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GLP-1 *C*-terminal structures affect its blood glucose lowering-function

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Abstract: Glucagon-like peptide-1 (GLP-1), which is an endogenous insulinotropic peptide that can stimulate islet cells to secret insulin, is a promising new drug candidate for the treatment of type 2 diabetes. However, due to the very short half-life of this peptide, the clinical value of GLP-1 is restricted. A GLP-1 peptide analog that had been altered by deletion of five amino acids from the *C*-terminus (sGLP-1) was selected and investigated *in vivo* for the therapeutic effect on GK rats with type II DM (T2DM). The results revealed that sGLP-1 exhibited decreased blood glucose-lowering ability compared to GLP-1 in the first week, as measured after once-daily administration. However, after drug administration for 2 weeks, the blood glucose-lowering effect of sGLP-1 became superior to that of GLP-1. sGLP-1 reduced apoptosis of the old islets, enhanced insulin production, and promoted new islets replication. sGLP-1 is a shorter but more efficient GLP-1 analog for type 2 diabetes management. Because sGLP-1 prolonged the proliferation and recovery of islet cells, the ability to maintain blood glucose (BG) within a normal range was still present 2 weeks after drug withdrawal. These results confirmed the importance of the *C*-terminus of GLP-1 molecule, and further demonstrated that GLP-1 (7–37) can be truncated till the 32nd amino acid to have a better long-term BG lowing function. This result may imply for the presence of glucagon family clearance receptors *in vivo* and demonstrates that the *C*-terminus participates in GLP-1 clearance. Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: GLP-1; diabetes; islet; apoptosis

INTRODUCTION

Type 2 diabetes mellitus (T2DM) has complicated pathogenic causes and is characterized by an insulin secretory defect in the beta cells and insulin resistance in peripheral tissue. Stimulating beta cells to secrete insulin in order to control blood glucose (BG) levels is an important goal of drug treatment in T2DM patients. In recent years, the endogenous insulinotropic peptide glucagon-like peptide-1 (GLP-1) has received much attention. This peptide derives its name from the fact it is generated from proglucagon via a protease [1–4]. GLP-1 stimulates islet cells to secret insulin and has significant effects on insulin secretion when BG levels exceed 6 mmol/l. Indeed, of all human hormones capable of stimulating insulin secretion from islet cells, GLP-1 is the most potent. The insulin secretory effects of GLP-1 are elicited via the GLP-1 receptor. Stimulation of the GLP-1 receptor not only increases insulin secretion, but also inhibits beta cell apoptosis and stimulates beta cell proliferation, leading to improved glucose tolerance. These combined effects are very valuable in the treatment of T2DM, making GLP-1 the most promising new drug candidate for the treatment of T2DM [2,5–7].

GLP-1 exists in two forms in the human body, GLP-1-(7–36) NH(2) and GLP-1-(737). GLP-1- (7–36) NH (2) is a 30-amino acid peptide with an amidated *C*-terminus, and GLP-1-(7–37) is a 31-amino acid peptide. Both function to stimulate insulin secretion [8]. However, because of the very short half-life of GLP-1 *in vivo*, which is only 90–120 s, it has almost no medicinal value. This short half-life of GLP-1 is attributable to the degradation of the amino acid at the 8th position by dipeptidyl peptidase IV (DPP-IV) as well as the rapid clearance of GLP-1 *in vivo* [9,10]. Previous studies have focused on generating GLP-1 analogs in which the amino acid at the 8th position is substituted to resist DPP-IV degradation. The fusion of GLP-1 with a carrier protein, albumin for example, can also delay *in vivo* clearance [11–13].

Previous studies proposed that *C*-terminus of GLP-1(7–37) mediates GLP-1 receptor binding. Consequently, shortening of the *C*-terminus reduces ligand affinity [14–16]. Nevertheless, in our analysis of the glucagon family hormones, we have noted a certain homology in the configuration of *C*-terminal amino

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acids among BG-lowering GLP-1 and BG-elevating glucagons. We postulated that the homology portions at the upstream should have similar function, but the heterogenic portions at the downstream should be responsible for their special functions. Therefore, we made truncations from the downstream of GLP-1(7–37), and found that a GLP-1 analog with both A8 to G8 substitution and five amino acid truncation at the *C*-terminus is more efficient than GLP-1(7–37) analog with only G8 substitution in lowing BG level, and we call this *C*-terminus truncated GLP-1 analog G8 GLP-1 (7–32) as sGLP-1.

