) on filter plate. The aim of this paper is to check the effect of incubation time in the *in-vitro* brain permeability which was not sufficiently investigated in the earlier findings. In this study we have tested the permeability at various time points and the methods has been validated using 16 structurally diverse commercial drugs covering a broad range of physicochemical properties and absorption properties upon oral administration in humans. P_{app} values obtained from Porcine Brain Lipid (PBL) and Phosphatidylcholine lipid membrane were compared with literature reports. We have also run the same set of the 16 drugs for the log BB prediction using Qik Prop software and the predicted permeability values were compared with experimental log BB values.

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Materials and Methods

Materials

All the chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Permeability was conducted in Phosphate Buffer (PBS, KH_2PO_4 and K_2HPO_4, pH 7.4) in Multiscreen Millipore TM, plate MAIPN45 and MSSACCEPTOR acceptor plate (Millipore Corporation, Bedford, MA, USA). L-α-Phosphatidylcholine, Dodecane and Dimethyl Sulfoxide (DMSO) were purchased from Sigma Aldrich (St. Louis, MO). The porcine brain lipids were procured from Avanti Polar Lipids, Inc. (Alabaster, AL). All the solvents used in the experiments were of reagent grade. The drug quantification was done in the 96 well UV plates were procured from Corning (MA, USA).

Methods

Test compounds: The drugs chosen to validate the phospholipids vesicle based membrane model cover a wide range of physiochemical properties (molecular weight, Log P, Log D see Table 1). A set of 16 structurally diverse commercially drugs (which show effects on CNS with BBB penetration properties) were selected as a test candidates. Amongst them, 12 drugs belong to CNS^+ and 4 drugs to CNS^- category [11]. The stock solutions (10 mm) of all standard drugs were prepared by dissolving the drug in DMSO. The maximum absorbance (λ_{max}) of these test standards was measured using a 96 well plate UV spectrophotometer (250-750 nm). The working solutions of the test standards were obtained by dissolving the drug in phosphate buffer pH 7.4 on a sonication bath (Branson 1510, Branson Ultrasonic B.V, The Netherlands) followed by filtration through 0.22 µm filter (Millex-GS, Millipore, USA). The concentration of the different drug solutions had to be high enough that the amount of drug in the acceptor chamber during permeation studies could be quantified by means of UV-absorbance, and still be below the solubility limits.

PAMPA-PBL procedure: A PAMPA-PBL was performed in a 96 well sandwich plate format according to Schmidt and Lynch [12] with slight modification**.** The porcine brain lipid membrane was constituted by adding the 4 µL (20 mg/mL in dodecane) PBL membrane preparation on the PVDF filter in each well of 96 well plates. The test solutions (300 µL of 250 µM drugs) were added to each donor well while the acceptor wells were filled with 300 µL of PBS. The donor plate was placed on the top of the acceptor plate to create a sandwich. The assembly was incubated for different time intervals (2, 5, 8, 16 and 24 hours) at 25°C. After completion of incubation, the sandwich was disassembled and the acceptor solutions were transferred to a 96-well UV transparent plates (Corning) and the concentration of the drugs was measured spectrophotometrically (Spectramax 190; Molecular Device Corporation, California, USA) at the wavelength most appropriate for each drug. The experiments were performed at least in triplicate for every compound. The mean value and standard error mean are reported.

PAMPA- Phosphatidylcholine lipid procedure: The experimental procedure, conditions and analysis of PAMPA-Phosphatidylcholine lipid membrane model were similar to PAMPA-PBL model with the only difference of artificial lipid used i.e. Phosphatidylcholine lipid membrane. The lipid membrane of PAMPA-Phosphatidylcholine model was prepared by adding the 4 µL (20 mg/mL in dodecane) of Phosphatidylcholine membrane preparation on PVDF filter and same set of 16 different drugs molecules were tested for permeability assay.

Permeability calculations: Apparent permeability (P_{app}) was calculated for PAMPA-PBL and PAMPA-Phosphatidylcholine models y the following equation as given by Sugano et al. [8]

$$P_{app} = \left\{ (-C) \times In \left[1 - \frac{[Durg]_{accepter}}{[Durg]_{equlibrium}} \right] \right\} \times 10^{-6}$$

Where

$$\frac{[Durg]_{accepter}}{[Durg]_{equlibrium}} = \frac{[O.D]_{accepter}}{[O.D]_{equlibrium}}$$

$$C_{(cm/s)} = \left\{ \frac{V_D \times V_A}{V_D + V_A \times area \times time} \right\}$$

Where, V_D is the donor solution volume (µL), V_A is the acceptor solution volume (µL), A is the surface area of the filter (cm^2) and t is the incubation time.

