

PHOSPHOLIPIDOSIS

Cationic amphiphilic drugs (CADs), such as antibiotics, antidepressants, antihistaminics and other prescription drugs, have been identified as inducers of phospholipidosis in animal and cell culture models. Drug-induced phospholipidosis is characterized by the appearance of intracellular phospholipids in lysosomes. Even though a direct link between *in vitro* drug-induced phospholipidosis and adverse drug reactions in humans has not yet been firmly established, this does not exclude the possibility that drug-induced phospholipidosis is a contributing factor in toxicity. Currently, there are two prevailing hypotheses for the putative mechanisms involved in drug-induced phospholipidosis: 1) phospholipids disrupt the enzymatic activity of the phospholipases, or 2) a chemical entity binds to the phospholipids resulting in the formation of a complex that can not be enzymatically processed.

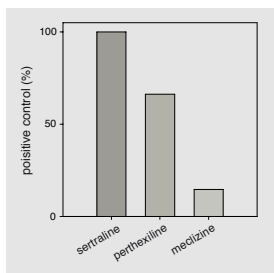
Detection of drug-induced phospholipidosis has been performed using electron microscopy, a time-consuming and labor intensive technique, and/or quantitative PCR. More recently, fluorescent dyes have been employed to assess phospholipidosis in a high throughput manner. Recently, a high throughput assay using LipidTox, fluorescent lipophilic dye, has been shown to be highly sensitive in detecting drug-induced phospholipidosis in HepG2 cells¹. This fluorescent probe methodology can be performed rapidly and requires only a small amount of test compound. Cerep offers a rapid high content analysis (HCA) assay to assess drug-induced phospholipidosis in HepG2 cells.

WHY SCREEN FOR DRUG-INDUCED PHOSPHOLIPIDOSIS ?

The Food and Drug Administration (FDA) has acknowledged that drug-induced phospholipidosis is an adverse drug reaction that warrants both additional guidelines and research into the molecular mechanisms that govern this biological response². Since the molecular mechanisms regulating drug-induced phospholipidosis have not been fully described in the literature, there is a strong impetus to understand phospholipidosis in the context of drug discovery. A number of clinical and experimental drugs have been shown to induce phospholipidosis in numerous animal and cell culture models³.

PHOSPHOLIPIDOSIS ASSAY

HepG2 cells are plated in 96-well plates in MEM growth medium and allowed to incubate overnight. Cells are treated with test compounds that have been added to assay medium (10% fetal bovine serum) containing LipidTox (fluorescent lipophilic dye). After a 48 h incubation period, cells are fixed and stained with Hoechst.



Plates are scanned with an automated fluorescent microscope (Cellomics ArrayScan 4.5). Image-analysis software is used to quantitate cell number and phospholipid accumulation.

The client compound data from the phospholipidosis assay are expressed as fold over background (1% DMSO, solvent) and % of the positive control (sertraline). Each plate also includes two additional reference compounds, perhexiline and meclizine (moderate and low inducers of phospholipidosis, respectively).

◀ Example of reference compound data (% of positive control) from a phospholipidosis assay. Three reference compounds, sertraline (high inducer), perhexiline (medium) and meclizine (low inducer) are included in every phospholipidosis assay.

CUSTOMIZATION

Compounds are tested at eight concentrations starting at 100 μ M (two-fold dilutions) in triplicate. Customized concentrations and replicates can be accommodated.

ADVANTAGES OF THE PHOSPHOLIPIDOSIS ASSAY

The phospholipidosis assay requires only a small amount of compound (2 mg) to test at the top concentration of 100 μ M (assuming a MW of 500).

RAPID TURNAROUND TIME

Results are delivered within two weeks upon receipt of the compounds at the Cerep testing site. Data are made available on line as soon as they are validated.

¹ Nioi, P., Perry, B.K., Wang, E.J., Gu, Y.Z., and Snyder, R.D. (2007) *In vitro* detection of drug-induced phospholipidosis using gene expression and fluorescent phospholipid based methodologies. *Toxicol Sci.* 99(1): 162-173.

² Berridge, B.R., Chatman, L.A., Odin, M., Schultze, A.E., Losco, P.E., Meehan, J.T., Peters, T., Vonderferck, S.L. (Society of Toxicologic Pathology Scientific and Regulatory Policy Committee Working Group). (2007) Phospholipidosis in nonclinical toxicity studies. *Toxicol Pathol.* 35(2): 325.

