17-β Hydroxysteroid Dehydrogenase 13 Inhibitors are Hepatoprotective and Anti-Inflammatory In a Mouse Model of Autoimmune Hepatitis

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BACKGROUND

Genome-wide association studies identified a loss of function gene variant (rs72613567:TA) for 17-β hydroxysteroid dehydrogenase 13 (HSD17B13), a hepatic lipid droplet-associated protein, linked to decreased risk for chronic liver diseases. HSD17B13-deficient TA variant carriers have delayed onset of autoimmune hepatitis (AIH)[1], and presence of the TA variant decreases an AIH polygenic risk score in an allele-dependent manner[2]. HSD17B13 inhibitors, previously shown to be anti-inflammatory *in vivo* with modulation of sphingolipids, were evaluated in a mouse model of AIH for anti-inflammatory and hepatoprotective effects.

Delayed Onset of AIH Observed In Patients with HSD17B13 Deficient TA Variant

Supplementary Table 6: Characteristics of patients and laboratory data at end of follow-up according to *HSD17B13* genotype

	Homozygous ancestral (TT)	Heterozygous insertion (T:TA)	Homozygous insertion (TA/TA)	p-value
Number of patients	135	78	24	
Age at last visit [years]	51.1 ± 1.4	50.7 ± 1.9	59.3 ± 3.3	0.06
Age at diagnosis [years]	37.2 ± 1.5	39.3 ± 2.0	48.3 ± 3.6	0.02
Duration of AIH [years]	13.3 ± 0.7	11.0 ± 1.0	10.9 ± 1.7	0.09
Liver biopsy - no fibrosis - fibrosis - cirrhosis	N=125 37 (29.6%) 52 (41.6%) 36 (28.8%)	N=70 16 (22.9%) 32 (45.7%) 22 (31.4%)	N=19 5 (26.3%) 9 (47.4%) 5 (26.3%)	0.9#
Liver biopsy - no steatosis - steatosis	98 (78.4%) 27 (21.6%)	56 (80%) 14 (20%)	16 (84.2%) 3 (15.8%)	0.8#
AST [x ULN]	1.60 ± 0.14	1.27 ± 0.18	1.38 ± 0.33	0.6
ALT [x ULN]	1.61 ± 0.18	1.08 ± 0.23	1.25 ± 0.41	0.2

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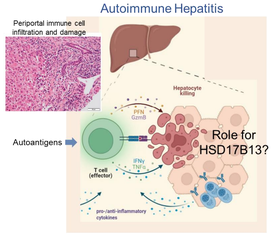
Autoimmune Hepatitis



Seminars in Immunopathology (2022) 44:411–427

Figure 1. Rationale for targeting HSD17B13 for Autoimmune Hepatitis.

1. Mederacke et al, *Aliment Pharmacol Ther* 2020 Jun 51(11); 1160-1168
2. Zandanell et al, *J Pers Med.* 2023 Mar 17;13(3):540; online
3. Schultheiß et al, *Semin Immunopathol* 2022; 44(4): 411–427.

METHODS

Multiple chemical series of HSD17B13 inhibitors (HSD17B13i) were identified and optimized for potency, selectivity, and pharmacokinetic properties.

In Vitro. HSD17B13 inhibition was monitored by Rapid Fire mass spectrometry in biochemical and cellular assays, which utilized either recombinantly expressed HSD17B13 or HEK293 stably expressing human or mouse HSD17B13, respectively. Leukotriene B4 served as the substrate for biochemical assays, whereas estradiol was used in cellular assays.

