Brief/Technical Note

Comparison of Drug Permeabilities and BCS Classification: Three Lipid-Component PAMPA System Method *versus* Caco-2 Monolayers

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INTRODUCTION

A three lipid-component parallel artificial membrane permeability assay (PAMPA) system was previously devised and evaluated (1). Denoted A-PAMPA for anionic-PAMPA, A-PAMPA was designed to mimic the lipid composition of the enterocyte's plasma membrane and included 1,2-dioleoyl-*sn*-glycero-3-[phospho-L-serine] (PS18:1) as an anionic lipid component. A-PAMPA also consists of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (PC18:1) and cholesterol. Metoprolol flux across A-PAMPA was measured, as well as across three other PAMPA systems (1). Results indicated that metoprolol transport across A-PAMPA was dominated by an ion-pair-mediated mechanism (i.e., metoprolol-PS18:1 complex).

The objective in the present study was to compare the performance of this three lipid-component PAMPA system to Caco-2 monolayers, in terms of screening for drug permeability values. Several development laboratories employ PAMPA membranes in candidate selection, in part because of other experimental approaches, such as Caco-2 and MDCK monolayers, are more labor-intensive and expensive (2–8). However, A-PAMPA has not been previously evaluated to identify drugs that are likely to exhibit high permeability in Caco-2 BCS testing.

MATERIALS AND METHODS

Materials. PC18:1 and PS18:1 were purchased from Avanti Polar Lipids (Alabaster, AL). Hydrophobic microfilter plates (polyvinylidene fluoride, 0.45 μm) were purchased

from Millipore Corporation (Bedford, MA). Caco-2 cells were obtained from ATCC (Rockville, MD). Drug compounds were either USP- or commercial-grade.

Permeability Measurements. A-PAMPA preparation and permeability methods follow previous descriptions (1,5). Hydrophobic filters were impregnated with 5 μL of membrane solution consisting of lipid in *n*-dodecane (5% *w/v*), where lipid consisted of PC18:1 (2.6% *w/v*), PS18:1 (0.9% *w/v*), and cholesterol (1.5% *w/v*). Permeability was calculated as described previously, using Eq. 3 in Palm *et al.* (7). Mass balance ranged from 85–100%.

Caco-2 studies followed the cell culture and permeability methods previously described (9,10). Permeability was calculated as described previously (9,10). Mass balance ranged between 80–100%.

RESULTS AND DISCUSSION

Comparison of Drug Permeabilities. Permeability across A-PAMPA and Caco-2 are given at Table I. Figure 1 plots Caco-2 compound permeability *versus* A-PAMPA compound permeability. There was generally good agreement between permeability values from the two methods (linear $r^2=0.82$, excluding coumarin and naproxen, which provided the highest permeabilities in both assays). Results here for A-PAMPA follow previous comparisons of PAMPA permeabilities to Caco-2 permeabilities (2,3,11–16). As suggestive of Fig. 1 and noted previously (8), A-PAMPA permeability values were generally several-fold lower than Caco-2 permeability values.

Corti *et al.* (16) showed a good correlation of PAMPA and Caco-2 permeabilities ($r^2=0.813$), where PAMPA membrane employed Lipoid E80 (1.70%), *n*-octanol (96.2%), and cholesterol (2.10%). Lipoid E80 is fat-free egg lecithin and consists of largely (about 80%) phosphatidyl choline (17). A-PAMPA has some compositional similarity to

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Table I. A-PAMPA and Caco-2 Permeability, Using Metoprolol Tartrate as a Reference Compound

