A Reliable High-Throughput Enzymatic Screen for Drug-Induced Phospholipidosis

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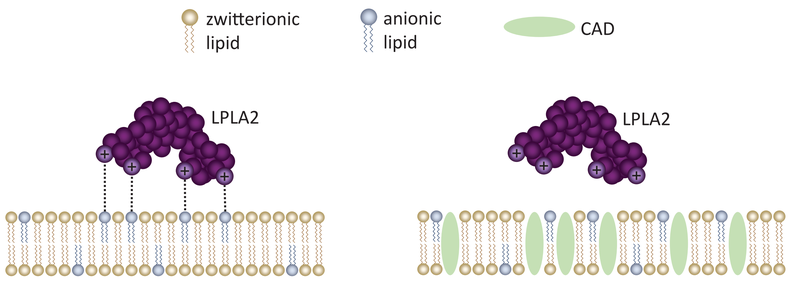
OVERVIEW

- FDA considers drug induced phospholipidosis (DIPL) a serious problem, it can delay pre-clinical & clinical drug development. Currently, there are no high throughput screening (HTS) methods for DIPL detection
- Based on the recently described mechanism for DIPL, we utilized lysosomal phospholipiase A2 (LPLA2) and the quenched fluorescent probe, LS-1, to test a selected library of 60 compounds and their tendency to promote DIPL. The library included some marketed cationic amphiphilic drugs (CADs), such as amiodarone
- We developed a reliable HTS and automation-compatible assay for detecting DIPL with 88% sensitivity and 100% specificity

INTRODUCTION

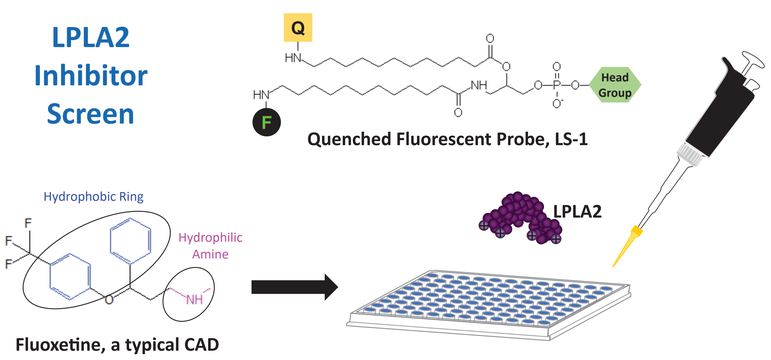


Four proposed hypotheses for the mechanism of DIPL:

- ❖ **CADs bind to phospholipids**
- ❖ CADs bind to lysosomal phospholipases
- ❖ CADs stimulate phospholipid synthesis in the cells
- ❖ CADs induce the dissociation of a lysosomal hydrolase from the lysosomal membrane

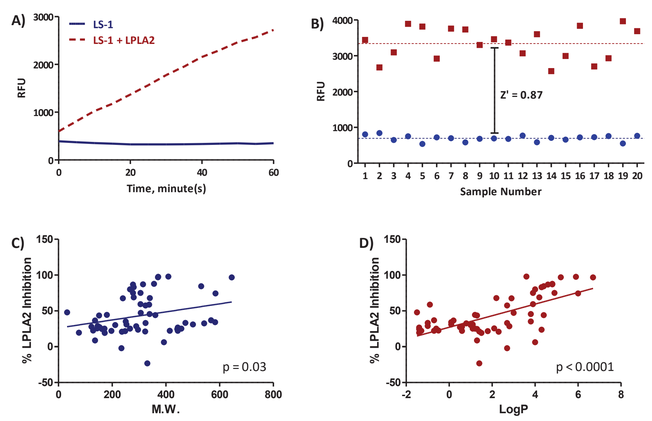
METHOD

LPLA2 Inhibitor Screen

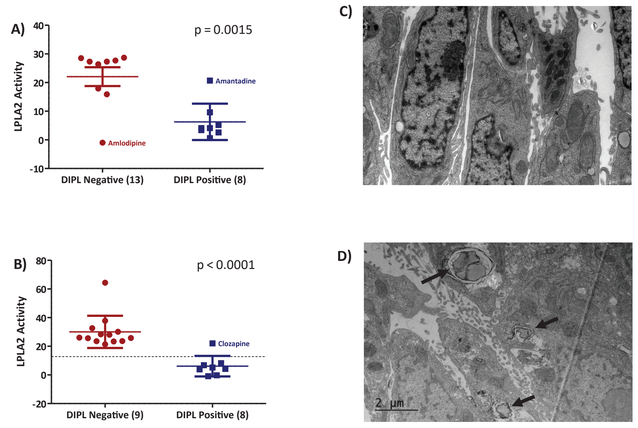


- 60 compounds were plated blindly in 384-well plates at Frontier Scientific
- Plates were then transferred to Echelon Biosciences for testing
- Plated compounds were pre-incubated with LS-1 in assay buffer for 1 hour with shaking
- LPLA2 was then added to start the reactions
- The fluorescence signal was recorded in kinetic mode at 490 nm excitation & 540 nm emission
- Data was analyzed blindly at Echelon Bioscience before compounds were decoded by Frontier Scientific

RESULTS



The quenched fluorescent probe, LS-1, allows continuous readings for kinetic enzyme studies (A) with a Z' factor of 0.87 (B). Significant correlation was observed between the percent LPLA2 inhibition and both the compounds molecular weight (C) and lipophilicity, LogP (D).



