Effect of Bovine Serum Albumin on Permeability, Efflux Ratio and Recovery of Compounds in MDCKII-MDR1 Monolayers

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Introduction

MDCKII-MDR1 monolayers, derived from Madin-Darby Canine Kidney cells and transfected with the human MDR1 gene, are widely used in drug discovery and development to study the permeability of potential drug compounds and determine substrates and inhibitors of the MDR1 (P-gp) efflux transporter. However, for many compounds, non-specific binding (NSB) to the monolayers and/or filter plate apparatus leads to a significant challenge in permeability assays, as it can compromise the accuracy and reliability of the results. To minimize NSB issues, the assay buffer is often supplemented with protein (e.g., bovine serum albumin (BSA) or fetal bovine serum). However, limited data are available for the incubation conditions. The objective of the present study was to evaluate the effect of BSA on the apparent permeability (P_{app}), efflux ratios (ER) and recovery under several conditions in the MDCKII-MDR1 monolayer test system.

Methods

The MDCKII-MDR1 cells were seeded onto the membrane inserts of 24 well Millicell plates and grown for 4 days. The bidirectional permeabilities of quinidine, metoprolol, digoxin, sulfasalazine, compound X and compound Y at 1 µM were examined in MDCKII-MDR1 monolayers. The assay were performed in the absence and presence of BSA in both apical and basolateral chambers under five conditions: 1) Buffer only (Hank's Balanced Salt Solution, pH 7.4, supplemented with 10 mM HEPES (HBSS-HEPES), 2) Buffer with 1% BSA, 3) Buffer with 4% BSA, 4) Pre-incubated with 1% BSA, and 5) Pre-incubated with 4% BSA and dosing and receiver solutions were buffer only.

The sample plates were incubated at 37 °C for 120 min. Samples were quantitatively measured by LC-MS/MS.

Results and Discussion

- In the absence of BSA, quinidine, metoprolol, digoxin and sulfasalazine showed high recovery (> 94%) indicating minimal NSB property for these compounds.
- BSA reduced P_{app} in both apical to basolateral (A-B) and B-A direction in various degree for these 4 compounds, resulted in similar ERs for treatments with or without BSA. The reduction of P_{app} was likely due to decrease in free fraction of compounds in donor wells. Interestingly, BSA at 1% and 4% exhibited similar effect on the pre-incubation versus incubation treatments.
- In the absence of BSA, compound X showed low recovery (21-42%), indicating its NSB property. In the presence of BSA, the recovery of compound X was remarkably improved.
- BSA increased the A-B P_{app} of compound X, but not the B-to-A P_{app}, suggesting that BSA may have effectively blocked the NSB of compound X to the basal chambers of the filter plate apparatus.
- In the absence of BSA, compound Y exhibited extremely low A-B and B-A P_{app}, but relative high recovery.
- In the presence of BSA, A-B and B-A P_{app} increased significantly, indicating that BSA in the receiver wells was critical for preventing compound Y loss due to NSB. Thus, P_{app} and ER can be accurately determined.

Conclusion

In conclusion, incubation or pre-incubation with BSA appears to provide an optimal assay condition for compounds with NSB property or very low P_{app} in the MDCKII-MDR1 monolayer test system.

Table 1. BSA Effect on Apparent Permeability, Efflux Ratio and Recovery of Test Compounds

