Engineered glucagon-like peptide-1-producing hepatocytes lower plasma glucose levels in mice

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Riedel MJ, Lee CW, Kieffer TJ. Engineered glucagon-like peptide-1-producing hepatocytes lower plasma glucose levels in mice. Am J Physiol Endocrinol Metab 296: E936–E944, 2009. First published February 3, 2009; doi:10.1152/ajpendo.90768.2008.—Glucagon-like peptide (GLP)-1 is an incretin hormone derived from processing of proglucagon and secreted from intestinal L-cells in response to nutrient ingestion. GLP-1 agonists have recently been developed and are now in clinical use for the treatment of type 2 diabetes. Rapid degradation of GLP-1 by enzymes including dipeptidyl-peptidase (DPP)-IV and neutral endopeptidase (NEP) 24.11, along with renal clearance, contribute to a short biochemical half-life, necessitating frequent injections to maintain therapeutic efficacy. Gene therapy may represent a promising alternative approach for achieving long-term increases in endogenous release of GLP-1. We have developed a novel strategy for glucose-regulated production of GLP-1 in hepatocytes by expressing a DPP-IV-resistant GLP-1 peptide in hepatocytes under control of the liver-type pyruvate kinase promoter. Adenoviral delivery of this construct to hepatocytes in vitro resulted in production and secretion of bioactive GLP-1 as measured by a luciferase-based bioassay developed to detect the NH2-terminally modified GLP-1 peptide engineered for this study. Transplantation of encapsulated hepatocytes into CD-1 mice resulted in an increase in plasma GLP-1 levels that was accompanied by a significant reduction in fasting plasma glucose levels. The results from this study demonstrate that a gene therapy approach designed to induce GLP-1 production in hepatocytes may represent a novel strategy for long-term secretion of bioactive GLP-1 for the treatment of type 2 diabetes.

diabetes; gene therapy; liver

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) is an incretin hormone derived from processing of proglucagon and secreted from intestinal L-cells in response to nutrient ingestion. GLP-1 exerts its physiological actions both centrally, by attenuating food and water intake, and in the periphery, where it affects multiple tissues including the stomach, liver, and endocrine pancreas (11). GLP-1 is a potent glucose-dependent insulin secretagogue and stimulates β-cell growth, survival, and differentiation, as well as insulin gene expression, while inhibiting glucagon secretion from α-cells (11, 20). Thus GLP-1 possesses a host of antidiabetic activities.

Studies have shown that some individuals with type 2 diabetes have reduced GLP-1 secretion (34, 36); yet a physiological response to exogenous GLP-1 remains intact (24). Although administration of synthetic GLP-1 analogs has been shown to reduce plasma glucose levels in diabetic individuals (5), the short half-life of these agents necessitates multiple injections to maintain therapeutic efficacy. Endogenous GLP-1 is degraded rapidly by the enzyme DPP-IV (12, 21). GLP-1 agonists that are resistant to DPP-IV have been developed to extend the half-life, but these pharmaceuticals still require frequent injections to maintain effective plasma concentrations. Engineering cells to express GLP-1 is an alternative therapeutic approach to replace exogenous delivery of GLP-1. We have developed a gene therapy strategy for the expression of DPP-IV-resistant GLP-1 in hepatocytes. Hepatocytes are an attractive target for antidiabetic gene therapy for multiple reasons. First, the liver is capable of regenerating to its normal mass following removal of its cells (25), an ideal characteristic for ex vivo gene therapy. Second, hepatocytes contain glucose-responsive genes including glucose transporter-2 (GLUT2), glucokinase, and liver-type pyruvate kinase (LPK) (23, 30). Finally, hepatocytes contain the machinery required for delivery of therapeutic proteins into the circulation (32). This study demonstrates that hepatocytes can be engineered to produce GLP-1 in a glucose-regulated manner and can increase circulating levels of bioactive GLP-1 sufficiently to reduce fasting blood glucose levels following a cellular transplant.

MATERIALS AND METHODS

LPK-GLP-1 gene engineering. An oligonucleotide sequence was generated to match that of human GLP-1 (7–37) with a single nucleotide change resulting in an alanine-to-glycine amino acid substitution at the eighth position to confer DPP-IV resistance to the generated peptide (7). This sequence was inserted downstream of the rat albumin secretory signal peptide and a furin cleavage site to allow GLP-1 to be constitutively secreted by hepatocytes and engineered for expression to be driven by the rat LPK promoter. The 194-bp fragment of the LPK promoter (−183 to +11 relative to the cap site) contains the L1/L3 and L4 sequences, which have been shown to confer liver specificity and glucose responsiveness, respectively (1, 4, 8). To increase mRNA stability of the generated gene, an SV40 splice site and an SV40 polyA signal were generated to flank the GLP-1 gene. Finally, to enhance gene expression, an SV40 enhancer element was inserted upstream of the LPK promoter (Fig. 1).

