Effects of Lipoprotein Binding and Plasma Dilution on the Accuracy of Ultracentrifugation Method in Plasma Protein Binding

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Background

Ultracentrifugation (UC) is the proposed methodology to study the protein binding of challenging compounds, such as targeted protein degraders, covalent inhibitors, and those with severe nonspecific binding properties. However, UC is prone to lipoprotein contamination that can result in artificially elevated fraction unbound (f_u) values compared to other methods. To quantitatively estimate the effect of binding to low-density lipoproteins/very-low density lipoproteins (LDL/VLDL) as well as plasma dilution on the data accuracy of ultracentrifugation method, a wide range of Log P(0.8-8.6), with or without report binding to LDL/VLDL, were selected, and the protein binding in human plasma was tested by the in house UC protocol. The results obtained were compared with literature values tested on the dialysis-based method or the other methods.

Methods

- Human plasma dilution of 50-fold, 20-fold, 10-fold, and neat plasma were tested for 4 highly lipophilic compounds and 7 moderately lipophilic compounds that bind to LDL/VLDL in human plasma. An additional 50-fold, 20-fold, and 10-fold dilution of human plasma was tested for 4 highly lipophilic compounds and 7 moderately lipophilic compounds that were not reported to bind to LDL/VLDL in human plasma.
- The criteria for lipophilicity were Log P≥5 for highly lipophilic compounds and Log P<5 for moderately lipophilic compounds.
- Drug-spiked plasma (2 μM) were pre-incubated at 37 ºC for 0.5 hours and then centrifuged at 37°C, 155000 ×g for 4 hours to pellet the plasma protein, enabling separation from the free compound in the supernatant. Values of f_u at each table represent the mean ± SD of 3 to 8 replicates from 1 to 3 experiments.

Results

Table 1. PPB of highly lipophilic compounds that bind to LDL/VLDL in human plasma.

All the f_u values in neat plasma were higher than in diluted plasma. F_u values of amiodarone in UC were at least 87-fold higher than reported values as well as in-house values from dialysis-based methods (data not shown). Data in diluted plasma were closer to reported values to different extents.

Compound	Bind to LDL/VLDL (Y/N)	LogP>5 (Y/N)	Plasma dilution fold	f_u Mean	SD	Reported f_u (reference)		Reported LogP (reference)		Fold Change (f_u/reported f_u)
						1	2	1	2	
amiodarone	Y	Y	Neat	0.1036	0.0092	0.0002	0.00014	7.81	7.82	609.7
			10-fold	0.0427	0.0049					251.1
			20-fold	0.0247	0.0021					145.3
			50-fold	0.0149	0.0031					87.8
halofantrine	Y	Y	Neat	0.1095	0.0132	0.0040	0.0090	8.06	8.60	16.8
			10-fold	0.0322	0.0036					5.0
			20-fold	0.0244	0.0036					3.7
			50-fold	0.0173	0.0032					2.7
nystatin	Y	Y	Neat	0.1623	0.0176	0.0800	0.1370	6.10	/	1.5
			10-fold	0.1184	0.0096					1.1
			20-fold	0.0673	0.0079					0.6
			50-fold	0.0498	0.0041					0.5
sertraline	Y	Y	Neat	0.0653	0.0167	0.0200	0.0350	5.10	/	2.4
			10-fold	0.0264	0.0026					1.0
			20-fold	0.0241	0.0009					0.9
			50-fold	0.0216	0.0010					0.8

Table 2. PPB of 4 highly lipophilic compounds with no lipoprotein binding evidence.

Higher f_u in neat plasma were also observed, similar to the trend for compounds in Table 1.

Compound	Bind to LDL/VLDL (Y/N)	LogP>5 (Y/N)	Plasma dilution fold	f_u Mean	SD	Reported f_u (reference)		Reported LogP (reference)		Fold Change (f_u/reported f_u)
						1	2	1	2	
Itraconazole	N	Y	Neat	0.0367	0.0096	0.0023	0.0025	5.66	6.20	15.3
			10-fold	0.0010	0.0002					0.4
			20-fold	0.0053	0.0010					2.2
			50-fold	0.0029	0.0002					1.2
lapatinib	N	Y	Neat	0.0085	0.0015	0.0012	0.0011	5.10	6.30	7.4
			10-fold	0.0009	0.0001					0.8
			20-fold	0.0039	0.0000					3.4
			50-fold	0.0020	0.0000					1.7
nelfinavir	N	Y	Neat	0.0107	0.0002	0.0066	0.0046	5.84	7.28	1.9
			10-fold	0.0117	0.0004					2.1
			20-fold	0.0093	0.0005					1.7
			50-fold	0.0039	0.0004					0.7
verlukast	N	Y	Neat	0.0018	0.0004	0.0006	0.0005	5.30	5.93	3.3
			10-fold	0.0011	0.0001					2.0
			20-fold	0.0010	0.0001					1.9
			50-fold	0.0010	0.0001					1.8

Table 3. PPB of moderate lipophilic compounds that bind to LDL/VLDL in human plasma

