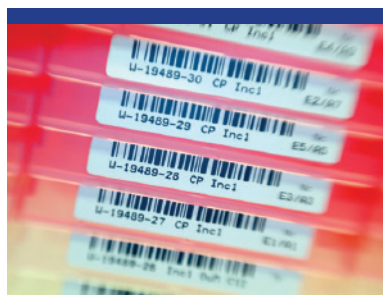


In vitro Toxicology

Drug Induced Phospholipidosis and Steatosis

Background Information



'Phospholipidosis alone, or appearing concurrently with other changes, could potentially delay the drug development process if not properly assessed with an effective risk management strategy.'

⁴Chatman LA, Morton D, Johnson TO and Anway SD (2009) *Toxicol Pathol* **37(7)**: 997-1005

- Phospholipidosis is a lysosomal storage disorder characterised by excessive accumulation of intracellular phospholipids in tissues, such as the liver, kidney and lung. It is well established¹ that a number of cationic amphiphilic drugs have the potential to induce phospholipidosis. These include antibacterials, antipsychotics, antidepressants, antianginals, antimalarials, antiarrhythmics, and cholesterol-lowering agents.
- Several mechanisms have been proposed for drug-induced phospholipidosis, including inhibition of the enzymatic activity of phospholipidases² and binding of the drug to the phospholipid to form drug-phospholipid complexes that cannot be broken down by phospholipidases¹.
- Phospholipidosis does not necessarily constitute toxicity, and can resolve by itself, but it predicts drug or metabolite accumulation that have led to liver, kidney, or respiratory failure. Consequently, in 2004 the FDA formed the Phospholipidosis Working Group to study the problem^{3,4} and recommend strategies for phospholipidosis screening studies.
- Steatosis can result as a consequence of drugs interfering with hepatic lipid processing, leading to accumulation of triglycerides within the liver cells. This condition may lead to harmful liver inflammation, or steatohepatitis. Discontinuation of the offending drug generally leads to reversal of steatosis.
- Accumulation of fatty acids as a consequence of steatosis have led to the recall of marketed drugs and revised therapeutic guidelines for the use of drugs (e.g. tetracycline and valproic acid)⁵.
- Drug-induced phospholipidosis and steatosis is determined by measuring the accumulation of specific fluorescent probes in HepG2 cells or primary hepatocytes using High Content Screening.

Protocol

Instruments

Cellomics ArrayScan® VTI (Thermo Scientific)

Analysis Method

High Content Screening

Toxicity Markers

Cell loss
Nuclear area
DNA structure
Phospholipidosis
Steatosis

Cell Types Available

HepG2, primary hepatocytes

Test Article Concentration

8 point assay (0.04, 0.1, 0.4, 1, 4, 10, 40, 100 µM)
(custom concentrations available)

Number of Replicates

3 replicates per concentration

Quality Controls

0.5% DMSO (vehicle control)
Sertraline (phospholipidosis positive control)
Cyclosporin A (steatosis positive control)

Test Article Requirements

50 µL of a 20mM solution or
equivalent amount in solid compound

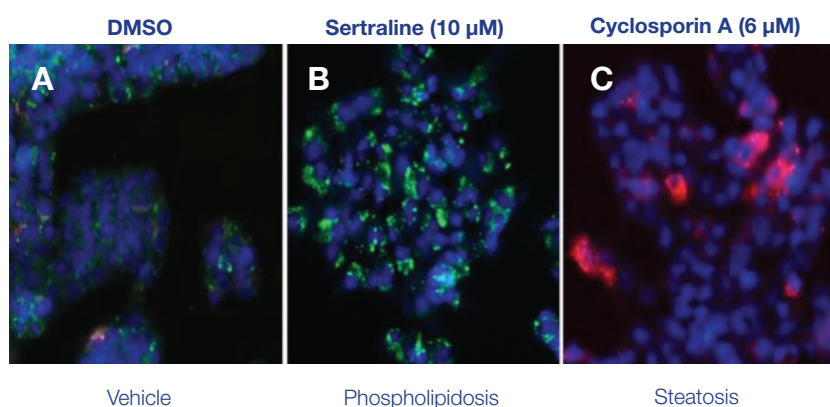
Data Delivery

Minimum effective concentration and AC₅₀ value for each measured parameter (cell loss, nuclear morphology, DNA fragmentation, phospholipidosis, and steatosis)

Drug-induced steatosis is a serious issue for the pharmaceutical industry as it could lead to liver failure and, therefore, to the withdrawal of the compound from the market⁶.

