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Liposomal formulations of glucagon-like peptide-1: Improved bioavailability and anti-diabetic effect

Junko Hanato[a], Kazuki Kuriyama[a], Takahiro Mizumoto[b,c], Kazuhiro Debari[d], Junya Hatanaka[a], Satomi Onoue[a,*], Shizuo Yamada[a]

[a] Department of Pharmacokinetics and Pharmacodynamics and Global Center of Excellence (COE) Program, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
[b] Department of Product Development, ILS Inc., Moriya, Ibaraki 302-0104, Japan
[c] American Peptide Company, 777 East Evelyn Ave., Sunnyvale, CA 94086, USA
[d] Laboratory of Electron Microscopy, Showa University School of Medicine, Shinagawa, Tokyo 142-8555, Japan

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ABSTRACT

Glucagon-like peptide-1 (GLP-1), an incretin hormone, is recognized to be potent drug candidate for treatment of diabetes, however its clinical application has been highly limited, because of rapid enzymatic degradation by dipeptidyl-peptidase IV. To protect GLP-1 from enzymatic degradation and improve pharmacological effects, liposomal formulations of GLP-1 were prepared using three types of lyophilized empty liposomes such as anionic, neutral and cationic liposomes. Electron microscopic and dynamic light scattering experiments indicated the uniform size distribution of GLP-1-loaded liposomes with mean diameter of 130–210 nm, and inclusion of GLP-1 did not affect the dispersibility and morphology of each liposome. Of all liposomal formulations tested, anionic liposomal formulation exhibited the highest encapsulation efficiency of GLP-1 (ca. 80%). In intraperitoneal glucose tolerance testing in rats, marked improvement of hypoglycemic effects were observed in anionic liposomal formulation of GLP-1 (100 nmol/kg) with 1.7-fold higher increase of insulin secretion, as compared to GLP-1 solution. In pharmacokinetic studies, intravenous administration of anionic liposomal formulation of GLP-1 (100 nmol/kg) resulted in 3.6-fold higher elevation of serum GLP-1 level as compared to GLP-1 injection. Upon these findings, anionic liposomal formulation of GLP-1 would provide the improved pharmacokinetics and insulinotropic action, possibly leading to efficacious anti-diabetic medication.

1. Introduction

Glucagon-like peptide-1 (GLP-1) is an endogenous insulinotropic peptide, secreted from L cells in the gastrointestinal tract (Orskov et al., 1986). The biologically active forms of GLP-1, such as GLP-1(7–36)-amide and GLP-1(7–37), have been demonstrated to possess multiple functions, including enhancement of glucose-dependent insulin secretion, stimulation of pro-insulin gene expression, suppression of glucagon secretion and gastric emptying (Drucker, 1998; Fehmann et al., 1995; Xiao et al., 2001). Because of the important roles in glucose metabolism, GLP-1 and its derivatives have been recognized as potent drug candidates for the treatment of type 2 diabetes (Holst, 2000; Siegel et al., 1999). However, there is a problem limiting the therapeutic potential of GLP-1 in diabetes treatment, and the major drawback of GLP-1 is the rapid enzymatic degradation by dipeptidyl-peptidase IV (DPP-IV) (Deacon et al., 1995). DPP-IV cleaves off the two N-terminal amino acid residues, and the chemical conversion of GLP-1 into the inactive metabolites occurs with a half-life of 1–1.5 min. To overcome the drawback of GLP-1, a number of efforts have been made, including development of metabolically stable analogs of GLP-1 (Edwards et al., 2001; Juhl et al., 2002) and DPP-IV inhibitors (Holst and Deacon, 1998). In contrast, there are a limited number of formulation studies on GLP-1 and its derivatives for prolonged duration of action and enhanced bioavailability (Choi et al., 2004; Gedulin et al., 2008; Lim et al., 2008).

Recently, novel drug delivery systems have been developed for clinical use of some neuropeptides such as VIP and PACAP (Onoue et al., 2007), in particular, liposomal and PEG-phospholipid micellar formulation for intravenous use were proposed to protect neuropeptides from enzymatic degradation (Ashok et al., 2004; Suzuki et al., 1995). The association of neuropeptides with liposomes was believed to curtail enzyme-induced inactivation of neuropeptides, and association of neuropeptides with biocompatible sterically stabilized liposome also circumvents peptide inactivation by catalytic

* Corresponding author. Tel.: +81 54 264 5633 fax: +81 54 264 5635.
E-mail address: onoue@u-shizuoka-ken.ac.jp (S. Onoue).