Compound	A-PAMPA permeability ($\pm$ SEM) $\times 10^6$ [cm/s]	Caco-2 permeability ($\pm$ SEM) $\times 10^6$ [cm/s]	BCS classification according to A-PAMPA	BCS classification according to Caco-2	Human percent dose absorbed (literature reference)
Acyclovir	0.0841 ($\pm$ 0.0019)	1.24 ($\pm$ 0.24)	Low	Low	20 (20,21)
Atenolol	2.41 ($\pm$ 0.38)	2.62 ($\pm$ 0.17)	High	Low	50 (21,22)
Bupropion	18.5 ($\pm$ 0.1)	66.4 ($\pm$ 0.6)	High	High	87 (23)
Carbamazepine	27.4 ($\pm$ 0.3)	95.7 ($\pm$ 5.1)	High	High	97 (24)
Chlorpheniramine	8.57 ($\pm$ 0.07)	16.0 ($\pm$ 1.9)	High	Low	80 (25)
Coumarin	48.4 ($\pm$ 0.2)	141 ($\pm$ 6)	High	High	100 (26)
Disopyramide	2.71 ($\pm$ 0.26)	4.24 ($\pm$ 0.05)	High	Low	85.3 (27,28)
Furosemide	0.572 ($\pm$ 0.135)	3.33 ($\pm$ 0.07)	Low	Low	61 (21,29)
Hydrochlorothiazide	0.497 ($\pm$ 0.040)	0.710 ($\pm$ 0.063)	Low	Low	70 (30)
Indomethacin	12.4 ($\pm$ 0.4)	38.4 ($\pm$ 2.2)	High	Low	100 (25,28)
Inulin	0.360 ($\pm$ 0.006)	0.921 ($\pm$ 0.101)	Low	Low	0 (31)
Ketoprofen	12.6 ($\pm$ 0.5)	50.5 ($\pm$ 0.5)	High	High	100 (23,30)
Mannitol	0.018 ($\pm$ 0.025)	2.81 ($\pm$ 0.16)	Low	Low	16 (32,33)
Metoprolol	1.53 ($\pm$ 0.05)	40.0 ($\pm$ 1.4)	High	High	95 (25)
Naproxen	41.8 ($\pm$ 0.2)	126 ($\pm$ 4)	High	High	98 (30)
Piroxicam	29.2 ($\pm$ 0.1)	116 ($\pm$ 1)	High	High	100 (8,24,34)
Propranolol	4.29 ($\pm$ 0.13)	49.5 ($\pm$ 1.2)	High	High	100 (21,33)
Ranitidine	0.672 ($\pm$ 0.087)	0.405 ($\pm$ 0.031)	Low	Low	52 (35)
Theophylline	8.58 ($\pm$ 0.06)	61.0 ($\pm$ 0.9)	High	High	96 (36)
Verapamil	9.40 ($\pm$ 0.09)	32.9 ($\pm$ 1.0)	High	Low	98 (30,37)

the membranes in Corti *et al.* (16). However, since oleic acid is a modest fatty acid component in Lipoid E80 (about 28% of all fatty acid), the phosphatidyl choline in Lipoid E80 is largely not PC18:1. Lipoid E80 also lacks PS18:1, which was included in the present three lipid-component PAMPA system as an anionic lipid component and an important contributor to ion-pair-mediated drug transport (1).

Of the 21 compounds studied by Corti *et al.* and the 20 compounds studied here, there were 11 common compounds tested in both studies: acyclovir, atenolol, furosemide, hydrochlorothiazide, ketoprofen, metoprolol tartrate, naproxen, propranolol, ranitidine, theophylline, and verapamil. Among these drugs, naproxen showed the highest permeability here and in Corti *et al.* Likewise, acyclovir showed the lowest permeability. In general, permeability values from Corti *et al.* were several-fold larger than values from A-PAMPA here. Permeability values from A-PAMPA exhibited a wider range,

which differed 500-fold between naproxen and acyclovir. Naproxen and acyclovir permeabilities in Corti *et al.* differed less than sixfold, although differences were easily detected.

PAMPA to Anticipate BCS Results. These results suggest that three lipid-component PAMPA system can be used to identify drugs that are likely to exhibit high permeability in Caco-2 BCS testing (18). Prior to subjecting a compound to BCS-based permeability assessment, it is typically desirable to know whether the compound is likely to show high permeability. Because a BCS-regulatory submission requires a validated permeability method, a more facile method, such as A-PAMPA, has potential role in rapidly assessing whether a drug will likely show high permeability across Caco-2 monolayers.

