Establishment of a Novel Funnel Model for Evaluating Blood-Brain Barrier Penetration *In Vitro*

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Poster#: P39

Abstract

The blood-brain barrier (BBB) Parallel Artificial Membrane Permeability Assay (PAMPA) model, renowned for its capacity to predict passive penetration of substances across the BBB, is frequently utilized in the early stages of Central Nervous System (CNS) drug development. MDR1-MDCK I cell from the National Institutes of Health (NIH), is mainly adopted for the exclusion of P-glycoprotein (P-gp, MDR1) substrates for CNS drug development[1]. Despite the complementary strengths and limitations, the two models have traditionally been applied independently. To improve the efficiency and accuracy of prediction, DMPK department of WuXi AppTec has established a novel funnel model that combines the BBB PAMPA model and the MDR1-MDCK I cell model, providing a more efficient and precise tool for BBB penetration screening in the early stages of CNS drug development.

Objective

The objective of this study was to establish a funnel model that combines the BBB PAMPA model and the MDR1-MDCK I cell model for BBB penetration screening in the early stages of CNS drug development.

Methods

Twenty-four commercially available drugs with diverse CNS penetration characteristics were selected, of which 10 were brain penetrated (CNS+) and 14 were brain restricted (CNS-) (Table 1). Experiments were carried out in the BBB PAMPA model and the MDR1-MDCK I cell model to determine the cut-off values for passive BBB permeability and P-gp efflux ratio, respectively, to establish the funnel model.

An additional set of 22 commercially available drugs was selected to validate the funnel model. First, drugs with poor passive brain penetration were excluded using the BBB PAMPA model, and then potential P-gp substrates were removed using the MDR1-MDCK I cell model. Finally, the predictive performance was analyzed based on the known BBB penetration characteristics of these drugs.

Results

1. The Pe value of 15.0 nm/s was defined as the cut-off value of permeability for “CNS+” and “CNS-” in BBB PAMPA model. All commercially available drugs except the four efflux substrates (red triangle) could be correctly classified (20/24, 83.3%) (Figure 1a).
2. The efflux ratio of 4.00 was defined as the cut-off value for “P-gp+” (P-gp substrates) and “P-gp-” (poor or non-P-gp substrates) in MDR1-MDCK I cell model. All commercially available drugs except the four with limited BBB penetration (red square) could be correctly classified (20/24, 83.3%) (Figure 1b) .

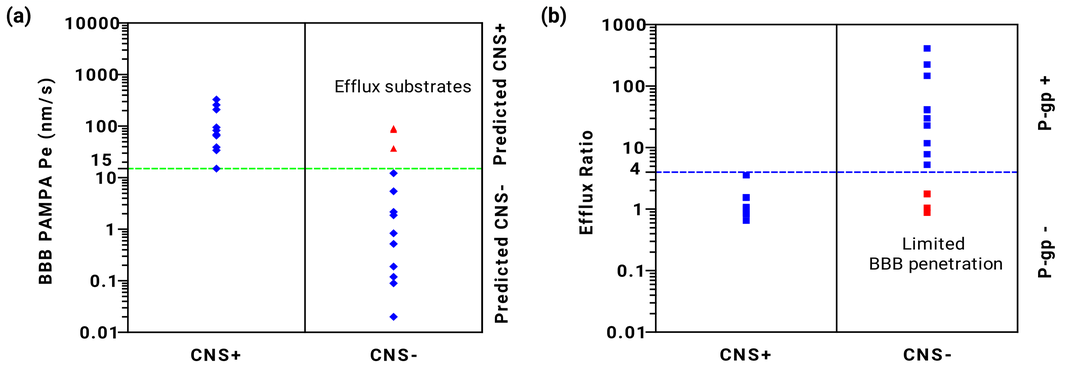

Figure 1. Correlation of CNS+/- with mean Pe (nm/s) values generated from BBB PAMPA (a) and mean efflux ratio values generated from MDR1-MDCK I cells (b) for 24 commercially available drugs (n=3)

Conclusion

The funnel model, in combination with BBB PAMPA and MDR1-MDCK I cells, can serve as an efficient screening tool for evaluating BBB penetration in the early stages of CNS drug development.

