Clinical Relevance of an Animal Model of Non-Alcoholic Steatohepatitis (NASH) and Digital Pathology with Artificial Intelligence (DP-AI) Analyses of Hepatic Fibrosis

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Background and Aims

Clinical relevance of animal models for NASH is crucial for the assessment of in vivo efficacy of compounds in preclinical development. The validity of efficacy assessment in animal models rests on histopathology of not only steatosis, ballooning and inflammation but also fibrosis. However, hepatic fibrosis varies from model to model, despite the adoption of fibrosis staging system for human, such as the Brunt system. In the current study, we applied an automated quantitative DP-AI system to a selected mouse model of NASH to evaluate the hepatic fibrosis and the antifibrotic activity of obeticholic acid (OCA), in particular the fibrous septa that are typical in the late stage of NASH patients.

Methods

In the mouse NASH model, after animals develop steatosis on feeding of high-fat diet (HFD), they are treated with carbon tetrachloride (CCL4) to induce hepatic fibrosis. Test compounds are administered during the CCL4 induction. The liver histopathology is analyzed for steatosis, ballooning, inflammation and fibrosis by the standard means. For DP-AI analyses, Second-Harmonic Generation (SHG)/Two-Photon Excitation Fluorescent (TPEF) microscopy is used for imaging of unstained liver sections. Collagen fibers (including septa) are identified and quantified by an AI-based algorithm that recognizes the portal tract (PT) and the central vein (CV) and examines collagen bundles therebetween, with reference to septa diagnosed in the human NASH livers.

Conclusions

Our results demonstrate that the septa identified by the DP-AI system in the HFD+CCL4 model are NASH related, and they are the target of antifibrotic activities of NASH compounds with demonstrated clinical efficacies. Such septa could represent the bridging fibrosis as observed the NASH patients. The HFD+CCL4 model is therefore clinically relevant in terms of histopathological presentation and targets of NASH compounds.

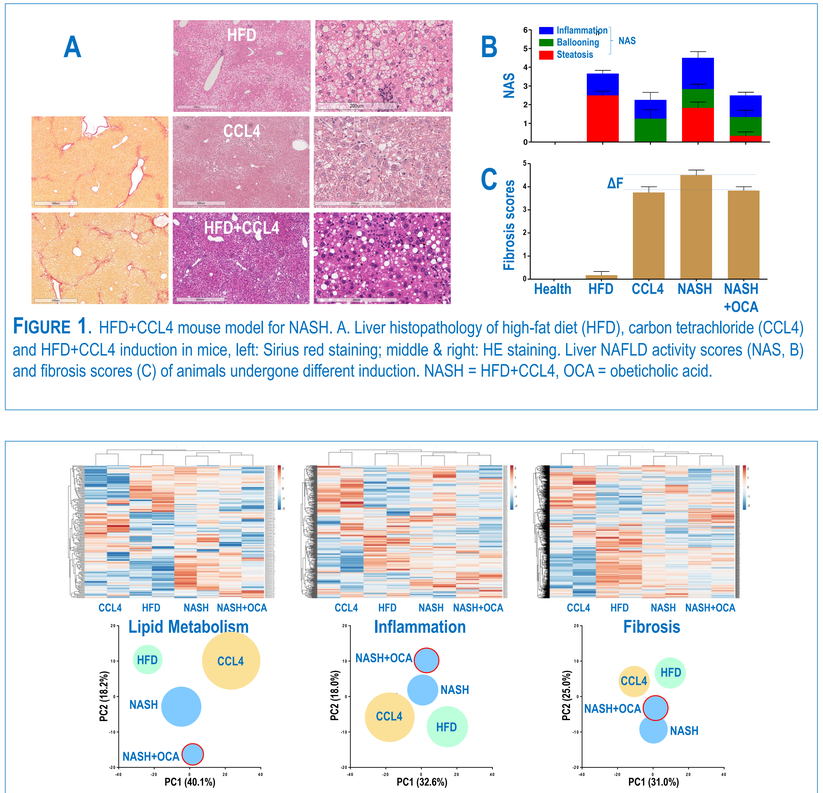


Figure 1. HFD+CCL4 mouse model for NASH. A. Liver histopathology of high-fat diet (HFD), carbon tetrachloride (CCL4) and HFD+CCL4 induction in mice, left: Sirius red staining; middle & right: HE staining. Liver NAFLD activity scores (NAS, B) and fibrosis scores (C) of animals undergone different induction. NASH = HFD+CCL4, OCA = obeticholic acid.

Figure 2. Gene expression (RNA seq) profiling of genes involved in lipid metabolism (left), inflammation (middle) and fibrosis (right) by clustering heat map (top) and principal component (PC) analysis (bottom).

