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Microscopic Techniques to Evaluate Phospholipidosis I*n Vitro*: Applications in Drug Discovery.

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Chronic administration of cationic amphiphilic drugs (CADs) can induce phospholipidosis (PL) in humans and animals. PL is characterized by the excessive intracellular accumulation of phospholipids within lysosomes to form lamellar concentric bodies (Fig.1) leading to lysosomal dysfunction and lysosomal lysis [1]. Four major hypotheses have been proposed for the mechanism of CAD-induced PL [2]. First, CADs bind to phospholipids and the complex becomes resistant to degradation. Second, these molecules may directly inhibit lysosomal phospholipase activity, the enzymes responsible for phospholipid catabolism. Third, they may influence the biosynthesis of phospholipids and, finally, they may inhibit the clearance of phospholipid-containing lysosomes by modulating processes involved in exocytosis. Small molecules sharing common chemical properties with CADs raise safety concerns due to the potential toxicological consequences associated with the accumulation of drugs in affected tissues. Thus, PL liabilities have led pharmaceutical scientists to establish early and predictive screens in drug discovery for PL.

In animal models, the induction time for PL may be a few days to several months, depending on the affinity of the drugs for susceptible tissues, and phospholipid accumulations can be detected using electron microscopy, the gold standard technique. Unfortunately, this approach is not amenable to early discovery in the pharmaceutical industry, which requires high throughput evaluation of early lead candidates using minimal amounts of compounds. In contrast, in cell cultures, phospholipids can accumulate intracellularly and induce lysosomal concentric lamellar body formation after only a few hours of exposure to phospholipidosis-inducing, agents making an *in vitro* approach more attractive. Fluorescent probes have been successfully used to monitor the accumulation of phospholipids in lysosomes (Fig. 2) using primary rat hepatocytes and computer assisted image analysis [4,5]. In this presentation, we will discuss the significance of PL to the pharmaceutical industry and how new quantitative microscopic techniques utilizing automated image analysis systems are being used in drug discovery. These techniques have been successfully employed during lead optimization and during compound prioritization to help rank order compounds to select candidates with optimal toxicologic profiles.

References

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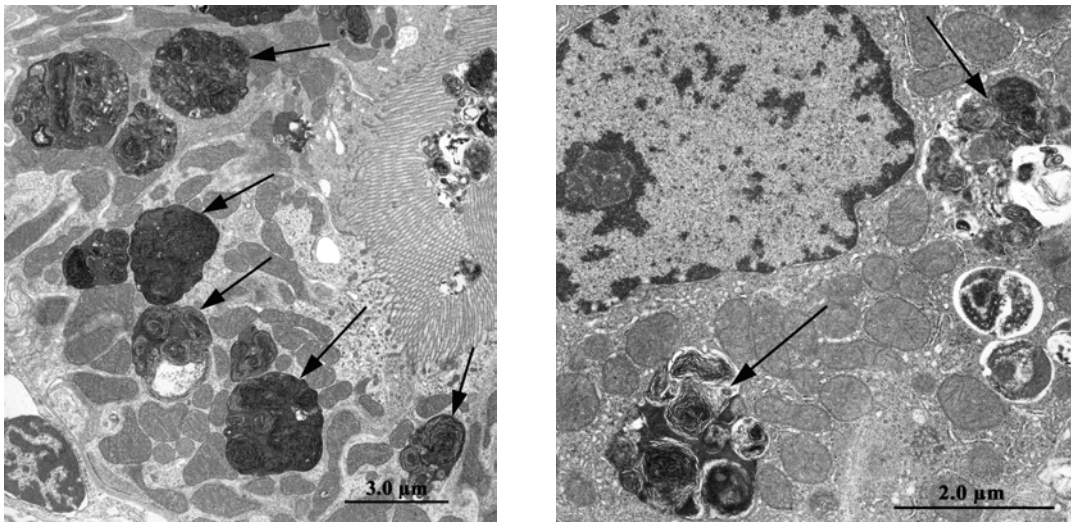

Fig. 1. Electron micrographs of lamellar bodies (arrows) in kidney proximal tubule epithelial cells (left), and hepatocytes (right) of a rat following administration of an anticancer compound.

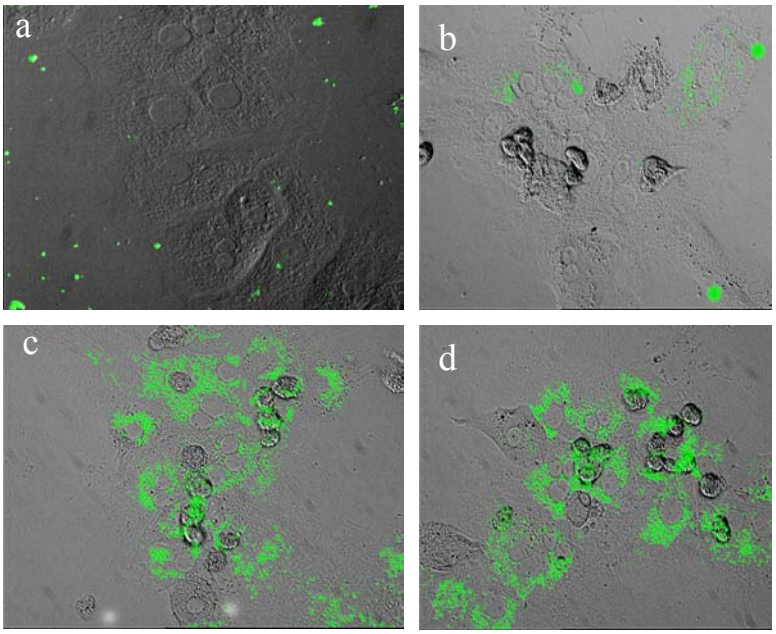

Fig. 2. Dose-dependent accumulation of lysosomal phospholipids as monitored by N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-dipalmitoylphosphatidylethanolamine (NBD-PE) in primary rat hepatocytes exposed for 24 hrs to either vehicle (a, 0.5%DMSO) or amiodarone at 1.50 µM (b), 6.13 µM (c), and 12.50 µM (d).

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