MATERIALS AND METHODS

Reagents and GLP-1 Preparation

All chemicals used, including glucose, were of analytical reagent grade and were purchased from the Beijing Chemical and Reagent Company (Beijing, China). GLP-1 and *C*-terminal truncated GLP-1 analogs were synthesized using solid-phase peptide synthesis by Xian Lianmei Biotech Co., Ltd (Xian, China). Peptides were purified by liquid chromatography (>98% purity), lyophilized, and stored at −20 °C. The amino acid sequences of GLP-1 analogs were as follows:

GLP-1 (7–37): H**A**EGTFTSDVSSYLEGQAAKEFIAWLVKGRG
G8GLP-1: H**G**EGTFTSDVSSYLEGQAAKEFIAWLVKGRG
sGLP-1 : H**G**EGTFTSDVSSYLEGQAAKEFIAWL
(G8) GLP-1 (7–30): H**G**EGTFTSDVSSYLEGQAAKEFIA
(G8) GLP-1 (7–27): H**G**EGTFTSDVSSYLEGQAAKE

G8 indicates the A to G substitution at 8th amino acid of GLP-1(7–37), which made GLP-1(7–37) and the analogs resistant to DPP-IV degradation.

Animals

Eight-week-old female specific-pathogen-free BALB/c mice (18–20 g), 9–10 week-old male Wistar rats (180–220 g), and 9–10 week-old spontaneous diabetic GK rats (both sexes, 180–220 g) were purchased from the Laboratory Animal Center of Academy of Military Medical Science. The animals were maintained in a room air-conditioned to 25 °C ± 2 °C, with a relative humidity of 50 ± 20%, and were maintained on a 12-h light/dark cycle. All animals were treated humanely, and the study protocols were in accordance with the Regulations of Good Laboratory Practice for nonclinical laboratory studies of drugs issued by the National Scientific and Technologic Committee of People's Republic of China. The animals were housed and fed under standard conditions. Prior to experimentation, all animals underwent 12 h fast.

Screening of *C*-terminal Truncated GLP-1 Analogs

The glucose-lowering effects of *C*-terminal truncated GLP-1 analogs of various lengths were screened using the spontaneous type 2 diabetic model rat (GK rat). GK rats weighing 200 ± 10 g were first grouped based on mean BG levels (<0.5 mmol/l among groups) of 24 h fasting, measured with One touch Ultra (Johnson & Johnson, NJ, USA). The rats were administrated 0.5 ml of 2 μmol/l *C*-terminal truncated GLP-1 analog subcutaneously. Animals received injections every day for 2 weeks. The positive control group received G8GLP-1 and the negative group received normal saline solution. Fasting blood glucose (FBG) levels were measured prior to drug administration, as well as 7 and 14 days after the final sGLP-1 administration.

Glucose Tolerance Tests

BALB/c mice were randomly divided into the following three groups: sGLP-1- administrated group, G8GLP-1-administrated group, and normal saline solution-administrated group. The mice received subcutaneous 0.5 ml injections of 2 μmol/l sGLP-1 or G8GLP-1. The animals also received intra-peritoneal injections of 0.2 ml 400 g/l glucose at 0, 2, 4, 6, or 8 h after drug administration. Blood was collected from the tail veil 30 min after glucose injection for BG determinations.

High Sugar/Fat Diet and Streptozotocin (STZ)-Induced Type 2 Diabetes in Rats

Wistar rats were maintained on a high sugar/fat diet (20% sugar, 10% lard, 1% pig cholate, 2% cholesterol, and 67% basal rat chew) for 4 weeks. Then the animals received intra-peritoneal injections of 30 mg/kg Streptozotocin (STZ) (Sigma, Missouri, USA) once after 16–18 h of fasting and free watering. The rats were maintained on the same high sugar/fat diet (20% sugar, 10% lard, 1% pig cholate, 2% cholesterol, and 67% basal rat chew) for another 4 weeks to become diabetic models. This diabetic model was confirmed by BG measurements (with One touch Ultra, Johnson & Johnson, NJ, USA) and glucose tolerance tests. Then the rats were divided into the following groups based on 24 h fasting BG levels (measured with One touch Ultra, Johnson & Johnson, NJ, USA), and treated thus: healthy control group, diabetic control group (normal saline solution-treated), sGLP-1-treated diabetic group (a single 0.5 ml subcutaneous injection of 7, 14, 28 μmol/l/kg every day for 2 weeks), and GLP-1(7–37)-treated diabetic group (24 μmol/l/kg every day for 2 weeks). Blood samples were collected for FBG determination (with One touch Ultra, Johnson & Johnson, NJ, USA) before grouping, at day 7, and day 14 before the peptide injection. Levels of glycosylated hemoglobin were also determined (with the kit from Zhongsheng Biotech on SABA/18 Automatic Biochemistry Analyzer from Italy) at day 14. After sacrificing rats, pancreas tissues were immediately collected and fixed with 10% neutral formalin.

