Hepatoprotective effects of a HSD17b13 inhibitor in the CDAA-HFD mouse model of advanced MASH with progressive fibrosis

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Background & Aim

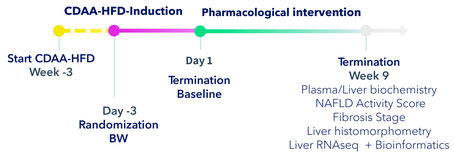
HSD17B13, a newly identified hepatocyte-specific, lipid droplet-associated protein, has recently been reported to be closely associated with development and progression of MASLD/MASH. Furthermore, inhibition of HSD17B13 has shown to protect against liver fibrosis in preclinical models of MASH.

The present study aimed to evaluate effects of a HSD17b13 inhibitor following interventional therapy in the non-obese choline-deficient L-amino-acid defined high-fat diet (CDAA-HFD) mouse model of advanced MASH with progressive fibrosis.

Methods

C57BL/6JRj mice were fed chow or CDAA-HFD (45 kcal% fat, 0.1% methionine, 1% cholesterol, 28 kcal% fructose) for 3 weeks prior to treatment start (*i.e.* before onset of fibrosis). Animals were randomized into treatment groups based on body weight. A baseline group (n=12) was terminated at study start. CDAA-HFD fed mice (n=9-12 per group) were administered (PO) HSD17b13 inhibitor (M5475, 30 mg/kg or 100 mg/kg) or vehicle for 9 weeks. Chow-fed mice (n=8) served as normal controls. Terminal endpoints included plasma biomarkers, liver biochemistry, NAFLD Activity Score (NAS), fibrosis stage, quantitative liver histology.

1 Study Outline



Group no.	Group	Name	Number of animals	Administration route	Dosing frequency	Dose
1	**Chow**	LEAN-CHOW	8	PO	QD	-
2	**Baseline**	CDAA-HFD	12	-	-	-
3	**Vehicle**	CDAA-HFD	12	PO	QD	-
4	**M5475 30 mg/kg**	CDAA-HFD	12	PO	QD	30 mg/kg
5	**M5475 100 mg/kg**	CDAA-HFD	10	PO	QD	100 mg/kg

2 Metabolic and biochemical parameters

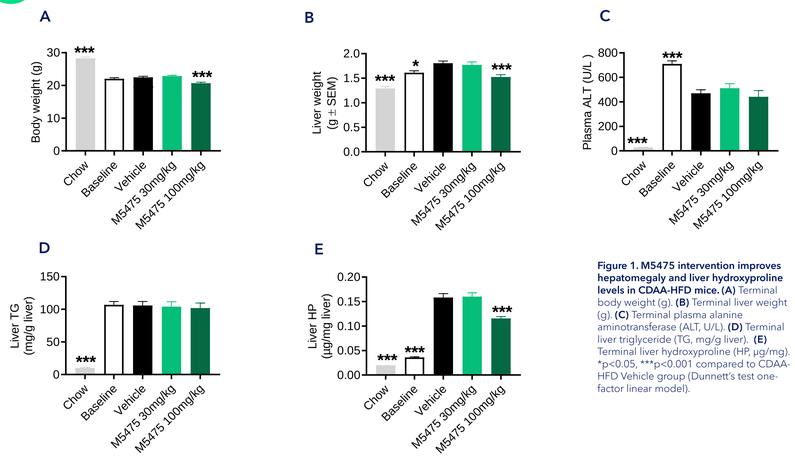
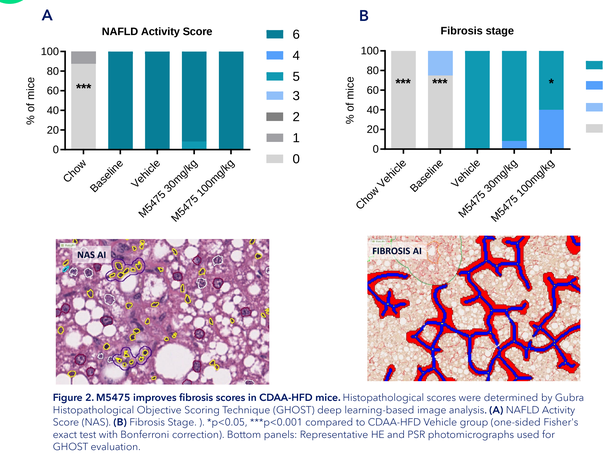


Figure 1. M5475 intervention improves hepatomegaly and liver hydroxyproline levels in CDAA-HFD mice. (A) Terminal body weight (g). **(B)** Terminal liver weight (g). **(C)** Terminal plasma alanine aminotransferase (ALT, U/L). **(D)** Terminal liver triglyceride (TG, mg/g liver). **(E)** Terminal liver hydroxyproline (HP, µg/mg). *p<0.05, ***p<0.001 compared to CDAA-HFD Vehicle group (Dunnett's test one-factor linear model).