AdLPK-GLP-1 was constructed by cloning the resulting GLP-1 transgene into the pShuttle vector, followed by amplification in Escherichia coli under kanamycin selection. After amplification, the GLP-1 transgene was removed and ligated into Adeno-X viral DNA, a replication incompetent human adenoviral type 5 (Ad5) genome, to produce pAdLPK-GLP-1.

Cell culture and infection. All cell lines used in this study were cultured in high glucose-Dulbecco’s modified Eagle’s medium (HG-DMEM) with the exception of the WRL-68 cell line (cultured in DMEM) with the exception of the WRL-68 cell line.

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MEM) and the INS-1 cell line (cultured in RPMI 1640 supplemented with 50 μmol/l β-mercaptoethanol). All cell lines were cultured in the presence of 10% fetal bovine serum, 100 U/ml penicillin G, and 100 μg/ml streptomycin at 37°C in a 5% CO2 atmosphere. IEC-6 cells were also supplemented with 0.1 U/ml human insulin (Novo Nordisk Canada, Mississauga, ON, Canada).

**β-Galactosidase assay.** WRL-68, Huh7, and HEPA 1-6 cells were infected with AdCMV-βGal at a multiplicity of infection (MOI) of 50. Seventy-two hours later, liver cell lines were fixed using 0.2% glutaraldehyde (Sigma-Aldrich Canada, Oakville, ON, Canada) in phosphate-buffered saline (PBS; Invitrogen, Burlington, ON, Canada) without CaCl2 (PBS–) for 5 min at room temperature. Cells were then washed with PBS– and incubated in 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) solution (1 mg/ml X-Gal, 5 mmol/l potassium ferricyanide, 5 mmol/l potassium ferrocyanide, 2 mmol/l MgCl2; all from Sigma-Aldrich Canada) for 1–2 h at 37°C. Cells were then counterstained with Nuclear Fast Red (Vector Laboratories, Burlingame, CA) and mounted with coverslips. Images of the cells were captured under ×100 magnification using an Axiovert 200 inverted microscope (Carl Zeiss Canada, Toronto, ON, Canada) connected to a camera (Retiga 2000R; Qimaging, Burnaby, BC, Canada) with a color monitor and imaging software (Openlab, Improvision, Waltham, MA).

**GLP-1 assays.** Following 48–72 h static incubations of AdLPK-GLP-1-infected or uninfected hepatocyte cell lines, medium samples were harvested and assayed for total GLP-1 content using a total GLP-1 radioimmunoassay (RIA) (GLP1T-36HK, Linco Research, St. Charles, MO). The antisera in this assay cross-react 100% with human GLP-1 (7–37), GLP-1 (7–30), and GLP-1 (9–36). The detection limit of this assay is 10 pmol/l. Values below 10 pmol/l are reported as not detected (ND). Active GLP-1 (7–37) and GLP-1 (7–36 amide) were measured in medium samples using an active GLP-1 ELISA (EGLP-35K, Linco Research). The antisera in this assay cross-react 100% with human GLP-1 (7–37) and GLP-1 (7–36 amide) but cannot detect GLP-1 (9–36). The detection limit of this assay is 2 pmol/l. Values below 2 pmol/l are reported as ND.

**GLP-1 bioassay.** HEK 293 cells stably expressing the human GLP-1 receptor (HEK-hGLP-1R) were generously provided by Dr. Jesper Gromada (Novo Nordisk A/S, Denmark). These cells were transfected with PHTS-CRE plasmid (Biomyx, San Diego, CA) using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. These cells (HEK-hGLP-1R-Luc) were grown in HG-DMEM supplemented with 800 μg/ml each of hygromycin and geneticin (Invitrogen). For GLP-1 measurements, HEK-hGLP-1R-Luc cells were plated in a 96-well plate at a density of 5 × 10^4 cells/well and incubated overnight at 37°C. The next day, the medium was aspirated and replaced with 100 μl of either GLP-1 standards, or medium samples from transduced cells. Following a 5-h incubation at 37°C, a luciferase assay was performed using the Bright-Glo luciferase assay kit (Promega, Madison, WI) according to the manufacturer’s instructions. Luminescence was measured using an LMax II microplate reader (Molecular Devices, Sunnyvale, CA).

