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

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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 hyperlipidaemic 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 hyperlipidaemic 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 fat (31% of calories as fat) diet to develop NASH. Icosabutate or EPA (18-fold higher than EPA) was administered for 6 weeks via gavage to mice starting at 8 weeks of age (n=6/group). NASH was assessed by day 0 (baseline), 7 and 28 (study end) in response to either 600mg EPA q.d. or the same diet deficient in choline and ketocholesterol (7-KC) or the same diet deficient in choline. To assess plasma lipo-proteinuria we used a urinary proteinuria assay (U/P). The changes are designed to reduce hepatic non-oxidised (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) whereas 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).

Research

Background

Below left panel: Icosabutate, reduced a marker of hepatic oxidative stress, the reduced (GSH) to oxidised (GSSG) glutathione ratio, in both EPA and icosabutate treated mice, whereas EPA worsens GSH/GSSG in the CDAAS model (ref. 3).

Right panel: Icosabutate, but not EPA, improved hepatic fibrosis in the CDAAS NAFLD model (ref. 4). CII = MDF control.

Conclusions

• Icosabutate effectively reduces NASH (hepatic oxPLs) and atherosclerosis (plasma 7-KC) pathology-associated lipids in vivo in murine models.
• In the CDAAS 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

Results (A) NASH associated oxPLs in mice

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

Below: 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) whereas 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).

The changes in oxidised AA-containing oxPLs in mice treated with icosabutate (left) are significantly reduced versus baseline and placebo (right).

Below left: Icosabutate reduces hepatic AA-oxPLs in CD fed mice with fibrosis NASH

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

Below right: The CD diet reduces hepatic PC secondary to choline deficiency (far left).

The changes are designed to reduce hepatic non-oxidised (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) whereas 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 fed mice with fibrosis NASH

In the CD diet fed mice, hepatic AA-oxPLs are significantly reduced in EPA treated mice (left) 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.

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

Plasma GGT

Icosabutate (n=54)

Placebo (n=56)

-43% -43% -38% -32%