Phospholipid Substrates for Lysosomal Phospholipase A2 (LPLA2)

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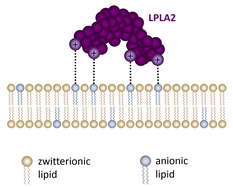
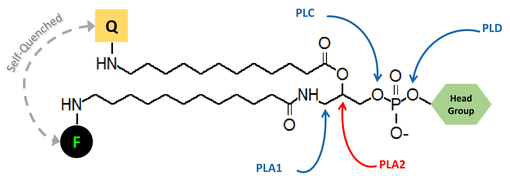
1. OVERVIEW

- **Lysosomal phospholipase A2 (LPLA2) is involved in both drug-induced phospholipidosis (DIPL) & drug-induced lupus (DIL)**
- **Evaluated phospholipid charge & structure in relation to LPLA2 activity**
- **Studied plasma LPLA2 substrate profile using a self-quenched fluorogenic probe specifically designed for LPLA2 in acidic environment**

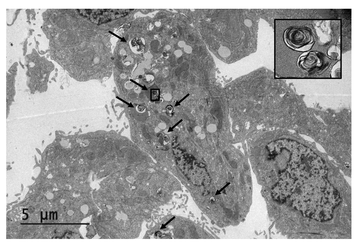
2. LYSOSOMAL PHOSPHOLIPASE A2 (LPLA2)

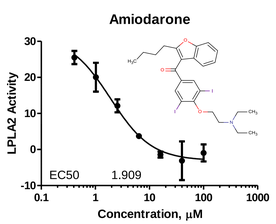
- **LPLA2:**
  - **Localized in lysosome with optimal pH at 4.5**
  - Has both PLA1 and PLA2 activity
  - Also has transacylase activity when an acceptor, such as N-acetyl-sphingosine (NAS), is present
- **Detection method:**
  - A self-quenched fluorogenic probe is synthesized specifically for PLA2 activity
  - The self-quenched fluorogenic probe is incorporated into liposomes and used as a substrate for LPLA2
  - Reaction is performed under acidic condition



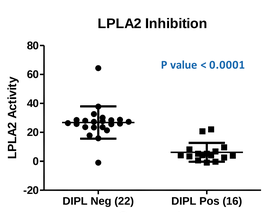


3. LPLA2 & DISEASE



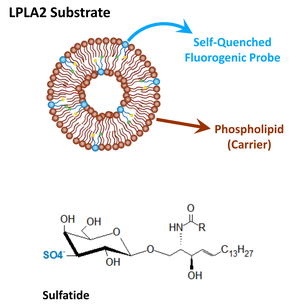


- **Drug-induced phospholipidosis (DIPL)**
  - Condition of excessive accumulation of intracellular phospholipids caused by common cationic amphiphilic drugs (CADs) on the market
  - **CADs significantly inhibit LPLA2 activity in vitro**

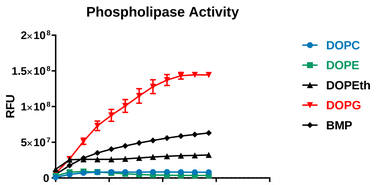


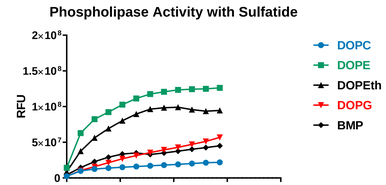
- **Drug-induced lupus (DIPL)**
  - Systemic autoimmune disease when immune system attacks own tissues and organs
  - 5-10% lupus is triggered by long-term drug use
  - **LPLA2 KO mice express phenotypes similar to lupus**