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neutralizing antibodies, leading to enhancement of pharmacological effects (Rubinstein et al., 2001). In practice, liposomal formulation strategies have already been applied to interleukin-2 (Bergers et al., 1993), vasoactive intestinal peptide (Gololobov et al., 1998), salmon calcitonin (Yamabe et al., 2003) and insulin (Wu et al., 2004). Thus, a growing body of experimental evidence suggests that liposomal formulation strategy might be efficacious for protecting GLP-1 from enzymatic digestion, possibly resulting in improved pharmacokinetics (PK) and pharmacodynamics. However, there has been very limited number of reports describing practical application of the formulation technique to GLP-1 for the treatment of type 2 diabetes.

The present investigation aimed to develop the liposomal formulations of GLP-1 for improving the pharmacological effect of GLP-1. The physicochemical properties of liposomal GLP-1 formulations were assessed by transmission electron microscopy (TEM) observation, dynamic light scattering (DLS) for size distribution analysis, and determinations of encapsulation efficiency. Pharmacokinetic profiles after intravenous administration of GLP-1 and liposomal GLP-1 formulations were also characterized in rats, as well as the pharmacological effects with emphasis on insulin secretion and glucose metabolism by intraperitoneal glucose tolerance testing.

2. Materials and methods

2.1. Peptides

Glucagon-like peptide 1 (GLP-1) was chemically synthesized by the solid-phase strategy employing optimal side-chain protection as reported previously (Merrifield, 1969). The purity (>98%) of each tested peptide was checked by Waters Acuity UPLC/ESI-MS system (Waters, Milford, MA), that include the binary solvent manager, sampler manager, column compartment, Tunable UV detector with a detection wavelength of 254 nm and square-law detector, connected with Waters MassLynx software. An Acuity UPLC BEH C18 column (particle size: 1.7 μm, column size: Ø 2.1 mm × 50 mm; Waters) was used. The pure peptides showed the expected molar ratio of the constituent amino acids in amino acid analysis with an L-8500 amino acid analyzer (Hitachi, Tokyo, Japan). Molecular mass was confirmed with a MALDI-TOF mass spectrometer (Kratos, Manchester, UK).

2.2. Liposomal formulations

Liposomal formulations was prepared by rehydration of freeze-dried empty liposomes with an aqueous drug solution (FDEL method) as established by Kikuchi et al. (1999). Dried empty liposomal formulation (COATSOME® EL), prepared by conventional lipid-film method (Yachi et al., 1996), was purchased from NOF Corporation (Tokyo, Japan), that includes DSPE-PG8G (distearoylphosphatidylethanolamine-polyglyceline), DPPC (dipalmitoylphosphatidylchorine), DPPC (dipalmitoylaphosphatidylglycerol), cholesterol or stearyl amine. The components of liposomes utilized in this study are listed in Table 1. GLP-1 (calculated isoelectric point: 5.53) was dissolved in 20 mM sodium phosphate buffer (pH7.4) at the final concentration of 10^{-4} M, and addition of 2 mL of GLP-1 solutions to a vial containing the dried empty liposomes resulted in preparation of liposomal GLP-1 formulations, such as anionic liposomal GLP-1 (AL-GLP-1), nonionic liposomal GLP-1 (NL-GLP-1) and cationic liposomal GLP-1 (CL-GLP-1).

2.3. Transmission electron microscopy (TEM)

An aliquot (2 μL) of the liposomal GLP-1 solutions was placed on a carbon-coated Formvar 200 mesh nickel grid. The sample was allowed to stand for 15–30 s, and then any excess solution was removed by blotting. The samples were negatively stained with 2% (w/v) uranyl acetate and allowed to dry. The samples were then visualized under as H-7600 transmission electron microscope (Hitachi, Japan) operating at 75 kV.

2.4. Vesicle size

Mean particle sizes of liposomes were measured by a dynamic light scattering, ELS-Z (Otsuka Electronics, Osaka, Japan). Liposomal suspensions were diluted 10-fold with 20 mM sodium phosphate buffer (pH 7.4) before the measurement. The determination was repeated three times per sample. The sizes of liposomes were measured again 1 week after storage at ca. 25 °C.

2.5. Encapsulation efficiency

Encapsulation efficiency of GLP-1 entrapped by liposomes was determined using Centrifree® (MilliPore, Ireland) ultrafiltration device with a molecular weight cutoff of 30,000 Da, according to previously reported method with some modification (Huang et al., 2005; Mayer and St-Onge, 1995). Immediately after the end of preparing the liposomes, the product was filtered using Centrifree® at 1000 × *g* for 20 min in centrifuge (Hitachi, Tokyo, Japan) in order to separate the liposomal GLP-1 from the free form. The ultrafiltrate solutions were analyzed by UPLC/ESI-MS to determine the amount of free GLP-1. The entrapment capacity of liposomes was calculated as follows: $[(A - B)/A] \times 100$, where A is the amount of total GLP-1, and B is the amount of free GLP-1 in the ultrafiltrate.

