Coadministration of a GLP-1/Glucagon Receptor Agonist With an FXR Agonist and ACC Inhibitor Reverses Nonalcoholic Steatohepatitis in Diet-Induced and Biopsy-Confirmed Mice

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Presented at American Diabetes Association 80th Scientific Sessions: a Virtual Experience, June 12–16, 2020

Introduction

- GLP1 (CLO) is a fast-acting X receptor (FXR) agonist in clinical development for non-alcoholic steatohepatitis (NASH) and is in phase 3 development for non-alcoholic fatty liver disease (NAFLD). CLO is well tolerated and reduces liver fat by magnetic resonance imaging–pion density fat fraction, and improves markers of liver injury in patients with NASH and Type 2 diabetes in a phase 3 trial.
- Preclinical studies have shown that GLP1 and glucagon receptor agonists improve liver function in rodent models of NAFLD.
- Preclinical effects of CILO/ACCi/SEMA have been evaluated in preclinical models, ACC inhibition via tool FIR analog GS-834356 (pending).
- The preclinical effects of CILO/GlcG/ACCi have been evaluated in the amylin liver (AMLN) trans-fat diet-induced obese mouse model and the glucagon-like peptide-1 (GLP1) receptor agonist semaglutide (SEMA) on liver fat and biomarkers of liver injury and fibrosis are also being evaluated in patients with NAFLD (NCT02389704).

Objective

- To explore the effects of 12 wk of treatment with GLP1:GcG and/or CLO and/or ACCi on metabolic and liver pathology in AMLN DIO-NASH

Methods

AMLN DIO-NASH Study Design

- Animals were randomized to treatment groups based on baseline liver steatosis (score 12) and fibrosis (stage 2).
- All groups were fed a diet with either vehicle (0.2% sodium carboxymethylcellulose, 1% ethanol, 86.5% mid-fat lard) or lead agent, and with either vehicle (0.005%, poloxamer 201, 20 mg/kg sodium phosphate, 70 mg sodium chloride) or test agent, both at 5 mL/kg, for 12 wk.
- Body weight was measured daily; blood was collected via tail vein during Week 12 after 4 h for measurement of liver and plasma biomarkers.
- Monocytes were analyzed at the end of the study via immunofluorescence and liver tissue was collected, weighed, and subjected to TG and TC measurement or histological analysis.
- Histology and immunohistochemistry were performed using published protocols.
- Hematoxylin and eosin (H&E) staining was used to determine steatosis and NASH Activity Score (NAS; sum of 0–2 for steatosis and lobular inflammation, and 0–2 for ballooning).
- Individual changes in pre- vs post-treatment were evaluated by unpaired 2-tailed Student's t-test and presented as mean ± standard error of mean.
- Statistical analyses were performed using GraphPad Prism 8.1.2 for Windows (GraphPad Software, San Diego, CA).
- For NAS and fibrosis responder rate comparisons, Fisher exact test was used.
- All other graphs and analyses were generated using Prism 8.1.2 for Windows (GraphPad Software).

Results

Body Weight

- Body weight was significantly reduced (15%) by GLP1:GcG treatment.
- Further body weight loss was observed (22%) in GLP1:GcG + CLO group, but not with addition of ACCi or in triple-combination group.

Liver Weight and Triglyceride Content at Week 12

- Liver weight and TG content were significantly reduced by GLP1:GcG and in the triple-combination group.
- The addition of ACCi did not improve liver pathology.
- The combination of CILO + ACCi + GLP1:GcG was the most effective treatment; there was no additional benefit with dual-combination or triple therapy.

NASH Activity

- AMLN DIO-NASH demonstrated increased NAS, primarily driven by steatosis and inflammation.
- GLP1:GcG treatment significantly reduced NAS, which was minimalized by addition of CLO or ACCi.
- CILO + ACCi combination similarly improved NAS.
- Improvement in NAS by 23 points required ≥1 pharmacology.

α-SMA as Marker of Fibrogenesis

- AMLN DIO-NASH demonstrated increased α-SMA deposition and clinically derived fibrosis staging.
- GLP1:GcG treatment did not significantly reduce Col1a1 % fractional area or lead to significant number of mice with improved fibrosis (p=0.075).†
- Both CILO + ACCi and triple-combination therapy lead to highest numbers of animals with improved fibrosis (n=8/11 (73%) and n=11/13 (85%), respectively), and the greatest reductions in Col1a1 % fractional area.

Fibrosis

- AMLN DIO-NASH demonstrated increased fibrosis and clinically derived fibrosis staging.
- CILO + ACCi and triple-combination therapy lead to highest numbers of animals with improved fibrosis (n=8/11 (73%) and n=11/13 (85%), respectively).
- Col1a1 % fractional area was reduced to ≤90% of vehicle in all treatment groups.

Conclusions

- GLP1:GcG effectively reduced hepatic lipids, and the addition of CILO and ACCi led to greater reductions in NAS and fibrosis stage in AMLN DIO-NASH.
- AMLN DIO-NASH exhibited increased plasma levels of ALT, AST, and TC, with TG being below chow levels.
- GLP1:GcG and ACCi significantly reduced ALT, which was not further decreased by addition of other agents.
- Plasma TG was slightly elevated in ACCi groups and lower in CILO groups.
- Plasma TC was elevated by diet and reduced in all treatment groups.

* p <0.05 vs vehicle; † p <0.05 vs CLO; ‡ p <0.05 vs ACCi; § p <0.05 vs vehicle + ACCi; || p <0.05 vs vehicle + CLO + ACCi.

Acknowledgments

This study was supported as part of a collaboration between Gilead and Novo Nordisk.