Cpd	Treatment	A-B P_{app} (10^{-6} cm/s)	B-A P_{app} (10^{-6} cm/s)	Efflux Ration	A-B Recovery%	B-A Recovery%
Quinidine	Buffer	1.9	93	50	95	115
	+ 1%BSA	1.1	62	54	100	110
	+ 4%BSA	1.2	35	29	97	95
	Pre-1%BSA	1.1	54	51	98	101
	Pre-4%BSA	1.1	37	33	96	95
Metoprolol	Buffer	31	40	1.3	94	99
	+ 1%BSA	25	30	1.2	97	100
	+ 4%BSA	24	28	1.2	110	104
	Pre-1%BSA	18	22	1.2	90	91
	Pre-4%BSA	19	16	0.87	98	93
Digoxin	Buffer	0.27	27	99	99	117
	+ 1%BSA	0.23	17	72	104	97
	+ 4%BSA	0.24	16	69	100	96
	Pre-1%BSA	0.17	15	87	98	91
	Pre-4%BSA	0.15	11	74	96	94
Sulfasalazin	Buffer	0.51	0.30	0.59	98	97
	+ 1%BSA	0.048	0.035	0.73	97	97
	+ 4%BSA	0.035	0.036	1.1	98	97
	Pre-1%BSA	0.036	0.045	1.2	100	100
	Pre-4%BSA	0.044	0.056	1.3	102	99
Compound X	Buffer	1.5	1.5	1.0	21	42
	+ 1%BSA	2.6	1.3	0.48	68	83
	+ 4%BSA	2.6	1.3	0.50	63	82
	Pre-1%BSA	3.4	0.73	0.21	79	89
	Pre-4%BSA	3.3	0.72	0.22	82	89
Compound Y	Buffer	0.00024	0.000038	0.16	86	77
	+ 1%BSA	0.015	0.044	2.9	93	67
	+ 4%BSA	0.015	0.055	3.7	103	59
	Pre-1%BSA	0.015	0.025	1.6	88	83
	Pre-4%BSA	0.011	0.027	2.5	89	80

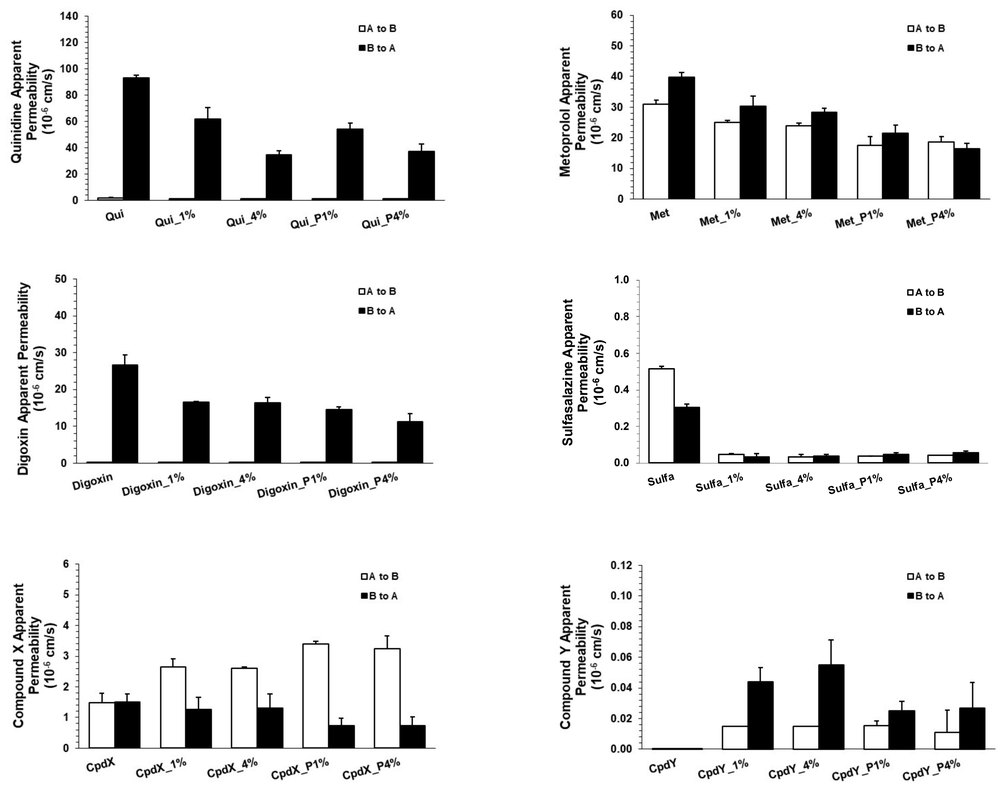

Figure 1. BSA Effect on A-B and B-A P_{app} of Test Compounds

Reference

1. Neuhoff, S., *et al*. J. Drug Del. Sci. Tech. **2007**; 17:259-266.

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