Histologic and Immune Histochemical Analysis

Tissue specimens were embedded in paraffin, and 4-μm-thick sections were prepared. Sections were deparaffinized in multiple changes of xylene and were rehydrated through a series of descending ethanol concentrations.

Hematoxylin and eosin (HE) staining was performed, and morphological changes were evaluated microscopically.

The slides were stained with single or double first antibodies in PBS at 37 °C overnight, after 3 × 15 min washes in PBS, and then stained with conjugated single or double secondary antibodies in PBS at 37 °C for 2 h. The slides were washed 3 times for 15 min each, and then analyzed under Olympus fluorescence microscope.

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Statistics

The statistical package SPSS 10.0 (SPSS incorporated, Chicago) was used for all analyses. Comparison of group means was performed by ANOVA. $P < 0.05$ was accepted as significant. All values were expressed as mean ± SD.

RESULTS

The BG-Lowering Effect of *C*-terminus Deletion GLP-1 Analogs in GK Rats with Type II DM

The FBG values in GK rats following injection of various GLP-1 analogs is presented in Table 1. One week after drug administration, there was a trend toward decreased FBG values in animals receiving G8GLP-1, or sGLP-1. Of these analogs, the BG-lowering effect of G8GLP-1 was the most obvious. FBG values did not decrease in animals receiving the GLP-1 analogue, which contained the largest *C*-terminus deletion G8GLP-1(7–30). Two weeks after drug administration, FBG values in animals receiving G8GLP-1, sGLP-1, and G8GLP-1(7–30) were significantly decreased compared to control ($^{*}P < 0.05$, $^{**}P < 0.01$, respectively). Moreover, FBG values had returned to normal range in animals receiving either G8GLP-1 or sGLP-1. The BG-lowering effect of sGLP-1 showed a trend towards a greater BG-lowering effect compared to G8GLP-1.

Comparison of Efficiency of G8GLP-1 and sGLP-1, as Determined by Glucose Tolerance in Mice

BG values in G8GLP-1 and sGLP-1-treated BALB/c mice 30 min following glucose injection are shown in Figure 1. At 0 or 2 h after drug administration, G8GLP-1 exhibited greater BG-lowering effects than sGLP-1. However, sGLP-1 retained its BG-lowering effect for at least 6 h after drug administration. In contrast, the effects of G8GLP-1 were apparent 4 h after drug administration. These results show that sGLP-1, which lacks five amino acids at *C*-terminus, has a longer-lasting efficiency than that of G8GLP-1.

The Effect of sGLP-1 on the BG of Rats with Type II DM Induced by a High Glucose/Fat Diet and STZ

T2DM was successfully established by a high glucose/fat diet and a low dose of STZ as seen by elevated BG levels and decreased glucose tolerance (Table 2). In animals receiving sGLP-1 (7, 14, 28 μmol/l/kg), BG levels were decreased at 2 weeks ($^{*}P < 0.05$, *vs* diabetic control). In the high-dose group (24 μmol/l/kg), glucosylated hemoglobin (GHb) was also markedly decreased at 2 weeks ($^{**}P < 0.01$; Table 3). Pathohistological analyses revealed that, in diabetic animals, pancreatic volume (PV) decreased and islet number was significantly reduced (Table 4, Figure 2). Also, adipose degeneration was observed in pancreatic acini epithelia. In contrast, the sGLP group was characterized by a much larger PV and an increased number of islets (Table 4, Figure 2). Moreover, these effects were dose-dependent. The morphology of the G8GLP group was indistinguishable from that of the diabetic control group (Table 4, Figure 2).

Encouraged by superior BG-lowering effect of sGLP-1 in a 2-week regimen, especially towards the end of the 2 weeks, we further explored the therapeutic effect of sGLP-1 over G8 GLP-1 on GK rats with T2DM

Table 1 Blood glucose[a]-lowering effects of *C*-terminus deletion GLP-1 analogues

Time after treatment	G8GLP-1	sGLP-1	G8GLP-1 (7–30)	G8GLP-1 (7–27)	Control
1 week	8.08 ± 0.65	8.21 ± 0.59	8.58 ± 0.87	8.90 ± 0.67	8.75 ± 0.72
2 weeks	5.64 ± 1.40*	3.90 ± 0.74**	7.64 ± 0.87*	8.90 ± 0.67	9.23 ± 1.01

[a] Blood glucose concentrations are expressed in units of mmol/l.
$n = 30$; * $P < 0.05$ or ** $P < 0.001$ *versus* control.