Effect of time on permeability: Diffusion of the tested compounds in the 96 well plate, from the donor to acceptor compartments for the PAMPA-PBL and PAMPA-Phosphatidylcholine lipid assays was monitored at various time points (2, 5, 8, 16, 24 hours). Drugs were added to 96 well plates in three replicates for a set time points. After completion of the incubation, the acceptor plate was separated and the diffused drug was analyzed by UV spectrophotometer. The P_{app} calculated for each time points are listed in Table 3**.**

***In-Silico* studies:** All 16 drugs were evaluated for properties predictions by using QikProp V 1.3 software. Monte Carlo statistical mechanics simulation [13] was used to generate descriptors like Solute-Water Coulomb and Lennard- Jones energy, Solute internal energy, Dipole moment, Solute Accessible Surface Area (SASA), Hydrophobic (FOSA) and Hydrophilic (FISA) component, Donor and acceptor hydrogen bonds, non conjugated amines and amides and No. of rotatable bonds. There were total 5 significant descriptors to calcute Log BB (brain/blood concentration ratio) value vis., FOSA, FISA, amine, dipole moment and rotatable bond. The FOSA and non conjugated amine increase the concentration of drug in brain. The increased polarity of drug will reflect as an increase in the hydrophilic surface area, dipole moment, and flexibility of drug.

Hence, it will lead to increase the concentration of the drug in blood. The QPlogBB values generated from an *In-silico* method were compared with the experimental log BB [14] values are shown in Table 2, Figure 4**.**

QPlogBB (QikProplogBB) value was calculated based on following equation [13]

QPlogBB = (0.0013 **x** FOSA) - (0.004332 **x** FISA) + (0.6337 **x** amine) - (0.0751 **x** Dipole moment) - (0.1369 **x** rotatable bonds) + 0.04192.

Components	PAMPA-PBL[a]	PAMPA-Phosphatidylcholine
Phosphatidylcholine	12.6	100
Phosphatidylethanolamine	33.1	-
Phosphatidylinositol	4.1	-
Phosphatidylserine	18.5	-
Phosphatidic acid	0.8	-
Others (cerebrosides, sulfatides,pigments)	30.9	-

[a]From Avanti Polar lipids (Alabaster, AL, USA)

Table 1: Lipid composition of artificial membranes used in PAMPA models.

Name of the Compounds	Literature CNS penetration classification [11]	Molecular Weight (Mw)	log*P*	p*Ka*	log*D*	PAMPA-PBL P_{app} (10^{-6} Cm/S) (Mean ± SEM)	PAMPA-Phosphatidylcholine P_{app} (10^{-6} Cm/S) (Mean ± SEM)	*In silico* prediction (QPlog BB)	Experimental (log BB) [14]
Olanzapine	CNS+	312.4	2		1.8	30.76 ± 0.78	27.77 ± 1.3	0.814	-
Duloxetine	CNS+	297.4	4	9.34	1.32	28.33 ± 0.36	31.44 ± 0.68	0.469	-
Carbamazepine	CNS+	236.3	2.45	9.3	1.78	23.32 ± 0.12	14.97 ± 0.42	-0.2	-0.14
Diphenhydramine	CNS+	255.4	3.27	9.0	1.61	22.78 ± 0.04	17.56 ± 1.63	0.54	-
Desipramine	CNS+	266.4	4.9	10.1	1.28	20.88 ± 0.21	23.63 ± 0.42	0.5	1.2
Diazepam	CNS+	284.7	2.9	3.3	2.8	20.76 ± 0.63	21.04 ± 1.55	0.258	0.52
Alprazolam	CNS+	308.8	4.9	2.8	1.26	11.81 ± 0.32	8.65 ± 0.54	0.09	0.04
Imipramine	CNS+	280.4	4.8	9.4	2.4	13.34 ± 0.15	11.93 ± 0.32	0.662	1.07
Promazine	CNS+	284.4	4.3	4.2	2.52	8.97 ± 0.13	9.77 ± 0.23	0.733	1.23
Caffeine	CNS+	194.2	-0.5	0.6	0.02	4.73 ± 0.09	4.87 ± 0.07	-0.262	-0.06
Amitriphyline	CNS+	277.4	4.9	9.4	2.77	5.03 ± 0.18	4.51 ± 0.11	0.722	0.89
Chlorpromazine	CNS+	318.9	4.8	9.3	3.38	2.62 ± 0.06	2.62 ± 0.06	0.916	1.06
Dopamine	CNS-	153.2	0.9	8.93	-0.80	1.03 ± 0.09	1.38 ± 0.10	-1.669	-
Atenolol	CNS-	266.3	0.5	9.6	-1.29	0.87 ± 0.17	1.08 ± 0.01	-1.152	-0.87
Ofloxacin	CNS-	361.4	2.1		-0.62	0.72 ± 0.02	0.16 ± 0.03	-1.482	-
Norfloxacin	CNS-	319.3	0.42		-0.81	0.39 ± 0.10	0.28 ± 0.03	-1.699	-

