

PHOSPHOLIPIDOSIS

Cationic amphiphilic drugs (CADs), such as antibiotics, antidepressants, antihistamines 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, a 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?

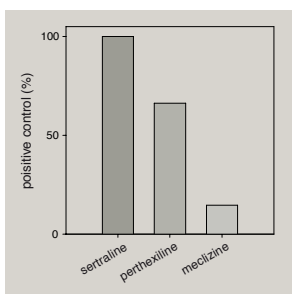
The Food and Drug Administration (FDA) has acknowledged that drug-induced phospholipidosis is an adverse drug reaction that warrants both additional guidelines and research into the molecular mechanisms that govern this biological response². Since the molecular mechanisms regulating drug-induced phospholipidosis 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 (Thermo Fisher Cellomics ArrayScan 4.5). Image-analysis software is used to quantitate cell number and phospholipid accumulation.

Compounds are tested in triplicate at either a single concentration (30 μ M by default) or multiple concentrations (0.03, 0.1, 0.3, 1, 3, 10, 30, and 100 μ M by default). Three reference compounds, sertraline, perhexiline, and meclizine (high, moderate, and low inducers of phospholipidosis, respectively) are included in each assay. The test compound data is expressed as fold over background (1% DMSO) and % of the positive control (sertraline), which is calculated using the following equation:



$$\% \text{ positive control} = 100 \times \frac{\text{RFU}_{\text{compound}} - \text{RFU}_{\text{background}}}{\text{RFU}_{\text{sertraline}} - \text{RFU}_{\text{background}}}$$

Where RFU = Relative Fluorescence Units

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

ADVANTAGES OF THE PHOSPHOLIPIDOSIS ASSAY

The phospholipidosis assay requires only a small amount of compound (1 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.

■ PHOSPHOLIPIDOSIS COMPOUND TRAINING SET

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

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 separate assays. In general, antidepressants were moderate to high inducers of phospholipidosis with the exception of citalopram and imipramine.

REFERENCES

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