Quantitative Assessment of Liver Septal Fibrosis Severity Using Morphometric Analysis

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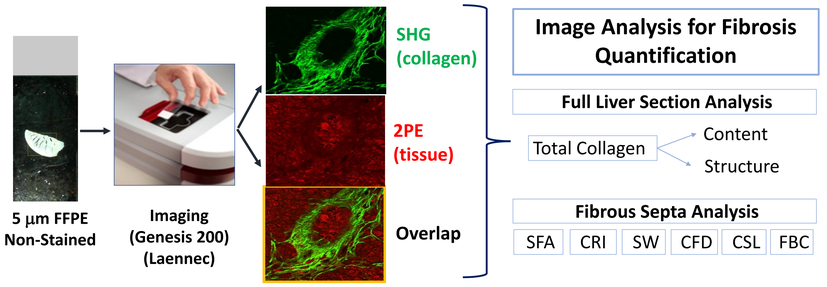
BACKGROUND

Liver fibrosis is associated with excessive accumulation of extracellular matrix in response to persistent injury, inflammation, and abnormal wound healing. Bridging fibrous septa can be found during advanced liver fibrosis, including human NASH diseases and animal liver fibrosis models. A common method to assess liver fibrosis is by conventional staining followed by histopathological evaluation for fibrosis scoring and staging. This assessment has limitations, as it uses a narrow range for scoring, qualitative fibrosis evaluation, and it is also prone to observer variations. Here, we introduce a sensitive method for quantitative assessment of fibrosis with an emphasis on the quantifiable fibrous septal phenotypes imaged by Second Harmonic Generation (SHG). We present a thioacetamide (TAA)-induced liver fibrosis animal study to provide a more in-depth look at the changes of the fibrous septa as fibrosis advances and regresses.

METHOD

TISSUE PREPARATION, INSTRUMENTATION, AND WORKFLOW



- 5-200µM FFPE or Frozen sections
- Stain-Free and label- free imaging
- Fully quantitative for collagen
- High Resolution (0.4 uM @ 20x)
- Non-destructive (tissue resusable)

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

THIOACETAMIDE- INDUCED LIVER FIBROSIS MODEL

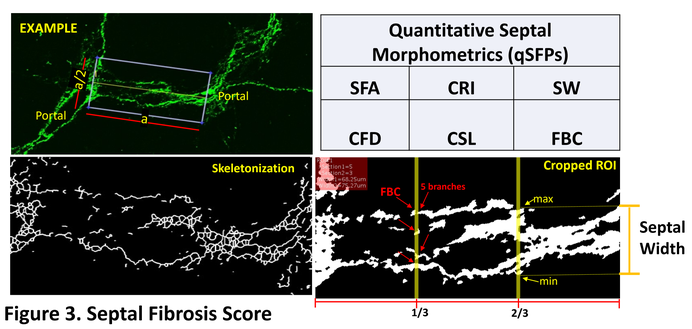
Rats are treated (n=5/gp) with saline or thioacetamide (TAA, 100mg/kg) for 4 and 8 wks with PBS or with a small molecule anti-fibrotic ALK-5 (TGF-beta type I receptor kinase) inhibitor.

Group 1	Group 2	Group 3	Group 4
Control	TAA (4 wk)	TAA (8 wk)	TAA (8wk) + ALK5 Inhibitor (ALK5)

LIVER FIBROSIS IMAGE ANALYSIS

Figure 1. Full Liver Section Analysis
-Histology images (SHG/TPE)
-Collagen quantitation and 2D Fibrosis Chart

Figure 2. Fibrous Septal Analysis (quantitative septal fibrosis parameters, qSFPs)
-Septal Fiber Area% (SFA): area of collagen in the region of interest
-Collagen Reticulation Index (CRI): collagen fiber network complexity
-Septal Width (SW): width at mid 1/3rd length of septa
-Fiber Branch Count (FBC): # branches or intersections at mid 1/3rd length of septa
-Collagen Segment Length (CSL): average length of the septal branches
-Collagen Fiber Density (CFD): collagen density within fiber based on collagen intensity