Table 2 Glucose tolerance in rats with type II DM induced by high glucose and high fat diet + STZ

Experimental group	Blood glucose (mmol/l)			
	Before glucose administration	30 min after glucose injection	60 min after glucose injection	120 min after glucose injection
Healthy animals	4.0 ± 0.4	6.1 ± 1.3	7.0 ± 0.9	7.3 ± 0.5
Diabetic animals	10.0 ± 7.1	21.5 ± 7.7	21.3 ± 9.4	17.9 ± 7.5

$n = 30$

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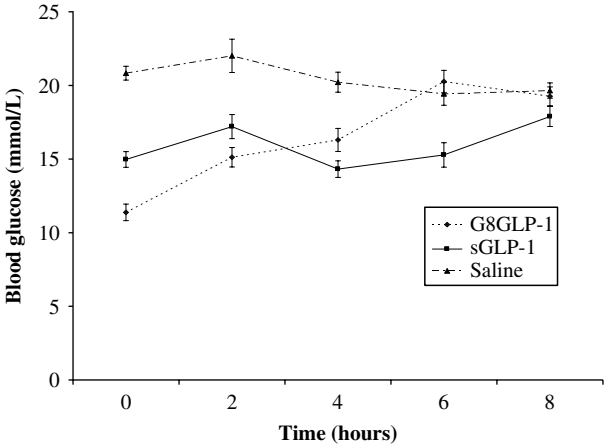

Figure 1 The effect of sGLP-1 on glucose tolerance in healthy mice. Glucose was administered at 0, 2, 4, 6, and 8 h after sGLP-1 or GLP-1 administration (0.5 ml of 2 μmol/l for each). Blood glucose concentrations were measured 30 min later. $n = 30$.

with an 8-week regimen of sGLP-1. Animals received daily subcutaneous 0.5 ml injections of 2 μmol/l for 2 weeks, the same as in the 2-week regimen. Animals subsequently received sGLP-1 injections (0.5 ml of 2 μmol/l) once every 2 days for 2 weeks. The change from daily to once every 2 days at the same dosage is to test the possibility of reducing the peptide used without loss of efficiency. Based on the success achieved in the once every 2 days for 2 weeks experiment, we went on to try out the possibility of reducing the peptide injection frequency. For the remaining 4 weeks, animals received 0.5 ml of 4 μmol/l sGLP-1 twice a week (Monday and Friday) such that the total weekly dose remained the same as in the previous 2 weeks. Please note that the dosage change from 0.5 ml of 2 μmol/l to 0.5 ml of 4 μmol/l is necessary while changing over from once every 2 days to twice a week. As shown in Table 5, after an initial 2 weeks of treatment, BG levels returned to normal. BG values remained within normal range over the next 6 weeks. Two weeks after sGLP-1 withdrawal, BG values began to increase but were still within normal range. In marked contrast, BG values were significantly elevated over the 8-week period in control animals.

sGLP-1 Reduced Islet Apoptosis Enhanced Insulin Production and Promoted Islet Proliferation

After 4 weeks of continuous applications of 12 μmol/l/kg sGLP-1, there were more and bigger insulin-positive

Table 3 The effect of sGLP-1 on levels of blood glucose and glucosylated hemoglobin (GHb) in rats with type II DM induced by high glucose/high fat diet and STZ

Experimental group		Blood glucose (mmol/l)			GHb (%)
		Before drug administration	1 week after drug administration	2 weeks after drug administration	2 weeks after drug administration
Healthy animal		3.48 ± 0.79	3.34 ± 0.57	3.37 ± 0.39	3.42 ± 0.44
Diabetic control		9.45 ± 5.17	11.62 ± 8.05	13.42 ± 8.45	5.89 ± 0.75
sGLP-1	3 μmol/l/kg	9.72 ± 5.57	8.93 ± 3.32	6.15 ± 0.87*	5.52 ± 1.61
	6 μmol/l/kg	9.55 ± 2.22	10.38 ± 5.62	5.68 ± 0.69*	4.76 ± 1.22
	12 μmol/l/kg	9.38 ± 3.91	8.08 ± 2.89	5.88 ± 2.79*	3.37 ± 0.36**
G8GLP-1	12 μmol/l/kg	9.43 ± 2.48	11.35 ± 6.49	5.97 ± 1.00*	3.64 ± 0.65**

$n = 30$; * $P < 0.05$ or ** $P < 0.001$ *versus* diabetic control (saline-treated).

