A NOVEL, HOST-DIRECTED, SMALL MOLECULE INHIBITOR OF HCV DISPLAYS SUSTAINED PHARMACOLOGICAL INHIBITION OF LIVER FATTY ACID SYNTHASE

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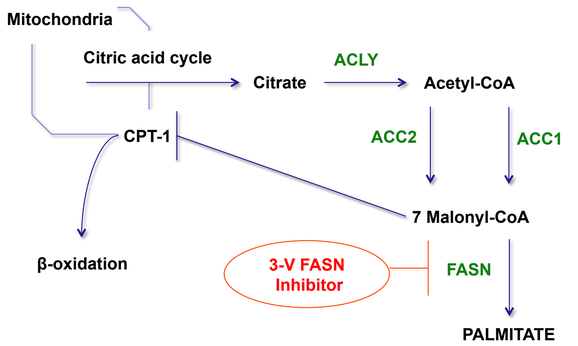
Abstract

BACKGROUND: Optimized new therapies for HCV are needed that have pan genotypic activity and a high barrier to viral resistance. To meet these challenges, 3-V Biosciences has developed a small molecule inhibitor of HCV that targets the host's fatty acid synthase (FASN) enzyme (Abstract 88). HCV infection increases the expression of FASN, the host enzyme responsible for the production of palmitate, and down regulation of FASN inhibits critical viral processes including entry into cells, RNA replication, and particle assembly. FASN inhibitors are expected to have pan-genotypic activity and pose a high barrier to viral resistance due to interference with multiple stages of the HCV lifecycle.

OBJECTIVE: The objective of this study is to characterize the pharmaceutical properties of a novel, small molecule inhibitor of FASN as a treatment for chronic HCV infection.

RESULTS: 3-V Bioscience's FASN small molecule inhibits the human FASN enzyme in biochemical and cell based assays with an IC_{50} of 0.049 µM and 0.025 µM, respectively. Activity against the HCV Gt1b replicon parallels FASN inhibition with an EC_{50} of 0.060 µM and no observed cytotoxicity. A 10 mpk oral dose of this compound is rapidly absorbed and highly bioavailable (~60%) in rats and dogs with an apparent half-life of 3.2 h and 3.9 h, respectively. Pharmacodynamic activity in rats is exposure-dependent: a 60 mpk dose causes complete inhibition of palmitate synthesis 12h after administration while a single 30 mpk oral dose causes >50 % inhibition at 12h. Palmitate synthesis remains suppressed by ~60% at 24 hours following a 60 mpk dose, consistent with a once-daily oral dosing regimen. The results of this current work demonstrate that 3-V Bioscience's FASN inhibitor can inhibit *de novo* fatty acid synthesis *in vivo* and has pharmaceutical properties necessary for clinical development.

In Vitro Activity Against Human, Rat, and Dog FASN



Species	Cell Line	Biochemical (µM)*	Palmitate (µM)†
Human	HeLa Ohio	0.049	0.025
Human	Huh-7	0.049	0.049
Dog	MDCK NBL-2	Not Tested	0.27
Rat	NMU	0.11	0.090

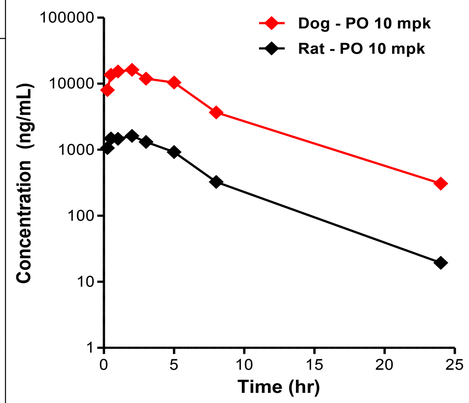
* Biochemical assay: measures the activity of human (crude extract from SKBr3 cells) or rat (purified from rat liver) FASN by measuring the release of coenzyme A with the dye CPM

† Palmitate assay: To measure *de novo* synthesis of palmitate by FASN, a stable labeled substrate ($^{13}C_2$ acetate) was added to cells and $^{13}C_2$ labeled palmitate was measured by LC-MS.

