Decline in NASH- and atherosclerosis-associated oxidised phospholipids and 7-ketocholesterol in response to icosabutate therapy

David A Fraser[1], Xiaoyu Wang[2], Yong Ook Kim[2], Marta Iruarrizaga-Lejarreta[3], Tore Skjaeret[1], Cristina Alonso[3], John Kastelein[4], Detlef Schuppan[2, 5]

1 NorthSea Therapeutics, 1105 BP Amsterdam, The Netherlands; 2 Institute of Translational Immunology, University Medical Center, Mainz, Germany; 3 OWL Metabolomics, Parque Tecnológico de Bizkaia, Derio, Spain; 4 Department of Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; 5 Division of Gastroenterology, Beth Israel Deaconess Medical Center, Boston, MA, USA. Disclosure: D. Schuppan acts as a consultant for NorthSea Therapeutics

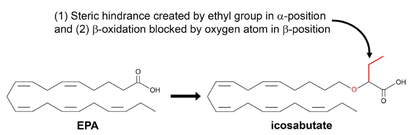
Introduction

Oxidative stress plays a central role in the pathology of both non-alcoholic steatohepatitis (NASH) and atherosclerosis. We have recently shown that icosabutate, a liver-targeted, structurally engineered eicosapentaenoic acid (EPA) derivative resistant to β-oxidation and membrane incorporation, significantly reduces hepatic oxidised glutathione in multiple murine NASH models (in association with significant anti-fibrotic effects) and normalises elevated plasma gamma-glutamyltransferase (GGT) in hyperlipidemic humans. To investigate if these findings extend to pathology-associated oxidised lipids, we assessed (A) hepatic concentrations of NASH associated oxidised phospholipids (oxPLs) (ref. 1) in icosabutate versus EPA treated mice and (B) plasma concentrations of atherosclerosis associated 7-ketocholesterol (7-KC) (ref. 2) in hypercholesterolemic humans after 28 days treatment with either 600mg q.d. icosabutate or placebo. In hyperlipidemic subjects treated with statins, we also measured changes in plasma GGT, irrespective of baseline values, in response to 12-weeks treatment with 600mg q.d. icosabutate or placebo.

Methods

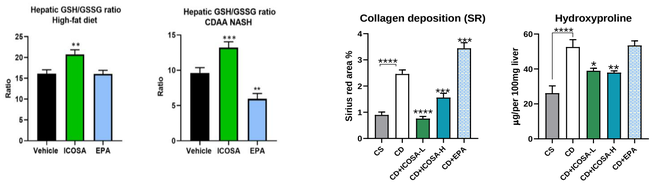
9-week old C56BL/6J mice were fed a high sucrose (40% of calories), amino acid defined and moderately high-fat (31% of calories as fat) diet (MFD), or the same diet deficient in choline and supplemented with low (0.2%) cholesterol for 12 weeks (CD). Mice in both the MFD and CD groups received treatment with either low (56mg/kg, ICOSA-L) or high (112mg/kg, ICOSA-H) dose icosabutate or EPA (eicosapentaenoic acid, equimolar to ICOSA-H) for the final 6 weeks. Changes in hepatic concentrations of oxPLs (phosphatidylcholine) containing either oxidised linoleic acid (LA; 16:0/18:2-OH) or arachidonic acid (AA; 16:0/20:4-OH) isomers were measured via LC-MS/MS, and differences versus baseline were assessed via ANOVA with Dunnet's correction. In hyperlipidemic subjects temporarily withdrawn from statins (NCT02364635), plasma concentrations of 7-KC were assessed at day 0 (baseline), 7 and 28 (study end) in response to either 600mg q.d. icosabutate (n=15) or placebo (n=5) and differences versus baseline were assessed via ANOVA as a univariate procedure. Data are presented as mean (SEM), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Background



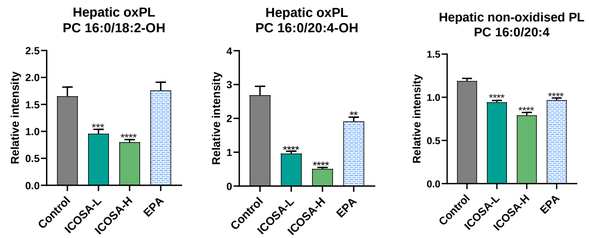
Left: Chemical structure of EPA compared with icosabutate. The changes are designed to prevent use as an energy source and to avoid incorporation into complex lipids.

Below left panel: Icosabutate, reduced a marker of hepatic oxidative stress, the reduced (GSH) to oxidised (GSSG) glutathione ratio, in both MFD and CD diet fed mice, whereas EPA worsens GSH/GSSG in the CDAA model (see ref. 3).
Right panel: Icosabutate, but not EPA, improved hepatic fibrosis in the CDAA NASH model (see ref 4). CS = MFD control.