2.6. Pharmacokinetic and pharmacological characterization of liposomal GLP-1 formulations

2.6.1. Animals

For liposomal GLP-1 formulation studies, male Wistar rats (SLC, Shizuoka, Japan), weighing about 300–350 g, were acclimated under standardized environment with free access to standard food and water. All studies were conducted in accordance with the approval of Experimental Animal Ethical Committee of University of Shizuoka.

2.6.2. Intraperitoneal glucose tolerance test (IPGTT)

The hypoglycemic efficacies of the liposomal GLP-1 formulations were investigated using an IPGTT in rats. Briefly, 2 h fasted rats were injected with a glucose solution (2 g/kg, at time 0 min, i.p.), and GLP-1 formulations (100 μmol/L GLP-1) were given intravenously, at the doses of 100 nmol/kg, at 15 min before glucose injection.

Table 1
Compositions and characteristics of liposomes.

Liposome	Lipid compositions (μmol/vial)[a]	Property
COATSOME EL-01-PA (AL)	DSPE-PG8G:DPPC:cholesterol:DPPG = 4.2:11.4:15.2:11.4	Anionic
COATSOME EL-01-N (NL)	DPPC:cholesterol:DPPG = 5.4:4.0:0.6	Nonionic
COATSOME EL-01-C (CL)	DPPC:cholesterol:stearyl amine = 2.6:2.0:0.4	Cationic

[a] Information provided from manufacturer. DSPE, distearoylphosphatidylethanolamine; PG8G, polyglyceline; DPPC, dipalmitoylphosphatidylchorine; and DPPG, dipalmitoylaphosphatidylglycerol.

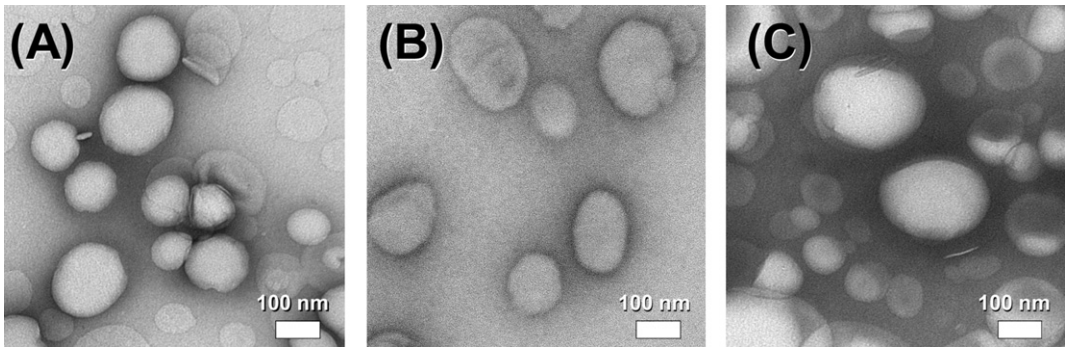

Fig. 1. Transmission electron microscopic image of GLP-1 formulations; AL-GLP-1 (A), NL-GLP-1 (B) and CL-GLP-1 (C).

Blood was collected from tail vein at various periods. Blood samples were prepared for each analysis by centrifuging appropriate volume at 10,000 × *g* for 10 min at 4 °C followed by transfer of the supernatant to another tube. Blood glucose level was determined by commercially available kits, Glucose CII-test Wako, according to the manufacturer's instructions. Briefly, 5 μL of blood serum was mixed with reaction mixture containing 0.13 units/mL mutarotase, 9.0 units/mL glucose oxidase, 0.65 units/mL peroxidase, 0.50 mmol/mL 4-aminoantipirine and 2.7 units/mL ascorbate oxidase. After incubating at 37 °C for 5 min, the absorbance (490 nm) was measured with a Multilabel Counter (PerkinElmer, Norwalk, CT, U.S.A.). The insulin concentration in each sample was measured by an enzyme immunoassay kit (Morinaga Insulin measurement kit, Morinaga, Yokohama, Japan). Briefly, 5 μL of blood serum and guinea pig anti-insulin antibody were incubated in anti-insulin monoclonal antibody solid-phased plate overnight. After the addition of anti-guinea pig enzyme-labeled anti-IgG antibody to the plate, the mixture was reacted with orthophenylenediamine substrate. The absorbance at 490 nm was measured with a Multilabel Counter (PerkinElmer).

