Homogeneous Time Resolved Fluorescence assay for alpha-Smooth Muscle Actin enables fast quantification of liver fibrosis: validation in mouse models treated with elafibranor

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OBJECTIVES

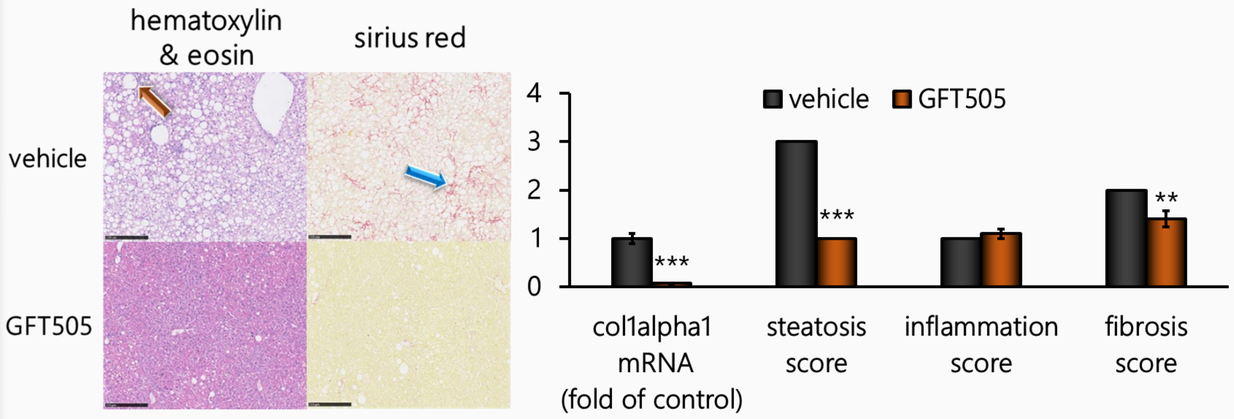
Evaluation of anti-fibrotic drugs in preclinical animal models requires histological analysis (e.g. Sirius Red or Masson Trichrome staining and immuno-staining) to demonstrate drug efficacy. As an alternative to this time-consuming methodology and to get a more accurate and rapid quantification of liver fibrosis, we introduced a Homogeneous Time Resolved Fluorescence (HTRF®) assay for the measurement of hepatic α-Smooth Muscle Actin (α-SMA) under 24 hours. Liver samples from mouse models treated with elafibranor, a PPAR-alpha/delta dual agonist, were used to validate the assay.

METHODS

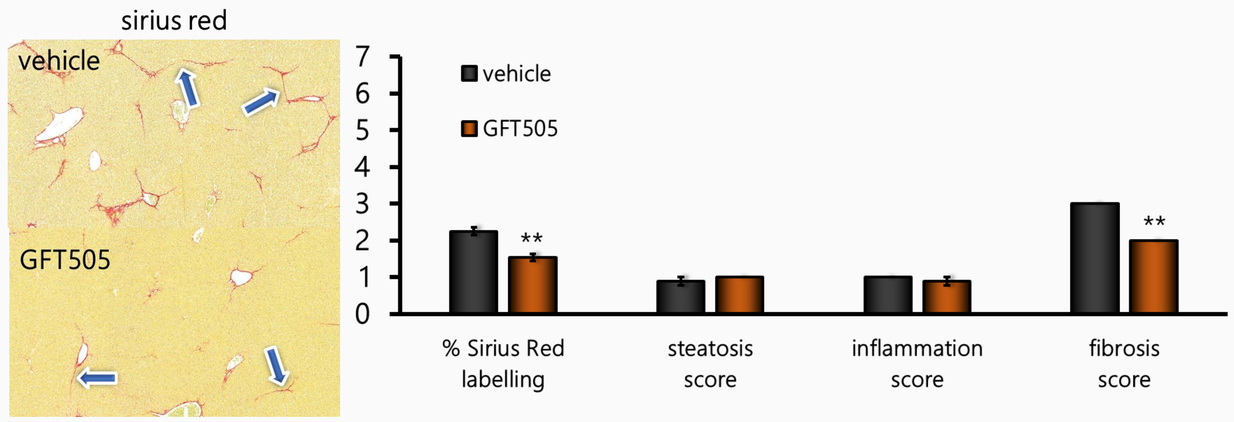
25-week Diet-Induced NASH (DIN) or 4-week CCl4-injected mice were treated with vehicle or elafibranor (GFT505) for 18 weeks (DIN mice) or 2 weeks (CCl4-injected mice). At the end of the treatment period, several liver samples were dissected from the left lateral lobe for histological analysis (Sirius Red) and quantification of α-SMA using either the novel HTRF® assay or by western blot (WB) analysis. Samples for HTRF® analysis were processed following Cisbio's procedures and guidelines for cell signaling assays on tissues. Data are shown as mean ± SEM, n=10 per treatment group. Statistical analysis was performed using an unpaired, two-tailed Student t-test. A $p<0.05$ was considered significant.

