INI-822: TARGETING HSD17B13, A GENETICALLY VALIDATED TARGET FOR CHRONIC LIVER DISEASE, WITH A SMALL MOLECULE INHIBITOR IN MODELS OF NASH

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Introduction

HSD17B13 is a lipid droplet enzyme whose substrates include bioactive lipids Oxylipins are bioactive lipids derived from polyunsaturated fatty acids that act as signaling molecules and have been found to be substrates of HSD17B13[1]. Inactive alleles of HSD17B13 are associated with:

- Decreased risk of developing NASH and cirrhosis[1]
- Decreased inflammation and fibrosis[2]
- Increased hepatic phosphatidylcholines containing polyunsaturated fatty acids[3]

HSD17B13 interacts with proteins on the mitochondria and endoplasmic reticulum[4]. These data suggest a role for HSD17B13 in mediating bioactive lipid flux in NASH and other diseases characterized by hepatocyte lipid accumulation.

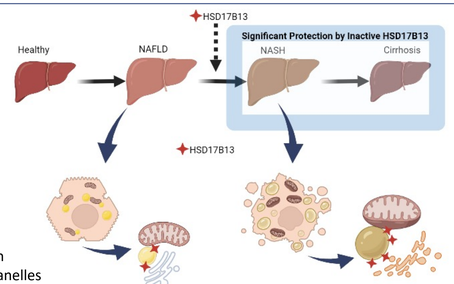
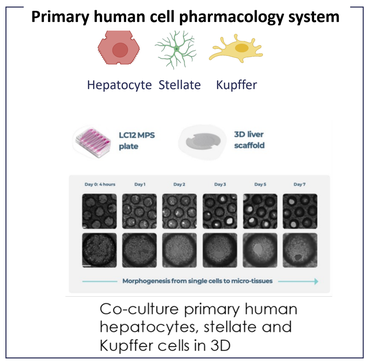


Figure 1. HSD17B13 in disease, cells and organelles

Methods and Materials

INI-822 was evaluated for inhibition of purified human HSD17B13-oxidation of multiple substrates by quantitation of product formation. Selectivity was determined by biochemical assays for HSD17B family members and broad off-target screens. Triplicate co-cultures containing primary human hepatocytes homozygous for the active HSD17B13 allele, Kupffer cells, and stellate cells were prepared in the LC12 microphysiological system (CNBio) in high fat media with or without INI-822. Fibrosis in the co-culture was analyzed by immunohistochemistry. INI-822 was dosed orally in 8 to 10 week-old Zucker obese rats. INI-822 concentrations, metabolomics (Biocrates) and lipidomics for oxylipins (West Coast Metabolomics Core/ UC Davis) were performed by LC/MS on media from the liver-on-a-chip and plasma from Zucker obese rats.



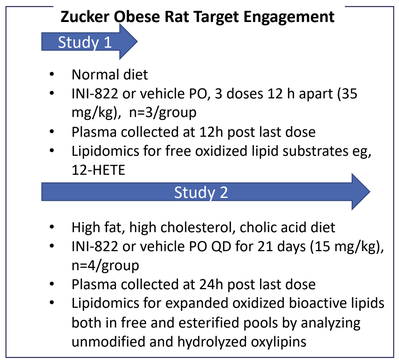


Figure 2. Liver-on-a-chip and Zucker obese rat study outlines

Results

INI-822 is potent and selective
Well tolerated and stable for in vitro and in vivo testing

INI-822 inhibits HSD17B13 with low nM potency. INI-822 has >100-fold selectivity over other HSD17B family members as well as safety and ADME targets including hERG. Hepatocyte stability supports testing in the primary liver-on-a-chip systems. INI-822 has low clearance and good oral bioavailability in mice, rats and dogs; modeling supports pharmacokinetics suitable for daily oral dosing. Pilot 7-day toxicology studies in 2 species are completed.

B13 Ki E2 (nM)	35 ± 4
Selectivity for HSD17B13 over HSD17B2, B3, B11, B14	>100 fold
Safety44/hERG/ non-GLP Ames/non-GLP Micronucleus	No significant off target

INI-822 Decreases Fibrotic Proteins in a Primary Human Liver-on-a-Chip System

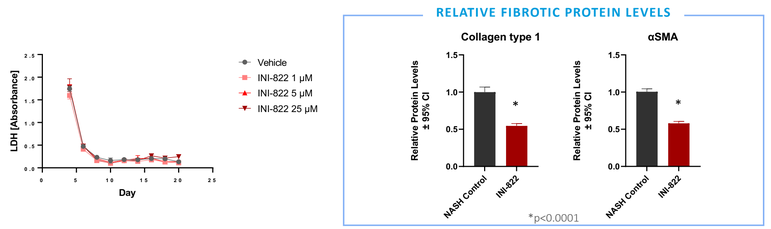


Figure 3. Liver-on-a-chip 20 Days in High Fat Media, 16 Days with INI-822
Vehicle and INI-822 allowed for high albumin levels consistent with retained hepatic characteristics and did not result in increased LDH release. INI-822 at 25 µM had >40% decrease of fibrotic proteins. In follow up study, INI-822 significantly decreased αSMA and collagen type1 at 1 and 5 µM (p<0.0001 for both). INI-822 free fractions measured as low as 45 nM in anti-fibrotic conditions. Study was performed twice in triplicate with 200 individual assessments on fibrotic protein levels.