Table 2: The physicochemical properties and permeability values of the drugs used in the validation of the PAMPA Porcine brain lipids and Phosphatidylcholine vesicle membrane models.

Name of the Compounds	Porcine brain lipid (PBL) P_{app} (10^{-6} Cm/S) (Mean ± SEM)					Phosphatidylcholine Lipid P_{app} (10^{-6} Cm/S) (Mean ± SEM)				
	2 hours	5 hours	8 hours	16 hours	24 hours	2 hours	5 hours	8 hours	16 hours	24 hours
Olanzapine	**18.40** ± 0.10	**30.76** ± 0.78	**30.11** ± 0.41	**30.02** ± 0.43	**30.86** ± 1.0	**19.43** ± 0.20	**27.77** ± 1.3	**27.29** ± 0.38	**27.64** ± 2.02	**27.66** ± 0.39
Duloxetine	**19.91** ± 0.00	**28.33** ± 0.36	**28.16** ± 1.30	**28.00** ± 3.3	**28.62** ± 1.3	**17.23** ± 0.34	**31.44** ± 0.68	**30.04** ± 1.43	**30.42** ± 0.25	**30.87** ± 1.4
Carbamazepine	**16.52** ± 0.3	**25.32** ± 0.12	**25.19** ± 0.49	**24.95** ± 0.17	**24.64** ± 0.6	**8.24** ± 0.05	**14.97** ± 0.42	**14.94** ± 0.10	**14.32** ± 0.15	**14.99** ± 0.13
Diphenhydramine	**14.55** ± 1.8	**22.78** ± 0.04	**22.20** ± 0.79	**22.08** ± 1.4	**22.31** ± 0.04	**11.6** ± 0.009	**17.96** ± 1.63	**18.06** ± 0.23	**17.86** ± 1.17	**18.17** ± 1.39
Desipramine	**13.07** ± 0.53	**20.88** ± 0.21	**20.34** ± 0.32	**21.9** ± 0.41	**21.95** ± 0.28	**16.53** ± 0.12	**23.63** ± 0.42	**22.71** ± 0.23	**22.86** ± 1.98	**22.53** ± 0.66
Diazepam	**11.93** ± 1.9	**20.76** ± 0.63	**20.88** ± 0.19	**20.59** ± 0.5	**20.35** ± 0.37	**12.93** ± 0.22	**20.04** ± 1.55	**19.40** ± 0.57	**20.8** ± 0.64	**20.49** ± 0.39
Alprazolam	**7.35** ± 0.48	**11.81** ± 0.32	**11.34** ± 0.25	**11.17** ± 0.19	**11.5** ± 0.20	**6.25** ± 0.54	**8.65** ± 0.54	**8.30** ± 0.44	**8.88** ± 0.53	**8.16** ± 0.24
Imipramine	**8.24** ± 0.65	**12.34** ± 0.15	**12.41** ± 0.60	**12.04** ± 0.17	**12.09** ± 0.17	**7.23** ± 0.19	**11.93** ± 0.32	**11.21** ± 0.29	**11.72** ± 0.31	**10.81** ± 0.08
Promazine	**5.14** ± 0.29	**8.97** ± 0.13	**9.05** ± 0.16	**8.64** ± 0.17	**8.86** ± 0.15	**5.07** ± 0.10	**8.77** ± 0.23	**8.76** ± 0.53	**8.71** ± 0.22	**8.54** ± 0.12
Caffeine	**2.98** ± 0.06	**4.73** ± 0.09	**5.13** ± 0.01	**4.83** ± 0.05	**4.98** ± 0.05	**2.65** ± 0.10	**4.87** ± 0.07	**4.94** ± 0.06	**4.93** ± 0.11	**4.73** ±0.02
Amitriphyline	**3.16** ± 0.08	**5.03** ± 0.18	**4.84** ± 0.02	**4.91** ± 0.04	**4.95** ± 0.02	**2.29** ± 0.13	**4.51** ± 0.11	**4.27** ± 0.07	**4.40** ± 0.06	**4.31** ± 0.06
Chlorpromazine	**1.32** ± 0.003	**2.92** ± 0.06	**3.00** ± 0.03	**2.96** ± 0.04	**2.81** ± 0.04	**1.26** ± 0.16	**2.22** ± 0.06	**2.40** ± 0.02	**2.18** ± 0.05	**2.12** ± 0.02
Dopamine	**1.02** ± 0.07	**1.03** ± 0.09	**0.98** ± 0.02	**1.07** ± 0.07	**1.04** ± 0.05	**1.30** ± 0.30	**1.38** ± 0.10	**1.30** ± 0.01	**1.35** ± 0.07	**1.32** ± 0.06
Atenolol	**0.86** ± 0.02	**0.97** ± 0.17	**0.98** ± 0.00	**0.95** ± 0.05	**0.97** ± 0.04	**0.92** ± 0.003	**1.08** ± 0.01	**0.99** ± 0.12	**1.01** ± 0.08	**0.98** ± 0.03
Ofloxacin	**0.73** ± 0.05	**0.72** ± 0.02	**0.73** ± 0.02	**0.71** ± 0.024	**0.71** ± 0.01	**0.11** ± 0.03	**0.10** ± 0.03	**0.13** ± 0.34	**0.10** ± 0.00	**0.11** ± 0.00
Norfloxacin	**0.44** ± 0.01	**0.39** ± 0.10	**0.42** ± 0.02	**0.39** ± 0.013	**0.41** ± 0.02	**0.26** ± 0.00	**0.28** ± 0.03	**0.30** ± 0.02	**0.29** ± 0.01	**0.31** ± 0.02