2.6.3. UPLC/ESI-MS analysis

For determination of serum GLP-1 level, samples (20 μL) were initially pretreated with 20 μL of acetonitrile (Kanto Chemical, Tokyo, Japan), followed by centrifugation at 10,000 × *g*. The deproteinized supernatants were assessed by Waters Acuity UPLC/ESI-MS system (Waters, Milford, MA), that include the binary solvent manager, sample manager, column compartment, Tunable UV detector with a detection wavelength of 210 nm and square-law detector, connected with Waters MassLynx software. An Acuity UPLC BEH C18 column (particle size: 1.7 μm, column size: Ø 2.1 mm × 50 mm; Waters), held at 30 °C, was used. The UPLC mobile phase consisted of 0.05% trifluoroacetic acid in water (A) and acetonitrile (Kanto Chemical Co., Inc.). While maintaining a constant flow rate of 0.25 mL/min, the samples were eluted using the following gradient: 0 min, 30% A; 3 min, 55% A; 3.01 min, 90% A; and 5 min, 30% A. The mass spectrometer instrument was operated in positive electrospray ionization mode using cone voltages of 60 mV with a selected ion recording (SIR) of 1029 amu. The source and desolvation temperatures were set at 120 and 400 °C, respectively, and the cone gas flow 50 L/h and the desolvation gas flow 900 L/h.

2.7. Statistical analysis

For statistical comparisons, Student's *t*-test or one-way analysis of variance (ANOVA) by Dunnett's *post hoc* test was used. A *p* value of less than 0.05 was considered significant for all analyses.

3. Results and discussion

3.1. Physicochemical properties of liposomal GLP-1

TEM observations of liposomal GLP-1 formulations revealed that three types of unilamellar liposomes were spherical and uniformly dispersed with mean diameters of 100–200 nm (Fig. 1). No significant transition of liposomal morphology was observed for at least 7 days (data not shown). These findings were consistent with DLS data, in which vesicle sizes of AL-, NL- and CL-GLP-1 were estimated to be 131, 148 and 208 nm, respectively (Table 2). The polydispersity index of the liposomal GLP-1 formulations was ranging from 0.12 to 0.24, indicating that these formulations were moderately homogeneous. Empty liposomal formulations also exhibit similar morphologies without aggregation and fusion (data not shown), so that inclusion of GLP-1 might not affect the physicochemical properties of liposome. Addition of GLP-1 led to only slight reduction in vesicle size of AL and NL by 8.4% and 4.5%, respectively. However, there was 15.5% reduction of vesicle size after inclusion of GLP-1 into CL, suggesting modified environment of liposomal membrane. On the basis of the data obtained, inclusion of GLP-1 did not affect the physicochemical properties of liposome, such as liposomal morphology, dispersibility and stability, except for vesicle size.

3.2. Anti-hyperglycemic effects of various liposomal GLP-1formulations

A half-life of GLP-1 in plasma was found to be less than 2 min, due to rapid degradation by some enzymes, mainly DPP-IV (Vilsboll et al., 2003), however liposomal GLP-1 formulations might exhibit

Table 2
Physicochemical properties of GLP-1 formulations.

Formulations	Vesicle size (nm)	Polydispersity index	Encapsulation efficiency (%)
Anionic liposome (AL)	143 ± 0.7	0.17 ± 0.02	–
AL-GLP-1	131 ± 0.1	0.14 ± 0.01	80.2
Nonionic liposome (NL)	155 ± 3.8	0.10 ± 0.01	–
NL-GLP-1	148 ± 2.8	0.12 ± 0.02	40.3
Cationic liposome (CL)	246 ± 13.4	0.20 ± 0.01	–
CL-GLP-1	208 ± 2.9	0.24 ± 0.01	27.8

Data represents mean ± S.E. of 3 experiments.

