A robust high throughput physicochemical screen for phospholipidosis

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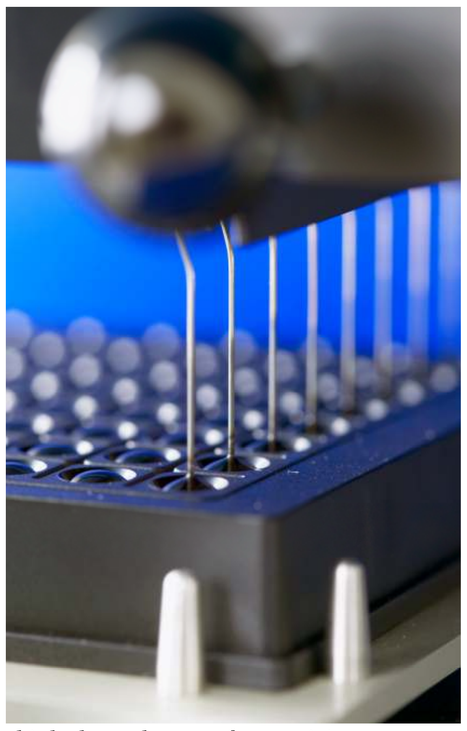
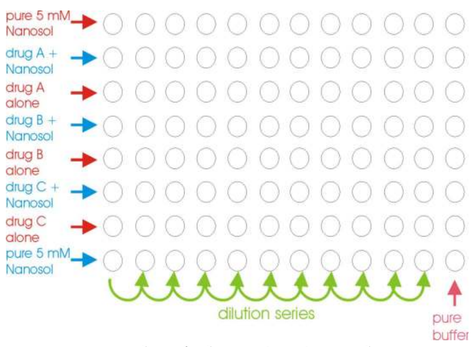
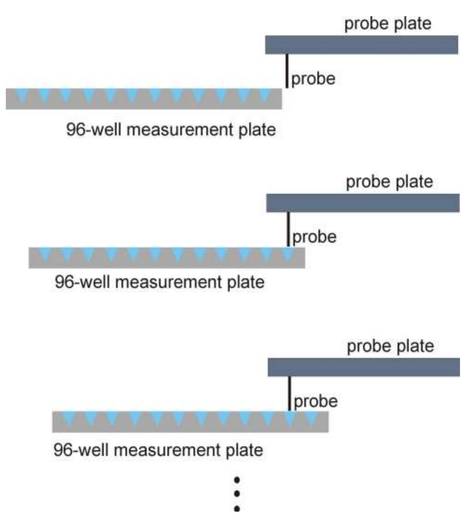
Method



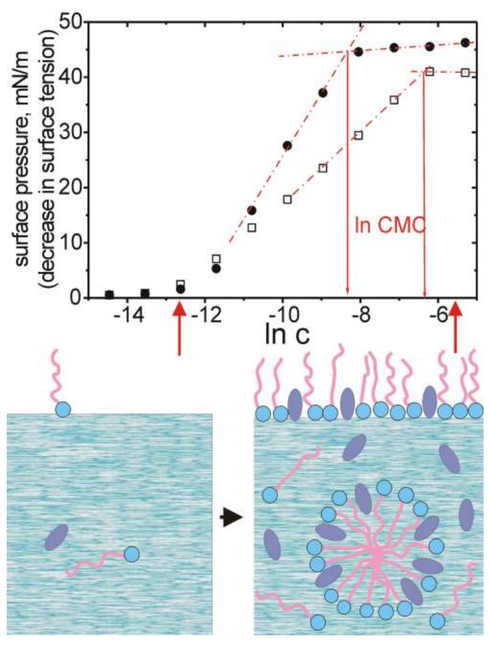
high-throughput surface activity measurements from 96-well plates by Kibron Delta-8



an example of plate pipeting scheme



surface tension measurement sequence



the principle of CMC measurement by surface pressure measurement: 1) surface pressure increases (=tension decreases) as long as bulk concentration increases, 2) when CMC is reached, all excess substance forms micelles, and surface pressure no longer increases

Introduction

Some drugs induce abnormal accumulation of phospholipids into lysosomes, resulting in lamellar bodies.[1] Typically, drugs inducing phospholipidosis are cationic and amphiphilic, and the mechanistic basis for the accumulation of the drugs is most likely the inhibition of lysosomal phospholipases by drug-phospholipid complexation.[1] It remains unknown whether this side effect of these drugs is related to any adverse effects the drugs might have, only for gentamicin a causal relationship between phospholipidosis and nephrotoxicity has been suggested.[1] Nevertheless, various methods to evaluate and screen the phospholipidosis-inducing potency of drugs have been presented, but many of them require cultured cells, which limits their throughput and adds to their cost.[2-4]

Our aim was to develop a method that

- is physicohemical and does not require cell cultures
- has a very high throughput (>10000 drugs/year/instrument)
- screens the causative interaction between drugs and phospholipids
- does not require a large amount of the compound
- provides fast feedback for synthesis

Methods

The decrease in the critical micellar concentration (CMC) of Kibron PLD Nanosol reagent in the presence of various drugs was used as an indicator of drug-phospholipid complexation.

