Icosabutate induces a potent reduction in hepatic oxidative stress in multiple rodent models of metabolic stress and fibrosing NASH

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Introduction

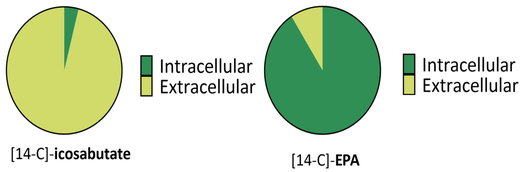
- Long-chain n-3 fatty acids and their oxygenated metabolites act as ligands and substrate for beneficial signaling pathways regulating hepatic inflammation and metabolism.
- However, their accumulation into cellular membranes and proneness to peroxidation could reduce their therapeutic potential in conditions associated with excessive oxidative stress, such as NASH.
- Icosabutate is a structurally-engineered eicosapentaenoic acid (EPA) derivative designed to resist both incorporation into complex cellular lipids (including cell membranes) and β-oxidation.
- Potent anti-fibrotic effects were observed after oral icosabutate treatment in multiple rodent models of NASH, whereas no effects were seen with either EPA, a FXR agonist (obeticholic acid) or a PPAR-γ agonist (rosiglitazone).
- Effects on hepatic oxidative stress and the arachidonic cascade as a potential differentiating factor between treatments was investigated.

Methods

- Partitioning of [14C]icosabutate or [14C]EPA into cellular lipids in Huh7 cells was investigated over 24h in 3 separate experiments.
- Oxidised (GSSG) and reduced (GSH) glutathione along with oxygenated arachidonic (AA) metabolites were measured in liver samples collected from 4 mouse models: (1) High-fat diet-12 week high-fat (31% total calories) choline-sufficient diet fed mice treated last 6 weeks with 112 mg/kg icosabutate or 91 mg/kg EPA (both 0.3mmol/kg); (2) CDAA NASH-12 week high-fat choline-deficient amino-acid defined diet treated last 6 weeks with 112 mg/kg icosabutate or EPA; (3) *ob/ob*-NASH diet-induced and biopsy-confirmed model of fibrotic NASH treated with icosabutate (135mg/kg) or OCA (30mg/kg) for 8 weeks; (4) APOE*3Leiden.CETP mice fed a 24% fat, 1% cholesterol diet for 20 weeks treated with either 112mg/kg icosabutate or 13mg/kg rosiglitazone (ROSI) by gavage.
- n=9 (icosabutate), n=8 (EPA) mice per group in HFD and CDAA NASH model, n=12 in *ob/ob*-NASH and APOE*3L.CETP NASH models. Data presented as mean values ± S.E.M. *p<0.05, **p<0.01, ***p<0.001, p<0.0001 vs vehicle.

Results

Cellular partitioning of icosabutate vs. EPA in hepatocytes *in vitro*

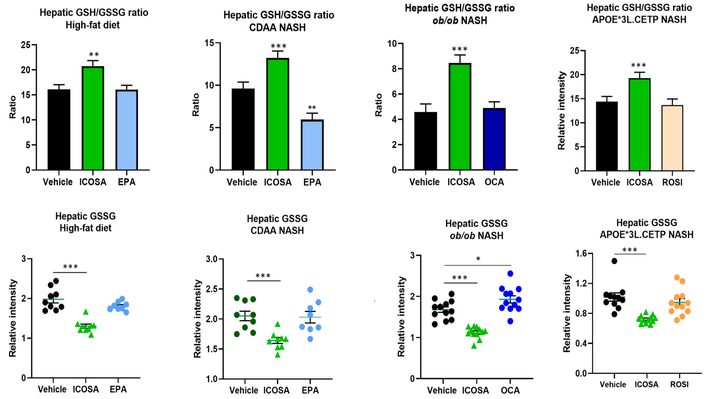


Left: In contrast to EPA, only a fraction of icosabutate/icosabutate metabolites have accumulated in HUH7 cells after 24h

	30µM		100µM	
	EPA	Icosabutate	EPA	Icosabutate
FFA	1.3	0.96	2.89	1.91
TAG	22.37	1.07	114.72	4.92
PL	33.26	0.74	70.31	2.99
CE	8.14	1.11	21.74	1.92
Total lipid	**65.07**	**3.88**	**209.65**	**11.74**

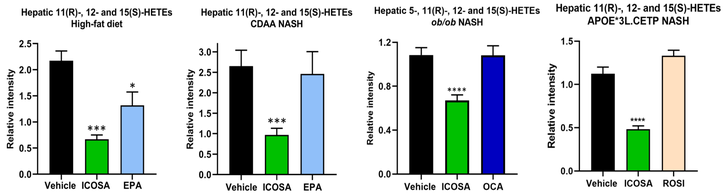
Left: Icosabutate/icosabutate metabolites avoid incorporation into complex lipids of HUH7 cells after 24h incubation values in nmol/mg protein).