Table 3: Average apparent permeability (P_{app}) value at different incubation times.

Results and Discussion

Shorter Incubation time

Incubation time with PBL previously reported was 18 hours [11] and later its decrease has been set for other solvents down to 2 hours by using the constant agitation at 200 rpm [15]. In this present study we have optimized the BBB permeability assay an *In vitro* higher throughput model for the determination of the permeability in two different lipids viz., PBL and Phosphatidylcholine. We have attempted permeability studies at various time points like 2, 5, 8, 16 and 24 hours (Table 3), negligible difference was observed in the permeability values (P_{app}) after 5 hours of incubation for both (PAMPA-PBL and PAMPA-Phosphatidylcholine) models. A set of 16 structurally diverse, commercially available drugs were used to validate both PAMPA models and both are capable to identify CNS^+ (High brain penetration) and CNS^- (Low brain penetration) drugs. Each model characterized all the compounds as per the literature [16] classification. The P_{app} value of the drugs was separated into two P_{app} ranges. The compounds which have P_{app} value greater than 4×10^{-6} Cm/S are CNS^+ and the compounds which have P_{app} value lower than 2×10^{-6} Cm/S are CNS^-. Moreover, the only difference in the experimental setup between two models is the lipid membrane. While the PAMPA-PBL model uses a more complex porcine brain extract to mimic the blood–brain barrier, the membrane barrier associated with the PAMPA-Phosphatidylcholine model, consists of 2% (w/v) L-α-Phosphatidylcholine dissolved in dodecane.

The correlation of the P_{app} value of the PAMPA–PBL and PAMPA Phosphatidylcholine assays with the literature reports showed good linearity of r^2 of 0.9487 and 0.930 respectively; Figures 1 and 2.

***In-silico* permeability prediction:** Penetration of BBB, into the CNS, is a complicated procedure involving a number of physiochemical properties (17-19). For permeability through the BBB, QikProp predicts QPlogBB; the brain blood partition coefficient. For the assessment of QPlogBB, a set of 16 drug molecules, in which 12 drugs pass through the BBB and enter the CNS and 4 Non CNS drugs as negative control was used.

