CB4209 and CB4211 Reduce the NAFLD Activity Score in the STAM Model of NASH, Reduce Triglyceride Levels, and Induce Selective Fat Mass Loss in DIO Mice

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Background

CB4209 and CB4211 are novel, improved peptide analogs of MOTS-c, a mitochondrially encoded peptide with a potential role in maintaining metabolic homeostasis. Metabolic dysfunction is a common underlying factor in the pathogenesis of many age-related diseases including, non-alcoholic fatty liver disease and steatohepatitis (NAFLD and NASH), obesity, type 2 diabetes, neurodegenerative disease, and cancer. The potential utility of CB4209 and CB4211 for the treatment of NASH, obesity, and type 2 diabetes was evaluated in preclinical models using cultured adipocytes, DIO mice, and the STAM® mouse model of NASH.

Methods

In Vitro Lipolysis

CB4209 and CB4211 were evaluated in vitro for effects on stimulated lipolysis in differentiated 3T3-L1 cells or primary human adipocytes. Cells were incubated with peptides (25 µM) for 24 h, stimulated with isoproterenol (ISO; 1 nM) or forskolin (FSK; 1 µM), and free fatty acid levels in media were determined after 3 h by FFA assay kit (Zen-Bio) (CV <5%).

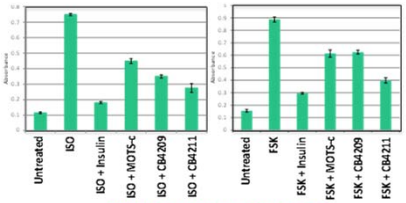
DIO Mouse Studies

Peptides were administered alone or in combination with liraglutide (10 nmol/kg) in various regimens (intra-peritoneal vs subcutaneous, 5 vs. 15 mg/kg, once vs. twice daily) for 10 - 21 days to 28 wk old male DIO mice maintained on a high fat diet (60% kcal from fat). Assessments: body weight, blood glucose, qNMR, food intake, serum biomarkers, and liver histopathology compared to vehicle and liraglutide-treated controls.

Figure 1. In Vitro Lipolysis Assay

Inhibition of Stimulated Adipocyte Lipolysis

3T3-L1 Mouse Adipocytes



Primary Human Adipocytes

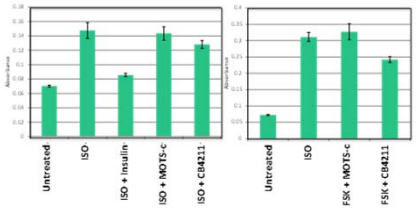
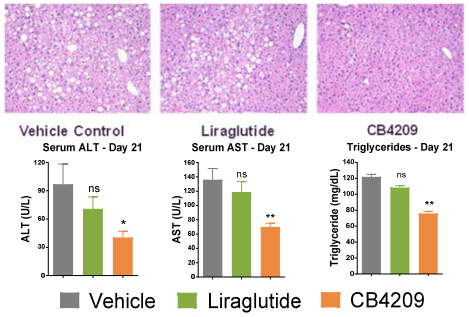
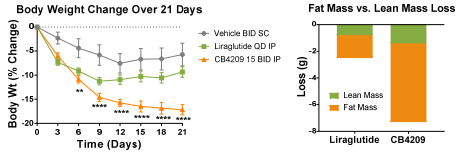


Figure 2. DIO Mouse Model

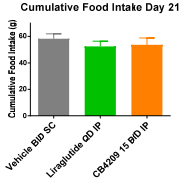
Liver Histopathology and Biomarkers at Day 21