Icosabutate, but not EPA, obeticholic acid or rosiglitazone, reduces hepatic oxidative stress in differentiated metabolic overload/NASH models

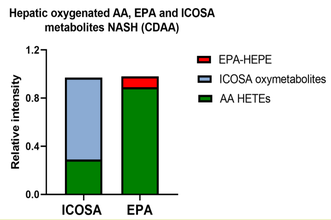


Above: Icosabutate (ICOSA), but not EPA, OCA or rosiglitazone, significantly improves the GSH/GSSG ratio via a reduction in hepatic GSSG concentrations in all 4 metabolic overload/ NASH models. EPA has a neutral effect in a moderate stress (high-fat) model but worsens the GSH/GSSG ratio in the more severe CDAA NASH model secondary to glutathione depletion, whilst OCA worsens hepatic GSSG in a *ob/ob* NASH model.

Icosabutate potently reduces hepatic oxygenated arachidonic acid metabolites (HETEs) in moderate and severe (NASH) metabolic overload models

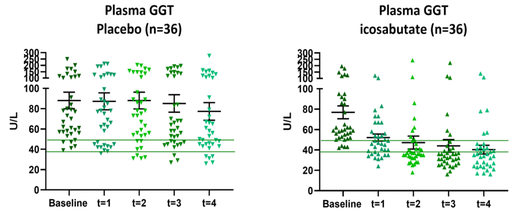


Above: Icosabutate (ICOSA) reduces hepatic concentrations of pro-inflammatory HETEs in 4 differentiated metabolic overload/NASH models. AA-HETEs are major substrates for the NASH/liver disease associated enzyme HSD17B13 (see suppl. data in *Abul-Husn NS et al., A Protein-Truncating HSD17B13 Variant and Protection from Chronic Liver Disease. N Engl J Med. 2018;378(12):1096-106).*



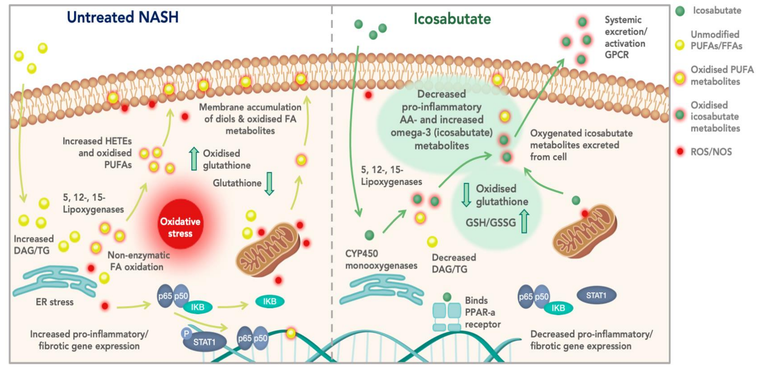
Left: Icosabutate (ICOSA) markedly decreases hepatic oxygenated AA metabolites (green) whilst increasing oxygenated omega-3 ICOSA (epoxide/hydroxy) metabolites (blue). EPA has modest effects on the concentration of oxygenated EPA (red) or AA metabolites.

Effects of up to 12 weeks treatment with oral icosabutate 600mg q.d. on elevated plasma GGT in overweight/obese, dyslipidemic humans



*The decrease in plasma GGT was significant at t=1 (p<0.05) and all other time points for icosabutate (p<0.0001) vs baseline. Placebo was reduced at t=4 only (p<0.01)

Above: Marked and rapid decreases* in elevated plasma GGT (a marker of oxidative stress) in overweight/obese dyslipidemic subjects suggest hepatic antioxidant effects in rodents are translatable to humans. Data are from 3 clinical trials where subjects were treated for up to 12 weeks with 600mg/day icosabutate. Horizontal lines represent abnormal thresholds (male/female).



Schematic overview of icosabutate's regulation of hepatic eicosanoid metabolism and oxidative stress in NASH. Icosabutate enters via the portal vein, remaining as a free-acid intracellularly. A profound switch from oxygenated omega-6 (AA) to omega-3 (icosabutate) metabolites occurs in conjunction with a decrease in oxidative stress. Icosabutate (and its oxygenated metabolites) avoid accumulation in peroxidation-prone membranes and exit the cell where they can also bind GPCRs and/or be excreted.

Conclusions

- Icosabutate does not accumulate in hepatocytes and acts as a potent hepatic antioxidant in both mild metabolic overload (HFD) and NASH (CDAA, *ob/ob*-NASH, APOE*3L.CETP) models.
- Icosabutate increases the generation of hepatic oxygenated omega-3 (icosabutate) metabolites with concomitant reductions in detrimental oxygenated AA metabolites.
- A reduction in hepatic oxidative stress and/or pro-inflammatory arachidonic acid metabolites may contribute to the potent anti-fibrotic effects of icosabutate observed in multiple rodent NASH models and differentiates icosabutate from unmodified EPA, FXR and PPAR-γ agonism.
- The rapid and marked reductions in plasma GGT (a marker of cellular oxidative stress) in overweight/obese dyslipidemic subjects support the plausibility that the reductions in hepatic oxidative stress in NASH rodent models are occurring in humans treated with icosabutate.

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