P2Y13 receptor agonist CER-209, an anti-atherosclerotic compound, decreases liver steatosis in vivo

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Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are increasing as a consequence of growing worldwide obesity prevalence. NAFLD is the most prevalent chronic liver disease, affecting 25–40% of the general population, and approximately one-third of patients with NAFLD will progress to NASH. More recently, NAFLD guidelines recommend weight loss, lifestyle changes, and the introduction of exercise programs as therapies. Increased physical activity, control of hyperglycemia and treatment of hyperlipidemia with statins stabilize liver lipids through regulation of low-density lipoprotein (LDL) cholesterol. However, lipid transport (RLT), driven by high-density lipoprotein (HDL) metabolism, controls the transfer of cholesterol from non-hepatic cells to the liver, where it is excreted from the body in the form of bile acids and unesterified cholesterol. The capacity of HDL particles to mobilize cholesterol from atherosclerotic plaque confers protection against heart failure and death. HDL14-dro- and HDL2 lowers cholesterol and triglyceride concentrations in a new NAFLD/NASH mouse model. Here we used a new in vivo mouse model of fatty liver and steatohepatitis; however, this has never been studied in NAFLD and NASH. We observed a significant reduction of the liver enzymes ALT and AST in the plasma due to CER-209 treatment. Those effects on the restoration of the liver integrity are in favor of a strong potential for CER-209 to treat liver disease such as NAFLD and/or NASH.

Regression of Atherosclerosis after CER-209 treatment in HFD rabbit model was confirmed. At randomization (1 week of diet), mean plasma ALT and AST levels were 249 and 286 U/L, respectively. *p<0.05 and **p<0.01 vs. vehicle.

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**CONCLUSION**

Recently, we demonstrated in vivo in mice that CER-209 acts as a key partner in HDL metabolism and the RLT process, and thereby promotes atherosclerosis protection. These data support a mechanism in which stimulation of HDL uptake or endocytosis by the liver, via activation of the P2Y13 receptor, promotes cholesterol excretion by the liver, secretion of cholesterol in the gallbladder and elimination in the faeces. In the present study, we demonstrated that CER-209 lowers triglyceride and cholesterol concentrations in a new NAFLD/NASH model. CER-209 treatment resulted in a decrease in the steatosis that is observed in diet induced mouse model. As expected with such short-term treatment, body weight and inflammatory markers was not changed, however, there was a tendency to decrease in the measured amount of steatosis. The findings were in accordance with our observation that cholesterol and triglyceride content of the liver also decreased with CER-209 treatment. It is noteworthy that liver or animal weights were not observed to change with treatment in any of our animal studies.

Materials and Methods

Ten-week-old male C57BL/6 mice were purchased at 5 weeks of age from Charles River Laboratories and were maintained under specific pathogen-free conditions in accordance with the Guide for the Care and Use of Laboratory Animals. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals. All animals were maintained in a specific-pathogen-free environment and were provided with food and water ad libitum. Mice were randomly assigned to 5 homogenous treatment groups (n=10/group) according to their body weight. Mice were then treated orally QD with vehicle, 60% high fat/1.25% cholesterol/0.5% cholic acid diet over a short-term period to determine whether CER-209, a hepatic G protein-coupled receptor agonist belonging to a new class of P2Y13 receptor (P2Y13r) agonists and designed to improve HDL metabolism (i.e. HDL elimination), could also have a marked effect on liver metabolism. We have recently demonstrated in vivo CER-209 in a canine P2Y13r receptor is key partner in the HDL metabolism and reverse cholesterol transport process, and thereby provide strong support for the hypothesis that these data support a mechanism where the stimula
tion of HDL uptake or endocytosis by the liver via P2Y13 pathway activation promotes cholesterol excretion by the liver, secretion in the gallbladder and final fecal elimination.

**Figure 1. Body weight and body weight gain during the 2-week treatment period.**

60% high fat, 2% cholesterol, 2% cholic acid diet with 20% 2-hydroxypropyl betacyclodextrin oral gavage over 1 week induced a 1.8g reduction in body weight. At randomization (1 week of diet), mean body weight was 24g.

**Figure 2. Plasma ALT and AST levels at randomization and at the end of treatment period.**

ALT, AST and body weight were determined in plasma of all mice. Body weight, ALT and AST were significantly decreased during the 2-week treatment period.

**Figure 3. Liver weight, hepatic total cholesterol, triglycerides, fatty acids and phospholipids at the end of treatment period.**

At the end of the 2-week treatment period, liver weight was unchanged by both CER-209 and obeticholic acid. Hepatic total cholesterol and phospholipids tended to be reduced with CER-209, but not significantly in opposite of triglycerides and FFA which were significantly reduced at 1 mg/kg and 3 mg/kg doses respectively.

**Figure 4. Hepatic gene expression for alpha-SMA, collagen, TIMP1 and luman-ectin at the end of treatment period.**

Collagen 1 alpha-1 (Col1a1), collagen alpha-2 (Col1a2), collagen alpha-3 (Col1a3) and collagen alpha-4 (Col1a4) tended to be reduced with CER-209 treatment. Those effects on the restoration of the liver integrity are in favor of a strong potential for CER-209 to treat liver disease such as NAFLD and/or NASH.

**Figure 5. Representative staining for oil red O, hematoxylin & eosin and Sirius red at the end of treatment period.**

Oil red O staining of the liver showed a significant decrease of triglycerides and cholesterol accumulation.

**Figure 6. Steatosis, inflammation, fibrosis, and total NAS score at the end of treatment period.**

NAS score was significantly decreased with CER-209 treatment and no treatment difference was observed in collagen or fat droplet.