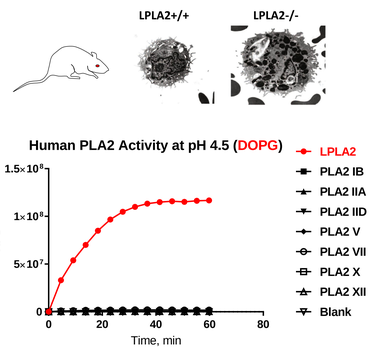
4. LPLA2 ACTIVITY & MEMBRANE PROPERTIES



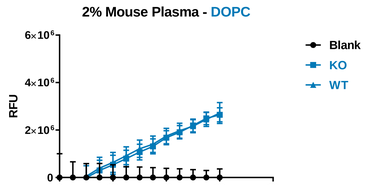
- **The self-quenched fluorogenic probe incorporated into negative charged phospholipids such as DOPG & DOPEth results in significantly higher LPLA2 activity**
- **No significant LPLA2 activity when the self-quenched fluorogenic probe incorporated into neutral phospholipids such as DOPC & DOPE**
- **The self-quenched fluorogenic probe also shows strong LPLA2 activity when incorporated in the bis(monoacylglycerol)phosphate (BMP), a special late endosome lipid**
- **The highly negatively charged lipid, sulfatide, significantly enhances the LPLA2 activity towards the self-quenched fluorogenic probe incorporated into neutral phospholipids such as DOPE**

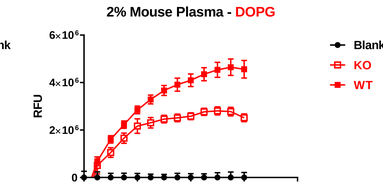




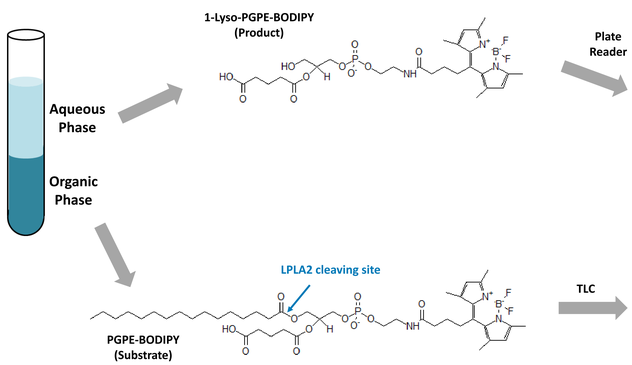


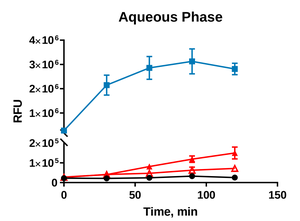
- **LPLA2 KO mice were generated by systemic deletion of exon 5 (catalytic site) of the LPLA2 gene**
- **Some non-specific PLA2 activity was detected in both mouse WT & KO plasma**
- **A negatively charged phospholipid carrier in the self-quenched fluorogenic probe liposome substrate is critical for specific LPLA2 activity detection**
- **No other PLA2s except LPLA2 metabolize the self-quenched fluorogenic probe incorporated in negatively charged phospholipid (DOPG)**

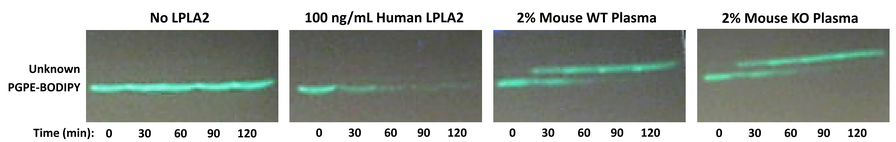




5. LPLA2 sn-1 PHOSPHOLIPASE ACTIVITY







- **Substrate (PGPE-BODIPY incorporated in DODPC) depletion observed in both recombinant human LPLA2 and mouse plasma**
- **Human recombinant LPLA2 cleaves only at the sn-1 position on truncated & oxidized phospholipid fluorescent probe**
- **Unknown product (possibly by PAF-AH cleaving at the sn-2 position) is formed in mouse plasma**
- **Mouse WT plasma shows higher aqueous product formation than mouse LPLA2 KO plasma**

6. CONCLUSIONS & ACKNOWLEDGEMENTS

- **CAD interfering with LPLA2 activity is a promising mechanism for DIPL**
- **Membrane charge & structure are critical for LPLA2 substrate engagement**
- **Mouse WT and KO plasma LPLA2 activity presents different substrate profile**

- **We appreciate Dr. James M Willard from FDA in sharing the Phospholipidosis Working Group database**
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