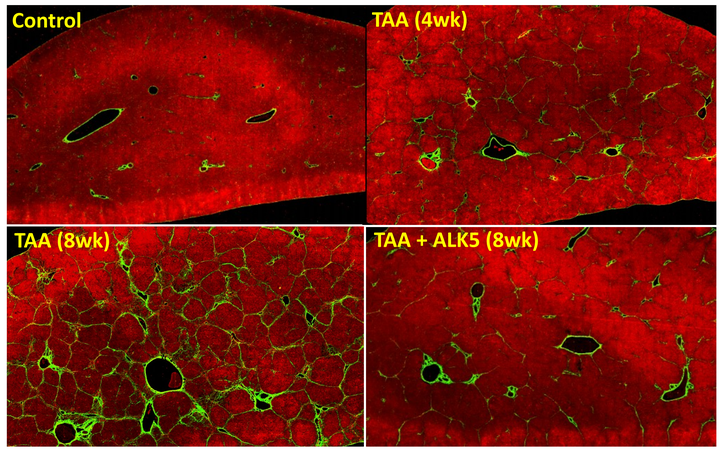
Quantitative Septal Morphometrics (qSFPs)		
SFA	CRI	SW
CFD	CSL	FBC

Figure 3. Septal Fibrosis Score
- A continuous quantifier of septal fibrosis using composite qSFPs

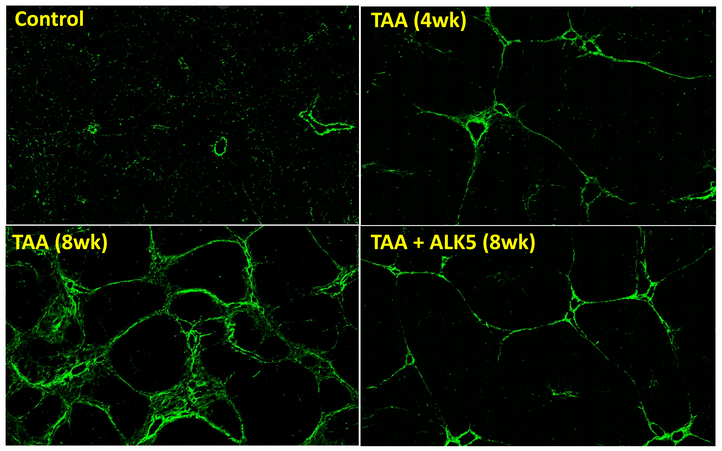
RESULTS

Figure 1. TAA-treated rats induce liver fibrosis and septa development in histological sections.

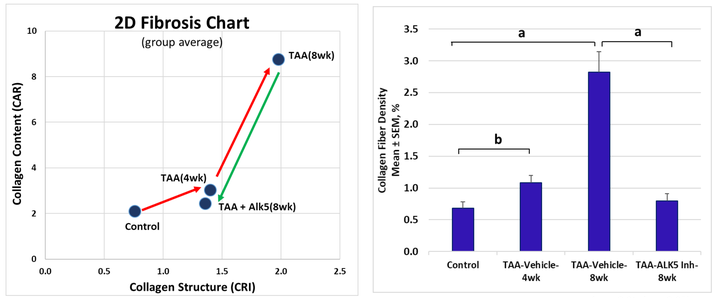
A. SHG/ 2PE livers. Collagen (green), Tissue (red)



