Second Harmonic Generation (SHG)/Two-photon Excitation Fluorescence (TPEF) Microscopy Imaging and Quantitative Assessment of Septal Fibrosis in Choline Deficient High Fat Diet (CDHFD) Rat Model of Nonalcoholic Steatohepatitis (NASH)

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Animal models of NASH are crucial in elucidating NASH pathogenesis and examining the therapeutic effects of various agents. However, there are very few preclinical models that exhibit features of late-stage fibrosis within a short time, including septae formation that is typical in F3 noncirrhotic and F4 cirrhotic human liver. In addition, there are few quantitative methods to assess the changes of septal fibrosis.

The purpose of this study was to develop an automated quantitative system for septa fibrosis and evaluate it in a CDHFD preclinical rat model of NASH that mimics histological changes in F3 noncirrhotic patients.

EXPERIMENT OVERVIEW

Wistar Han rats were fed CDHFD (Research Diet#A16092003), or control diet (Research Diet#A12450K) for 12 weeks as previously described[4]. The Acc inhibitor (ND630) was dosed from week 6 onwards in 2 separate studies. Liver tissue specimens were processed into formalin fixed paraffin embedded (FFPE) slides.

Stain-free SHG/TPEF microscopy (Genesis®200, HistoIndex, Singapore) was used for imaging, while an artificial intelligence (AI)-based system analyzed septa collagen in the unstained tissues. The algorithm first recognized the portal tract (PT) and central vein (CV) zones, and then examined each bundle of collagen in between using histological diagnosis of septa in human as a gold standard reference. Thereafter, the septae formation and reduction in these studies were detected, quantified and compared among different groups.

FFPE slides were stained with Picrosirius Red (PSR), whole slides were scanned using Leica Versa 200. PSR+ percentage analysis was performed in Leica Aperio ImageScope using the Color Deconvolution module.

Figure 1: Correlation between PSR+ and %SHG area

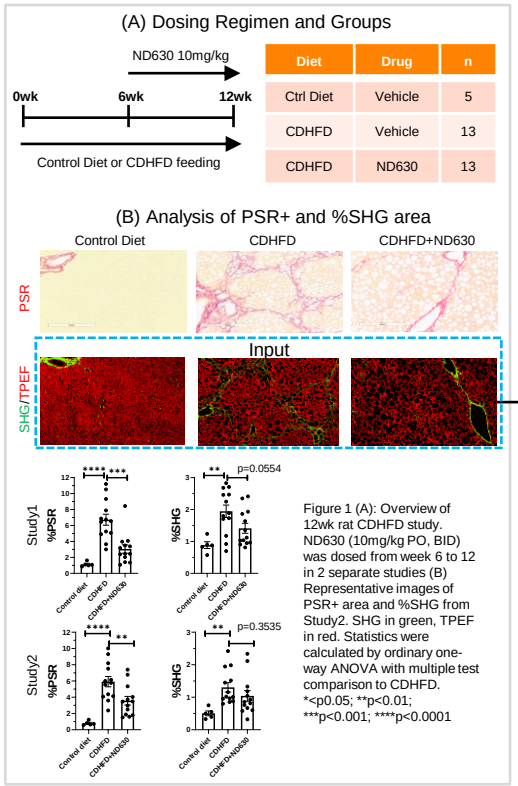

Figure 1 (A): Overview of 12wk rat CDHFD study. ND630 (10mg/kg PO, BID) was dosed from week 6 to 12 in 2 separate studies (B) Representative images of PSR+ area and %SHG from Study2. SHG in green, TPEF in red. Statistics were calculated by ordinary one-way ANOVA with multiple test comparison to CDHFD. *<p0.05; **p<0.01; ***p<0.001; ****p<0.0001

Figure 2: Septal area identification

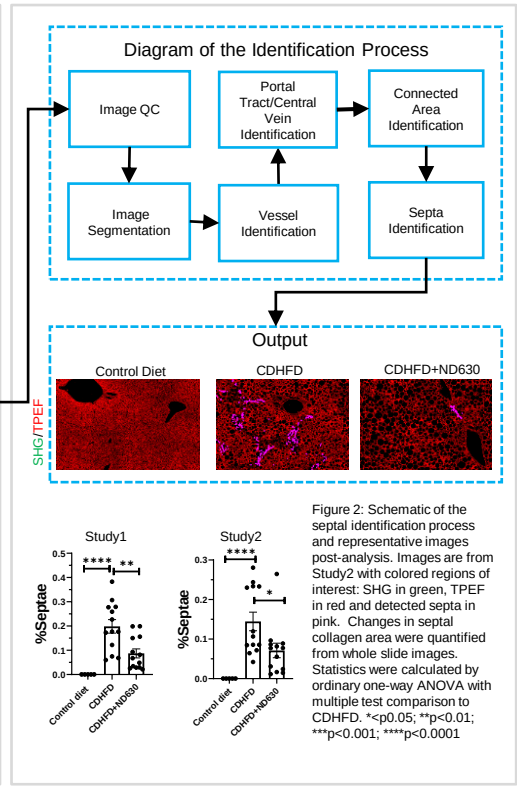

Figure 2: Schematic of the septal identification process and representative images post-analysis. Images are from Study2 with colored regions of interest: SHG in green, TPEF in red and detected septa in pink. Changes in septal collagen area were quantified from whole slide images. Statistics were calculated by ordinary one-way ANOVA with multiple test comparison to CDHFD. *<p0.05; **p<0.01; ***p<0.001; ****p<0.0001

1. In both studies, we observed a significant increase in the %SHG measured collagen area and %PSR in the CDFHD Vehicle rats compared to control diets.
2. CDHFD vehicle animals in both studies exhibited significant of septae between PT and CV zones, though the majority were delicate ones that are usually observed as regressive septae in human F3 patients. Control diet fed animals did not exhibit septae formation.
3. ND630 significantly reduced PSR+ area and septal collagen area and showed a trend reduction in %SHG in both studies.

Septae formation is observed in the CDHFD rat model after 12 weeks on diet, and this model might be suitable for septal assessment of NASH and changes with therapeutic agents. Septae in CDHFD rats at the 12th week on diet resemble the regressive septae in human F3 patients. Longer diet induction may be necessary to observe progressive septae. Further evaluation will be necessary to elucidate differential effects of ND630 on %SHG and %Septae.

An automated AI-based stain-free imaging system with SHG/TPEF could be useful for monitoring liver fibrosis activity in a fully quantitative manner, especially septal changes in animal models of NASH.

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R.M and H.Y are employees of Takeda Pharmaceuticals and own stock in Takeda. X.T, Q.Y, A.L and G.H are employees of HistoIndex or its subsidiary, X.T holds stock options and G.H owns stock in HistoIndex.

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