17 compounds with known TEM results were used to develop the assay cutoff parameter (A). Follow up TEM images of HepG2 cells with amantadine (C) & amlodipine (D) treatment confirmed the LPLA2 activity results to be correct. An additional 21 compounds were used to further validate the assay (B) demonstrating 88% sensitivity & 100% specificity.

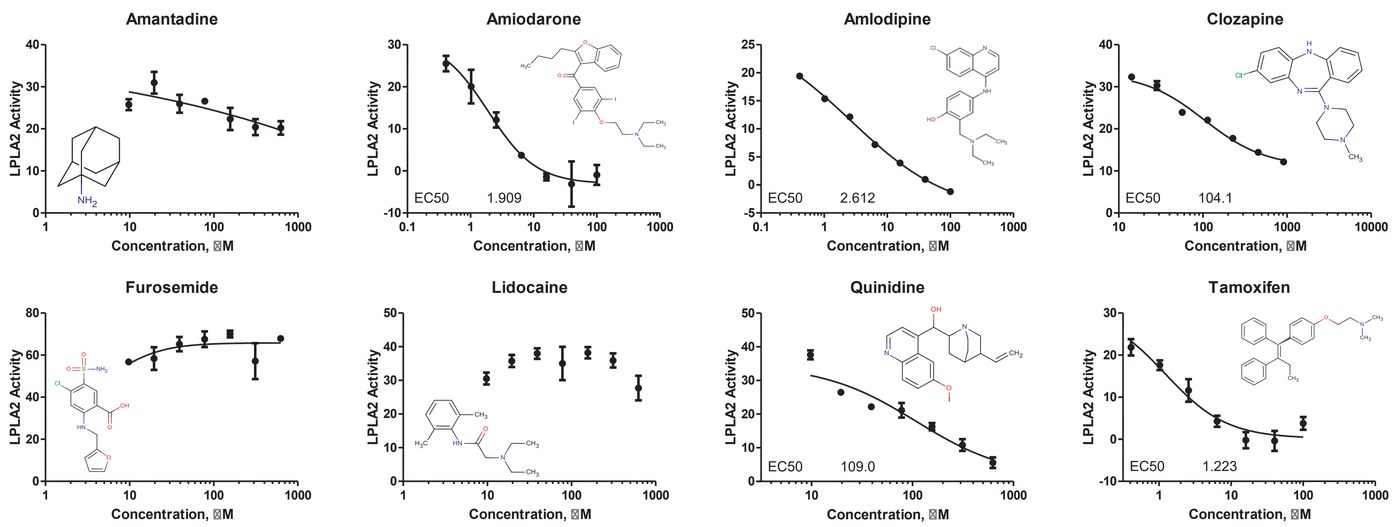


Table 1. Pairwise Comparison		Accuracy	Sensitivity	Specificity
Echelon LPLA2 Inhibitor Screen	n = 28	**93%**	**87%**	**100%**
NBD-PC/PE Cell Based Assay		75%	87%	62%
Echelon LPLA2 Inhibitor Screen	n = 19	**95%**	**92%**	**100%**
LysoTracker Cell Based Assay		74%	67%	86%
Echelon LPLA2 Inhibitor Screen	n = 19	**95%**	**92%**	**100%**
LipidTox Cell Based Assay		68%	67%	71%
Echelon LPLA2 Inhibitor Screen	n = 34	**94%**	**88%**	**100%**
Ploemen *in silico* Assay		88%	81%	94%

8 compounds were further evaluated by generating full titration curves. The non-CAD compounds, furosemide & lidocaine, show no effect on LPLA2. Not all CADs produced valid IC50 values, such as amantadine, which matched the in-house TEM imaging result as a DIPL negative compound. This result suggests *in silico* models based on compound structure may not predict DIPL accurately.

Comparison between the LPLA2 Inhibitor Screen and other current DIPL detection assays demonstrates that the LPLA2 Inhibitor Screen is a more effective assay for DIPL prediction (Table 1).

CONCLUSIONS & ACKNOWLEDGEMENTS

- ❑ CAD interfering with LPLA2 activity is a promising mechanism for DIPL
- ❑ The Echelon HTS LPLA2 Inhibitor Screen is better than TEM for DIPL prediction
- ❑ We appreciate Dr. James M Willard from FDA in sharing the Phospholipidosis Working Group database
- ❑ We thank our collaborators, Dr. James A. Shayman & Dr. Piotr Rzepecki, for advice
- ❑ This study is supported by FDA, SBIR Contract, 5R44FD004052

LPLA2 Inhibitor Screen is available from Echelon Biosciences, Inc.
For more information, please visit our website (www.echelon-inc.com)

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