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:
  - Located in lysosome with optimal pH at 4.5
  - Has both PLA1 and PLA2 activity
  - Also has transacylase activity when an acceptor, such as IV-acyl-lipid, 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 (DIL)
  - Systemic autoimmune disease when immune system attacks own tissues and organs
  - 5-15% 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 & DOPE 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 bi(monomono)glycerophosphate (BMP), a special late endosome lipid
- The highly negatively charged lipid, suitable, significantly enhances the LPLA2 activity towards the self-quenched fluorogenic probe incorporated into neutral phospholipids such as DOPE

5. LPLA2 sn-1 PHOSPHOLIPASE ACTIVITY

- Substrate (PGP-BOC-DPY) incorporated in DOPE-C5 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 PLA1) detected when the self-quenched fluorogenic probe is incorporated in negatively charged phospholipids (DOPG)

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

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