Oral Pharmacokinetics in Rats and Dogs

Dogs and rats display similar apparent half-lives after oral doses of 10 mg/kg, however exposures are 10 X higher in dogs

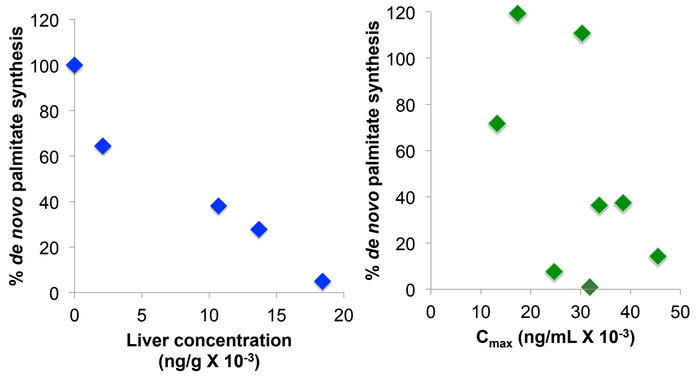
Species	Rat	Dog
Dose (mpk)	10	10
Half-life (hr)	3.2	3.9
T_{max} (hr)	1.2	1.5
C_{max} (ng/mL)	1780	16400
AUC_{last} (hr*ng/mL)	11300	118000
% F	59.6	57.8



Dose and Exposure Relationships to Inhibition of *de novo* Palmitate Synthesis *In Vivo*

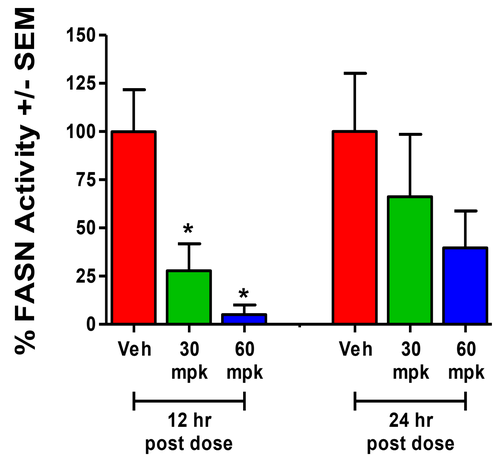
Inhibition of de novo palmitate synthesis correlates with liver concentration

Trend toward inhibition of de novo palmitate synthesis with high C_{max}

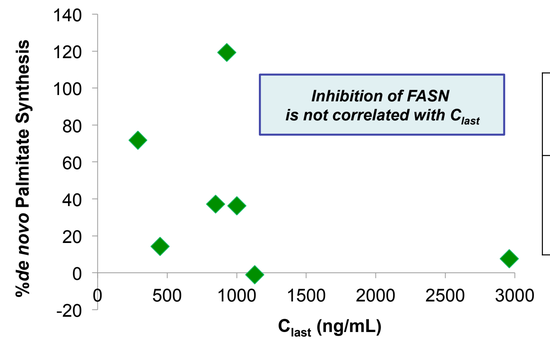


Rats were dosed with vehicle or doses of 3-V FASN inhibitor between 10 and 90mpk. 2 h prior to sacrifice, rats were injected with $^{13}C_2$ acetate tracer. $^{13}C_2$–palmitate was measured by LC-MS.

Sustained Inhibition of Palmitate Synthesis After a Single Dose



* p<0.05 by Mann–Whitney



Dose (mpk)	Half-life (hr)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{last} (hr*ng/mL)	C_{last} (ng/mL)
30	2.9	6.8	18500	208000	438
60	2.4	4.8	36300	419000	117

Rats were dosed with vehicle or doses of 30 or 60 mg/kg 3-V FASN inhibitor. 2 h prior to sacrifice, either 10 or 22 hours post dose, rats were injected with $^{13}C_2$ acetate. $^{13}C_2$–palmitate was measured by LC-MS

Conclusions

- 3-V's small molecule FASN inhibitor is a potent inhibitor of human fatty acid synthase
- A single oral dose can inhibit *de novo* palmitate synthesis up to 24 hours *in vivo*
- Inhibition is reversible *in vivo*
- Reduction of *de novo* palmitate synthesis correlates with liver concentration of the drug
- The drug's profile meets the criteria to advance into Phase 1 clinical studies in 2013

Disclosure: All authors are current or former employees of 3-V Biosciences, Inc.

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