Body Weight and qNMR at Day 21



Food Intake



Dosing Regimen

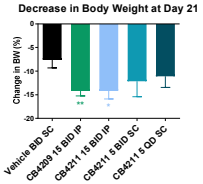


Figure 3. Synergy with Liraglutide

Liver Histopathology at Day 21

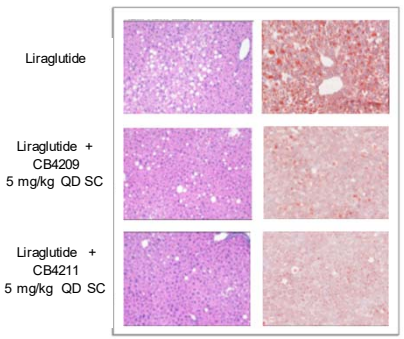


Figure 4. Synergy with Liraglutide

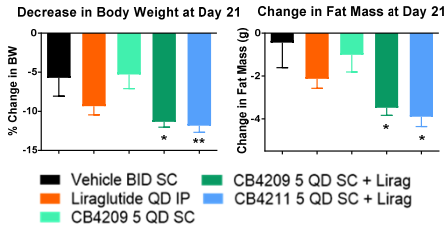
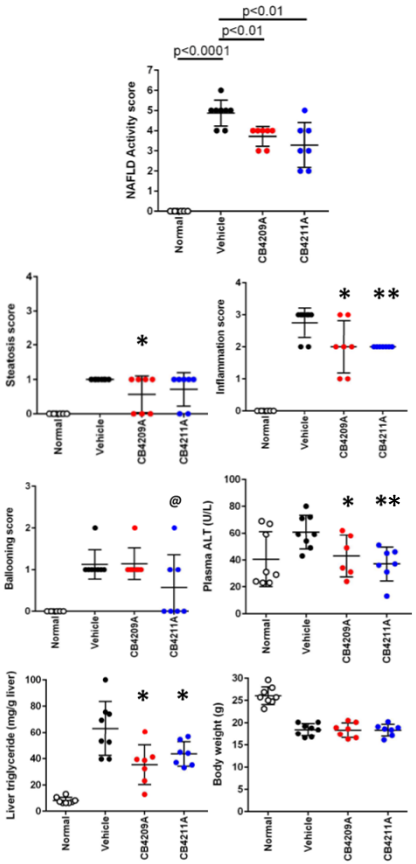


Figure 5. STAM® NASH Model



STAM® NASH Mouse Study

The peptides were evaluated in the STAM® mouse NASH model for 21 days (15 mg/kg IP BID), beginning at 6 wks of age and compared to vehicle treated STAM or normal mice. Assessments included NAS, body weight, serum biomarkers, and liver histopathology.

Healthy Mouse Study

CD-1 Mice (M/F 6 wks of age) received daily IP doses of CB4211 (dose range from 15 to 250 mg/kg/day) for 7 days.

Results

In Vitro Lipolysis

CB4209 and CB4211 significantly inhibited isoproterenol and forskolin-stimulated lipolysis in vitro in both species (Fig 1).

DIO Mouse Studies

In obese DIO mice, CB4209 and CB4211 significantly reduced body weight at 21 days and decreased liver steatosis vs vehicle control and liraglutide, with more selective fat mass loss than liraglutide (Fig 2). Serum ALT, AST, and triglyceride levels were significantly reduced. The peptides did not significantly decrease food intake. Once daily subcutaneous dosing of both peptides at 5 mg/kg showed a synergistic effect to liraglutide (GLP-1 agonist) on liver steatosis, body weight, and fat mass loss (Figs 3&4).

Healthy Mouse Study

Daily administration of CB4211 (intraperitoneal doses from 15 to 250 mg/kg/day) for 7 days did not cause body weight reduction in male or female healthy mice (data not shown).

STAM NASH Mouse Study

Both peptides significantly reduced NAFLD activity score (NAS) in STAM mice without affecting body weight (Fig. 5). NAS was decreased 24% by CB4209 and 33% by CB4211, versus vehicle treated animals (both P<0.01), without affecting body weight. Both peptides significantly reduced plasma ALT and liver triglyceride levels. Statistical significance: *P<0.05; **P<0.01 Student's t test; @P<0.05 Mann-Whitney.

Conclusions

CB4209 and CB4211 reduced secreted free fatty acid levels in cultured adipocytes. Both peptides were effective in reducing NAS in the STAM NASH model and produced significant and sustained weight loss in obese DIO mice, selectively decreasing fat mass and improving markers of liver damage. Effects were synergistic with the GLP-1 agonist liraglutide. No body weight loss occurred in healthy mice.

Disclosures

Kenneth Cundy, Kent Grindstaff, Rémi Magnan, and Wendy Luo: Employees and shareholders of CohBar. Taiishi Hasiguchi: Employee of SMC Laboratories. Diego Perez-Tilve: Research funds from Cohbar. Jenna Holland: None.