ADME Research Services Brief

Lysosomal Trapping

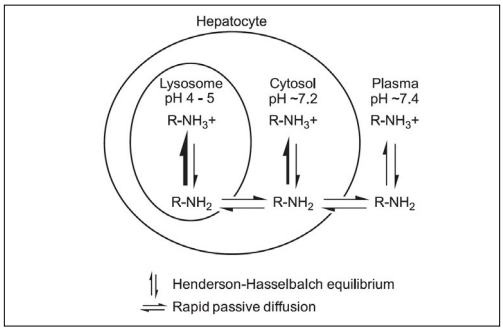
Evaluate inhibition and uptake of drug candidates into lysosomes

Lysosomal trapping is a physicochemical, that is, non-enzymatic and non-transporter mediated, process by which lysosomes sequester lipophilic amine or amphiphilic drugs also called cationic amphiphilic drugs (CADs). At physiological pH, these drugs readily cross the lysosomal membrane by passive diffusion where they become protonated due to the acidic environment. This restricts their diffusion back across the lysosomal membrane into the cytosolic space. As a result, many cationic drugs accumulate in the lysosomes and exhibit high liver-to-blood ratios. The accumulation of these drugs in the lysosomes is also associated with the development of drug-induced phospholipidosis, a condition that is characterized by the excess accumulation of phospholipids in tissues such as the liver, brain, kidney, and lung.

Competition for lysosomal trapping has been the subject of speculation as a potential mechanism for drug-drug interactions (DDIs). Many central nervous system (CNS) and cardiovascular drugs are lysosomotropic compounds i.e., they undergo lysosomal sequestration. It is possible that concomitant administration of lysosomotropic compounds could lead to elevated drug exposure levels when competition for lysosomal sequestration increases or the lysosomal pH is elevated due to amine accumulation.

BioIVT offers lysosomal trapping assays using either LysoTracker™ Red or cellular partitioning. Both assays evaluate inhibition and mechanistic uptake to predict lysosomotropic behavior and assess the potential risk and impact for pharmacokinetics, DDI, and other safety parameters.

Lysosomal trapping illustrated on a lipophilic amine compound ($R\text{-}NH_2$) (Kazmi *et al.*, Drug Metab Dispos. 2013; 41(4):897-905)



Study Design

Study Design Using LysoTracker Red

Element	Feature	Standard
Design	Immortalized Fa2N-4 hepatocytes	✔
	Fluorescent probe LysoTracker Red DND-99	✔
	Drug candidate (test article) concentrations	7
	Time point (30 min)	1
	Analysis using fluorescent plate reader	3
	Positive control propranolol	✔
	Negative control (solvent)	✔
Deliverables	LysoTracker Red DND-99 inhibition IC_{50} curve	✔
	Data Summary Report	✔

Study Design Measuring Cellular Partitioning

Element	Feature	Custom
Design	Immortalized Fa2N-4 hepatocytes	✔
	Test article incubation ± NH_4Cl (or other inhibitor)	✔
	Test article concentrations	custom
	Number of time points / incubation time	custom
	Quantification of test article by LC-MS/MS	✔
	Solvent control, positive control (propranolol)	✔
	Cytotoxicity using lactate dehydrogenase release (optional)	✔
Deliverables	% of control, ion-trapping dependent uptake as % of total cell uptake	✔
	Data Summary Report	✔

Lysosomal Trapping

Methodological Considerations and Test Systems

Lysosome-rich immortalized Fa2N-4 hepatocytes are used as the *in vitro* test system to evaluate lysosomal trapping of lipophilic amine or amphiphilic drugs. These transformed cells are ideal as they propagate in culture and readily attach to collagen plates but do not retain significant metabolism or transporter activity.

BioIVT offers two different methods to assess lysosomal trapping:

- **LysoTracker™ Red DND-99:** The fluorescent probe has been shown to be highly specific for lysosomal accumulation. Co-incubation with lysosomotropic compounds will block the accumulation of LysoTracker Red and an IC_{50} can be determined. This design is cost-effective and suitable for evaluating more than one drug candidate early in drug development.
- **Measuring Cellular Partitioning:** The accumulation of the test article is measured in the presence and absence of ammonium chloride (or another lysosomotropic compound) using LC-MS/MS. Lysosomal partitioning is determined as percent of control.

Study Deliverables

BioIVT's lysosomal trapping study reports using LysoTracker Red include the graphical and tabular illustration of the inhibition curve data and the IC_{50} is calculated when possible (Figs. 1 and 2, Table 1).

When measuring cellular partitioning, the report includes the results for accumulation, % of control and ion-trapping dependent uptake as % of total cell uptake in graphical and tabular form.

Fig. 1. Effect of known lysosomotropic compounds on LysoTracker Red fluorescence and calculation of IC_{50} values (Kazmi *et al.*, Drug Metab Dispos. 2013; 41(4):897-905)

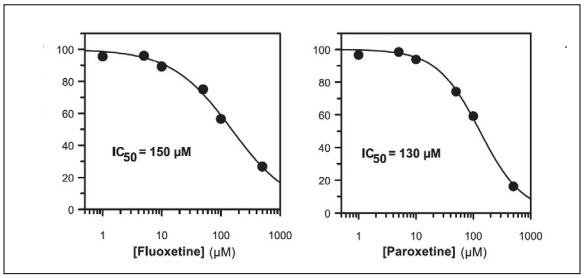


Fig. 2. Cellular partitioning data for propranolol, imipramine, and atorvastatin illustrating lysosomal trapping for propranolol and imipramine (Kazmi *et al.*, Drug Metab Dispos. 2013; 41(4):897-905)

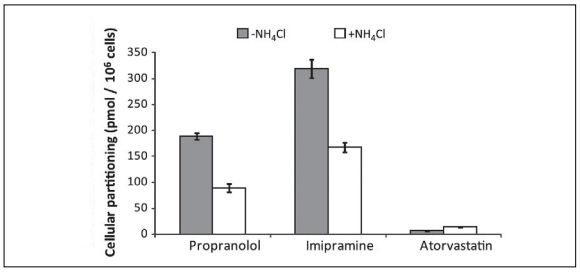


Table 1. Lysosomal trapping data from measuring cellular partitioning. No lysosomal trapping was observed for drug candidate 1 and moderate trapping for drug candidate 2. Propranolol served as the positive control, incubation time was 30 min.

Substrate	Accumulation (pmol/10⁶ cells)		Accumulation (pmol/mg) (mean ± SD)		Percent of control (Accumulation)		Ion-trapping dependent uptake (pmol/10⁶ cells)	Ion-trapping dependent uptake (pmol/mg)	Ion-trapping dependent uptake as % of total cell uptake
	0 mM NH_4Cl	50 mM NH_4Cl	0 mM NH_4Cl	50 mM NH_4Cl	0 mM NH_4Cl	50 mM NH_4Cl			
Drug candidate 1	0.325 ± 0.015	0.314 ± 0.020	9.53 ± 0.43	9.21 ± 0.58	100	96.6	0.0109	0.320	3.37
Drug candidate 2	0.947 ± 0.025	0.599 ± 0.021	27.8 ± 0.7	17.6 ± 0.6	100	63.3	0.347	10.2	36.7
Propranolol	22.1 ± 0.5	10.9 ± 0.4	650 ± 15	320 ± 11	100	49.2	11.2	330	50.8

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