In Figure 3 all of the investigated drugs are lie within the indicated limits of -3< QPlogBB <1.2; the usual limits given for an experimentally derived BBB penetration ranges between -2.0 and +1.0. Drugs with log BB greater that 0.3 are characterized as excellent, whereas drugs with log BB less than -1.0 are considered poor (20). These limits are denoted in Figure 3**.** Fifty percent of the compounds are above the 0.3 threshold, twenty five percent of the compounds are in between

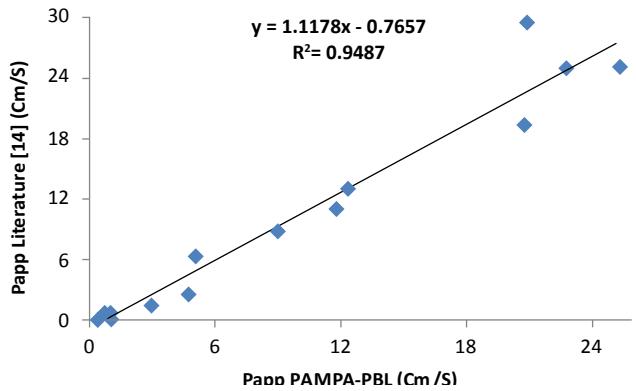

Figure 1: Correlation of the Apparent Permeability (Papp) values of PAMPA-PBL with values reported by Di et al. [11].

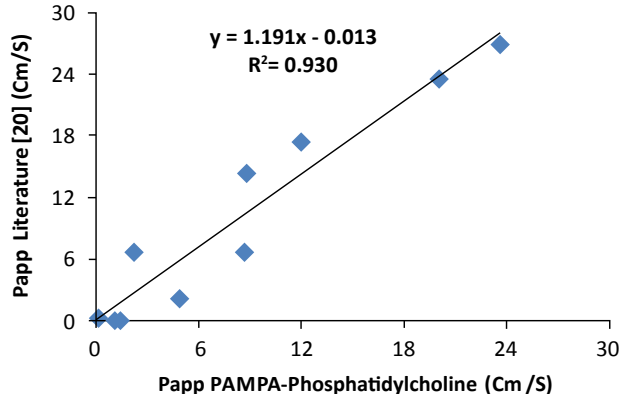

Figure 2: Correlation of the Apparent Permeability (P_{app}) of PAMPA-Phosphatidylcholine lipid with values reported by Mensh et al. [16].

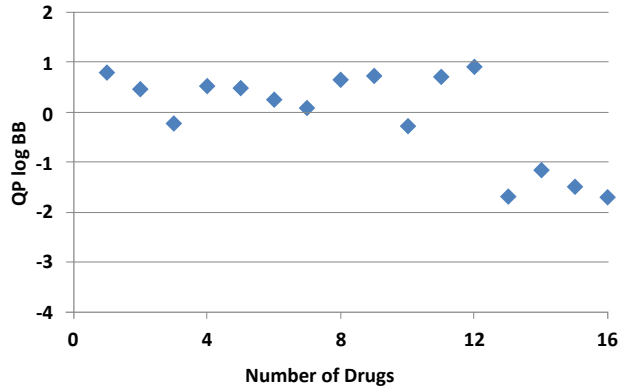

Figure 3: QP log BB for CNS and non CNS drugs.

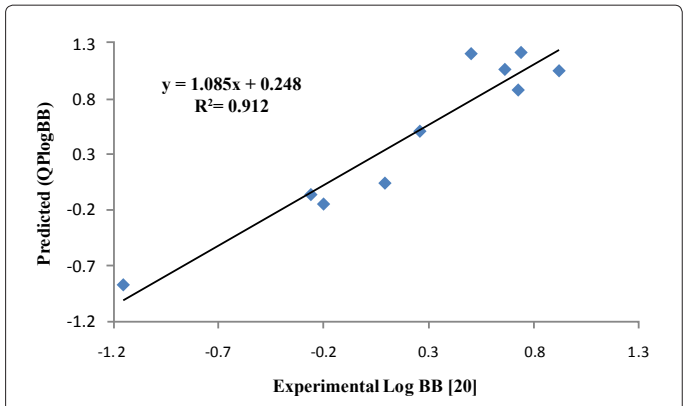

Figure 4: Correlation of the Predicted (QPlogBB) value with the Experimental logBB values reported by Karelson et al., [14].

