Morphometric Collagen Analysis Discerns Anti-fibrotic Effects of INT-767 and OCA in NASH Mouse Models

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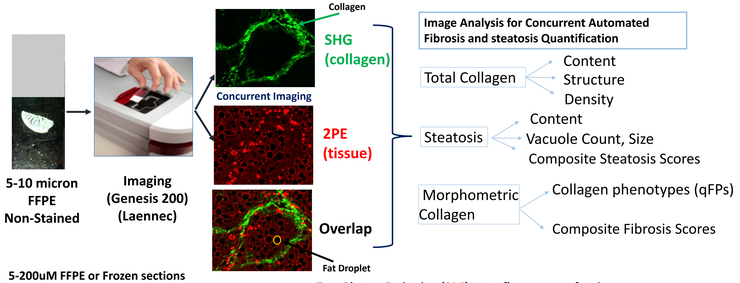
BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) is a highly prevalent liver disorder that can gradually progress to liver inflammation and fibrosis, including nonalcoholic steatohepatitis (NASH). The farnesoid X receptor (FXR) and transmembrane G protein-coupled receptor 5 (TGR5) regulate bile acid metabolism, inflammation, fibrosis, and inhibit NASH. Here, we assess the anti-fibrotic efficacy of INT-767 (a dual FXR/TGR5 agonist) and obeticholic acid (OCA) (an FXR agonist) in a diet-induced NASH mouse model with a focus on the morphometric quantification of the fibrosis phenotype imaged by Second Harmonic Generation (SHG).

METHOD

TISSUE PREPARATION, INSTRUMENTATION, AND WORKFLOW



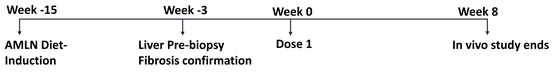
- 5-200uM FFPE or Frozen sections
- Stain-Free and Label- Free imaging
- Fully Quantitative collagen & fat
- High Resolution (0.4 uM @ 20x)
- Non-destructive (tissue can be save for further studies)

- Two-Photon Excitation (2PE): auto fluorescence for tissue morphology depict cellular structure and injury
- Second Harmonic Generation (SHG): specific for collagens I and III
- Machine- learning Image Analysis Software optimized and validated with pathologists

AMYLIN LIVER NASH FIBROSIS MODEL (AMLN)

Leptin-deficient (lep 0b/0b) mice were allowed ad libitum access to normal chow (low-fat diet w no fructose nor cholesterol) or to modified ALIOS diet (high trans fat (40%), fructose (22%), cholesterol (2%) in food pellets) to induce NASH. Mice were biopsied and confirmed to have steatosis (score 3) and fibrosis (stage 2-3) before treatment with vehicle, INT-767 (10 mg/kg), or OCA (30 mg/kg) for 8 wks. These investigational drugs are provided by Intercept to treat patients with NASH and liver fibrosis. Obeticholic acid (OCA, bile acid) is a FXR agonist. INT-767 is a dual FXR and TGR5 (bile acid receptors) agonist.

Study Design



Treatments	Strain	N	ADMIN	DOSE
NASH (Vehicle)	Lep $^{ob/ob}$	10	QD, PO	--
OCA	Lep $^{ob/ob}$	8	QD, PO	30 mg/kg
INT-767	Lep $^{ob/ob}$	10	QD, PO	10 mg/kg

Analysis Parameters
1. Fibrosis (basic analysis)
-Collagen Content: collagen area%
-Collagen Structure: Collagen Reticulation Index measures the complexity of collagen fiber network.
-Collagen Fiber Density: collagen density within fiber
2. Steatosis
-Fat Content: fat area%
-Fat Vacuole morphometrics
-Composite Steatosis Scores (CSS), a continuous phenotypic quantifier of Steatosis.
3. Fibrosis (advance analysis)
Morphometric Collagen Phenotypes
- quantifiable fibrosis parameters (qFPs): collagen fiber length, width, area, perimeter, density, etc
- Fibrosis phenotypic maps (heat charts) illustrate the normalized values of qFPs (healthy/green, fibrotic/red) for the entire group of animals.
- Composite Fibrosis Scores (CFS) is a continuous phenotypic quantifier of fibrosis. CFS is obtained by mathematically weighting and combining normalized qFPs into one composite score for each animal. This score evolves continuously as fibrosis progresses /regresses.

RESULTS

Figure 1. AMLN diet-feed mice induces hepatic fibrosis and steatosis in histological sections.
A. Collagen (SHG, green), Tissue (2PE, red), fat vacuoles (black circles)