Figure 1

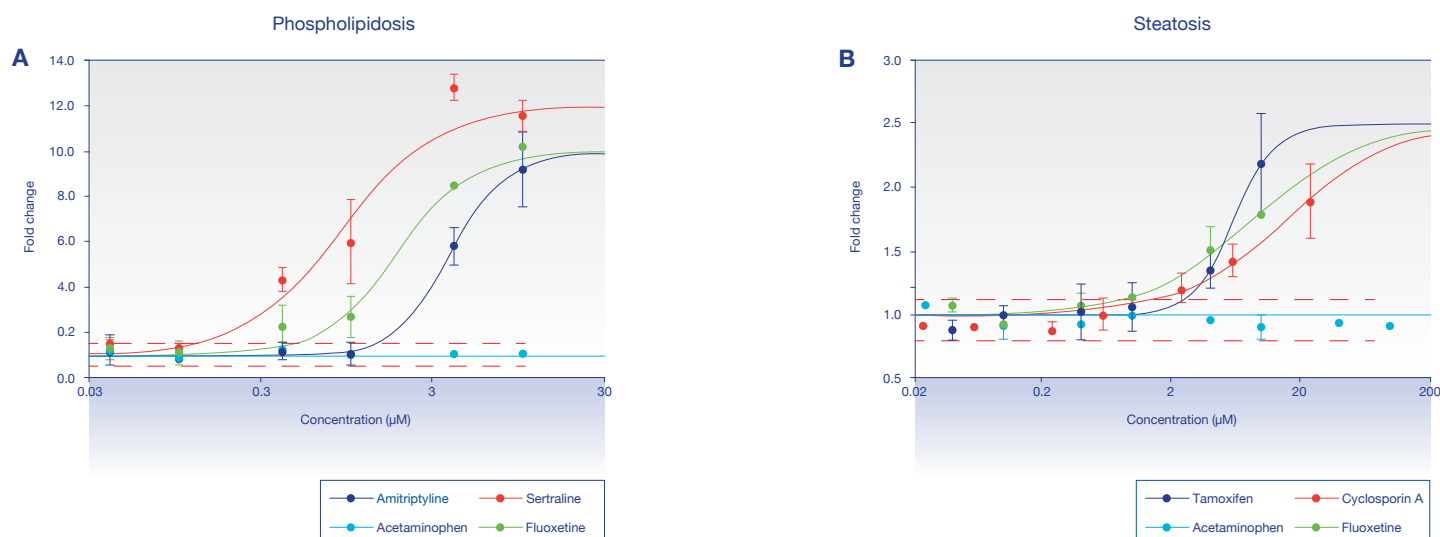
Simultaneous monitoring of multiple cellular responses (cell loss, nuclear morphology, DNA fragmentation, phospholipidosis, and steatosis) in HepG2 cells using High Content Screening.



The images for treated cells (exposed to sertraline (10 µM) or cyclosporin A (6 µM) over 72 hr) are representative of positive phospholipidosis (B) and steatosis (C) responses. Data are compared with those from vehicle control wells (A) (exposed to 0.5% DMSO over 72 hr). In addition to measuring these responses, adverse effects on cell health are determined by monitoring cell loss and nuclear morphology.

Figure 2

Representative data showing the observed responses to known compounds exhibiting positive phospholipidosis (A) and steatosis (B) responses over 72 hrs. Acetaminophen is used as a negative control in both cases. Data are compared with those from vehicle control wells (exposed to 0.5% DMSO over 72 hr). Dotted lines represent 2 standard deviations of the mean of the vehicle control data.



Drug-induced phospholipidosis and steatosis can be determined using High Content Screening. Measuring multiple parameters from a single analysis, allows the simultaneous determination of changes in cell loss, nuclear size and intensity, and the presence of phospholipidosis and steatosis (Figures 1 and 2).

References

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