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Metabolism

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Translational

Ezetimibe Stimulates Intestinal Glucagon-Like Peptide 1 Secretion Via the MEK/ERK Pathway Rather Than Dipeptidyl Peptidase 4 Inhibition

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ARTICLE INFO

Article history:
Received 15 September 2014
Accepted 4 February 2015

Keywords:
Ezetimibe
GLP-1
DPP-4
ERK
MEK

ABSTRACT

Objective. Ezetimibe is known as a Niemann-Pick C1-Like 1 (NPC1L1) inhibitor and has been used as an agent for hypercholesterolemia. In our previous study, ezetimibe administration improved glycemic control and increased glucagon like peptide-1 (GLP-1), an incretin hormone with anti-diabetic properties. However, the mechanisms by which ezetimibe stimulates GLP-1 secretion are not fully understood. Thus, the specific aim of this study was to investigate the mechanism(s) by which ezetimibe stimulates GLP-1 secretion.

Materials/methods. Male KK/H1J mice were divided into following groups: AIN-93G (NC), NC with ezetimibe (10 mg/kg/day), 45% high fat (HF) diet, and HF diet with ezetimibe. To investigate the role of ezetimibe in glucose homeostasis and GLP-1 secretion, an insulin tolerance test was performed and serum and intestinal GLP-1 levels and intestinal mRNA expression involved in GLP-1 synthesis were measured after 6 weeks of ezetimibe treatment. *In vivo* and *in vitro* dipeptidyl peptidase-4 (DPP-4) inhibition assays were employed to demonstrate the association between ezetimibe-induced GLP-1 change and DPP-4. The molecular mechanism by which ezetimibe affects GLP-1 secretion was evaluated by using human enteroendocrine NCI-H716 cells.

Results. Ezetimibe supplementation significantly ameliorated HF-increased glucose and insulin resistance in the type 2 diabetic KK/H1J mouse model. Serum and intestinal active GLP-1 levels were significantly increased by ezetimibe in HF-fed animals. However, mRNA expression of genes involved in intestinal GLP-1 synthesis was not altered. Furthermore, ezetimibe did not inhibit the activity of either *in vivo* or *in vitro* dipeptidyl peptidase-4 (DPP-4). The direct effects of ezetimibe on GLP-1 secretion and L cell secretory mechanisms were examined in human NCI-H716 intestinal cells. Ezetimibe significantly stimulated active GLP-1 secretion,

Abbreviations: DPP-4, dipeptidyl peptidase-4; ERK, extracellular signal-regulated kinase; GLP-1, glucagon like peptide-1; MEK, mitogen-activated protein/extracellular signal-regulated kinase (MAPK/ERK) kinase; NPC1L1, Niemann-Pick C1-Like 1; PC3, prohormone convertase 3; T2DM, type 2 diabetes mellitus.

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http://dx.doi.org/10.1016/j.metabol.2015.02.001
0026-0495/

which was accompanied by the activation of mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK)/extracellular signal-regulated kinase (ERK). Ezetimibe-increased GLP-1 secretion was abrogated by inhibiting the MEK/ERK pathway with PD98059.

Conclusion. These findings suggest a possible novel biological role of ezetimibe in glycemic control to stimulate intestinal GLP-1 secretion via the MEK/ERK signaling pathway.

1. Introduction

Type 2 diabetes mellitus (T2DM), characterized by insulin resistance and dysfunctional insulin action, is one of the most common metabolic disorders in the world [1]. T2DM is closely associated with comorbidities including obesity, dyslipidemia, and hypertension. Given this close link, the strategies of anti-diabetic drugs consist of not only achieving effective blood glucose control but also preventing its related long-term complications [2]. However, most anti-hyperglycemic medications are aimed only at achieving normal glucose levels, and not treating comorbidities, and thus do not lead to long-term efficacy.

One of the newly introduced classes of drugs for the treatment of T2DM consists of glucagon-like peptide-1 (GLP-1)-based therapies, including GLP-1 receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors. GLP-1 is released from intestinal epithelial L cells [3–5], where it exerts anti-diabetic actions by increasing β-cell proliferation and insulin secretion from pancreatic β-cells and inhibiting glucagon release, food intake, and gastric emptying [6–8]. Impaired GLP-1 response is closely associated with insulin resistance [9]. In addition, DPP-4 is a serine protease that is responsible for GLP-1 degradation and inactivation [10]. Studies in both humans and animals have shown that specific inhibitors against DPP-4 increase circulating levels of GLP-1, resulting in improvement of glucose intolerance. In this way, DPP-4 inhibitors can be used as a treatment for T2DM [11–13].