Compound	Bind to LDL/VLDL (Y/N)	LogP>5 (Y/N)	Plasma dilution fold	f_u Mean	SD	Reported f_u (reference)		Reported LogP (reference)		Fold Change (f_u/reported f_u)
						1	2	1	2	
cyclosporine A	Y	N	Neat	0.0731	0.0137	0.0680	0.1000	2.92	4.12	0.9
			10-fold	0.0164	0.0018					0.3
			20-fold	0.0078	0.0013					0.1
			50-fold	0.0056	0.0004					0.1
amphotericin B	Y	N	Neat	0.0743	0.0150	<0.0500	<0.1000	1.16	0.80	1.0
			10-fold	0.0383	0.0063					0.5
			20-fold	0.0151	0.0022					0.2
			50-fold	0.0070	0.0005					0.1
clozapine	Y	N	Neat	0.0349	0.0088	0.0550	0.0530	3.20	/	0.6
			10-fold	0.0806	0.0019					1.5
			20-fold	0.1140	0.0071					2.1
			50-fold	0.0289	0.0022					0.5
amitriptyline	Y	N	Neat	0.0552	0.0028	0.0700	0.0760	4.92	/	0.8
			10-fold	0.1081	0.0044					1.5
			20-fold	0.1262	0.0274					1.7
			50-fold	0.0969	0.0059					1.3
aripiprazole	Y	N	Neat	0.0108	0.0019	0.0100	0.0100	4.50	/	1.1
			10-fold	0.0047	0.0001					0.5
			20-fold	0.0042	0.0002					0.4
			50-fold	0.0041	0.0002					0.4
fluoxetine	Y	N	Neat	0.0325	0.0059	0.0500	0.0510	4.05	/	0.6
			10-fold	0.0584	0.0021					1.2
			20-fold	0.0548	0.0013					1.1
			50-fold	0.0421	0.0019					0.8
paroxetine	Y	N	Neat	0.0540	0.0056	0.0500	0.0600	3.60	/	1.0
			10-fold	0.0788	0.0009					1.4
			20-fold	0.0678	0.0012					1.2
			50-fold	0.0472	0.0017					0.9

Comment: The fold change of amphotericin B was calculated with the mean of 0.05 and 0.1 as the reported values.

Table 4. PPB of moderate lipophilic compounds that do not bind to LDL/VLDL in human plasma

Compound	Bind to LDL/VLDL (Y/N)	LogP>5 (Y/N)	Plasma dilution fold	f_u Mean	SD	Reported f_u (reference)		Reported LogP (reference)		Fold Change (f_u/reported f_u)
						1	2	1	2	
warfarin	N	N	Neat	0.0063	0.0001	0.0150	0.0120	2.70	2.30	0.5
			10-fold	0.0076	0.0002					0.6
			20-fold	0.0081	0.0002					0.6
			50-fold	0.0089	0.0004					0.7
indomethacin	N	N	Neat	0.0032	0.0002	0.0033	0.0037	4.25	4.27	0.9
			10-fold	0.0048	0.0008					1.4
			20-fold	0.0033	0.0000					0.9
			50-fold	0.0030	0.0001					0.9
rosiglitazone	N	N	Neat	0.0023	0.0003	0.0017	0.0013	2.95	2.56	1.5
			10-fold	0.0020	0.0003					1.3
			20-fold	0.0028	0.0000					1.9
			50-fold	0.0026	0.0001					1.8
glyburide	N	N	Neat	0.0008	0.0000	0.0014	0.0011	3.79	3.08	0.6
			10-fold	0.0010	0.0001					0.8
			20-fold	0.0010	0.0000					0.8
			50-fold	0.0009	0.0000					0.7
tesaglitazar	N	N	Neat	0.0013	0.0003	0.00076	0.00078	3.14	2.64	1.7
			10-fold	0.0007	0.0001					1.0
			20-fold	0.0008	0.0001					1.0
			50-fold	0.0008	0.0001					1.0
ziprasidone	N	N	Neat	0.0035	0.0002	0.0013	0.0017	4.30	4.00	2.4
			10-fold	0.0027	0.0002					1.8
			20-fold	0.0023	0.0003					1.5
			50-fold	0.0023	0.0001					1.5
tolcapone	N	N	Neat	NA	NA	0.00061	0.0007	3.28	4.07	NA
			10-fold	0.0008	0.0003					1.3
			20-fold	0.0008	0.0001					1.3
			50-fold	0.0008	0.0001					1.3

- Table 3 and Table 4 indicate that 11 of the 14 compounds were within 2-fold of literature values in neat and 10% diluted plasma. For the rest of the compounds cyclosporine A, ziprasidone, and tolcapone, the fold changes were close to 2-fold.

Conclusion

- The present results indicate that lipophilicity could play an important role in the data accuracy in the ultracentrifugation method. F_u could be over-estimated using neat plasma for highly lipophilic compounds.
- Appropriate plasma dilution fold should be selected according to the compound's log P and estimated binding ratios when the UC method should be used for protein binding during drug discovery.
- In the late stages, it is recommended that a different method should be used to verify the accuracy of fu values determined by UC.

References

Please contact us to obtain detailed references due to limited space.

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