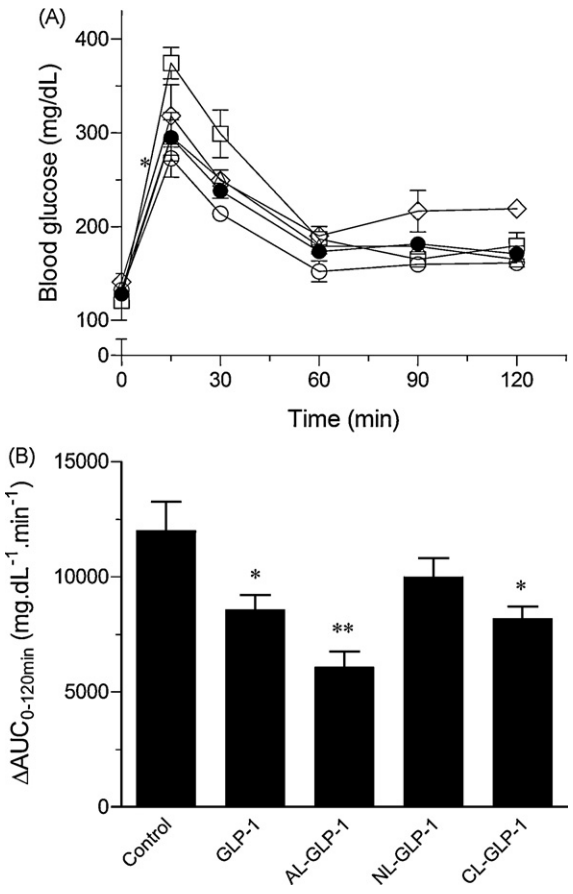

Fig. 2. Anti-diabetic activity of GLP-1 formulations in intraperitoneal glucose tolerance test using rats. (A) Blood glucose stabilization after intravenous administration of GLP-1 formulations (−15 min, 100 nmol/kg) and intraperitoneal glucose (0 min, 2 g/kg). Blood glucose concentrations were measured at indicated periods. Control (vehicle alone) (□); GLP-1 (●); AL-GLP-1 (○); NL-GLP-1 (◊); and CL-GLP-1 (△). (B) Calculated glucose AUC of the experimental groups. Data represents mean ± S.E. of 5–6 experiments. $^{**}P < 0.01$ and $^{*}P < 0.05$ with respect to the control group.

higher metabolic stability than GLP-1 itself. In the present study, the differences in hypoglycemic effect among liposomal GLP-1 formulations were clarified by intraperitoneal glucose tolerance testing (IPGTT) in rats. Following the intraperitoneal glucose injection in rats, the blood glucose concentration in control rats increased and reached maximum level at 15 min (Fig. 2A). The glucose levels of control rats, however, declined to the basal level at 60 min. At 15 min after glucose challenge, blood glucose levels in GLP-1 or liposomal GLP-1-treated rats were found to be ca. 30% lower, as compared to control group. There was no significant difference in hypoglycemic effect between GLP-1 and NL-GLP-1, as evidenced by similar ΔAUC_{0-120} values, whereas both AL- and CL-GLP-1-treated groups exhibited significant reduction of AUC values as compared control group (Fig. 2B). Of all liposomal GLP-1 formulations tested, AL-GLP-1 exhibited the highest hypoglycemic effect, showing 49.2% reduction of ΔAUC_{0-120} compared with control. Thus, AL-GLP-1 could regulate blood glucose level much longer than other liposomal formulations and non-liposomal GLP-1, and these results would suggest that GLP-1 might retain on anionic liposome with protection from enzymatic digestion.

Previously, Yamabe and co-workers demonstrated that charge of the lipids had influence on the encapsulation efficiency of salmon calcitonin (Yamabe et al., 2003). Upon these findings, encapsulation efficiency of GLP-1 could also be affected by acidic components of the liposomes. In the present investigation, the drug-entrapment percentage in liposomal GLP-1 formulations was determined by dialysis method using UPLC–ESI-MS, and the encapsulation efficiencies of GLP-1 for liposomal formulations, including AL-GLP-1, NL-GLP-1 and CL-GLP-1, were calculated to be 80.2%, 40.3% and 27.8%, respectively (Table 2). Though never unequivocally proved, the encapsulation efficiency of GLP-1 seemed to be related to theoretical electric charge of total membrane components. Previously, 2D NMR spectral analysis of GLP-1 in SDS micelle revealed that large helical structure in C-terminus of GLP-1 would be responsible for interaction with the hydrophobic portion of the micelle or membrane (Thornton and Gorenstein, 1994). Theoretical isoelectric point of GLP-1 was calculated to be 5.53, however GLP-1 has some cationic amino acid residues, including arginine (p*K*a = 12.5) at residue 30 and lysine (p*K*a = 10.5) at residues 20 and 28, on the hydrophilic face of the C-terminal helix. The hydrophobic face of the C-terminal helix could be buried deeply into the micelle or membrane (Thornton and Gorenstein, 1994), then cationic amino acids on opposite face of the helix might interact with negatively charged phospholipids, including dipalmitoylaphosphatidylglycerol. This could be a part of reasons why anionic liposome exhibited the high encapsulation of GLP-1, providing an intense anti-hyperglycemic effect. These findings would suggest the presence of ionic interaction with phospholipid bilayers, which might be a crucial factor for the high encapsulation.