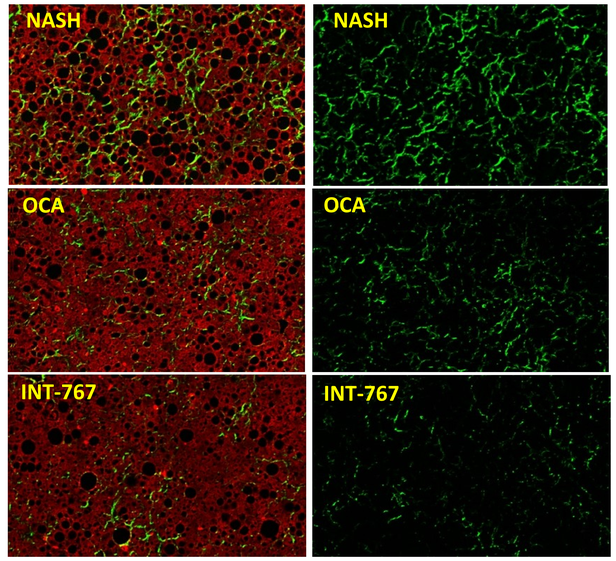


Figure 2. Quantitation of Liver Fibrosis

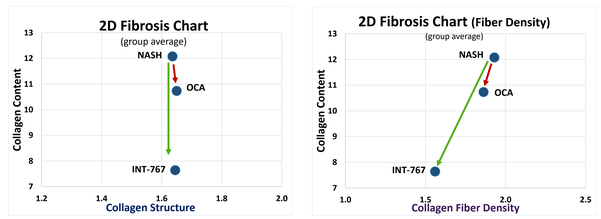


Figure 3. Quantitation of Liver Steatosis

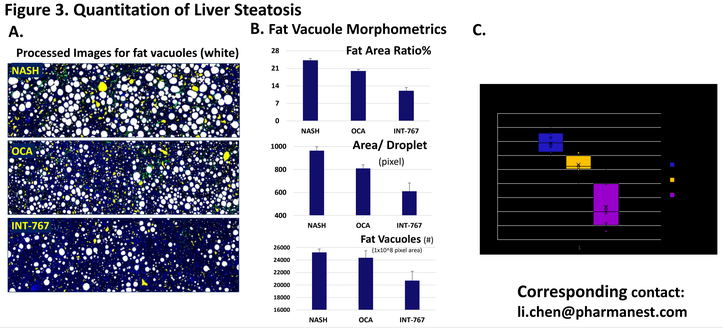


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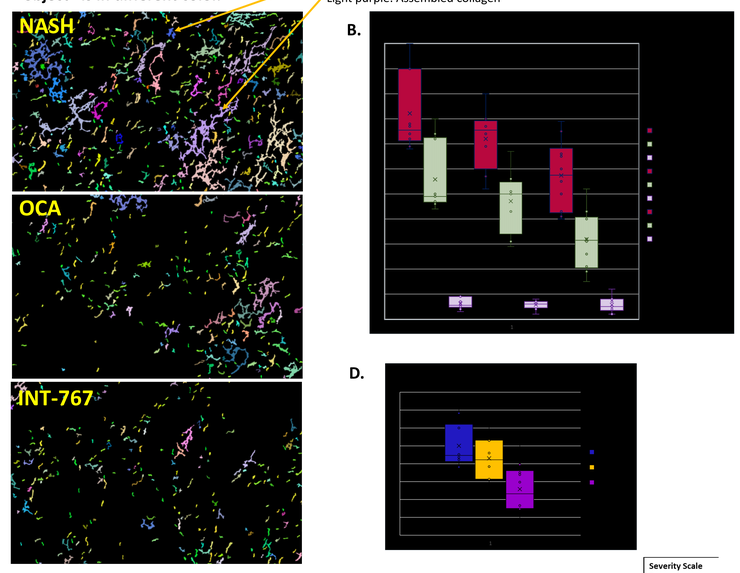
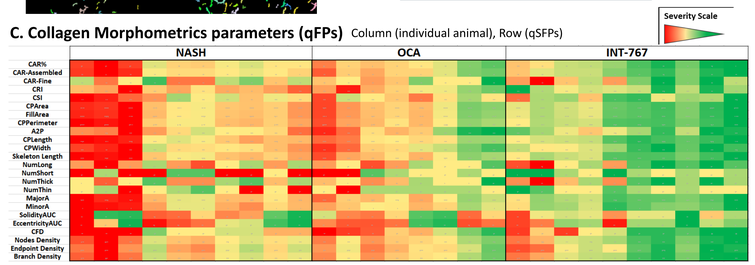
Figure 4. Liver Collagen Morphometric Phenotyping
A. Processed images: Each Collagen "object" is in different color.



C. Collagen Morphometrics parameters (qFPs) Column (individual animal), Row (qSFPs)



CONCLUSIONS

- INT-767 reduced liver collagen fiber area and fiber density, while OCA decreased it to a lesser degree. This can be expected due to the 3-fold higher potency of INT-767 compared to OCA in FXR activation. Neither drug affected the collagen reticulation structure.
- The qFPs, reported on heat charts, show highest values for Vehicle, mid values for OCA, and lowest values for INT-767. INT-767 is more effective than OCA in improving fibrosis area, fiber density, qFPs, and Composite Fibrosis Scores. Fibrosis Morphometric Phenotyping identifies non-responders to intervention and highlights the fact that more animals respond positively to INT-767 than OCA.
- On a side note, INT-767 also reduces steatosis and the Composite Steatosis Scores, while OCA decreases it to a lesser amount. SHG/2PE technology allows concurrent analysis of both fibrosis and steatosis.
- Thus, INT-767 has higher anti-fibrotic and anti-steatotic effects compared to OCA in ob/ob NASH mice.
- Morphometric analysis of SHG images is an effective label-free method to describe and quantify the severity and progression of liver fibrosis and differentiate pharmacological agents in their efficacy and group responses. These data enrich previous findings obtained using conventional methods.