RESULTS

1 Elafibranor reduces liver fibrosis in 25-week DIN obese/insulin resistant mice and in 4-week CCl4-injected mice

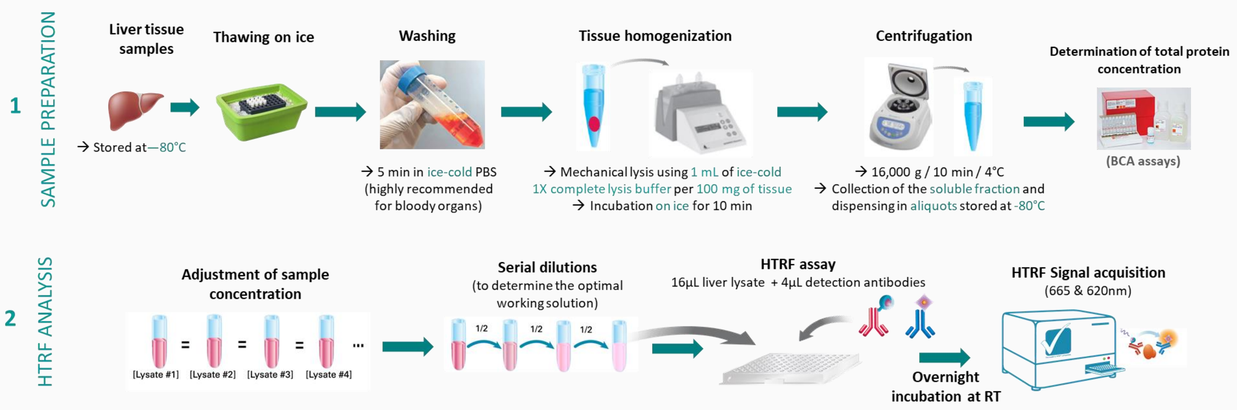


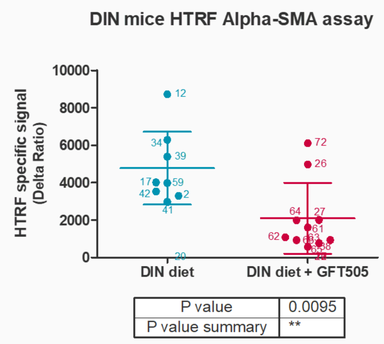
Representative hematoxylin & eosin and sirius red staining (brown arrow indicates steatosis, blue arrow indicates fibrosis, hepatic collagen type I alpha 1 chain (Col1a1) mRNA expression, hepatic steatosis, inflammation and fibrosis scores in 25-week DIN mice treated with vehicle or elafibranor (GFT505) 30mg/kg.
***p<0.01 and ***p<0.001 vs. vehicle.*

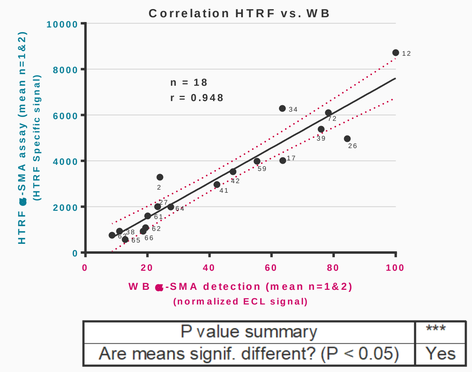


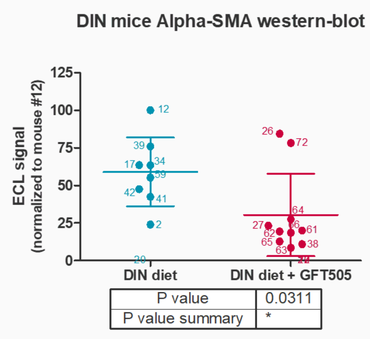
Representative sirius red staining (blue arrow indicates fibrosis), % Sirius Red staining, hepatic steatosis, inflammation and fibrosis scores in 4-week CCl4 injected mice treated with vehicle or elafibranor (GFT505) 30mg/kg.
***p<0.01 vs. vehicle.*

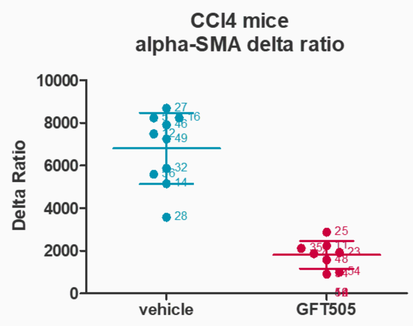
2 Elafibranor significantly reduces alpha-SMA protein levels in both 25-week DIN mice and 4-week CCl4 mice, as measured by HTRF®











Homogeneous Time Resolved Fluorescence (HTRF®) assay workflow: after sample preparation, total protein quantification and determination of optimal working solution, liver lysate are incubated overnight at room temperature with detection antibodies before HTRF® signal acquisition. This enables a quantification of alpha-SMA in less than 24 hours.

Left panel: alpha-SMA quantification by HTRF® or Western blot (ECL quantification) analysis in 25-week DIN mice treated with vehicle or elafibranor (GFT505) 30mg/kg. Right upper panel indicates robust correlation between HTRF® and Western blot assays. Of note, HTRF® showed superior inter-assay variability over western blot (4.4% vs. 23% mean CV). Right lower panel: alpha-SMA quantification by HTRF® in 4-week CCl4-injected mice treated with vehicle or elafibranor (GFT505) 30mg/kg.

CONCLUSION

- In contrast with time-consuming and less accurate methods such as histological or western blot analysis, this novel HTRF® assay enables a very rapid assessment of hepatic α-SMA protein levels.
- This tool will be helpful to evaluate anti-fibrotic effects of novel drugs targeting hepatic fibrosis.

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