Remogliflozin Etabonate Improves Fatty Liver Disease in Diet-Induced Obese Male Mice

Shigeru Nakano [*], Kenji Katsuno [*], Masayuki Isaji [†], Tatsuya Nagasawa [‡], Benjamin Buehrer [§], Susan Walker [‖], William O. Wilkison [¶], Bentley Cheatham [#]

[*]Discovery Research R&D, Kissei Pharmaceutical Co. Ltd., Nagano 399-8304, Japan, [†]Research and Development Division, Kissei Pharmaceutical Co. Ltd., Nagano 399-8304, Japan, [‡]Toxicology Research Laboratory, Kissei Pharmaceutical Co. Ltd., Nagano 399-8305, Japan, [§]ZenBio, Research Triangle Park, NC 27709, USA, [‖]Apex Biostatistics, New Hill, NC 27562, USA, [¶]Islet Sciences, Raleigh, NC 27615, USA and [#]BHV Pharma, RTP, NC 27709, USA

Background: **Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis (NASH) are serious conditions and are being diagnosed at an increased rate. The etiology of these hepatic disorders is not clear but involves insulin resistance and oxidative stress. Remogliflozin etabonate (Remo) is an inhibitor of the sodium glucose-dependent renal transporter 2 (SGLT2), and improves insulin sensitivity in type 2 diabetics. In the current study, we examined the effects of Remo in a diet-induced obese mouse model of NAFLD.** ***Methods:*** **After 11-weeks on High-Fat-Diet 32 (HFD32), C57BL/6J mice were obese and displayed characteristics consistent with NAFLD. Cohorts of obese animals were continued on HFD32 for an additional 4-week treatment period with or without Remo.** ***Results:*** **Treatment with Remo for 4 weeks markedly lowered both plasma alanine aminotransferase (76%) and aspartate aminotransferase (48%), and reduced both liver weight and hepatic triglyceride content by 42% and 40%, respectively. Remo also reduced hepatic mRNA content for tumor necrosis factor (TNF)-α (69%), and monocyte chemoattractant protein (MCP)-1 (69%). The diet-induced increase in thiobarbituric acid-reactive substances, a marker of oxidative stress, was reduced following treatment with Remo, as measured in both liver homogenates (22%) and serum (37%). Finally, the oxygen radical absorbance capacity (ORAC) in three different SGLT2 inhibitors was determined: remogliflozin, canagliflozin and dapagliflozin. Only remogliflozin had any significant ORAC activity.** ***Conclusions:*** **Remo significantly improved markers associated with NAFLD in this animal model, and may be an effective compound for the treatment of NASH and NAFLD due to its insulin-sensitizing and antioxidant properties. (J Clin Exp Hepatol 2015;5:190–198)**

NAFLD

Non-alcoholic fatty liver disease (NAFLD) is a chronic hepatic disorder that affects 25% of the population; including a significant portion of pediatric cases.[1] NAFLD can lead to non-alcoholic steatohepatitis (NASH), a more severe condition that includes damage such as fibrosis. Major risk factors for NAFLD include those for metabolic syndrome, especially obesity and insulin resistance.[1] While there are clinical trials investigating the use of anti-diabetic therapies,[2] antioxidants are also being pursued,[3] as well as farnesoid X receptor (FXR) agonists, such as the bile acid analog obeticholic acid.[4]

Although the exact mechanisms underlying progression of NAFLD are unclear, it likely involves an early lipotoxic response due to hepatic steatosis. The intrahepatic accumulation of free fatty acids (FFA) induces hepatic insulin resistance and both oxidative and endoplasmic reticulum (ER) stress, culminating in decreased hepatic function, apoptosis and fibrosis.[5] Elevated concentrations of intracellular FFA are accompanied by numerous alterations in the expression of genes involved in lipid metabolism, leading to increased *de novo* lipid synthesis and a concomitant decrease in secretion of FFA.[6–14] Chronic exposure to fatty acids also induces ER stress, promoting elevated expression of the ER chaperone Grp78, increased phosphorylation of both eIF2α and PERK, activation of caspase-3, as well as induction of other pro-apoptotic genes and stress-activated transcription factors.[15–19] Oxidative stress is also activated by FFA and is associated with a decline in glutathione, reduced superoxide dismutase activity, elevated lipid peroxidation and increased generation of reactive oxygen species (ROS).[13] Thus, it is likely that insulin resistance and cellular stress, resulting from hepatic steatosis, act collectively to promote NAFLD, a pro-fibrotic state and progression to NASH.

Keywords: hepatic steatosis, NAFLD, NASH, obesity, SGLT2
Received: 30.12.2014; *Accepted:* 27.02.2015; *Available online:* 28 April 2015
Address for correspondence: Bentley Cheatham, BHV Pharma, P.O. Box 13765, Research Triangle Park, NC 27709, USA. Tel.: +1 919 904 4248.
E-mail: Bentley@bhvpharma.com