Table 4 The effect of sGLP-1 on the pancreatic morphology of the rats with type II DM induced by high glucose and high fat diet and STZ

Experimental group	*n*	PV	Islet no	Inflammatory cell infiltration in pancreatic islets	Adipose degeneration in epithelia of pancreatic acini
Healthy animal	5	Large	Much	—	—
Diabetic control	5	Small	Little	—	+
sGLP-1 10 μg/kg	5	Large/small	Medium	—	—
20 μg/kg	5	Large	Much	—	—
40 μg/kg	5	Large	Much	—	—
G8GLP-1 40 μg/kg	5	Small	Little	—	—

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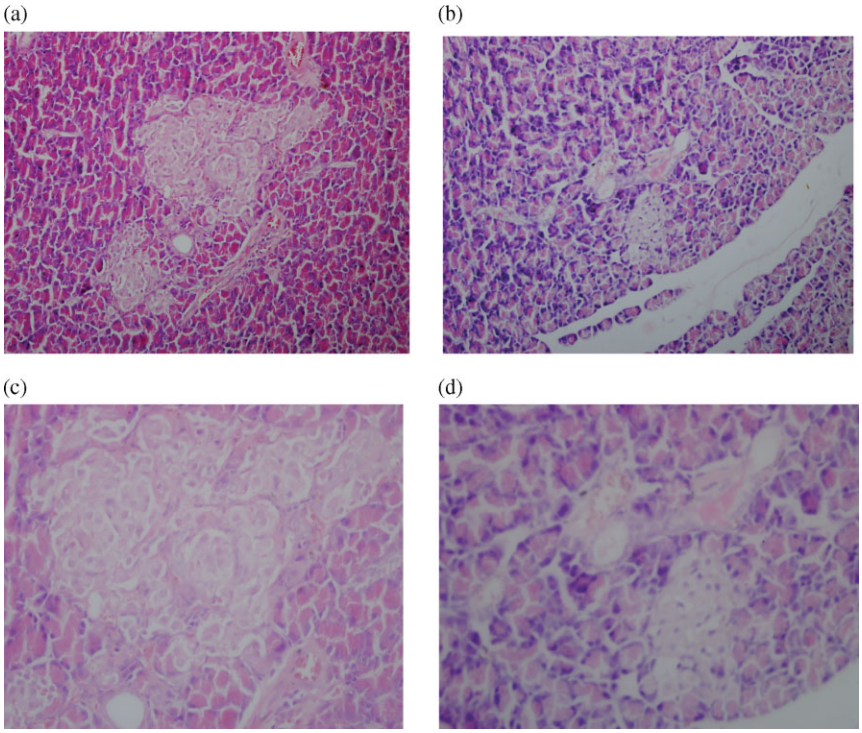

Figure 2 The effect of sGLP-1 on the pancreatic morphology of the rats with type II DM induced by high glucose/high fat diet and STZ. Representative images of H&E stained pancreatic tissue are shown for animals with T2DM. (a) (X200) and (c) (X400) were from a T2DM rat after 4 weeks of injection with sGLP-1 of 12 μmol/l/kg. (b) (X200) and (d) (X400) were from a T2DM rat after 4 weeks of mock injection with saline. Note the smaller pancreatic volume (PV) and the decreased number of islets in the control T2DM animal (b) and (d). Tissue from sGLP-1-treated animal was characterized by a larger PV and a greater number of islets (a) and (c).

Table 5 The therapeutic effect of sGLP-1 on GK rats with type II DM

Experimental group	Blood glucose (mmol/l)			
	2 weeks treatment	4 weeks treatment	8 weeks treatment	2 weeks after drug withdrawal
sGLP—1[a]	$5.99 \pm 0.38^{**}$	$5.92 \pm 0.34^{**}$	$5.52 \pm 0.46^{**}$	$7.18 \pm 0.62^{**}$
Control	16.50 ± 0.32	16.19 ± 0.33	20.80 ± 1.27	20.05 ± 0.84

[a] Animals received a 0.5 ml injection of 2 μmol/l daily for 2 weeks, a 0.5 ml injection of 2 μmol/l once every 2 days for an additional 2 weeks, and 0.5 ml injection of 4 μmol/twice a week for 4 weeks.
$n = 30$; ** $P < 0.001$ *versus* control.

cells in sGLP-1 treated groups (Figure 3(a), (c) and (e)) than in the control groups (Figure 3(b), (d) and (f)); and almost all the cells in the control groups were strongly Caspase-3 positive (Figure 3(b), (d) and (f)) compared with only the smaller numbers and weaker Caspase-3 positive cells (Figure 3(a), (c) and (e)). That means sGLP-1 reduced apoptosis and enhanced insulin production of T2D rat islet β cells.

Ki67 is a molecule that can be easily detected in growing cells in order to gain an understanding of the rate at which the cells within a tissue are growing. The immune fluoresce stains (Figure 4) revealed that there were more and bigger insulin-positive cells in sGLP-1-treated groups than in the control groups, and the insulin-positive cells are proliferating cells. That demonstrated that sGLP-1 improved islet β cell replication.