3.3. Pharmacokinetic behavior and insulinotropic action of AL-GLP-1

The AL-GLP-1 exhibited the marked enhancement of hypoglycemic effect in rats as compared to GLP-1, suggesting that the pharmacokinetic behavior of GLP-1 after intravenous administration of liposomal GLP-1 might be improved as well. To clarify the possible mechanisms in the enhanced pharmacological effect, the pharmacokinetic behavior of GLP-1 was characterized after intravenous administration of AL-GLP-1 and GLP-1 (Fig. 3). According to the time evolution of serum GLP-1 concentration, the maximum concentration (C_{max}) of serum GLP-1 was calculated to be 4.4 μg/mL at 15 min after intravenous administration of GLP-1 solution, then the serum concentration of GLP-1 decreased steadily. Intravenous administration of AL-GLP-1 resulted in 3.6-fold higher elevation of serum GLP-1 level, however the liposomal formulation exhibited rapid elimination as compared to GLP-1 solution. These results would suggest that anionic liposome could capture GLP-1 with protection from enzymatic digestion, possibly resulting

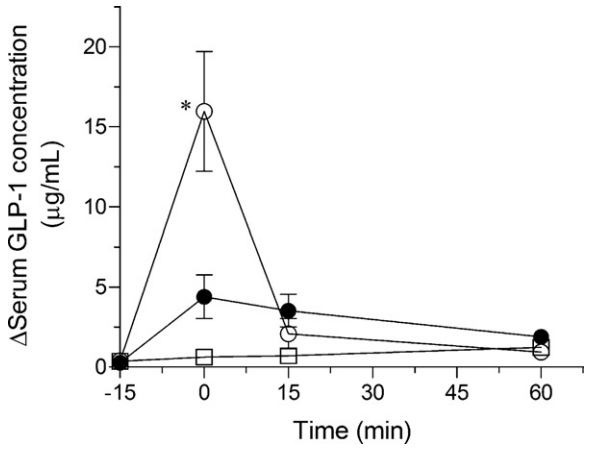

Fig. 3. Blood GLP-1 concentrations in rats after intravenous administration of GLP-1 formulations. Blood GLP-1 concentrations at indicated periods were measured after intravenous administration of GLP-1 or AL-GLP-1 (−15 min, 100 nmol/kg) and intraperitoneal glucose (0 min, 2 g/kg). Control (vehicle alone) (□); GLP-1 (●); and AL-GLP-1 (○). Data represents mean ± S.E. of 8–11 experiments. $^{*}P < 0.01$ with respect to the GLP-1 group.

Table 3
Insulin secretion stimulated by GLP-1 formulations in rats.

	Blood insulin level (pg/mL)
Control	245.6 ± 390.8
GLP-1	656.4 ± 124.2
AL-GLP-1	1083.8 ± 555.9

Blood insulin concentrations were determined at 15 min after intravenous administration of GLP-1 or AL-GLP-1 (100 nmol/kg) in rats. Data represent mean ± S.E. of 5–6 experiments.

in the enhanced pharmacological effect of GLP-1. Generally, intravenously administered liposomes tend to associate with various organs of the reticuloendothelial system (RES), mainly the liver and spleen, leading to rapid clearance from systemic circulation (Alving et al., 1978). In some liposomal drugs, the systemic elimination of intravenously administered liposomes could be explained by RES uptake, as well as systemic degradation of liposomes (Iga et al., 1993; Qi et al., 1995), and this might cause quick elimination of GLP-1 after injection of AL-GLP-1. The rapid decline of serum GLP-1, following the temporary rise in serum GLP-1 after administration of AL-GLP-1, might be related to RES uptake of liposomal GLP-1.

It is well established that glucose-dependent insulinotropic action is responsible for the anti-diabetic function of GLP-1 through the stimulation of adenylate cyclase and Ca^{2+} influx in pancreatic β-cells (Holst, 2007). In addition to PK profiling, insulinotropic action of AL-GLP-1 was investigated by measuring insulin secretion after intravenous administration of GLP-1 formulations. As determined by ELISA (Table 3), there appeared to be 1.7-fold difference in insulin level between rats pretreated with AL-GLP-1and GLP-1. These findings were consistent with the observed PK profiles of AL-GLP-1, so that the enhanced hypoglycemic effect of liposomal GLP-1 could be attributed to the improved PK behavior.

