A structurally engineered fatty acid, icosabutate, displays optimised absorption, distribution and metabolism properties for targeting hepatic inflammation and normalises elevated liver enzymes in dyslipidemic patients.

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INTRODUCTION

The use of unmodified fatty acids as efficacious oral drugs targeting inflammatory/fibrotic liver disease is inherently restricted by a marked dose-related increase in (auto) β-oxidation as an energy source (1), systemic distribution and incorporation into complex lipids. Icosabutate is a structurally engineered fatty acid (SEFA) designed to overcome these inherent limitations and may offer a novel approach to treat necroinflammation and subsequent fibrosis in NASH.

AIMS

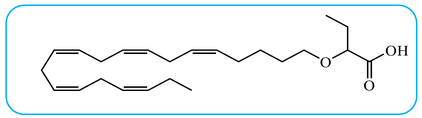
We tested the absorption, distribution, metabolism, excretion (ADME) properties of icosabutate, a structurally engineered fatty acid (SEFA) with distinct signalling properties, in addition to its effects upon hepatic inflammation and glucose metabolism in rodents. To further explore hepatoprotective properties we also evaluated the effects of 12 weeks icosabutate treatment (600mg QD) upon elevated liver enzymes in patients with hypertriglyceridemia (HTG).

METHODS

ADME properties of icosabutate were assessed in rodents *in vivo* and human hepatocytes *in vitro*. Treatment effects [introduced from week 7-12 of a 12-week choline deficient or choline sufficient amino acid defined modestly high-fat (31% of total calories) diet (CDAA and CSAA, resp.) in 9-week old C56BL/6J mice] of low-and high-dose icosabutate (56mg/kg ICOSA-L and 112mg/kg ICOSA-H orally) vs a GLP-1 agonist (Bydureon, 0.4mg/kg weekly) on hepatic inflammatory gene expression were also assessed. The liver enzyme response to 12-weeks treatment with icosabutate 600mg QD or placebo was also measured in HTG patients with elevated (> 1 to ≤ 3 X ULN) alanine aminotransferase (ALT) and gamma- glutamyl transferase (GGT) levels in a phase 2 study (CTN 4016 13201) investigating the hypolipidemic effects of icosabutate.

(1) STRUCTURE AND BACKGROUND

Icosabutate ($C_{24}H_{38}O_3$) **(below)** was selected from >30 *in vivo* tested SEFAs based on its potent and broad pharmacodynamic properties. The oxygen atom in the β-position and the ethyl group in the α-position serve to minimise β-oxidation and esterification with a goal of facilitating a high turnover of phospholipase A_2-independent signalling substrate in liver cells. In a recent phase 2 study, icosabutate at 600mg QD in severe HTG patients achieved highly significant reductions in plasma triglycerides, fasting plasma insulin and HOMA-IR (2).



(2) ADME

(a) After a single 50mg/kg oral administration to Wistar rats, [14-C]-icosabutate is absorbed almost entirely (>99%) via the portal vein **(below).**

Portal Vein Plasma (blood flow rate: 522ml/h)*			Mesenteric Lymph (flow rate: 0.5ml/h)			Icosabutate: Portal vein/Lymph
C_{max} (µg/mL)	Tmax (h)	$AUC_{0\text{-}24h}$ (µg.h/mL)	C_{max} (µg/mL)	Tmax (h)	$AUC_{0\text{-}24h}$ (µg.h/mL)	
17.6	2 (0.5-4)	106	8.43	3 (1-8)	45.8	1200:1

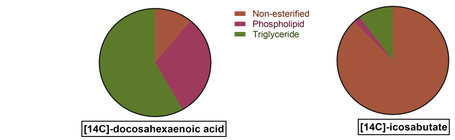
*Assume volume ratio of plasma:blood=1:2

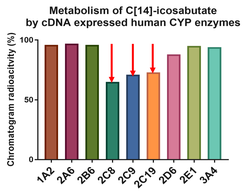
(b) Rapid hepatic accumulation, minimal systemic distribution (**below,** values µg equiv./g tissue) and rapid excretion (>95% complete at 48hrs, urinary 55.5%, faecal 39.5%) is observed after a 100mg/kg oral dose in rats.

	1 hr	2 hrs	4 hrs	8 hrs	1 day	3 days
Liver	78	134	174	145	26	2.6
Muscle	3.6	5.2	10	4.8	1.2	BLQ
Subcutaneous fat	9	14.6	25.3	15.4	7.1	4
Blood	24.6	36.2	60.9	28.1	2.6	BLQ

BLQ Below detection limits

(c) In Huh7 cells, minimal incorporation of [14C]-icosabutate and its metabolites into complex lipids at 24hrs is reflected in the high percentage of radioactivity (>85%) in the most bioactive non-esterified fraction as compared to unmodified [14C]-docosahexaenoic acid of which <10% is non-esterified **(below).**





(d) The marked decline in the concentrations of the parent compound, [14C]-icosabutate, in the presence of human CYP2C isoforms **(left)** supports a major role for CYP450 epoxygenases in the hepatic metabolism of icosabutate

