ALDEHYDE TRAPPING AGENT NS2 BLOCKS FORMATION OF FATTY ALDEHYDE ADDUCTS WITH PHOSPHATIDYLETHANOLAMINE AND SUGGESTS POTENTIAL THERAPEUTIC APPROACH FOR SJÖGREN-LARSSON SYNDROME

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Background

Sjögren-Larsson syndrome (SLS) is a rare autosomal recessive neuro-ichthyotic disorder caused by mutations in the *ALDH3A2* gene encoding fatty aldehyde dehydrogenase (FALDH), which catalyzes the oxidation of fatty aldehyde to fatty acid. Associated clinical features include ichthyosis, developmental delay/intellectual disability, spastic diplegia and a retinal crystalline maculopathy (Fig 1).

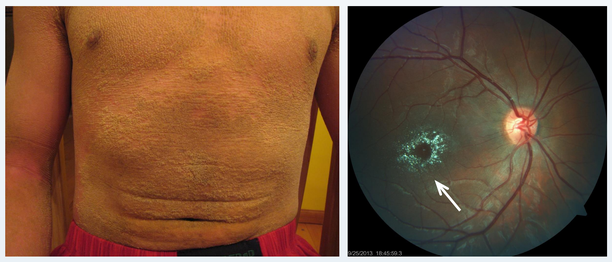


***Fig 1*. Ichthyosis (left) and retinal perimacular crystalline inclusions (right, arrow).**

The pathogenic mechanisms of SLS are thought to be related to accumulation of long-chain fatty aldehydes (C16:0-C18:0), which are derived from metabolism of several lipids (Fig 2). Most aldehydes are short-lived toxic molecules due to their propensity to form covalent Schiff base adducts with amino-containing molecules such as phosphatidylethanolamine (PE), proteins or other components of cellular membranes. Fatty aldehyde adducts are hypothesized to cause the symptoms in SLS.

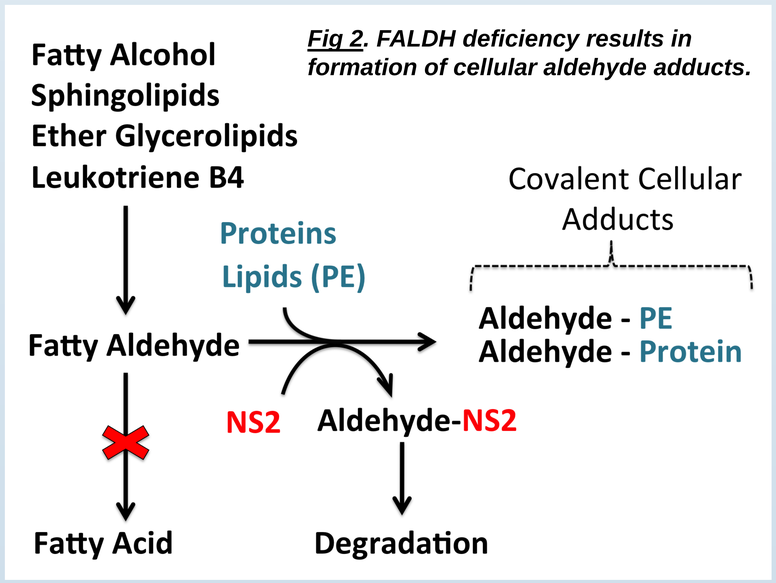


***Fig 2*. FALDH deficiency results in formation of cellular aldehyde adducts.**

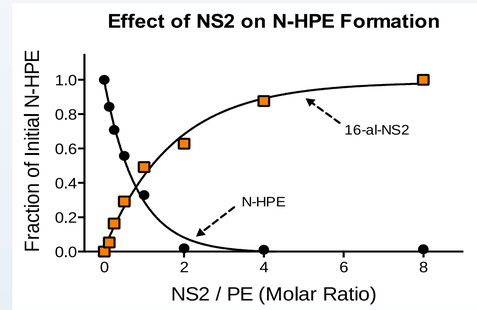
We therefore investigated the ability of a newly developed aldehyde-scavenging drug NS2 [Aldeyra Therapeutics] to act as a sacrificial target for fatty aldehydes and prevent formation of adducts with cellular PE.

Methods

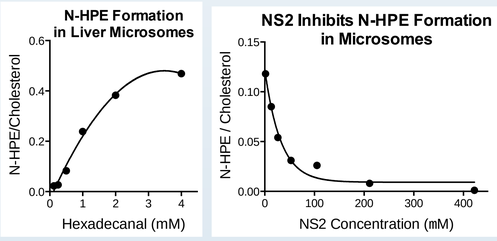
Hexadecanal (16:0-al) was incubated *in vitro* with: 1) PE in methanol solution, 2) mouse liver microsomes in PBS, and 3) cultured Chinese hamster ovary (CHO) cells. 16:0-al-PE Schiff base adducts were stabilized by treatment with a reducing agent sodium cyanoborohydride (NaBH3CN). After lipid extraction of the N-hexadecanoyl-PE (N-HPE) and alkaline hydrolysis, the unique hydrolysis product of N-HPE (N-hexadecanoyl-ethanolamine) was quantitated as its trimethylsilyl derivative using GC-MS with SIM (m/z 254).

Results

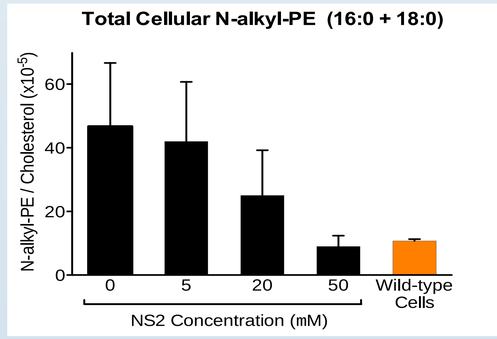
In methanol, 16:0-al formed Schiff base adducts with PE (N-HPE), which were inhibited by NS2 in a concentration dependent manner. Reduction in N-HPE formation was inversely related to the appearance of the competing 16:0-al-NS2 adduct.



Incubation of mouse liver microsomal membranes (containing endogenous PE) with 16:0-al in phosphate buffer resulted in formation of N-HPE in a concentration dependent manner. NS2 added to the reaction inhibited N-HPE formation.



FALDH-deficient CHO cells accumulated 5-fold more N-alkyl-PE (total 16:0 + 18:0) under standard growth conditions compared to wild-type cells. Mutant cells grown in the presence of NS2 for 4 days showed a concentration-dependent reduction in N-alkyl-PE content to levels seen in wild-type cells.



Conclusions

- **Long-chain fatty aldehyde adducts with PE are readily formed *in vitro* and accumulate in FALDH-deficient mammalian cells. It is probable that aldehyde adducts also form with other cellular lipids or proteins.**
- **By acting as a competitive target molecule, NS2 blocks formation of fatty aldehyde adducts with PE in biological membranes (liver microsomes) and intact cells.**
- **Aldehyde-trapping agents, such as NS2, may constitute a novel therapeutic approach for lowering aldehyde adducts in SLS.**

Acknowledgements

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