A liver-targeted structurally engineered fatty acid, icosabutate, potently reduces hepatic pro-fibrotic gene expression and improves glycemic control in an obese diet-induced mouse model of NASH

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Introduction

It was recently shown that icosabutate ($C_{24}H_{38}O_3$), a novel structurally-engineered eicosapentaenoic acid (EPA) derivative, induced potent anti-inflammatory and anti-fibrotic effects in a rodent NASH model without insulin resistance (CDAA model). As a significant proportion of NASH patients have type 2 diabetes, we investigated the effects of delayed onset treatment with icosabutate on hepatic fibrosis in 2 separate studies in an obese diet-induced mouse model (ob/ob) of NASH along with its effects upon glycemic control. To assess whether anti-fibrotic effects were dependent on paracrine signals from hepatocytes and/or macrophages, we also evaluated the proliferative effects of icosabutate in human stellate (LX-2) cells.

Methods

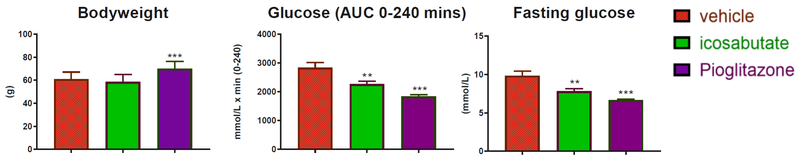
Study 1: 30 male B6.V-*Lepob*/JRj (*ob/ob*) mice (5 weeks old) were fed a diet high in fat (40% total calories, containing 18% trans-fat), 20% fructose, 2% cholesterol (AMLN diet) for 15 weeks then randomised into 3 *ob/ob*-NASH groups of 10 mice to receive either 112mg/kg icosabutate PO, 30mg/kg pioglitazone PO or no treatment (vehicle) for a further 4 weeks. An oral glucose tolerance test (GTT) was also performed at 3 weeks and assessment of hepatic fibrogenic gene expression was performed at 4 weeks.

Study 2: 36 male B6.V-*Lepob*/JRj (*ob/ob*) mice (5 weeks old) were fed the AMLN diet for 18 weeks then, after biopsy confirmation of fibrotic NASH, randomised into 3 *ob/ob*-NASH groups of 12 mice to receive either 135mg/kg icosabutate PO, 30mg/kg obeticholic (OCA) acid PO or no treatment (control) for a further 8 weeks. Assessment of multiple NASH relevant parameters and fibrosis was made at study end.

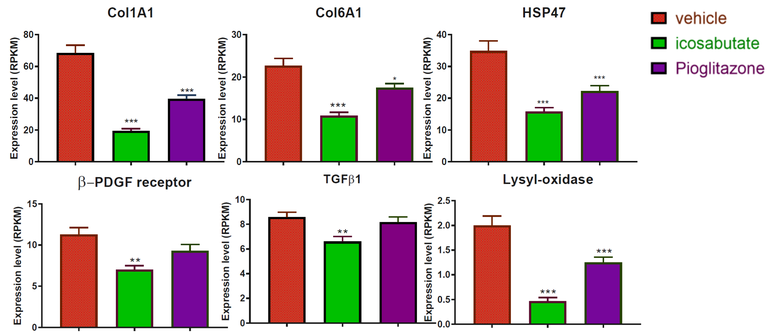
Study 3: To assess direct anti-fibrotic of icosabutate in human stellate cells, proliferative responses (BrdU incorporation) of LX-2 cells in serum free medium containing 25 μM BSA were measured after 24hrs co-incubation with either icosabutate (10-75 μM) or oleic acid (75 μM). Differences were assessed versus vehicle via one-way ANOVA with Dunnett's correction for multiple comparisons.

