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Phospholipid content is a major determinant of intracellular binding of drugs

Andrea Treyer[1], André Mateus[1], Hinnerk Boriss[2], Pär Matsson[1], Per Artursson[1]
1: Uppsala University, Department of Pharmacy, Uppsala, Sweden 2: Sovicell GmbH, Leipzig, Germany

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PURPOSE

Intracellular unbound drug concentration ($C_{u,cell}$) drives pharmacological and toxicological responses for targets inside cells. We have previously shown that $C_{u,cell}$ is not always correlated to extracellular unbound drug concentrations (e.g. plasma concentrations) (Mateus et al., Mol Pharmaceutics, 2013). However, the underlying mechanisms that drive cellular drug binding are poorly understood. The aim of this study was to evaluate drug binding to individual cellular components, such as neutral lipids and phospholipids.

CONCLUSION

For the first time, 3T3-L1 cells have been used as a cellular model with accumulation of both neutral lipids (steatotic model) and phospholipids (early stage of phospholipidosis). Our results suggest that phospholipid content is a major determinant of drug binding to cell homogenates. This finding will allow improving predictions on drug disposition at the target site and accumulation of drugs in tissues, where lipid content is known.

METHODS

To study the impact of neutral- and phospholipids on drug binding, the 3T3-L1 fibroblast cell line was induced to accumulate each of these components:

Neutral lipid model (NL) : a steatotic, adipocyte-like phenotype was generated by addition of a differentiation cocktail containing isobutylmethylxanthine. Nile-Red was used as a fluorescent dye to label neutral lipids.

Phospholipid model (PL) : the cells were exposed the cationic amphiphilic drug propranolol which is known to cause drug-induced phospholipidosis *in vitro*. NBD-PE, a fluorescent phospholipid, was added to the culture medium to visualize accumulation of phospholipids.

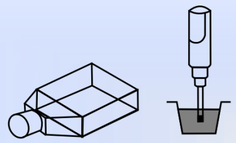
Quantification of phospholipids was performed using the enzymatic Wako Phospholipids C assay.

Drug binding to cell homogenates, $\mathbf{f_{u,cell}}$, and pure phosphatidylcholine (PC) in the TRANSIL membrane affinity kit, $\mathbf{f_{u,lipid}}$, were measured for a set of 25 compounds spread across the drug chemical space:

Drug charge	+	n	-
Range logD	-0.28 – 3.16	-0.15 – 4.98	-0.65 – 3.12
Range PSA	28.2 – 90.8	61.8 – 120	75.3 – 146
Range MW	248.7 – 414.5	194.2 – 628.8	276.7 – 551.6

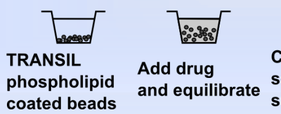
Experimental

$f_{u,cell}$



$$f_{u,cell} = \frac{1}{D \cdot (1/f_{u,hom} - 1) + 1}$$

$f_{u,PC}$



$$f_{u,PC} = \frac{C_{buffer}}{C_{buffer} + C_{membrane(PC)}}$$

Increase in lipids:
The fluorescent labelling of total neutral lipid content increased up to 5-fold in the neutral lipid model. The total phospholipid content was found to increase 1.5-fold in both cellular models (see figure 1 for staining and quantification).

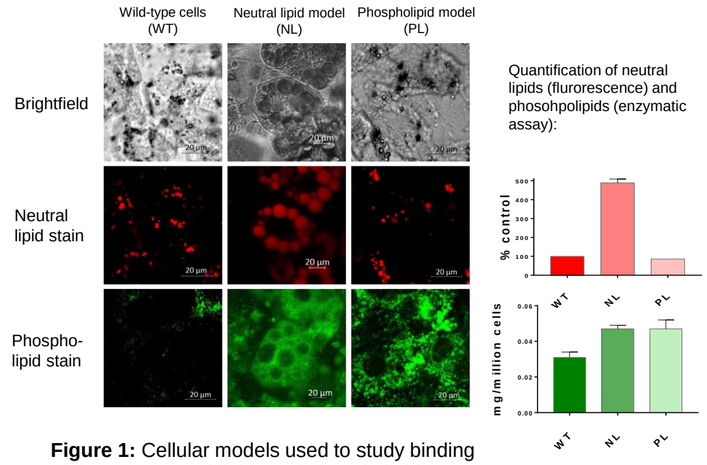


Figure 1: Cellular models used to study binding of drugs to neutral lipids and phospholipids.

Increase in binding:
Average difference of drug binding compared to wild-type cells was found to be 1.7-fold over the whole compound set (refer to figure 2 for changes according to cell type and charge of the compound). This increase corresponds to the observed increase in phospholipid content. The large increase in neutral lipids in the NL model was not reflected in the drug binding results.

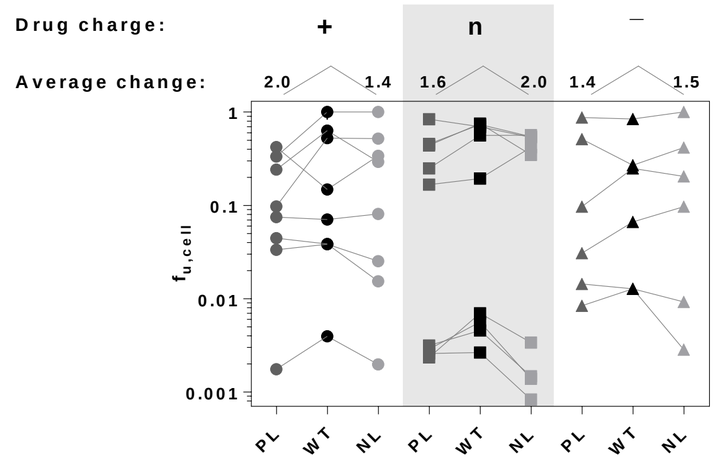
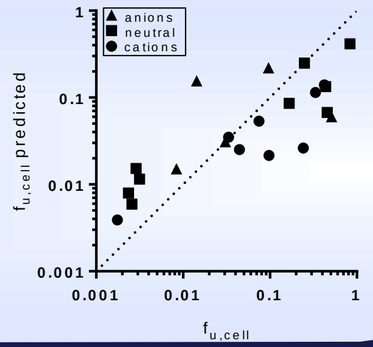


Figure 2: measured $f_{u,cell}$ in the different cellular models.

Predicted

$$f_{u,cell} = \frac{1}{\frac{1}{D} \cdot (1/f_{u,PC} - 1) + 1}$$



Cellular binding is explained by affinity of drugs to pure lipids:

Binding to pure phospholipid ($f_{u,lipid}$) showed a strong correlation with $f_{u,cell}$ and could be used to predict $f_{u,cell}$ in the phospholipid model with an accuracy of r = 0.8.

RESULTS

andrea.treyer@farmaci.uu.se

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