B. SHG livers (zoom in). Collagen (green)



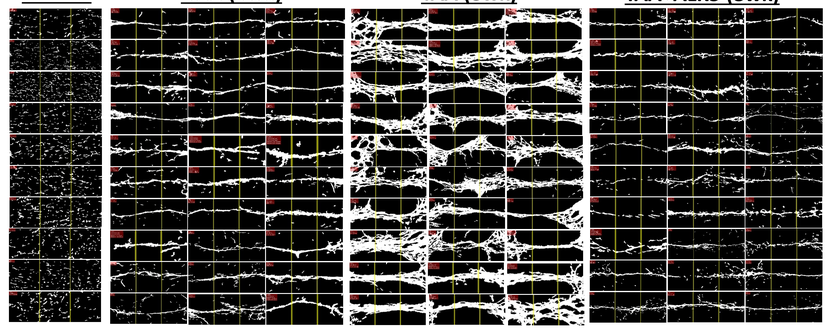
C. Quantitation of Liver Fibrosis



(a, p<0.01; b, p<0.05)

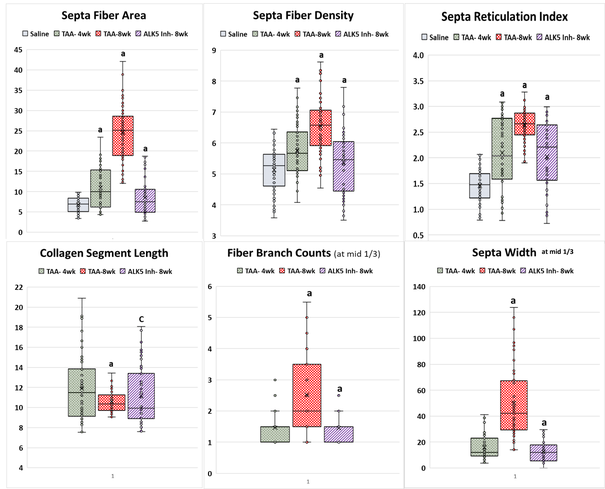
Figure 2. Fibrous Septal Analysis

A. TAA-treated rats induce septal progression shown in individual septal images.



B. Quantitation of Septal Morphometrics (qSFPs)

Individual animal (box) shows septal bridges (dots) in various stage of development



Heat Chart: Column (individual animal), Row (qSFPs)

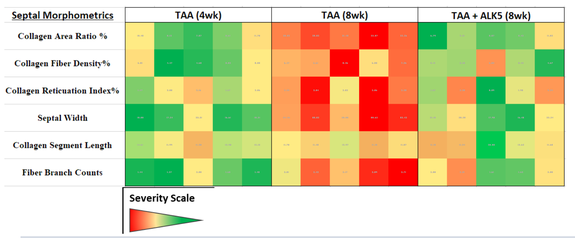
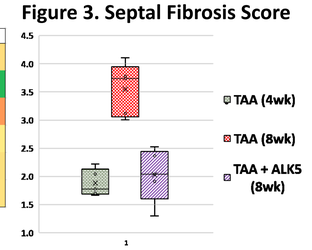


Figure 3. Septal Fibrosis Score



Conclusion

- Thioacetamide induced liver fibrosis by increasing liver collagen fiber area, structure, and density, while the anti-fibrotic ALK-5 inhibitor ameliorated this induction.
- The quantitative Septal Fibrosis Parameters (qSFPs) and Septal Fibrosis Score (SFS) result correlated with the liver collagen assessment. The qSFPs and SFS increased as TAA induced fibrosis progression from week 4 (mild fibrosis with short thin septa) to week 8 (advanced bridging fibrosis with long thick septa). These induction by TAA were reduced with the anti-fibrotic drug.
- These data demonstrate that qSFPs and composite Septal Fibrosis Score are an effective method using stain-free SHG/TPE imaging to measure the severity of liver septal fibrosis and its related regression with pharmacological agents.
- The Septal Fibrosis Score may provide a quantitative method to better classify and differentiate bridging fibrosis, in particular, the fibrosis F3-F4 stage in human liver diseases. Future studies will showcase this work in human NASH with fibrosis.

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