0.3 and -1 and final 25% have values of less than -1. The compounds which have less than -1, QPlogBB value are used as antibiotics and for cardiovascular disease and are not specifically designed to penetrate BBB. The correlation of the logBB value of the both predicted with the experimental reports showed good correlation of r^2 of 0.91

Conclusion

Both the PAMPA–PBL and PAMPA Phosphatidylcholine models have been successfully developed and optimized in high throughput format. We observed that the differences between the artificial lipids (PBL and Phosphatidylcholine) on the BBB classification of the tested compounds seem to be negligible. With the same experimental conditions, the Porcine Brain Lipids and Phosphatidylcholine displayed very good correlation for the selection of CNS^+ and CNS^- compounds. The comparison of two different set of data gives a better classification of the compounds. The *In-silico* data also showed fair correlation with the experimental log BB values.

Although, PAMPA can't be used as a surrogate assay for a cellular permeability assay, but this assay can be used for the prediction of BBB penetration in a more robust fashion. Our model gives near to 95% of the predictions which are in good accordance with the literature.

References

1. Hitchcock SA, Pennington LD (2006) Structure−brain exposure relationships. J Med Chem 49: 7559-7583.
2. Di L, Kerns EH, Carter TG (2008) Strategies to access blood-brain barrier penetration. Expert Opin Drug Discov 3: 677-687.
3. Pardridge WM (1998) CNS drug design based on principles of blood-brain barrier transport. J Neurochem 70: 1781-1792.
4. Tamai I, Tsuji A (2000) Transporter-mediated permeation of drugs across the blood-brain barrier. J Pharm Sci 89: 1371-1388.
5. Gumbleton M, Audus KL (2001) 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 90: 1681-1698.
6. Kansy M, Senner F, Gubernator K (1998) Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. J Med Chem 41: 1007-1010.
7. Seo PR, Teksin ZS, Kao JP, Polli JE (2006) Lipid composition effect on permeability across PAMPA. Eur J Pharm Sci 29: 259-268.
8. Sugano K, Hamada H, Machida M, Ushio H, Saitoh K, et al. (2001) Optimized conditions of bio-mimetic artificial membrane permeation assay. Int J Pharm 228: 181-188.
9. Avdeef A, Tsinman O (2006) PAMPA--a drug absorption in vitro model

13. Chemical selectivity due to membrane hydrogen bonding: in combo comparisons of HDM-, DOPC-, and DS-PAMPA models. Eur J Pharm Sci 28: 43-50.

10. Kerns EH, Di L, Petusky S, Farris M, Ley R, et al. (2004) Combined application of parallel artificial membrane permeability assay and Caco-2 permeability assay in drug discovery. J Pharm Sci 93: 1440-1453.

11. Di L, Kerns EH, Fan K, McConnell OJ, Carter GT (2003) High throughput artificial membrane permeability assay for blood–brain barrier. Eur J Med Chem 38: 223-232.

12. Schmidt D, Lynch J (2003) Evaluation of the reproducibility of Parallel Artificial Membrane Permeability Assay (PAMPA). Millipore corporation application note, Literature Notes AN1728EN00.

13. Duffy EM, Jorgensen WL (2001) Prediction of pharmaceutically important properties from Monte Carlo simulations. Chemical Data Analysis in the Large: The Challenge of Automation Age 83-87.

14. Karelson M, Dobchev D, Tamm T, Tulp I, Janes J, et al. (2008) Correlation of blood-brain barrier penetration and human serum albumin binding with theoretical descriptors. ARKIVOC XVI 2008: 38-60.

15. Carrara S, Reali V, Misiano P, Dondio G, Bigogno C (2007) Evaluation of in vitro brain penetration: Optimized PAMPA and MDCKII-MDRI assay comparison. Int J Pharm 345: 125-133.

16. Mensch J, Melis A, Mackie C, Verreck G, Brewster ME, et al. (2010) Evaluation of various PAMPA model to identify the most discriminating method for the prediction of BBB permeability. Eur J Pharm Biopharm 74: 495-502.

17. Ajay, Bemis GW, Murcko MA (1999) Designing libraries with CNS activity. J Med Chem 42: 4942-4951.

18. Abraham MH (2004) The factors that influence permeation across then blood-brain barrier. Eur J Med Chem 39: 235-240.

19. Luco JM (1999) 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 Comp Sci 39: 396-404.

20. Jonsdottir SO, Jorgensen FS, Brunak S (2005) Prediction methods and databases within chemoinformatics: emphasis on drugs and drug candidates. Bioinformatics 21: 2145-2160.