In order to assess the utility of A-PAMPA to anticipate Caco-2 BCS classification results, each compound was classified as either high or low permeability. Table II shows the concordance between A-PAMPA classification results and Caco-2 classification results, employing metoprolol as the lower limit of high permeability (18). Overall, six compounds exhibited lower permeability than metoprolol in A-PAMPA, with the remaining 14 (including metoprolol itself) showing high permeability. Of the 20 compounds, 15 were correctly predicted by A-PAMPA. Six compounds predicted by A-

Fig. 1. Comparison between Caco-2 permeability and PAMPA permeability. The PAMPA membrane included an anionic component, such that this system is denoted A-PAMPA. In general, high permeability in PAMPA corresponded with high permeability across Caco-2 monolayers

Table II. Comparison of BCS Classification of A-PAMPA and Caco-2, Using Metoprolol Tartrate as a Reference Compound

		Caco-2 classification result	
		Low	High
A-PAMPA classification result	Low	6	0
	High	5	9

Entries in table denote number of compounds (out of 20)

Fig. 2. Plots of human percent dose absorbed *versus* A-PAMPA permeability and Caco-2 permeability. For each of the 20 compounds, A-PAMPA permeability was generally less than corresponding Caco-2 permeability, typically by several-fold

PAMPA to have low permeability showed low Caco-2 permeability. Nine compounds predicted by A-PAMPA to have high permeability (of 14 such compounds) showed high Caco-2 permeability. No compound was predicted by A-PAMPA to have low permeability but showed high Caco-2 permeability.

However, five compounds predicted by A-PAMPA to have high permeability showed low Caco-2 permeability and were atenolol, chlorpheneramine, disopyramide, indomethacin, and verapamil. As noted in Table I, the human percent dose absorbed of atenolol, chlorpheneramine, disopyramide, indomethacin, and verapamil are 50%, 80%, 85.3%, 100%, and 98%, respectively. Hence, the discordance between A-PAMPA and Caco-2 classifications for indomethacin and verapamil were Caco-2 classification error. Meanwhile, the discordance for atenolol is A-PAMPA classification error. The absorption of atenolol is low and incomplete (19). Likewise, but to a lesser extent given their fraction dose absorbed nears that of metoprolol, the discordance for chlorpheniramine and disopyramide was also A-PAMPA classification error.

From these five cases, A-PAMPA permeability “over-estimated” BCS permeability classification based on Caco-2 permeability. These results support the use of A-PAMPA to screen compounds for subsequent BCS classification, with the expectation that only compounds that are likely to exhibit high permeability are subjected to BCS Caco-2 assay. One possible explanation for the mis-specification of atenolol, chlorpheneramine, and disopyramide by A-PAMPA may be possible ion pairing between these weakly basic drug and PS18:1. Such ion pairing increases the permeability of metoprolol (1), but may perhaps have an even greater effect on atenolol, chlorpheneramine, and disopyramide.

Figure 2 plots human percent dose absorbed *versus* A-PAMPA permeability for the 20 compounds in Table I. Corresponding Caco-2 results are also plotted. The A-PAMPA profile is generally pushed towards the left, reflecting the lower A-PAMPA permeability of each compound, compared to Caco-2 permeability.

Based on human-fraction-dose absorbed data, A-PAMPA assay correctly classified all ten high permeability drugs as high permeability. Among the ten low-permeability drugs, four (atenolol, bupropion, chlorpheniramine, and

disopyramide) was incorrectly classified as high permeability. The Caco-2 assay incorrectly classified three drugs: bupropion, indomethacin, and verapamil.

CONCLUSIONS

A three lipid-component PAMPA system was previously devised to mimic the lipid composition of the enterocyte's plasma membrane. The objective was to compare A-PAMPA system to Caco-2 monolayers, in terms of screening for drug permeability values. Results show good agreement for 20 drugs between PAMPA permeabilities and Caco-2 permeabilities, including concordance between PAMPA BCS classification and Caco-2 BCS classification. There was a modest tendency for the PAMPA system to “over-estimate” BCS permeability classification, such that the system appears to have utility to anticipate compounds that are likely to exhibit high permeability in a BCS Caco-2 assay.

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