DISCUSSION

GLP-1 and Diabetes

GLP-1(7–37) is an intestinal insulinotropic peptide that can stimulate insulin secretion, inhibit glucagon release, decelerate gastric emptying, and increase beta

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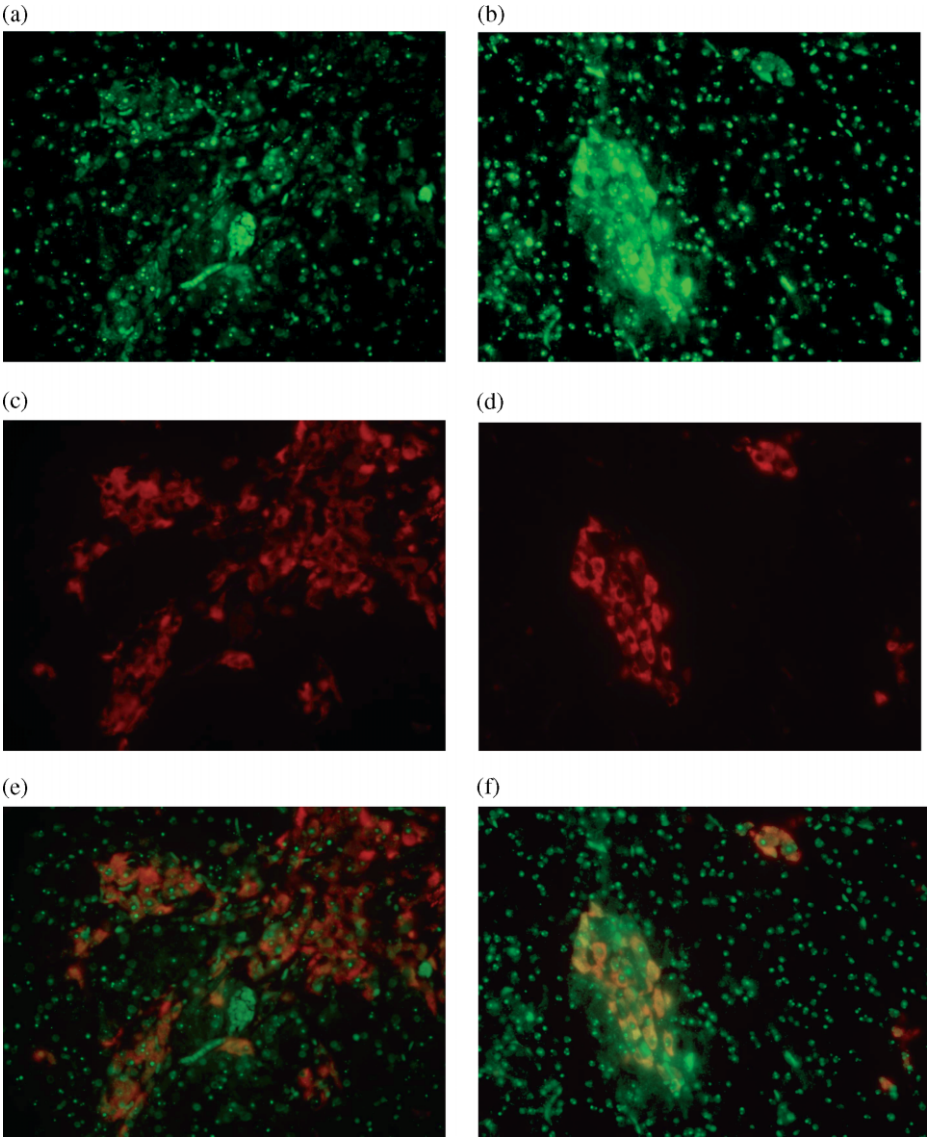

Figure 3 sGLP-1 reduced apoptosis and enhanced insulin production of T2DM rats islet β cells. (a), (c), and (e) are sGLP-1 groups after 4 weeks of continuous applications of 12 μmol/l/kg; (b), (d), and (f) are control groups after 4 weeks of continuous applications of mock injections of saline. (a) and (b) were stained with rabbit antirat Caspase-3 polyclonal antibody first, then with FITC-conjugated goat antirabbit secondary antibody (both from Zhongsan Jingqiao, Beijing); (c) and (d) were stained with mouse antirat insulin polyclonal antibody first, and then with TRITC-conjugated goat antimouse secondary antibody (both from Zhongsan Jingqiao, Beijing); (e) and (f) are anti-Caspase-3 and anti-insulin double stains. The immune fluoresce stains revealed that there were more and bigger insulin-positive cells in sGLP-1 treated groups than in the control groups, and almost all the cells in the control groups were strongly Caspase-3 positive compared with only the smaller numbers and weaker Caspase-3 positive cells.

cell numbers. The BG-lowering effect of GLP-1(7–37) is glucose-dependent but does not produce low BG. It has also been shown to improve blood lipid levels in T2DM patients [5–7,17–19].