Kibron Delta-8 high-throughput surface activity profiling instrument was used to measure the CMCs of the mixtures of PLD Nanosol and various drugs (purchased from Sigma-Aldrich). The measurement was done on dedicated 96-well plates.

Each analysis requires 175 µl of 5 mM PLD Nanosol reagent. A stock solution of drug in DMSO, water, or ethanol (or some other solvent) is then added to give 2.5 mM concentration of drug, and drug is also measure in another row without Nanosol, i.e. single analysis requires approx. 2x0.44 µmol of drug (corresponding to 0.3 mg when M_w=350 g/mol). As a control 96-well plate also included pure Nanosol and for purposes of calibration a pure buffer solution in the column measured first. Additional measurements varying the initial drug cocnentration, i.e. the drug/Nanosol molar ratio, were also done to evaluate $R_{1/2}$, the drug/Nanosol molar ratio where half of the total decrease in CMC was reached.

Following the measurements, PLD index was calculated as the ratio of the CMCs of drug-Nanosol mixture and pure Nanosol,

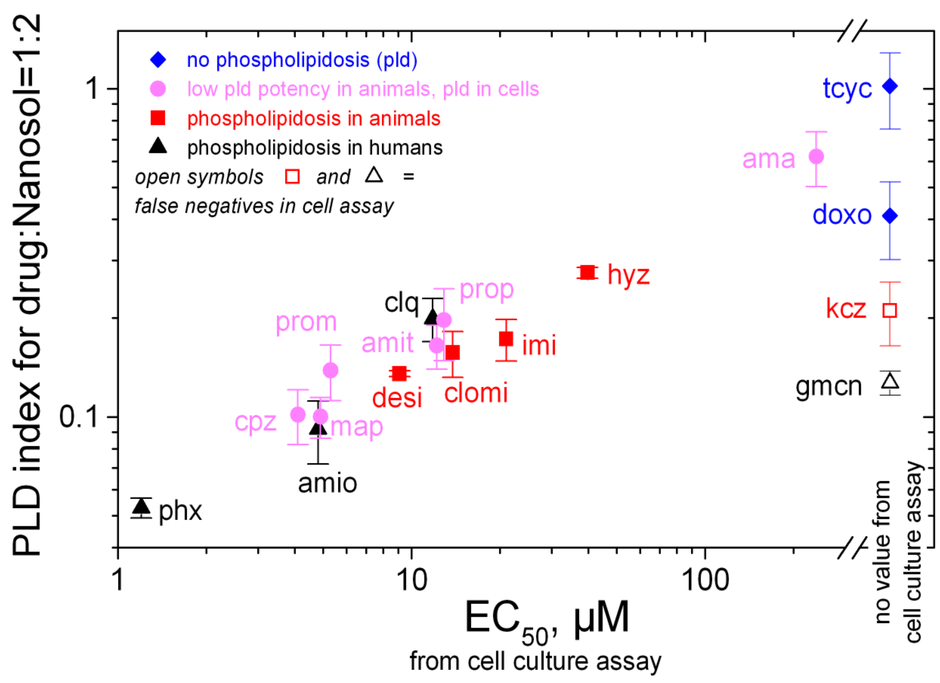
$$\text{PLD index} = \frac{\text{CMC of drug Nanosol mixture}}{\text{CMC of pure Nanosol}}$$

The drugs used were:

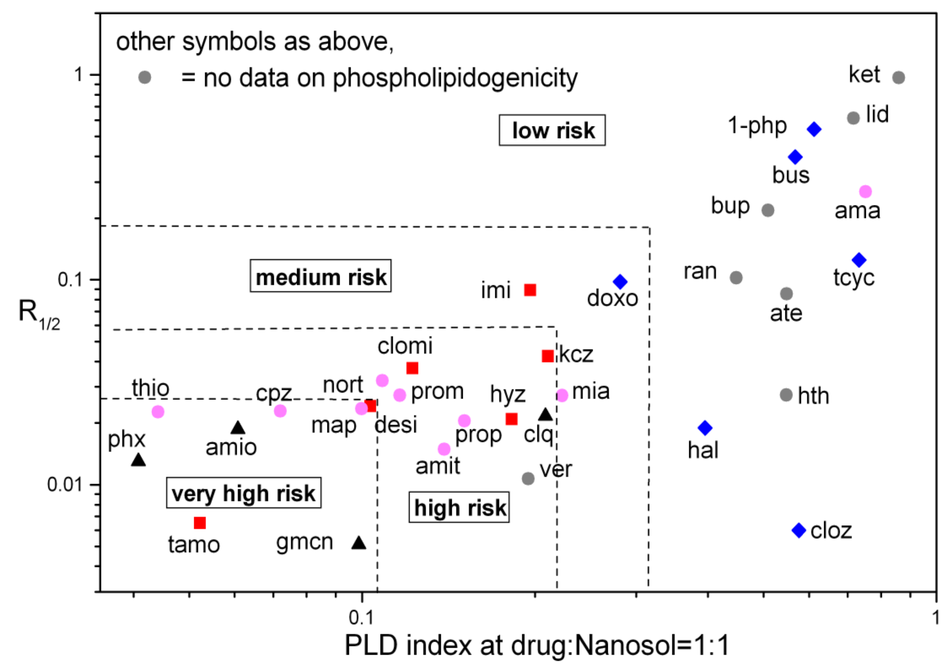
ama = amantadine
amio = amiodarone
amit = amitriptyline
ate = atenolol
bus = buspirone
bup = bupivacaine
clomi = clomipramine
cloz = clozapine
clq = chloroquine
cpz = chlorpromazine
desi = desipramine
doxo = doxorubicin
gmcn = gentamicin
hal = haloperidol
hth = hydroxythioridazine
hyz = hydroxyzine
imi = imipramine
kcz = ketoconazole
ket = ketoprofene
lid = lidocaine
map = maprotiline
mia = mianserin
nort = nortriptyline
1-php = 1-phenylpiperazine
phx = perhexiline
prom = promazine
prop = propranolol
ran = ranitidine
tamo = tamoxifene
thio = thioridazine
tcyc = tetracyclin
ver = verapamil

The data for phospholipidosis-inducing potency of the drugs were obtained from references 2 and 5.

Results



J. K. Morelli et al., 2006, *Cell Biol. Toxicol.* 22:15-27



Results and discussion

The results (upper figure) showed excellent agreement with cell culture screening data of Morelli et al.[2], with three exceptions

- tamoxifene that had a very low CMC of its own did not give good results (not shown)
- ketoconazole that had produced incalculable results in the cell culture assay had a PLD index that appears to agree with its phospholipidosis potency
- gentamicin, one of the most phospholipidogenic drugs, gives a false negative in cell culture assay, whereas PLD index correctly predicts high phospholipidogenicity

Reasonable results were also obtained by comparing the data to known data on phospholipidosis[5] (lower figure). However, such things as appearance of phospholipidosis in humans is obviously not only related to the potency of the drug to induce phospholipidosis, but also on the number of people using the drug. Nevertheless, PLD index at drug:Nanosol=1:2 appears to be sufficient.

In general, considering that cell culture assays appear to be no more or less reliable than the present assay regarding prediction of phospholipidosis in animals or humans, the biggest problem was the lack of quantitative reference data collected using a single method from animal testing.

Throughput and drug consumption were also satisfactory.

- if pipeting is automated, and analysis done in duplicate, 30 000 drugs / year / Delta-8 instrument can be screened
- drug consumption is < 1 mg / compound (0.6 mg for a 350 g/mol drug), and normal surface activity profile parameters obtained as a by-product can be used to predict absorption of drugs,[6] decreasing the need of drug for other analyses such as PAMPA

References

[1] Reasor, M. J. and S. Kacew (2001) *Exp. Biol. Med.* 226: 825–830.
[2] Morelli, J. K., M. Buehrle, F. Pognan, L. R. Barone, W. Fieles, and P. J. Ciaccio (2006) *Cell Biol. Toxicol.* 22: 15–27.
[3] Casartelli, A., M., et al. (2003) *Cell. Biol. Toxicol.* 19: 161–176.
[4] Sawada, H., K. Taniguchi, and K. Takami (2006) *Toxicol. in Vitro* 20: 1506–1513.
[5] Ploeman, J.–P. H. T. M., et al. (2004) *Eur. Toxic. Pathol.* 55: 347–355.
[6] K. Kiehm, M. Brewster, J. Peeters, and J.B. Dressman, In AAPS Annual Meeting and Exposition, Nashville, Nov 6-10, 2005