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

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 lipotoxicity.
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].

INCREASED SIRT1 AFTER TREATMENT WITH Ex-4 IMPROVES HEPATIC STEATOSIS

The silent mating type information regulation 2 homolog (sir-tuin, 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 (PPAR\(\alpha\)) [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 recovered after S1P treatment. Targeting S1P and prohibitin may be a possible strategy for improving β-cell function, but further investigation is needed.

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 sphingokinese 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.
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 intro-
duced as a novel therapeutic agent for diabetes mellitus. Hepatic
FGF21 expression is regulated by cyclic-AMP-responsive-ele-
ment-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 re-
duced plasma triglycerides and glucose levels [17], whereas the
liver-specific knockdown of FGF21 led to hepatic insulin resis-
tance by increasing gluconeogenesis and glycogenolysis [18].
FGF21 treatment also improved insulin resistance and fatty liv-
er in diet-induced obese mice [19]. Ex-4 enhanced the expres-
sion 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 experi-
ments and also observed significantly less expression of medi-
um-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 ex-
pression. 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 pro-
tein (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 tis-
sue, and white adipose tissue, whereas in humans it is specific
for the liver, and its expression levels in other tissues are ex-
remely 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-aminomidazole-4-carboxamide ribonucle-
etide (AICAR). Palmitic acid, tunicamycin, and T0901317 in-
creased liver X receptor-α (LXRα) and SREBP-1c mRNA ex-
ression. 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 in-
creased the phosphorylation of PPARα, and the effect of AICAR
was not observed in cells treated with a PPARα inhibitor. Met-
formin 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 bio-
markers for insulin resistance and nonalcoholic fatty liver dis-
ease. However, the effect of Ex-4 on the expression of hepato-
kines, SEPP1, and fetuin-A remains unknown. We performed
an experiment with HepG2 cells and observed that Ex-4 re-
duced the expression of hepatic SEPP1 and fetuin-A via im-
provement of palmitate-induced ER stress by AMPK [25].

**CONCLUSIONS**

GLP-1 exerts a protective effect on pancreatic β-cells and hepa-
tocytes via novel mechanisms against lipotoxicity-induced cel-
lar 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 re-
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