Research Article

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Sitagliptin *vs.* placebo for non-alcoholic fatty liver disease: A randomized controlled trial

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Background & Aims: Uncontrolled studies show sitagliptin, an oral DPP-4 inhibitor, may improve alanine aminotransferase and liver histology in non-alcoholic fatty liver disease (NAFLD) patients. We aimed to compare sitagliptin *vs.* the efficacy of a placebo in reducing liver fat measured by MRI-derived proton density-fat fraction (MRI-PDFF).

Methods: This randomized, double-blind, allocation-concealed, placebo-controlled trial included 50 NAFLD patients with prediabetes or early diabetes randomized to sitagliptin orally 100 mg/day or placebo for 24 weeks. Primary outcome was liver fat change measured by MRI-PDFF in colocalized regions of interest within each of nine liver segments. Additional advanced assessments included MR spectroscopy (MRS) for internal validation of MRI-PDFF's accuracy, and magnetic resonance elastography (MRE) and FIBROSpect® II to assess liver fibrosis.

Results: Sitagliptin was not significantly better than placebo in reducing liver fat measured by MRI-PDFF (mean difference between sitagliptin and placebo arms: −1.3%, $p = 0.4$). Compared to baseline, there were no significant differences in end-of-treatment MRI-PDFF for sitagliptin (18.1% to 16.9%, $p = 0.27$) or placebo (16.6% to 14.0%, $p = 0.07$). The groups had no significant differences for changes in alanine aminotransferase, aspartate aminotransferase, low-density lipoprotein, homeostatic model assessment insulin resistance, and MRE-derived liver stiffness. In both groups at baseline and post-treatment, MRI-PDFF and MRS showed robust correlation coefficients ranging from $r^2 = 0.96$ to $r^2 = 0.99$ ($p < 0.0001$), demonstrating the strong internal validity of the findings. FIBROSpect® II showed no changes in the sitagliptin group but was significantly increased in the placebo group ($p = 0.03$).

Conclusions: Sitagliptin was safe but not better than placebo in reducing liver fat in prediabetic or diabetic patients with NAFLD.

Lay summary: In a randomized, double-blind, placebo-controlled study, the anti-diabetic drug sitagliptin was no more effective than placebo for improving liver fat and liver fibrosis in patients with non-alcoholic fatty liver disease. This study demonstrates that non-invasive magnetic resonance imaging techniques, including magnetic resonance imaging-proton density-fat fraction and magnetic resonance elastography, can be used to assess treatment response in non-alcoholic fatty liver disease clinical trials.

Keywords: Sitagliptin; Fat mapping; MRI-proton-density-fat-fraction (PDFF); Lipid lowering therapy; NAFLD; Hepatic steatosis; Non-alcoholic steatohepatitis; Non-invasive assessment; Magnetic resonance elastography; Imaging; Biomarker; Fibrosis.

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Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; DPP-4, dipeptidyl peptidase 4; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic polypeptide; HbA1c, hemoglobin A1c; SREBP-1c, sterol regulatory element bind protein-1c; T2DM, type 2 diabetes mellitus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; MR, magnetic resonance; MRI, magnetic resonance imaging; MRI-PDFF, MRI-proton density-fat fraction; MRE, magnetic resonance elastography; ROI, regions of interest; MRS, MR spectroscopy; TIMP1, tissue inhibitor metalloproteinase I; CT, computed tomography.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in the United States and the Western world [1–4]. NAFLD is commonly associated with metabolic syndrome features, including obesity, dyslipidemia, and diabetes [5–7]. The presence of pre-diabetes and diabetes is associated with the progressive form of NAFLD also termed as non-alcoholic steatohepatitis (NASH) [8,9]. Several anti-diabetic therapies have been investigated in the treatment of NASH with varying success, including metformin [10–12], rosiglitazone [13,14], pioglitazone [15–17], and liraglutide [18].

Sitagliptin is an oral antihyperglycemic agent that competitively inhibits the enzyme dipeptidyl peptidase 4 (DPP-4), which

inactivates the hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) released in response to meals. By blocking GLP-1 and GIP breakdown, sitagliptin increases insulin secretion and suppresses glucagon release in the pancreas [19], which lowers blood glucose levels and improves hemoglobin A1c (HbA1c). Improvement in hyperglycemia and hyperinsulinemia results in the downregulation of sterol regulatory element binding protein-1c (SREBP-1c) and the blockage of fatty acid synthase [20], which should lead to improvement in liver fat and NASH. This provides mechanistic justification to conduct human trials with sitagliptin in NAFLD patients.

In clinical trials conducted in patients with type 2 diabetes mellitus (T2DM), sitagliptin has been shown to be effective in improving glycemic control, cholesterol, and lipoproteins [21–23] compared to placebo. Recent studies have shown that sitagliptin may improve serum alanine (ALT) and aspartate (AST) aminotransferase levels and gamma-glutamyl transpeptidase (GGT) in Japanese patients with T2DM [24]. In another uncontrolled pilot study, sitagliptin was shown to improve features of liver histology in 15 patients with T2DM over a 48 week period [25]. However, human trials on sitagliptin have been limited to date because of lack of placebo arm and allocation concealment.