Generally, type 2 diabetes has been identified as a constellation of metabolic disorders, including impaired control of hepatic glucose production and β-cell dysfunction (Hohmeier et al., 2003). Therefore, the anti-diabetic drugs with glucose-dependent insulinotropic effect have been required by patients with type 2 diabetes. On the basis of the present findings, showing the improved PK behavior and potent insulinotropic action of liposomal GLP-1 formulations, the liposomal formulations of GLP-1, especially anionic liposome, might be efficacious for clinical treatment of type 2 diabetes.

4. Conclusions

In the present study, liposomal formulations of GLP-1 were prepared with aim of improving pharmacological effects, and the physicochemical and pharmacological properties were characterized. Liposomal GLP-1 formulations with the diameter of 130–210 nm were found to be highly dispersible, and the highest encapsulation efficiency was observed in anionic liposomal GLP-1 formulation among all liposomal formulations tested. In particular, anionic liposomal GLP-1 formulation, containing DSPE-PG8G (10%), DPPC (27%), Cholesterol (36%) and DPPG (27%), also exhibited the marked improvement of pharmacological effects in rats, with increase of serum GLP-1 level and insulin secretion by 260% and 70%, respectively, as compared to GLP-1 solution. Upon these findings, liposomal formulations of GLP-1, in particular, anionic liposome might provide clinical benefit in patients with type 2 diabetes