Recent studies have shown that GPL-1(7–37) plays an important role in the differentiation and proliferation of beta cells. It is capable of inhibiting beta cell apoptosis, stimulating beta cell proliferation, and inducing differentiation of stem cells into pancreatic endocrine cells and thus, re-establishing insulin secretion in impaired islet cells. These functions make GPL-1(7–37) an ideal treatment for DM and have led to intense interest in this peptide [20,21]. However, the very short *in vivo* half-life of GLP-1(7–37) has hampered its clinic use. Thus, efforts have been made to identify more stable analogs of GPL-1(7–37).

Structure and Function of GLP-1 Analogs

One such analog, Exendin-4, is a 39 amino acid peptide isolated from the salivary secretions of the

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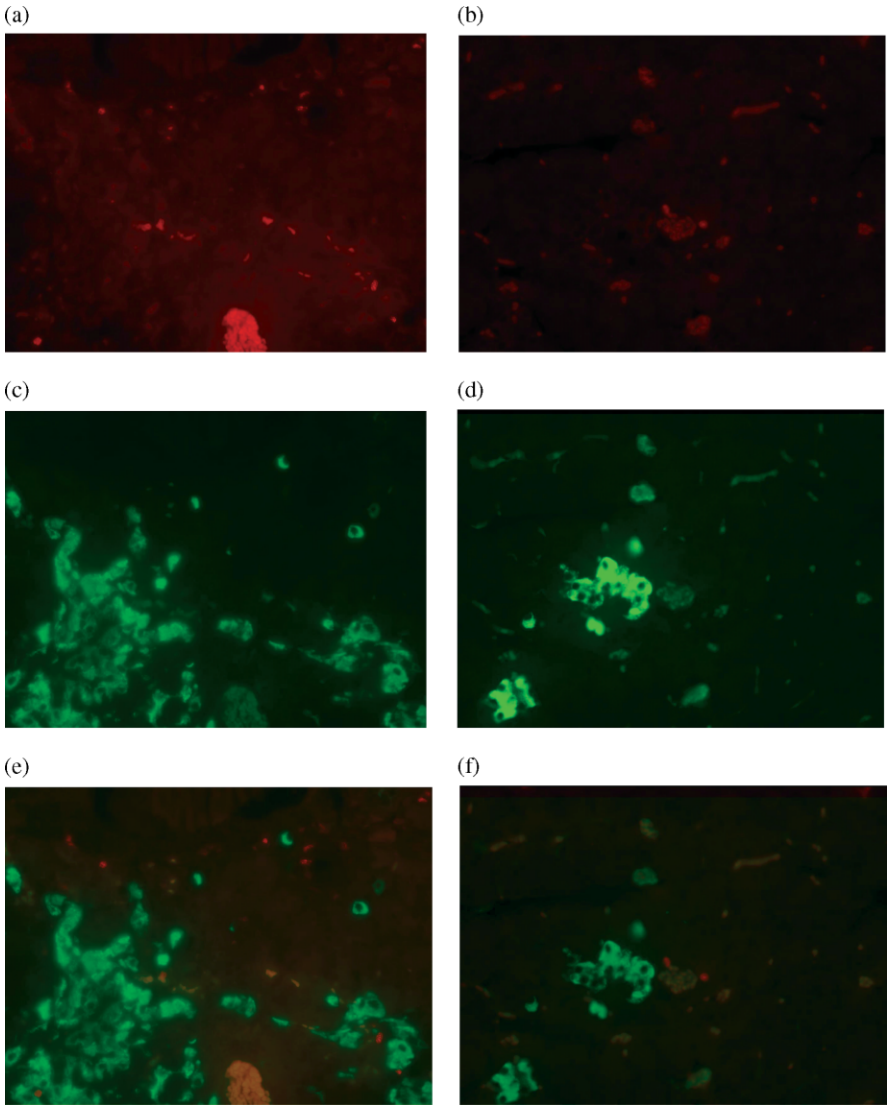

Figure 4 sGLP-1 improved islet β cell replication. (a), (c), and (e) are sGLP-1 groups after 4 weeks of continuous applications of 12 μmol/l/kg; (b), (d), and (f) are control groups after 4 weeks of continuous applications of mock injections of saline. (a) and (b) were stained with mouse anti-Ki-67 monoclonal antibody (Labvision Corp, Fremont, CA, USA) first, then with TRITC-conjugated goat antimouse secondary antibody (Zhongsan Jingqiao, Beijing); (c) and (d) were stained with mouse antirat insulin polyclonal antibody first, and then with FITC-conjugated goat antirabbit secondary antibody (both from Zhongsan Jingqiao, Beijing);(e) and (f) are anti-Ki-67 and anti-insulin double stains. Ki67 is a molecule that can be easily detected in growing cells in order to gain an understanding of the rate at which the cells within a tissue are growing. The immune fluoresce stains revealed that there were more and bigger insulin-positive cells in sGLP-1-treated groups than in the control groups, and the insulin-positive cells are proliferating cells.

