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New Potential Targets of Glucagon-Like Peptide 1 Receptor Agonists in Pancreatic β-Cells and Hepatocytes

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It is well known that both insulin resistance and decreased insulin secretory capacity are important factors in the pathogenesis of type 2 diabetes mellitus (T2DM). In addition to genetic factors, obesity and lipotoxicity can increase the risk of T2DM. Glucagon-like peptide 1 (GLP-1) receptor agonists are novel antidiabetic drugs with multiple effects. They can stimulate glucose-dependent insulin secretion, inhibit postprandial glucagon release, delay gastric emptying, and induce pancreatic β-cell proliferation. They can also reduce the weight of patients with T2DM and relieve lipotoxicity at the cellular level. Many intracellular targets of GLP-1 have been found, but more remain to be identified. Elucidating these targets could be a basis for developing new potential drugs. My colleagues and I have investigated new targets of GLP-1, with a particular focus on pancreatic β-cell lines and hepatic cell lines. Herein, I summarize the recent work from my laboratory, with profound gratitude for receiving the prestigious 2016 Namgok Award.

Keywords: Glucagon-like peptide-1 receptor; Diabetes mellitus; Insulin; Glucagon

The Namgok Award is the highest scientific award of the Korean Endocrine Society, and is given to honor an individual who has made excellent contributions to progress in the field of endocrinology and metabolism. The Namgok Award is named after the pen name of Professor Hun Ki Min, who founded the Korean Endocrine Society in 1982.

Professor Won-Young Lee received the Namgok Award at the Autumn Symposium of the Korean Endocrine Society in October 2016.

INTRODUCTION

Glucagon-like peptide-1 (GLP-1) is an incretin secreted by L-cells in the small intestine in response to food intake [1]. The main roles of GLP-1 are to stimulate glucose-dependent insulin secretion, to inhibit postprandial glucagon release, to delay gastric emptying, and to induce pancreatic β-cell proliferation [2].

Recent studies have reported that exendin-4 (Ex-4), a GLP-1 receptor agonist, was able to reduce hepatic steatosis by enhancing fatty acid oxidation and hepatic insulin signaling in human hepatocytes and *ob/ob* mice [3,4]. Some studies have shown that Ex-4 and liraglutide, another GLP-1 receptor agonist, improved fatty liver by stimulating autophagy. Treatment with Ex-4 and liraglutide resulted in reduced endoplasmic reticulum (ER) stress-associated apoptosis in human hepatocytes treated with palmitate and in mice fed a high-fat diet [5]. We have investigated new mechanisms of GLP-1 receptor agonists in pancreatic β-cells and hepatocytes in the context of relieving lipo-

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toxicity. Herein, the pleiotropic effects of GLP-1—reducing pancreatic β-cell dysfunction and fatty liver—are presented.

Ex-4 CAN REPRESS STEROL REGULATORY ELEMENT-BINDING PROTEIN 1c, WITH A PROTECTIVE EFFECT IN PANCREATIC β-CELLS

Ex-4 has novel abilities to protect β-cells against various forms of toxicity. However, the protective mechanism of Ex-4 needs to be investigated more thoroughly. We studied the protective effect that Ex-4 exerts against lipotoxicity by downregulating sterol regulatory element-binding protein (SREBP)-1c, a transcription factor involved in fat and cholesterol synthesis. SREBP-1c knockdown prevented the lipotoxic effects of palmitate on insulin secretion and apoptosis. We also observed that the protective effect of Ex-4 against palmitate-induced β-cell dysfunction was mediated by the inhibitory effect of phosphoinositide 3-kinase/Akt signaling on SREBP-1c [6].