Abbreviations: AAPH: 2,2′-azobis-2-methyl-propanimidamide dihydrochloride; ALT: Alanine aminotransferase; AST: aspartate aminotransferase; DIO: Diet-induced obesity; ER: Endoplasmic reticulum; FFA: Free fatty acids; FXR: Farnesoid X receptor; HFD32: High fat diet 32; MCP-1: Monocyte chemoattractant protein-1; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; ORAC: Oxygen radical absorbance capacity; Remo: Remogliflozin etabonate; ROS: Reactive oxygen species; SGLT2: sodium glucose-dependent renal transporter 2; TBARS: Thiobarbituric acid-reactive substances; TG: Triglyceride; TNF-α: Tumor necrosis factor alpha; Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

http://dx.doi.org/10.1016/j.jceh.2015.02.005

 © 2015, INASL

Inhibition of the renal specific sodium/glucose transporter 2 (SGLT2) is a contemporary therapeutic approach for the treatment of type 2 diabetes.[20] The mechanism of action for this class of compounds is to inhibit the reabsorption of urinary glucose, resulting in excretion of glucose and lowering of plasma glucose. Remogliflozin etabonate (Remo) is a novel inhibitor of SGLT2. The efficacy of Remo in the treatment of type 2 diabetes has been established in several phase II clinical trials.[21–26] Remo and other compounds in this class are effective in improving glucose homeostasis, insulin sensitivity, and beta cell function, as well as reducing body weight.[27–29] In this study, we have examined the potential benefits of Remo on hepatic steatosis and associated parameters of NAFLD in a diet-induced obese (DIO) mouse model.

METHODS

Drugs

Remogliflozin etabonate (4-[(4-isopropoxyphenyl)-methyl]-1-isopropyl-5-methyl-1H-pyrazol-3-yl 6-*O*-ethoxycarbonyl-β-D-glucopyranoside) was synthesized by Kissei Pharmaceutical Co. Ltd. (Matumoto, Japan). Bezafibrate was obtained from Chugai Pharmaceutical Co. Ltd. (Tokyo, Japan).

Vertebrate Animals

All animal experiments were conducted in accordance with the guidelines for animal care and welfare issued by Kissei Pharmaceutical Co. Ltd., which have been approved by The Japanese Pharmacological Society. C57BL/6J mice (4-weeks old) were housed individually with free access to water. The mice were fed a diet of normal chow or High-Fat-Diet 32 (HFD32; Clea, Japan) for 11-weeks. HFD32 contains 25% protein, 29% carbohydrate and 32% fat (with saturated, monounsaturated and polyunsaturated fatty acids at 7, 22 and 4 g/100 g chow, respectively). HFD32 has a caloric value of 507 kcal/100 g. The mice (15 weeks of age) were then randomly divided into 6–7 mice/group. These groups were maintained for 4-weeks on the following diets: group 1 (n = 7) was maintained continuously on a normal diet (Normal); group 2 (n = 7) was given HFD32 (Control group); group 3 (n = 7) received HFD32 supplemented with 0.01% (wt/wt) Remo; group 5 (n = 7), HFD32 with 0.03% Remo; and group 7 (n = 6), HFD32 with 0.2% bezafibrate. The animals receiving Remo ate slightly more than the untreated Control group. Thus, based on the average daily food consumption in the Control group, group 4 and group 6 (n = 7 each) were pair-fed (PF), receiving an equal amount of HFD32 chow as Control group, but containing either 0.01% Remo (group 4) or 0.03% Remo (group 6). Average daily drug intake was determined by multiplying drug concentration in chow by food consumption. Control and Control-PF groups are mice maintained on HFD32, but not treated with Remo.

Glucose, Alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST) Levels

Blood samples were collected from a tail vein into a heparinized hematocrit tube. Plasma ALT, AST and glucose levels were measured by using commercially available kits (Transaminase C-II test, Glucose C-II test; Wako Pure Chemical Industries, Osaka, Japan).

Liver Weights and Triglyceride (TG) Content

At the end of the treatment period liver tissue was removed, weighed and stored at −80 °C. A sample of liver tissue was homogenized and subjected to lipid extraction according to the method of Folch et al.[30] Hepatic TG content was measured by using a commercially available kit (TG E-test, Wako Pure Chemical Industries) and normalized for sample weight.

Thiobarbituric Acid Reactive Substances (TBARS) Assay

Serum samples and liver homogenates were used to determine levels of serum and hepatic TBARS, measured by using an OXI-TEK TBARS Assay kit (ALEXIS JAPAN, Tokyo, Japan). The content of hepatic TBARS was normalized for protein concentration.

Liver Histopathology and Fatty Droplet Area

Livers were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 3–4 μm. For the evaluation of hepatic steatosis, sections were stained with Oil red O. The average area of intracellular fatty droplets was determined as follows: livers were fixed in 10% buffered formalin. Frozen sections of the liver, which were stained with Oil Red O, were prepared for the evaluation of hepatic steatosis. The average area (μm^2) of the fat droplets within hepatocytes in 5 fields (each approximately 93000 μm^2) for each liver was measured with the aid of an image analyzer (IPAP-WIN, Sumika TechnosCo., Osaka, Japan).

Determination of mRNA Levels

Total RNA was isolated and purified from liver by means of an RNeasy Plus Mini Kit (QIAGEN) with DNase I treatment. The mRNA levels corresponding to various target genes were determined by real-time quantitative RT-PCR using 7500 Fast Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The primers for mouse TNF-α and MCP-1 were as previously described.[31,32]

Oxygen Radical Absorbance Capacity (ORAC) Assay

An ORAC antioxidant assay kit (Zen-Bio, NC) was used to determine the antioxidant capacity of dapagliflozin, canagliflozin, remogliflozin etabonate and remogliflozin. The assay measures the ability of the test compounds to block

Download English Version:

https://daneshyari.com/en/article/3338551

Download Persian Version:

https://daneshyari.com/article/3338551

Daneshyari.com