In Vivo. 8-week-old male C57BL/6J mice were pretreated with HSD17B13i by oral gavage for 3 days, followed by retro-orbital vein delivery of concanavalin (ConA) one hour after last dose. Liver, spleen, and plasma were collected at 6 hours post-ConA injection. Plasma ALT levels were measured by a colorimetric enzymatic assay. Inflammatory gene markers were evaluated in liver and spleen by qPCR. Plasma cytokines and chemokines were measured by mesoscale immunoassay. The HSD17B13i effect on sphingolipids was evaluated in mouse liver and primary human hepatocytes by mass spectrometry. Primary human hepatocytes deficient for HSD17B13 (rs72613567:TA) were used in rescue studies, where HSD17B13 was restored *via* infection of an adenoviral construct overexpressing HSD17B13.

Statistical analysis was performed with One Way ANOVA followed by Dunnett's multiple comparison test.

RESULTS

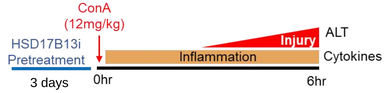
HSD17B13 Inhibitors are Potent and Selective

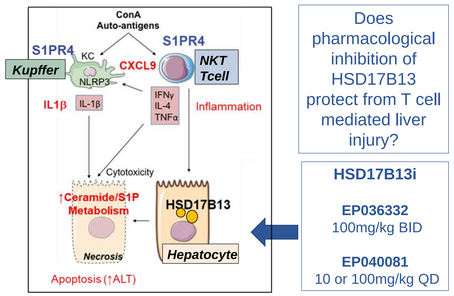
Table 1. Potency and Selectivity of Distinct Chemical Series: EP-036332 and EP-040081.

Assay		Series1: EP-036332	Series2: EP-040081
Biochemical Activity[1] Inhibition of product formation (RF/MS)	Human[1], IC_{50}	14 nM	79 nM
	Mouse[2], IC_{50}	2.5 nM	74 nM
Cellular Activity[2] Inhibition of product formation (RF/MS)	HEK293-Human, IC_{50}	47 nM	34 nM
	HEK293-Mouse, IC_{50}	55 nM	1083 nM
Selectivity[3] IC_{50} ratio (HSD17βx/ HSD17β13)	HSD17B1	>7000×	>1265×
	HSD17B2	4357×	322×
	HSD17B4	>7000×	982×
	HSD17B3, B5, B10, B11	>7000×	>1265×
	HSD11B1	>7000×	>630×

[1]RF/MS= Rapid Fire Mass Spectrometry with Leukotriene B4;
[2]RF/MS= Rapid Fire Mass Spectrometry with Estradiol-d_5;
[3]NADH Luminescence Assay

HSD17B13 Inhibitors In a T cell Mediated Injury Model





Does pharmacological inhibition of HSD17B13 protect from T cell mediated liver injury?

HSD17B13i

EP036332
100mg/kg BID

EP040081
10 or 100mg/kg QD

Figure 2. Concanavalin acute liver injury model study design and key end points evaluated +/- HSD17B13 inhibition.

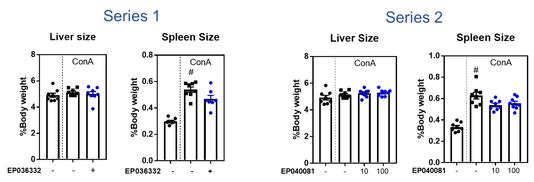


Figure 3. Liver and spleen size were not impacted by HSD17B13 inhibition (mg/kg). #p<0.05 vs uninjured + vehicle. n=8

RESULTS

HSD17B13 Inhibitors are Hepatoprotective and Anti-Inflammatory

HSD17B13i Decreases Plasma ALT and Circulating Inflammatory Cytokines

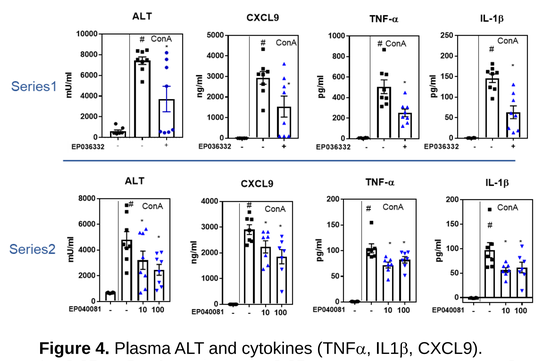


Figure 4. Plasma ALT and cytokines (TNFα, IL1β, CXCL9). *p<0.05 vs ConA + vehicle; #p<0.05 vs uninjured + vehicle. n=8