Ex-4 CAN INCREASE INTRACELLULAR SPHINGOSINE-1 PHOSPHATE LEVELS, THUS HAVING A PROTECTIVE EFFECT ON PANCREATIC β-CELLS

We performed an experiment to determine whether sphingosine-1 phosphate (S1P) had a protective role in a mouse insulinoma cell line (MIN6) and whether it was an essential factor for maintaining the mitochondrial membrane potential [7]. When we reduced intracellular S1P levels by treatment with a sphingokinase inhibitor, we observed decreased insulin secretory capacity and increased apoptotic signaling. Simultaneous treatment with S1P and a sphingokinase inhibitor removed the detrimental effects of the sphingokinase inhibitor. This illustrates the protective role of S1P in pancreatic β-cells. After treatment of MIN6 with S1P, we also observed the recovery of mitochondrial potential after it had been reduced by treatment with a sphingokinase inhibitor or palmitate. Next, we investigated the relationship of S1P treatment with prohibitin, a mitochondrial membrane protein. Prohibitin plays the role of a mitochondrial chaperone for the function of the respiratory chain and as a general structuring scaffold for optimal mitochondrial morphology and function. When prohibitin was silenced in MIN6, mitochondrial membrane potential and cellular adenosine triphosphate (ATP) content decreased. When MIN6 was treated with a sphingokinase inhibitor or palmitate, prohibitin expression decreased, but recovered after S1P treatment. Targeting S1P and prohibitin may be a possible strategy for improving β-cell function, but further investigation is needed.

INCREASED SIRT1 AFTER TREATMENT WITH Ex-4 IMPROVES HEPATIC STEATOSIS

The silent mating type information regulation 2 homolog (sirtuin, SIRT) family is composed of nicotinamide adenine dinucleotide (NAD^+)-dependent enzymes modifying various proteins by deacetylation. Seven members of the SIRT family have been reported in humans [8,9]. In particular, SIRT1 and SIRT6 are important in metabolic homeostasis. SIRT1 stimulates hepatic fatty acid oxidation by activating adenosine monophosphate (AMP)-activated protein kinase (AMPK) and peroxisomal proliferator-activated receptor α (PPARα) [10]. In contrast, SIRT1 deacetylase-deficient mice on a high-fat diet exhibited aggravation of fatty liver and insulin resistance [11]. Whether Ex-4 can affect SIRT1 expression as a mechanism of improving fatty liver has not been previously reported. Therefore, we performed experiments assessing whether the protective effects of Ex-4 on fatty liver were mediated through SIRT in high-fat diet-induced obese C57BL/6J mice and cell culture models. We found that increasing SIRT1 by Ex-4 treatment protected mice against high-fat diet-induced steatohepatitis by stimulating fatty acid oxidation [12].

Ex-4 DECREASES ENDOPLASMIC RETICULUM STRESS THROUGH A SIRT1-DEPENDENT MECHANISM

We also attempted to determine whether Ex-4 attenuated palmitate-induced ER stress via SIRT1 in HepG2 cells. Palmitate treatment enhanced the expression of activating transcription factor 6 (ATF6), inositol-requiring kinase 1α (IRE1α), and C/EBP homologous protein (CHOP) mRNA. Ex-4 decreased the expression of P-IRE1α, ATF6, X-box binding protein-1, and CHOP, and increased the expression of sarco/ER Ca^{2+}-ATPase 2b (SERCA2b). A significant decrease in the hepatic expression of the p53 upregulated modulator of apoptosis (PUMA), cytochrome c, and cleaved caspase-3 were found in hepatocytes treated with Ex-4. Inhibition of SIRT1 by nicotinamide and small interfering RNA (siRNA) significantly enhanced the expression of ER stress marker genes in cells treated with both palmitate and Ex-4. In conclusion, increased SIRT1 expression

after Ex-4 treatment was found to reduce palmitate-induced ER stress and mitochondrial dysfunction in hepatocytes [13].