Gila monster in Mexico. It has 50% homology to GLP-1(7–37). Very low concentrations of Exendin-4 can promote the pancreas to synthesize and secrete insulin [22,23], making it effective in controlling BG levels in DM patients. Other analogs include Albugon (HGS) and NN2211 (Liraglutide; Novo-Nordisk). Albugon is an albumin-fusion protein containing a mutant form of GLP-1(7–37), which exhibits increased resistance to DPP-IV, and has a prolonged half-life. NN2211 contains a 16-carbon fatty-acid side chain attached at position 26. Although this side chain slightly reduces the activity of GLP-1(7–37), it prolongs the half-life of GLP-1(7–37) by binding to plasmid albumin after injection [11–13]. Both of them delayed the clearance of GLP-1(7–37) *in vivo* by association with albumin either through binding to albumin or directly fusing with it.

Our sGLP-1 has both substitution of A to G at 8th amino acid, and a deletion of five amino acids from the *C*-terminus of GLP-1(7–37). The deletion of five amino acids from the *C*-terminus of GLP-1(7–37) did not abrogate activity. Indeed, compared to the other analogues, deletion of five amino acids at the *C*-terminus reduced short-term activity but enhanced activity after

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2 weeks of application as show in Figure 1. Glucose tolerance tests confirmed that sGLP-1 activity was significantly prolonged *in vivo* compared to that of G8GLP, suggesting that the shortened *C*-terminus may have prolonged the half-life of sGLP-1. It is interesting to have a careful looking of the report by Gefel *et al* [24]. In an *in vitro* insulin release test they noticed that GLP-1(7–37) was a potent insulin secretagogue at concentration of 10^{-11} M, GLP-1(7–34) had no stimulatory effect on insulin release at 10^{-10} M, but had a partial effect at 10^{-9} M and was as effective as GLP(7–37) at 10^{-8} M, but GLP-1(7–33) had no effect even at 10^{-8} M. In a cAMP formation test GLP-1(7–34) was less potent than GLP-1(7–37) at a concentration of 5×10^{-9} M, GLP-1(7–33) had only about 0.1% the potency of GLP-1(7–37). Because GLP-1(7–33) had no effects, they did not test GLP-1 analogs further. In this study, by using a *in vivo* test, we not only confirmed their above observations about the importance of the *C*-terminus of GLP-1 (7–37), but we also demonstrated that further truncation of G8GLP-1(7–37) till the 32nd amino acid was still functional, and it is much better for long-term BG-lowering applications.

However, one very important point we should like to emphasize is that even with *in vivo* tests, if we only test the short-term effect of the GLP-1(7–37) analogs, we may not be able to find sGLP-1. This strategy may have general applications in screening candidates for repeated long-term usage. A candidate with lower immediate effect after one dosage of *in vivo* or *in vitro* application may benefit more for prolonged effect after repeated dosages.

GLP-1(7–37) can stimulate the proliferation of pancreatic beta cells *in vitro* [20,21]. Administration of sGLP-1 to rats with DM not only decreased both BG and GHb levels, but also reduced apoptosis of the old islets, promoted proliferation of the new islets, resulted in a more normal histological appearance of pancreatic tissue, and enhanced insulin production. This included a larger PV and an increased number of islets. G8GLP-1 did not result in any of these improvements in our tests. Importantly, the ability of sGLP-1 to return BG levels to normal range in GK rats with T2DM could be maintained with a twice-a-week administration regimen. Moreover, even 2 weeks after drug withdrawal, rats still exhibited BG levels that were within normal range. These results suggest that, although stimulating pancreatic beta cells to produce insulin is important, stimulating the proliferation of pancreatic beta cells is even more critical and ideal in DM treatment. In this way, the progress of diabetes can be reversed.

CONCLUSIONS

Although shortening the *C*-terminus of GLP-1(7–37) (sGLP-1) weakens BG-lowering activity, it may be due to decreased receptor-binding ability; however, the stimulation of islet cell proliferation is extended, leading to prolonged BG-lowering effects, and may be due to the lengthened *in vivo* clearance. These findings point to a promising outlook for new drug development. Future studies will explore the mechanisms underlying GLP-1(7–37) clearance and will address the possibility of clearance receptors for the glucagon family peptides.

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