Results

Study 1: Treatment with icosabutate or pioglitazone in AMLN fed (4-weeks) *ob/ob* mice



Above. Icosabutate improves both glucose (AUC) in response to an oral glucose load (middle) and fasting glucose (right) at 4 weeks. Unlike the PPAR-γ agonist, pioglitazone, the improvements in glycemic control with icosabutate are not associated with an increase in bodyweight. **p<0.01, ***p<0.001 vs vehicle.



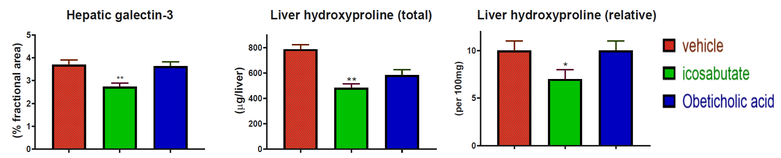
Above. Icosabutate significantly reduces hepatic transcripts of pivotal genes regulating fibrogenesis in AMLN fed (4-weeks) *ob/ob* mice. **p<0.01, ***p<0.001 vs vehicle.

Study 2: Treatment with icosabutate or obeticholic acid in AMLN fed (8-weeks) *ob/ob* mice with biopsy confirmed fibrotic NASH

Above. Both obeticholic acid and icosabutate reduced total hepatic lipids whereas only icosabutate significantly reduced plasma ALT. **p<0.01, ***p<0.001 vs vehicle.

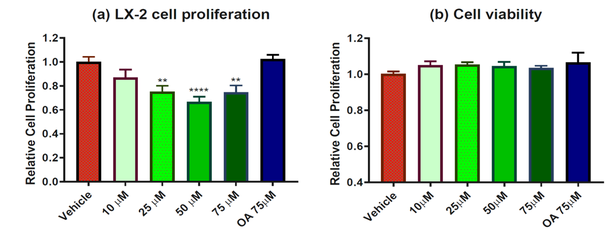
Representative images of liver stained with anti-α-SMA (AbCam, cat. no. ab124964) at the end of treatment period (magnification 20x, scale bar = 100 μm)

Above. Reduction in hepatic α-SMA content (a myofibroblast marker) after icosabutate treatment as assessed via immunohistochemical stain at 8 wks.
Below. (a, b) Icosabutate significantly reduces hepatic content of α-SMA at study end vs. vehicle and (c) also reduces α-SMA vs. baseline biopsy by 45% (% fractional area) assessed via IHC . **p<0.01, ***p<0.001 vs vehicle.



Above. Icosabutate, but not obeticholic acid, significantly reduced hepatic inflammation (galectin-3, immunohistochemical determination) and hepatic collagen both as total and relative content. **p<0.01, ***p<0.001 vs vehicle.

Study 3: Icosabutate reduces proliferative responses in human stellate cells



(a) Icosabutate (20, 50 and 75μM) significantly reduced proliferation of LX-2 cells by 25-34% at 24h whereas oleic acid (OA) was without effect; **p < 0.005, ****p < 0.0001 vs. vehicle (b) A non-significant increase in cell viability (MTS assay) demonstrating the anti-proliferative effects are not secondary to cytotoxicity. Results are presented as normalised mean values ± S.E.M. of 5 independent experiments performed in triplicate for icosabutate and 2 independent experiments for OA.

Conclusions

- **In a biopsy confirmed fibrotic NASH model, oral icosabutate reduced hepatic fibrosis in association with a 45% decrease in myofibroblast content in pre- versus post-treatment liver biopsies**
- **Robust anti-proliferative effects in human stellate (LX-2) cells *in vitro* confirm a direct anti-fibrotic effect of icosabutate independent of effects on hepatocytes and macrophages**
- **Liver injury (plasma ALT) and hepatic inflammation (galectin-3) are also reduced after icosabutate treatment.**
- **With established improvements in atherogenic lipids and glycemic control in humans, the direct and robust anti-proliferative and anti-fibrotic effects of icosabutate further underlie its clinical potential for the treatment of human NASH.**

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