Ex-4 CONTROLS FIBROBLAST GROWTH FACTOR 21 AND LIPID METABOLISM IN THE LIVER

Hepatokine fibroblast growth factor 21 (FGF21) has been introduced as a novel therapeutic agent for diabetes mellitus. Hepatic FGF21 expression is regulated by cyclic-AMP-responsive-element-binding protein H (CREBH) and PPARα, and it regulates lipid metabolism [14,15]. FGF21 functions as a potent activator of glucose uptake in adipocytes via glucose transporter 1 [16]. In both *ob/ob* and *db/db* mice, the administration of FGF21 reduced plasma triglycerides and glucose levels [17], whereas the liver-specific knockdown of FGF21 led to hepatic insulin resistance by increasing gluconeogenesis and glycogenolysis [18]. FGF21 treatment also improved insulin resistance and fatty liver in diet-induced obese mice [19]. Ex-4 enhanced the expression of FGF21 and its receptors in high-fat diet-induced obese mice. Recombinant FGF21 treatment also reduced lipid content in palmitic acid-treated HepG2 cells. We performed experiments and also observed significantly less expression of medium-chain acyl-coenzyme A dehydrogenase (MCAD) and PPARα in hepatocytes transfected with FGF21 siRNA [20]. In cells treated with Ex-4, inhibition of SIRT1, but not SIRT6, by siRNA significantly reduced the expression of FGF21 mRNA, whereas FGF21 inhibition was not found to reduce SIRT1 expression. These data indicate that Ex-4 may improve hepatic steatosis by increasing SIRT1-mediated FGF21 [20].

AMPK REDUCES THE EXPRESSION OF LIVER X RECEPTOR/SREBP-1 SIGNALING-INDUCED ANGPTL8 IN HepG2 CELLS

Angiopoietin like protein 8 (ANGPTL8), which is encoded by the *C19orf80* (human) gene or the *Gm6484* (mouse) gene, and is also known as β-trophin, refeeding-induced fat and liver protein (RIFL), hepatocellular carcinoma-associated protein TD26 homolog (TD26), or lipasin [21]. It is a secretory protein that plays multiple roles in glucose and lipid metabolism. In mice, ANGPTL8 is mainly expressed in the liver, brown adipose tissue, and white adipose tissue, whereas in humans it is specific for the liver, and its expression levels in other tissues are extremely low [21-23]. Since the regulators of ANGPTL8 gene are still not clear, we investigated the inhibitory mechanism of ANGPTL8 expression by AMPK in HepG2 cells exposed to palmitic acid, tunicamycin, or T0901317 [24]. The expression of ANGPTL8 was significantly enhanced in HepG2 cells treated with palmitic acid, tunicamycin, or T0901317, and was reduced in cells exposed to 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR). Palmitic acid, tunicamycin, and T0901317 increased liver X receptor-α (LXRα) and SREBP-1c mRNA expression. The inhibitory effect of AICAR on the expression of T0901317-induced ANGPTL8 was most strongly observed in cells that were transfected with SREBP-1 siRNA. AICAR increased the phosphorylation of PPARα, and the effect of AICAR was not observed in cells treated with a PPARα inhibitor. Metformin affected ANGPTL8 expression similarly to AICAR. These data indicate that AMPK reduced the expression of LXR/SREBP-1 signal-induced ANGPTL8 in HepG2 cells [24].

Ex-4 INHIBITS SELENOPROTEIN P AND FETUIN IN HepG2 CELLS

Selenoprotein P (SEPP1) and fetuin-A, both circulating liver-derived glycoproteins, have been suggested as potential biomarkers for insulin resistance and nonalcoholic fatty liver disease. However, the effect of Ex-4 on the expression of hepatokines, SEPP1, and fetuin-A remains unknown. We performed an experiment with HepG2 cells and observed that Ex-4 reduced the expression of hepatic SEPP1 and fetuin-A via improvement of palmitate-induced ER stress by AMPK [25].

CONCLUSIONS

GLP-1 exerts a protective effect on pancreatic β-cells and hepatocytes via novel mechanisms against lipotoxicity-induced cellular dysfunction. More research to investigate the novel targets of GLP-1 is needed.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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