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References

Alving, C.R., Steck, E.A., Chapman Jr., W.L., Waits, V.B., Hendricks, L.D., Swartz Jr., G.M., Hanson, W.L., 1978. Therapy of leishmaniasis: superior efficacies of liposome-encapsulated drugs. Proc. Natl. Acad. Sci. U.S.A. 75, 2959–2963.
Ashok, B., Rubinstein, I., Tsueshita, T., Onyuksel, H., 2004. Effects of peptide molecular mass and PEG chain length on the vasoreactivity of VIP and PACAP(1–38) in pegylated phospholipid micelles. Peptides 25, 1253–1258.
Bergers, J.J., Den Otter, W., Dullens, H.F., Kerkvliet, C.T., Crommelin, D.J., 1993. Interleukin-2-containing liposomes: interaction of interleukin-2 with liposomal bilayers and preliminary studies on application in cancer vaccines. Pharm. Res. 10, 1715–1721.
Choi, S., Baudys, M., Kim, S.W., 2004. Control of blood glucose by novel GLP-1 delivery using biodegradable triblock copolymer of PLGA-PEG-PLGA in type 2 diabetic rats. Pharm. Res. 21, 827–831.
Deacon, C.F., Johnsen, A.H., Holst, J.J., 1995. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. J. Clin. Endocrinol. Metab. 80, 952–957.
Drucker, D.J., 1998. Glucagon-like peptides. Diabetes 47, 159–169.
Edwards, C.M., Stanley, S.A., Davis, R., Brynes, A.E., Frost, G.S., Seal, L.J., Ghatei, M.A., Bloom, S.R., 2001. Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. Am. J. Physiol. Endocrinol. Metab. 281, E155–161.
Fehmann, H.C., Goke, R., Goke, B., 1995. Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. Endocr. Rev. 16, 390–410.
Gedulin, B.R., Smith, P.A., Jodka, C.M., Chen, K., Bhavsar, S., Nielsen, L.L., Parkes, D.G., Young, A.A., 2008. Pharmacokinetics and pharmacodynamics of exenatide following alternate routes of administration. Int. J. Pharm. 356, 231–238.
Gololobov, G., Noda, Y., Sherman, S., Rubinstein, I., Baranowska-Kortylewicz, J., Paul, S., 1998. Stabilization of vasoactive intestinal peptide by lipids. J. Pharmacol. Exp. Ther. 285, 753–758.
Hohmeier, H.E., Tran, V.V., Chen, G., Gasa, R., Newgard, C.B., 2003. Inflammatory mechanisms in diabetes: lessons from the beta-cell. Int. J. Obes. Relat. Metab. Disord. 27, S12–16.
Holst, J.J., 2000. Gut hormones as pharmaceuticals. From enteroglucagon to GLP-1 and GLP-2. Regul. Pept. 93, 45–51.
Holst, J.J., 2007. The physiology of glucagon-like peptide 1. Physiol. Rev. 87, 1409–1439.
Holst, J.J., Deacon, C.F., 1998. Inhibition of the activity of dipeptidyl-peptidase IV as a treatment for type 2 diabetes. Diabetes 47, 1663–1670.
Huang, Y.Z., Gao, J.Q., Liang, W.Q., Nakagawa, S., 2005. Preparation and characterization of liposomes encapsulating chitosan nanoparticles. Biol. Pharm. Bull. 28, 387–390.
Iga, K., Ogawa, Y., Toguchi, H., 1993. Rates of systemic degradation and reticuloendothelial system (RES) uptake of thermosensitive liposome encapsulating cisplatin in rats. Pharm. Res. 10, 1332–1337.
Juhl, C.B., Hollingdal, M., Sturis, J., Jakobsen, G., Agerso, H., Veldhuis, J., Porksen, N., Schmitz, O., 2002. Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes. Diabetes 51, 424–429.
Kikuchi, H., Suzuki, N., Ebihara, K., Morita, H., Ishii, Y., Kikuchi, A., Sugaya, S., Serikawa, T., Tanaka, K., 1999. Gene delivery using liposome technology. J. Control. Release. 62, 269–277.
Lim, S.B., Rubinstein, I., Onyuksel, H., 2008. Freeze drying of peptide drugs self-associated with long-circulating, biocompatible and biodegradable sterically stabilized phospholipid nanomicelles. Int. J. Pharm. 356, 345–350.
Mayer, L.D., St-Onge, G., 1995. Determination of free and liposome-associated doxorubicin and vincristine levels in plasma under equilibrium conditions employing ultrafiltration techniques. Anal. Biochem. 232, 149–157.
Merrifield, R.B., 1969. Solid-phase peptide synthesis. Adv. Enzymol. Relat. Areas Mol. Biol. 32, 221–296.
Onoue, S., Yamada, S., Yajima, T., 2007. Bioactive analogues and drug delivery systems of vasoactive intestinal peptide (VIP) for the treatment of asthma/COPD. Peptides 28, 1640–1650.
Orskov, C., Holst, J.J., Knuhtsen, S., Baldissera, F.G., Poulsen, S.S., Nielsen, O.V., 1986. Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, are secreted separately from pig small intestine but not pancreas. Endocrinology 119, 1467–1475.
Qi, X.R., Maitani, Y., Nagai, T., 1995. Rates of systemic degradation and reticuloendothelial system uptake of calcein in the dipalmitoylphosphatidylcholine liposomes with soybean-derived sterols in mice. Pharm. Res. 12, 49–52.
Rubinstein, I., Dagar, S., Sethi, V., Krishnadas, A., Onyuksel, H., 2001. Liposomal VIP potentiates DNA synthesis in cultured oral keratinocytes. Peptides 22, 671–675.
Siegel, E.G., Gallwitz, B., Scharf, G., Mentlein, R., Morys-Wortmann, C., Folsch, U.R., Schrezenmeir, J., Drescher, K., Schmidt, W.E., 1999. Biological activity of GLP-1-analogues with N-terminal modifications. Regul. Pept. 79, 93–102.
Suzuki, H., Noda, Y., Paul, S., Gao, X.P., Rubinstein, I., 1995. Encapsulation of vasoactive intestinal peptide into liposomes: effects on vasodilation in vivo. Life Sci. 57, 1451–1457.
Thornton, K., Gorenstein, D.G., 1994. Structure of glucagon-like peptide (7–36) amide in a dodecylphosphocholine micelle as determined by 2D NMR. Biochemistry 33, 3532–3539.

Vilsboll, T., Agerso, H., Krarup, T., Holst, J.J., 2003. Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects. J. Clin. Endocrinol. Metab. 88, 220–224.

Wu, Z.H., Ping, Q.N., Wei, Y., Lai, J.M., 2004. Hypoglycemic efficacy of chitosan-coated insulin liposomes after oral administration in mice. Acta Pharmacol. Sin. 25, 966–972.

Xiao, Q., Giguere, J., Parisien, M., Jeng, W., St-Pierre, S.A., Brubaker, P.L., Wheeler, M.B., 2001. Biological activities of glucagon-like peptide-1 analogues in vitro and in vivo. Biochemistry 40, 2860–2869.

Yachi, K., Harashima, H., Kikuchi, H., Sudo, R., Yamauchi, H., Ebihara, K., Matsuo, H., Funato, K., Kiwada, H., 1996. Biopharmaceutical evaluation of the liposomes prepared by rehydration of freeze-dried empty liposomes (FDELs) with an aqueous solution of a drug. Biopharm. Drug Dispos. 17, 589–605.

Yamabe, K., Kato, Y., Onishi, H., Machida, Y., 2003. Potentiality of double liposomes containing salmon calcitonin as an oral dosage form. J. Control. Release 89, 429–436.