HSD17B13 Inhibitors Modulate Gene Markers of T cell Activation

HSD17B13i Attenuates Inflammatory Gene Markers In Liver

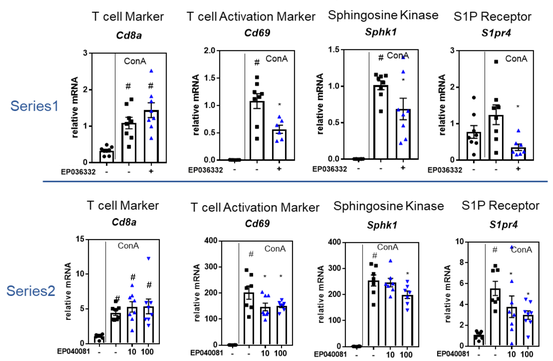
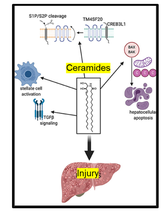


Figure 5. Liver gene expression. T cell gene marker (*Cd8*) was not altered by HSD17B13i in ConA treated livers, however, markers of immune cell activation (*Cd69*, *S1pr4, Sphk1*) were decreased. *p<0.05 vs ConA + vehicle; #p<0.05 vs uninjured + vehicle. n=8.

RESULTS

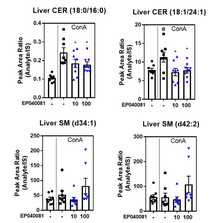
HSD17B13 Inhibitors Decrease Liver Ceramides In T cell Mediated Injury Model

Ceramide Is a Lipid Mediator of Cell Death



Poss et al, 2020 Frontiers In Endocrinology

HSD17B13 Inhibition Results in a Favorable Sphingolipid Profile



HSD17B13 Inhibitor Decreases Expression of *FasL*, an apoptotic inducer.

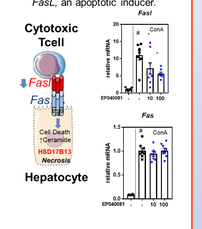
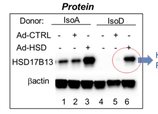


Figure 6. Sphingolipid analysis (CER=ceramide; SM=sphingomyelin) and gene markers of cell death (*Fasl*, *Fas*) in mouse livers +/- HSD17B13i. *p<0.05 vs ConA + vehicle; #p<0.05 vs uninjured + vehicle. n=8

Ceramide Levels are Regulated by HSD17B13 Inhibition in Human Hepatocytes

HSD17B13 Rescue In Primary Human Hepatocytes From IsoD Donor



HSD17B13 Inhibitor Decreases Liver Ceramide but Not Sphingomyelin

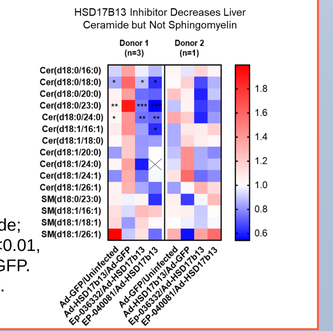


Figure 7. Sphingolipid analysis of primary human hepatocytes +/- HSD17B13i (5 μM). (CER=ceramide; SM=sphingomyelin). *p<0.05, **p<0.01, ***p<0.001 vs Ad-HSD17B13/Ad-GFP. Pooled from 3 studies (n=4/group).

CONCLUSION

Hepatoprotection by HSD17B13 inhibition in a model of autoimmune hepatitis is characterized by a favorable bioactive lipid profile that parallels a decrease in markers of cytotoxic immune cell activation and cell death.