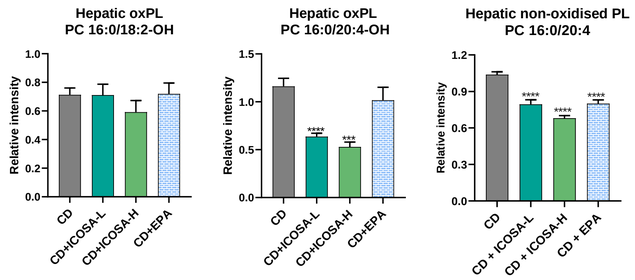
Results (A) NASH associated oxPLs in mice

Icosabutate reduces the predominant hepatic oxPLs (phosphatidylcholine) in MFD fed mice



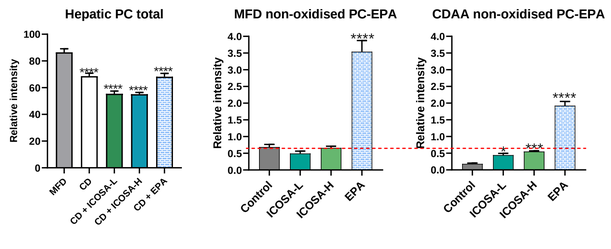
Above: In MFD fed mice, icosabutate significantly reduces oxPLs containing both oxidised LA (by 42 and 52% at low- and high-dose respectively, far left) and oxidised AA (by 64 and 81% at low- and high-dose respectively, middle) whilst EPA reduces PL containing oxidised AA (by 28%). The reductions in oxidised AA-containing oxPLs were associated with a less pronounced but significant decrease in non-oxidised AA containing PLs (far right).

Icosabutate reduces hepatic AA-oxPLs in CD diet fed mice with fibrosing NASH



Left: In CD diet fed mice with fibrosing NASH, icosabutate significantly reduces oxPLs containing oxidised AA (by 46 and 55% at low- and high-dose respectively) whilst EPA has no significant effect (middle).

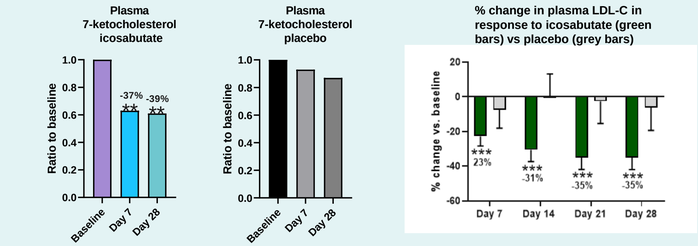
The CD diet reduces total hepatic PC and induces a disproportionate decrease in hepatic non-oxidised EPA relative to AA



Left: The CD diet reduces hepatic PC secondary to choline deficiency (far left). Note the % decrease in PC-EPA induced by the CD diet (red line) is disproportionate to both the decrease in total PC (far left) and PC-AA (above), but not in icosabutate treated mice.

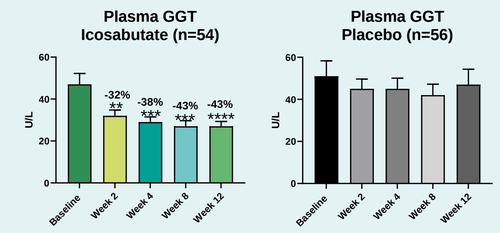
(B) Atherosclerosis associated 7-KC in humans

Icosabutate (600mg q.d.) rapidly reduces plasma 7-KC and reduces LDL cholesterol in hypercholesterolemic subjects



Above: Icosabutate 600mg q.d. for 28 days (n=15) reduces plasma 7-ketocholesterol (7-KC) in hypercholesterolemic (mean baseline non-HDL-C 180mg/dl) at day 7 and 28 (days 14 and 21 not measured). The reduction in 7-KC initially exceeds the decrease in LDL-C (right) at day 7 and corresponds with the day 28 LDL-C reduction of 35% (NCT02364635, see ref. 5).

12 weeks treatment with oral icosabutate 600mg q.d. reduces plasma GGT in overweight/obese, dyslipidemic humans



Left: Marked and rapid decreases in plasma GGT (a marker of hepatic oxidative stress) in overweight/obese dyslipidemic subjects treated with statins (NCT0197178, see ref.6). The percentage decreases are comparable to the previously reported decreases seen in subjects with elevated baseline plasma GGT (see ref. 7).

Conclusions

- **Icosabutate effectively reduces NASH (hepatic oxPLs) and atherosclerosis (plasma 7-KC) pathology-associated oxidised lipids in mice and humans.**
- **In the CD NASH mouse model the decreases in hepatic oxPLs occur in conjunction with significant reductions in fibrosis, neither of which are observed with EPA**
- **The decreases of hepatic oxPLs and plasma 7-KC can be partially explained by reductions in peroxidation prone lipids in liver and plasma respectively.**
- **These findings the therapeutic potential of icosabutate for the treatment of both NASH and atherosclerosis via a reduction in deleterious oxidised lipids.**

References

1. Sun X, Seidman JS, Zhao P, Troutman TD, Spann NJ, Que X, et al. Neutralization of Oxidized Phospholipids Ameliorates Non-alcoholic Steatohepatitis. Cell Metab 2020;31:189-206 e188.
2. Brown AJ, Jessup W. Oxysterols and atherosclerosis. Atherosclerosis. 1999; *142*:1–283.
3. https://www.northseatherapeutics.com/wp-content/uploads/2019/04/SAT_346_ILC2019.pdf
4. https://www.northseatherapeutics.com/wp-content/uploads/2018/11/poster-1-CDAA-AASLD-2018.pdf
5. Qin Y, Hallén J, Skjæret T, Odden E, Fraser D, Nyheim H, et al. Abstract 11889: Phase Ib Study of Icosabutate, a Novel Structurally Enhanced Fatty Acid, in Subjects With Hypercholesterolemia. Circulation 2014;130:A11889-A11889.
6. Kastelein JJ, Hallen J, Vige R, Fraser DA, Zhou R, Hustvedt SO, et al. Icosabutate, a Structurally Engineered Fatty Acid, Improves the Cardiovascular Risk Profile in Statin-Treated Patients with Residual Hypertriglyceridemia. Cardiology 2016;135:3-12.
7. https://www.northseatherapeutics.com/wp-content/uploads/2019/04/LBP_10_ILC2019.pdf