Ezetimibe has been used as a monotherapy or in combination with statins to treat hypercholesterolemia [14,15]. Ezetimibe inhibits intestinal cholesterol transport from lumen to enterocytes by specific binding to its molecular target, Niemann-Pick C1-like 1 (NPC1L1) [16]. In addition to the effects of ezetimibe on hypercholesterolemia, it also ameliorates other metabolic disorders such as hepatic steatosis and diabetes. In both human and animal studies, ezetimibe treatment and NPC1L1 deletion improve non-alcoholic fatty liver [17–21]. Accompanying these beneficial effects on fatty liver, ezetimibe improves insulin sensitivity and glucose intolerance [22,23]. In our previous study, we demonstrated that chronic ezetimibe administration improves glucose intolerance and increases circulating level of GLP-1 and abundance of β-cells [24]. However, the molecular mechanisms by which the NPC1L1 inhibitor, ezetimibe induces GLP-1 secretion resulting in favorable glycemic control have not been fully delineated.

In this study, we investigated the direct effects of ezetimibe on blood and intestinal GLP-1 stimulation in a DPP-4 independent but MEK/ERK pathway-dependent manner. We measured insulin tolerance, fasting plasma glucose, and levels of bioactive blood and intestinal GLP-1 in a diabetic animal model. We also used the human NCI-H716 L-cell model to investigate the direct effects of ezetimibe on GLP-1 secretion and its associated signaling.

2. Materials and Methods

2.1. Chemicals and Reagents

Ezetimibe was provided from Merck Sharp & Dohme (Rahway, NJ). DPP-4 inhibitor, gemigliptin was obtained from LG Life Sciences (Seoul, Korea). H-Glycyl-prolyl-7-amino-4-methylcoumarin (H-Gly-Pro-AMC) and H-glycyl-prolyl-p-nitroanilide (H-Gly-Pro-pNA) were purchased from Bachem (Torrance, CA). Human recombinant DPP-4 was purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Animal Experiments

All animal experimental protocols were approved by the Animal Experimentation Ethics Committee of the Sungkyunkwan University, Kangbuk Samsung Hospital. Male KK/H1J mice (Jackson Laboratory, Bar Harbor, ME), a mouse model that exhibits mild hyperglycemia, hyperinsulinemia, and obesity [25] were housed with a standard 12 h light:12 h dark cycle. To investigate the role of ezetimibe in glycemic control and GLP-1 secretion, mice were fed either AIN-93G (NC) or a 45% high fat (HF; Research Diet, New Brunswick, NJ) diet supplemented with ezetimibe (10 mg/kg/day). After 6 weeks, mice were fasted overnight and killed by CO_2 followed by cardiac puncture. Tissues were immediately dissected, weighed, and stored at −80 °C until further analysis. A second *in vivo* animal study was conducted in a viral pathogen-free facility at LG Life Sciences in accordance with the Ethics Committee for Animal Experiments of LG Life Sciences to delineate whether ezetimibe directly inhibits DPP-4 activity. Briefly, 8-week old male Sprague-Dawley rats (Orient Bio, Seoul, Korea) were fasted overnight and orally administrated ezetimibe (50 mg/kg body weight), DPP-4 inhibitor (0.1 or 1 mg gemigliptin/kg body weight), or distilled water as a control. For the determination of plasma DPP-4 activity, blood samples (approximately 0.2 mL) were collected via the tail vein of each rat before (0 h) and at 1, 4, 8, and 24 h after oral administration.

2.3. Measurement of Insulin Sensitivity and Blood Biochemical Parameters

Insulin tolerance test (ITT) was carried out after 6-h fasting. For ITT, mice were received an intraperitoneal injection of human insulin (0.75 mU/kg body weight) and blood glucose levels were measured at baseline and at 15, 30, 45, 60, 90 and 120 min after injection using an automated glucocard X-Meter (Arkray, Kyoto, Japan). The area under the curve (AUC) was calculated and the difference (ΔAUC) was reported. For plasma active GLP-1 measurements, blood samples were collected in tubes containing EDTA and DPP-4 inhibitor (10 μL/mL; Millipore, St. Charles, MO). Active GLP-1 (7-36 amide and 7-37) was analyzed by ELISA method (Millipore, St. Charles, MO).