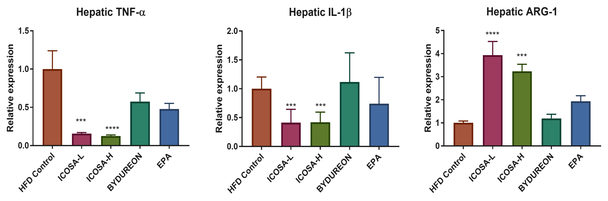
(3) EFFECTS ON GLUCOSE METABOLISM IN OBESE ZUCKER RATS

Icosabutate (135mg/kg) significantly improves plasma insulin, glucose and HbA1c in obese Zucker rats (*p<0.05 versus baseline). A synthetic PPAR-α agonist, fenofibrate (100mg/kg), had no effect on glucose, insulin or HbA1c **(below).**

	Control	Icosabutate	Fenofibrate
Body weight (g)	572.8 ± 9.3	587.5 ± 11.3	530.6 ± 11.0*
Blood glucose (mg/dL)	212.4 ± 31.5	129.3 ± 4.0*	287.6 ± 39.3
Plasma insulin (ng/mL)	17.2 ± 2.8	9.4 ± 0.6*	13.4 ± 1.6
Blood HbA1c (%)	7.8 ± 0.7	5.6 ± 0.4*	8.2 ± 0.6
Plasma adiponectin (µg/mL)	41.7 ± 2.4	36.3 ± 3.9	33.0 ± 3.7*
Plasma ALT (U/L)	125.0 ± 13.5	107.4 ± 6.5	147.9 ± 22.3

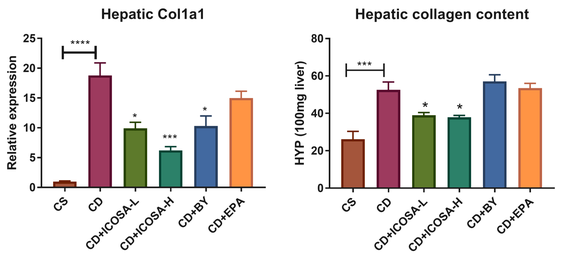
(4) EFFECTS UPON HEPATIC INFLAMMATION IN HIGH FAT FED MICE

Icosabutate, introduced from week 7-12 of the CSAA diet (HFD), induced potent decreases in hepatic TNF-α (p<0.005 and <0.0001 for ICOSA-L and ICOSA-H, respectively) and IL-1β transcripts (both p<0.01) and increased ARG-1 (M2 macrophage marker) expression (p<0.0001 and p<0.0005 for ICOSA-L and –H respectively vs. control). The GLP-1 agonist Bydureon and eicosapentaenoic acid (EPA) at equimolar conc. to ICOSA-H had no significant effect on any transcript **(below).**



(5) EFFECTS ON MARKERS OF FIBROSIS IN CHOLINE DEFICIENT DIET FED MICE

Icosabutate, introduced from week 7-12 of the CDAA diet (CD), significantly reduced both hepatic Col1a1 transcripts (p=0.017 ICOSA-L and p=0.0001 ICOSA-H) and collagen content [hydroxyproline, (HYP)] versus control CD mice (p=0.027 ICOSA-L and p=0.013 ICOSA-H). Bydureon (BY) reduced Col1a1 expression (p=0.024) but had no effect on collagen content, whilst eicosapentaenoic (EPA) acid had no effect on any parameter **(below).**

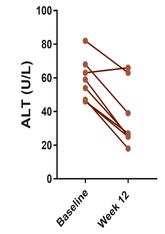


(6) EFFECTS UPON LIVER ENZYMES IN DYSLIPIDEMIC PATIENTS

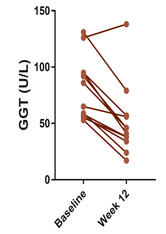
In patients with HTG and elevated liver enzymes, 12 weeks treatment with icosabutate (icosa) significantly reduced median ALT (U/L) and GGT (U/L) by 53% (p=0.015) and 42% (p=0.0015) respectively. Elevated liver enzymes in patients receiving placebo remained unchanged (p=0.98 and p=0.55 for ALT and GGT, respectively) **(below, individual responses under).**

	ALT icosa (n=8)	ALT placebo (n=10)	GGT icosa (n=12)	GGT placebo (n=15)
Baseline	56±4	54.5±4	75.5±8	101±14
Week 12	26.5±7*	58±3	43.5±9***	101±17
% change	-53	+6	-42	0

Serum ALT in response to icosabutate in HTG patients



Serum GGT in response to icosabutate in HTG patients



CONCLUSIONS

- **Icosabutate overcomes the inherent ADME limitations of unmodified fatty acids as oral drugs targeting metabolic and inflammatory pathways in the liver and demonstrates potent anti-inflammatory, insulin sensitising and anti-fibrotic effects.**
- **A 600mg/day dose normalised elevated liver enzymes in dyslipidemic patients.**
- **Icosabutate may offer a novel, potent and differentiated therapeutic approach to inflammatory and fibrotic disorders of the liver, including NASH.**

ACKNOWLEDGEMENTS

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