The aim of this study was to evaluate the efficacy of sitagliptin *vs.* placebo in high risk patients with well-characterized, imaging quantified, NAFLD in reducing liver fat as measured by an accurate and well-validated, robust, quantitative magnetic resonance (MR) imaging (MRI)-based biomarker: proton density-fat fraction (MRI-PDFF). Additionally, we evaluated the efficacy of sitagliptin *vs.* placebo in reducing liver fibrosis over a 24-week period using both advanced magnetic resonance elastography (MRE) techniques and biomarker-based FIBROSpect® II testing. MRE has been shown to be effective in the non-invasive measurement of hepatic stiffness as a surrogate for hepatic fibrosis in NAFLD patients [26–31]. FIBROSpect® II has also been shown to be a highly accurate, non-invasive test to diagnose hepatic fibrosis [32,33].

Materials and methods

Study design and patient population

We conducted an investigator initiated, randomized, double-blind, allocation-concealed, placebo-controlled clinical trial to examine the efficacy of sitagliptin at 100 mg/day orally *vs.* identical placebo given over 24 weeks to improve hepatic steatosis as measured by MRI-PDFF, a validated, accurate, and quantitative biomarker for hepatic steatosis. The trial was conducted in strict accordance with CONSORT guidelines (see supplementary materials for CONSORT checklist). The patient population for the trial was derived from the San Diego Integrated NAFLD Research Consortium – a city-wide network established by the principal investigator (Rohit Loomba, RL) that includes four sites: University College San Diego (UCSD) Medical Center, Balboa Naval Medical Center, Kaiser Permanente Medical Center, and Sharp Health System. Patients deemed eligible were referred to the UCSD NAFLD Research Center [28,34–38] for screening into the trial. The trial was conducted at the UCSD Clinical and Translational Research Institute and all imaging was performed at the UCSD Liver Imaging Group MRI laboratory. FIBROSpect® II testing was performed by PROMETHEUS® Therapeutics & Diagnostics (San Diego, USA). The trial was registered at clinicaltrials.gov (registration number: NCT01963845) and the trial protocol received food and drug administration (FDA) approval under an investigational new drug application held by RL. This clinical trial protocol was approved by the human subjects institutional review board at UCSD, and all patients provided a written informed consent at the initial visit.

Inclusion criteria

Patients were included if they were ⩾18 years of age, had ALT above upper limits of normal (19 U/L for women, 30 U/L for men), had documented hepatic steatosis (⩾5% on MRI-PDFF), were either prediabetic or controlled diabetic patients (HbA1c 5.7%-8.0%), and provided written informed consent.

Exclusion criteria

Patients were excluded if they met any of the following exclusion criteria: uncontrolled diabetes (HbA1c>8.0), alcohol intake >30 g/day (3 drinks per day) within the previous 10 years or >10 g/day within the previous year; evidence of other forms of liver disease, including hepatitis B (positive serum hepatitis B surface antigen), hepatitis C (positive hepatitis C viral RNA), autoimmune hepatitis (positive autoimmune serology and consistent biopsy), alpha-1 antitrypsin deficiency (low alpha-1 antitrypsin levels and consistent biopsy), hemochromatosis (homozygosity or heterozygosity on genetic analysis and 3+ or 4+ iron staining on biopsy), Wilson's disease (ceruloplasmin with consistent biopsy), drug-induced liver disease based on exposure and history, and biliary duct obstruction based on imaging studies; evidence of decompensated cirrhosis (Child-Pugh score >7 points); advanced liver disease (platelet count <75,000 mm^2, prothrombin time >16 s, or a history of bleeding disorders); history of gastrointestinal bypass or use of drugs known to cause hepatic steatosis; recent initiation or change of anti-diabetic drugs, including insulin, sulfonylureas, or thiazolidinediones, or recent initiation of sitagliptin (or other drugs in the same class), within 90 days of randomization; history of acute pancreatitis within 5 years (except gallstone pancreatitis); evidence of hepatocellular carcinoma; positive human immunodeficiency virus test; active substance abuse; pregnant or trying to become pregnant; renal insufficiency; significant systemic illnesses; contraindications to sitagliptin use; and inability to undergo MRI.

Baseline assessment at screening

All patients underwent a baseline assessment before randomization, including detailed medical history and physical exam (see supplementary material for details).

Randomization and allocation concealment

The UCSD Investigational Drug Services randomized the patients into either sitagliptin or placebo groups in blocks of four in a 1:1 ratio using computer-generated numbers. Blinding and allocation concealment was rigorously maintained by independent pharmacists at the UCSD Investigational Drug Services who dispensed active or placebo treatment pills that were identical in appearance to one another. The pills were prepackaged in identical bottles and also labeled based on the computer-generated randomization numbers. The allocation sequence remained concealed throughout the trial from all study investigators. Un-blinding of treatment allocation was done only after all study procedures were completed in all study patients. The trial dataset was locked and directly analyzed by the study statistician using pre-specified data analysis plan.

Study visits

After careful assessment at the baseline visit, patients meeting all inclusion and exclusion criteria were randomized to receive sitagliptin 100 mg orally daily or placebo for 24 weeks. Patients returned to the research clinic for follow-up visits at weeks 4, 12, and 24 (see supplementary material for details).

Primary and secondary outcomes

The primary outcome was change in liver fat quantified by MRI-PDFF in colocalized regions of interest (ROI) within each of the nine liver segments. The secondary outcomes were insulin sensitivity improvement as determined by homeostatic model assessment insulin resistance (HOMA-IR), change in serum AST and ALT values, and change in low density lipoprotein (LDL). Changes in liver stiffness as quantified by MRE and FIBROSpect® II were exploratory endpoints.