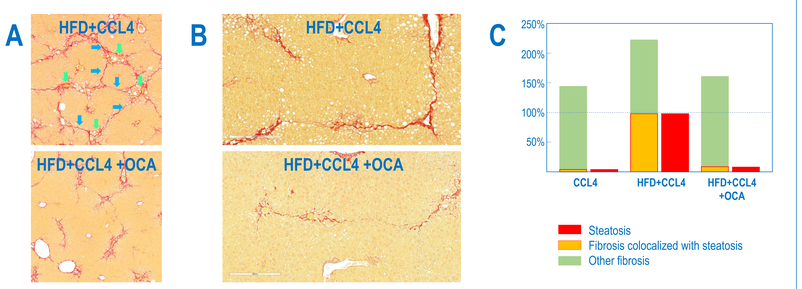
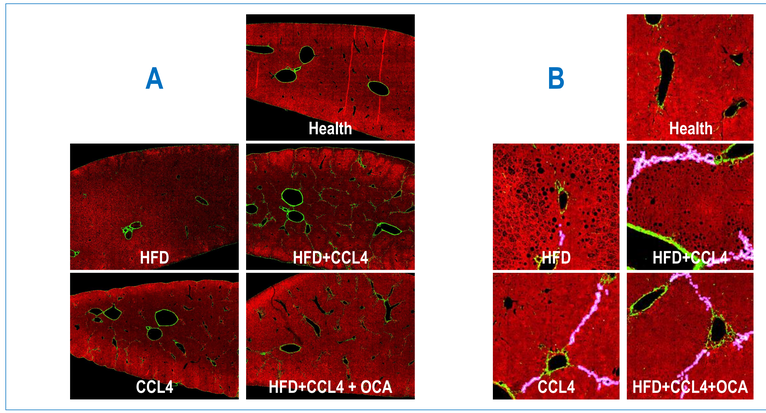


Figure 3. A. The HFD+CCL4 model induces perilobular bridging (blue arrows) and periportal fibrosis (green) that are the anti-fibrotic efficacy of OCA. B and C. In this model, the fibrosis colocalized with steatosis is also the target of OCA. In C, the fibrosis (quantified by SHG%) colocalized with steatosis in the HFD+CCL4 livers is set at 100%, and other fibrosis in the same livers or from other animals are normalized. Bothe fibrosis and steatosis are quantified by DP-AI.



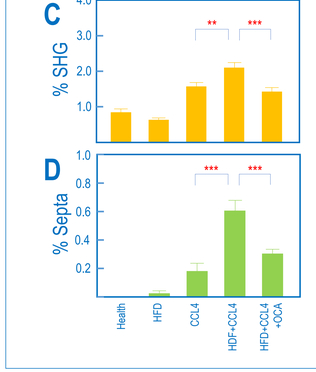


Figure 4. The SHG/TPEF images and the quantification of hepatic fibrosis in the HFD+CCL4 mouse model. A and B (above). Images of liver sections (A) and selected regions of interest (B) from labelled sources for illustration of SHG (in green), TPEF (in red) and septa (in pink, in B). C and C. The total collagen areas (C) and septal collagen areas (D) in whole livers from labelled animal groups, with statistical significance indicated.

While CCL4 alone induced significant fibrosis, an extra level of fibrosis was induced by the combination, as determined by SHG% (of total area). With the treatment of OCA, the extra fibrosis was suppressed (C). Although a basal level of fibrosis was observed in both the healthy control and the HDF induction by SHG% (C), no or minimal septa were present in either (D). The induction of septa was greatly increased when HDF and CCL4 were combined (septa% vis-à-vis SHG% in C and D). The OCA treatment reduced septa in the HDF+CCL4 model by ~50%, quantitatively much greater than that determined by SHG% (C and D).

Table 1. Clinical relevance of the HFD+CCL4 model for NASH. Clinical compounds of different MOAs were tested in the animal model, and their efficacies on improvement of NAS and fibrosis are summarized.

Compounds	Targets	Efficacy Test Results		Clinical Trial Status
		NAS	Fibrosis	
Obeticholic acid (INT-747)	FXR	Yes	Yes	P3, Completed
Selonsertib (GS4997)	ASK1	No	No	P3, Failed
Elafibranor (GFT-505)	PPAR-α/δ	No	Yes	P3, Failed
Cenicriviroc (TAK-652)	CCR2/CCR5	No	No	P3, Terminated
Resmetirom (MGL-3196)	THR-β	Yes	Yes	P3, Ongoing, positive
VK2809	THR-β	Yes	Yes	P2b, Ongoing
Firsocostat (ND630)	ACC1/2	Yes	Yes	P2, Discontinued (due to increase blood TG)
Liraglutide	GLP1	Yes	No	P2, Completed
tropifexor (LJN452)	FXR	Yes	Yes	P2, Ongoing
PXS 4728A	SSAO/VAP-1	No	No	P2, Discontinued
Lanifibranor (IVA337)	PPAR-α/δ/γ	Yes	Yes	P3, Ongoing
Aramchol	SCD-1	No	No	P3, Ongoing

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