³ Reasor, M.J., Hastings, K.L., and Ulrich, R.G. (2006) Drug-induced phospholipidosis: issues and future directions. *Expert Opin Drug Saf.* 5(4): 567-583.

PHOSPHOLIPIDOSIS COMPOUND TRAINING SET

Compound	Drug class	% of positive control ± SE ¹	Conc. (µM) ²	
High	sertraline ³	antidepressant	100 ± 0.03	3.13
	fluoxetine	antidepressant	66.5 ± 11.31	2.5 - 5
	erythromycin	antibiotic	61.9 ± 15.39	25 - 50
	chlorpromazine	antipsychotic	54.7 ± 9.50	2.5 - 5
	tamoxifen	chemotherapeutic	53.0 ± 5.00	3.13 - 6.25
	ketoconazole	antifungal	52.0 ± 3.78	12.5
	amitriptyline	antidepressant	49.8 ± 8.98	10 - 20
	triparanol	cholesterol synthesis inhibitor	44.9 ± 13.71	1.56 - 3.13
Moderate	maprotiline	antidepressant	42.2 ± 4.29	6.25
	haloperidol	antipsychotic	42.1 ± 4.74	12.5
	amiodarone	antiarrhythmic	38.4 ± 3.29	6.25
	doxepin	antidepressant	36.4 ± 5.26	25
	perhexiline ³	antianginal	36.0 ± 4.40	3.13
	propranolol	hypertension	36.0 ± 10.94	12.5 - 25
	hydroxyzine	antihistaminic	35.8 ± 12.32	25
	bromhexine	mucolytic	30.1 ± 13.39	12.5 - 50
	ambroxol	mucolytic	29.7 ± 9.53	50
	azithromycin	antibiotic	26.7 ± 1.96	25
Low	chloroquine	antimalarial	22.8 ± 3.64	5 - 10
	tilorone	antiviral	20.2 ± 0.69	3.13
	citalopram	antidepressant	19.6 ± 3.90	50 - 100
	clozapine	antipsychotic	18.9 ± 1.34	12.5
	meclizine ³	antihistaminic	10.5 ± 3.73	12.5 - 25
	labetalol	hypertension	5.3 ± 2.81	12.5 - 25
	amikacin	antibiotic	4.9 ± 0.90	12.5 - 25
	quinidine	antiarrhythmic	3.2 ± 1.43	12.5 - 25
	imipramine	antidepressant	2.1 ± 1.04	3.13 - 6.25
	Negative controls ⁴	menadione	vitamin K precursor	1.0 ± 0.43
acetaminophen		analgesic	0.3 ± 0.09	50 - 100
cyclosporin A		immunosuppressant	0.3 ± 0.26	3.13 - 6.25
ofloxacin		antibiotic	0.3 ± 0.27	25 - 50
sotalol		antiarrhythmic	0.3 ± 0.26	12.5 - 50
valproic acid		anticonvulsant	0.2 ± 0.21	100

¹ SE: standard error

² Conc. (µM): Maximum test concentrations that did not exhibit cytotoxicity.

³ Reference compounds

⁴ Negative controls: nonphospholipidogenic compounds

The HCA phospholipidosis assay is a rapid and sensitive method for screening compounds that may cause phospholipidosis. The training set of 33 compounds from various drug classes was comprised of 27 compounds known to induce phospholipidosis. This methodology accurately identified compounds that have been shown in the literature to induce phospholipidosis. Nonphospholipidogenic compounds (marked with subscript ⁴ in the above table) serve as negative controls. The data in the table are from four experiments. In general, antidepressants were moderate to high inducers of phospholipidosis with the exception of citalopram and imipramine. The % of the positive control was calculated using the following calculation:

$$\% \text{ positive control} = 100 \times \frac{RFU_{\text{compound}} - RFU_{\text{background}}}{RFU_{\text{sertraline}} - RFU_{\text{background}}} \quad \text{Where } RFU = \text{Relative Fluorescence Units}$$

Data from client studies are expressed as % of the positive control (sertraline) and fold over background (1% DMSO).



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