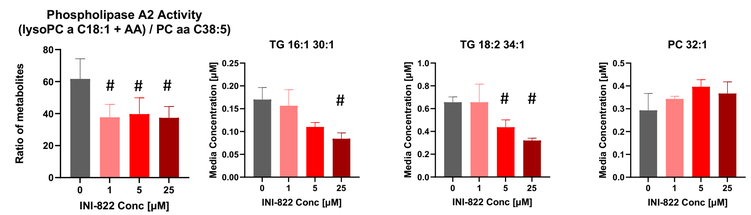
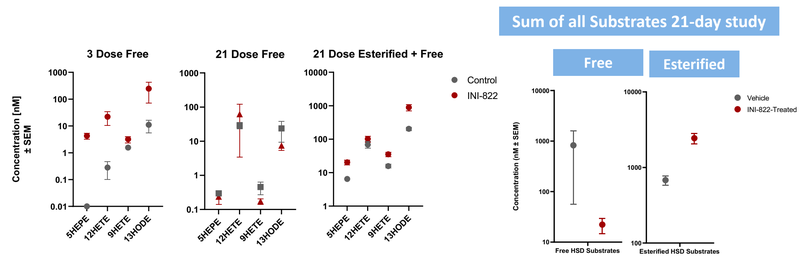


Figure 4. Metabolomics of the media show an evolution of the culture over time in the high fat media. Metabolomics of media from Day 20 show a decrease in product/substrate for PLA2, decreases in individual triglycerides and trends in increased individual phosphatidylcholines. Study was performed twice in triplicate. #p<0.05

Results

INI-822 Increases Free Substrates Acutely and Increases Esterified Substrates Following 3 Weeks Dosing in Zucker Obese Rats



INI-822 Alters Bioactive Lipids Following 3 Weeks Dosing in Zucker Obese Rats on High Fat High Cholesterol Diet

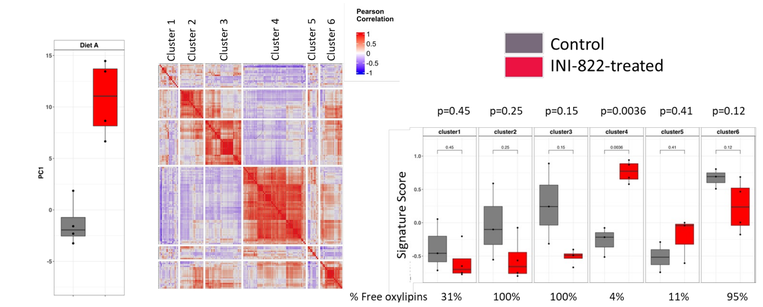


Figure 5. INI-822 oral dosing in Zucker rats increased bioactive oxylipin substrates in the esterified pool. Acutely, free several known substrates were elevated, but upon 3-week dosing, esterified 12-HETE and esterified substrates were elevated, and free substrates were decreased compared to plasma from vehicle treated rats. Data not shown: triglycerides were unchanged in response to INI-822. Principle component analysis identified one PC changed in response to INI-822. Hierarchical cluster analysis identified 6 clusters of bioactive lipids that segregated together. Cluster 4 which contains known HSD17B13 substrates was significantly elevated in response to INI-822 dosing and was composed dominantly with total (esterified + free) oxylipins.

Conclusions

- INI-822 has been selected as a drug development candidate based on the drug-like properties and improvement of fibrotic protein levels in a human-based system.
- INI-822 decreased fibrotic protein levels in a primary human liver-on-a-chip.
- INI-822 led to changes in the metabolomics profile of the media in the LoC system with decreased triglycerides and increased phospholipids.
- In vivo assessments of HSD17B13 substrates and products with and without inhibition by INI-822 confirm an increase in substrate levels that is initially free oxylipins and subsequently esterified.
- Inhibition of HSD17B13 over 3 weeks results in an increased pool of esterified bioactive lipids overall, suggesting a sequestration of bioactive lipids in response to drug.

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Disclosures: All authors are employees of Inipharm

References

Some images developed in BioRender
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