3 NAFLD Activity Score and Fibrosis Stage



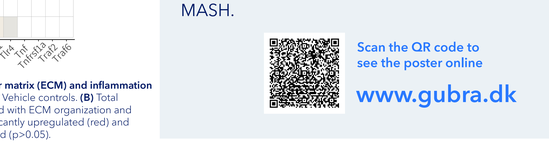


Figure 2. M5475 improves fibrosis scores in CDAA-HFD mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis Stage.). *p<0.05, ***p<0.001 compared to CDAA-HFD Vehicle group (one-sided Fisher's exact test with Bonferroni correction). Bottom panels: Representative HE and PSR photomicrographs used for GHOST evaluation.

4 Quantitative histological markers of steatosis, inflammation and fibrogenesis

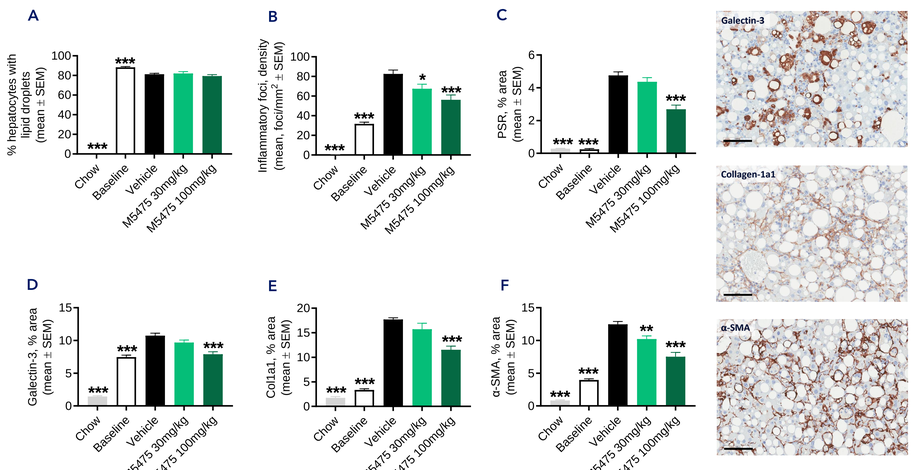


Figure 3. M5475 intervention improves quantitative histological markers of inflammation and fibrosis in CDAA-HFD mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables and conventional IHC image analysis. **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of PSR. **(D)** % area of galectin-3. **(E)** % area of collagen-1a1. **(F)** % area of alpha-smooth muscle actin (α-SMA). Mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 compared to CDAA-HFD Vehicle group (Dunnett's test one-factor linear model). Right panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs (scale bar, 100 µm).

5 Liver transcriptome profile

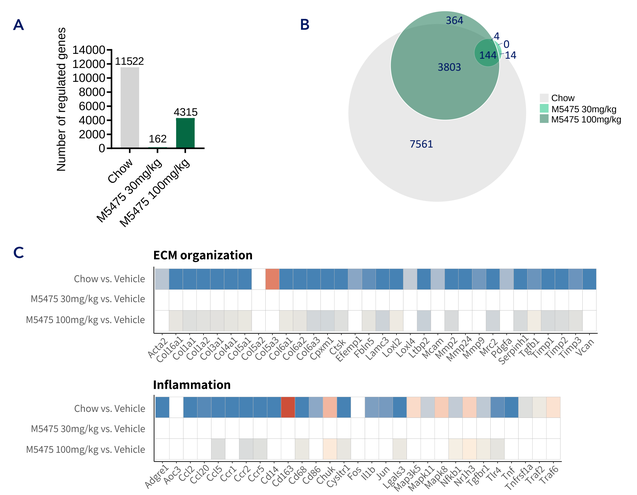


Figure 4. M5475 intervention marginally influences candidate gene expression markers of extracellular matrix (ECM) and inflammation in CDAA-HFD mice. (A) Total number of differentially expressed genes (DEGs) compared to CDAA-HFD Vehicle controls. **(B)** Total number of DEGs compared to CDAA-HFD Vehicle controls. **(C)** Regulation of candidate genes associated with ECM organization and inflammation (log2-fold change compared to CDAA-HFD vehicle group). Color gradients indicate significantly upregulated (red) and downregulated (blue) genes, respectively (p<0.05). White boxes indicate genes not significantly regulated (p>0.05).

Conclusion

Outcomes of HSD17b13 inhibitor (M5475) therapy in CDAA-HFD mice:

+ Improved hepatomegaly and liver hydroxyproline levels with marginal body weight loss
+ No effect on NAFLD Activity Score
+ Reduced fibrosis stage
+ Reduced quantitative histological markers of inflammation and fibrosis
+ Marginal effects on candidate genes involved in ECM remodelling and immune system

In conclusion, M5475 elicits anti-inflammatory action and prevents progression of liver fibrosis in the non-obese CDAA-HFD mouse model of MASH.

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