Table 1. Pe values and efflux ratios of the 24 commercially available drugs (n=3)

Compound ID	Pe (nm/s) from BBB PAMPA (mean ± SD)	Efflux ratio from MDR1-MDCK I (mean ± SD)	CNS +/-
Amitriptyline	33.9 ± 13.2	1.55 ± 0.799	+
Buspirone	211 ± 22.6	1.56 ± 0.751	+
Carbamazepine	66.0 ± 0.984	1.03 ± 0.193	+
Chlorpromazine	15.1 ± 10.5	1.53 ± 1.10	+
Clozapine	95.4 ± 30.3	1.56 ± 0.642	+
Corticosterone	39.3 ± 3.68	3.57 ± 0.595	+
Lidocaine	330 ± 37.2	0.83 ± 0.0701	+
Progesterone	68.9 ± 24.9	0.652 ± 0.145	+
Propranolol	83.0 ± 9.74	1.08 ± 0.0818	+
Testosterone	257 ± 44.0	0.856 ± 0.0765	+
Cimetidine	0.121 ± 0.0574	29.9 ± 9.00	–
Colchicine	0.841 ± 0.0899	11.8 ± 2.18	–
Digoxin	0.0213 ± 0.00413	22.9 ± 4.54	–
Dipyridamole	44.0 ± 6.89	412 ± 40.3	–
Etoposide	2.17 ± 0.441	7.81 ± 0.511	–
Loperamide	12.2 ± 6.52	225 ± 93.5	–
Nadolol	0.185 ± 0.00	5.26 ± 1.23	–
Naproxen	1.88 ± 0.180	0.883 ± 0.179	–
Prazosin	37.0 ± 4.92	40.8 ± 19.7	–
Probenecid	0.0902 ± 0.00275	1.75 ± 0.408	–
Quinidine	85.5 ± 8.69	147 ± 75.8	–
Theophylline	0.517 ± 0.0411	1.05 ± 0.0857	–
Verapamil	90.3 ± 21.1	41.8 ± 2.25	–
Warfarin	5.49 ± 0.446	1.79 ± 0.374	–

3. All commercially available drugs can be correctly classified by using the combined funnel model (Figure 2). Ten “CNS-” drugs were excluded due to limited BBB penetration (red dot), and 4 “CNS-” drugs were excluded due to high P-gp efflux (blue dot).

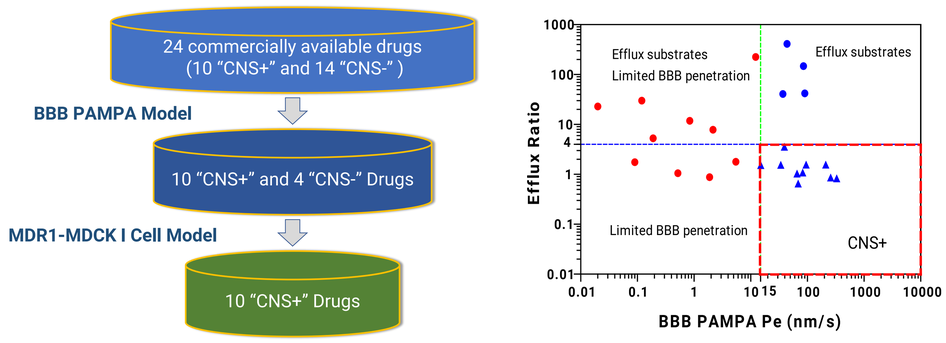

Figure 2. Schematic diagram of the funnel model and correlation of mean *in vitro* P-gp efflux ratios from MDR1-MDCK I cells, mean Pe values from BBB PAMPA, and CNS penetration for 24 commercially available drugs (n=3)

4. All the additional set of 22 commercially available drugs were accurately classified, except for sertraline, which exhibited a slightly lower Pe value of 12.7 nm/s in the BBB PAMPA assay, and gabapentin, which is known to be actively transported by LAT1[2]. The results demonstrated strong predictive accuracy (20/22, 90.9%) and a good *in vitro* to *in vivo* correlation (Figure 3).

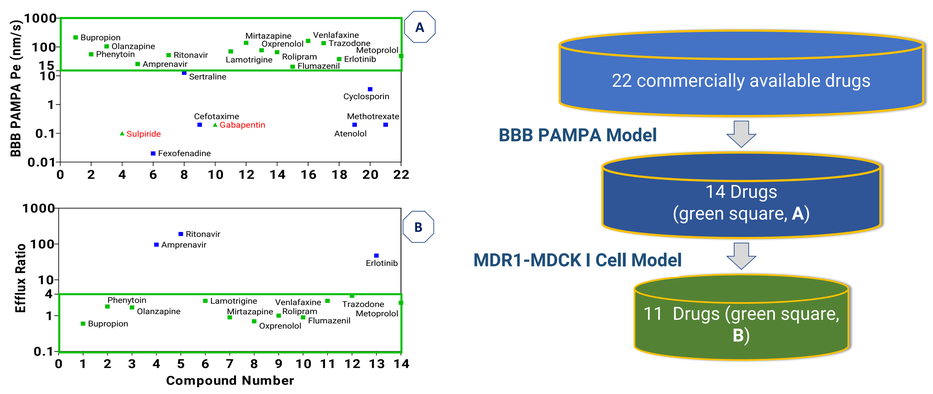

Figure 3. Schematic diagram of the funnel model applied to the additional set of 22 commercially available drugs

References

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