**Peptides.** GLP-1 (7–36 amide) and [Ser6GLP-1 (7–37)] were purchased from American Peptide (Sunnyvale, CA) and reconstituted in sterile saline as a 100 μmol/l stock. [Gly8GLP-1 (7–37)] was a generous gift from Dr. Bernard Thorens (Institute of Pharmacology and Toxicology, Lausanne, Switzerland). All GLP-1 peptides were diluted in HG-DMEM containing 1% bovine serum albumin (BSA, Invitrogen) to concentrations indicated in the text.

**Animals.** Male CD-1 mice age 8–10 wk, were acquired from the University of British Columbia Animal Care Facility. All experiments were approved by the University of British Columbia Animal Care Committee. Mice were maintained on a standard 12:12-h light-dark cycle and received a standard diet. Blood glucose and body weight were monitored two to three times weekly after a 4-h morning fast. Blood glucose monitoring and survival blood sampling were carried out on restrained unanesthetized mice via the saphenous vein.

**Encapsulation and transplantation of cells.** Cells were grown to ~80% confluence. After trypsinization, cells were washed in PBS–. Cells were resuspended in a mixture of 1.5% sodium alginate (IE-1010; Inotech Biosystems International, Rockville, MD) and morpholinepropanesulfonic acid, transferred to a sterile encapsulator (Inotech Biosystems International), and encapsulated according to the manufacturer’s instructions. Capsules (~700 μm) were washed in PBS– and loaded to sterile syringes attached to 14-G catheters. Recipient mice were anesthetized using isoflurane, and an incision was made midway between the ventral midline and the animal’s side, ~1.5 cm anterior of the hindleg. Capsules were injected to the intraperitoneal cavity, and the musculature and skin were closed with a running suture and a wound clip, respectively. Animals received ~3.8 × 10^6 cells in a total volume of 3 ml. Transplants were completed within 1.5 h of cell encapsulation.

**Statistical analysis.** All results are presented as means ± SE. Statistical significance was assessed using the unpaired Student’s t-test or one-way ANOVA as appropriate, with P values <0.05 considered significant.

**RESULTS**

**Adenoviral mediated infection of hepatocyte cell lines.** To determine the efficiency of adenoviral mediated gene delivery to hepatocyte cell lines in vitro, the fetal human hepatic cell line WRL-68, the adult human hepatoma cell line HuH7, and the mouse hepatoma cell line HEPA 1-6 were transduced with AdCMV-βGal at an MOI of 50. Expression of βGal was allowed to proceed for 72 h, after which, cells were fixed and incubated with X-gal. Each cell line exhibited robust β-galactosidase activity (Fig. 2).

**Evaluation of GLP-1 production from AdLPK-GLP-1-transduced hepatocytes.** To assess hepatocyte production and secretion of GLP-1, the same three hepatocyte cell lines were transduced with AdLPK-GLP-1 at increasing MOIs. Postinfection (72 h), media samples were harvested and assayed for the presence of immunoreactive (IR)-GLP-1 using a total GLP-1 RIA (Fig. 3, A–C). Nontransduced cells did not produce detectable levels of GLP-1. At an MOI of 500, each cell line produced significant IR-GLP-1, whereas at an MOI of 50 only the HEPA 1-6 cells produced measurable levels of GLP-1. Despite receiving the same MOI of virus, each cell line produced significantly different levels of GLP-1, with the rodent HEPA 1-6 cell line producing nearly eightfold higher levels of GLP-1 at an MOI of 500 than the human WRL-68

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Fig. 1. Schematic representation of the liver pyruvate kinase (LPK) promoter-driven glucagon-like peptide-1 (GLP-1) construct engineered in this study. The SV40 enhancer, splice site, and polyA signal were added to improve transcription efficiency and message stability. The presence of the albumin secretory signal peptide ensured that [Gly8]-GLP-1 would be processed in the constitutive secretion pathway of hepatocytes.
hepatocyte line (2.55 ± 0.22 vs. 0.33 ± 0.01 nmol/l IR-GLP-1) and over 26-fold higher levels of GLP-1 compared with the human Huh7 hepatocyte cell line (0.10 ± 0.01 nmol/l IR-GLP-1 at an MOI of 50).

To examine the stability of GLP-1 production, the WRL-68 and HEPA 1-6 cell lines were transduced with AdLPK-GLP-1 and media samples collected at the intervals specified in Fig. 3, D and E. Significant levels of GLP-1 could be detected in both cell lines at 48 h postinfection, with maximal GLP-1 production occurring at 72 h postinfection. Both cell lines exhibited a decrease in GLP-1 secretion after this time point, with production returning to baseline levels 2 wk following infection.

To examine the specificity of GLP-1 expression via the engineered LPK-GLP-1 construct, the intestinal IEC-6, β-cell INS-1, and embryonic fibroblast 3T3-L1 cell lines were transduced with AdLPK-GLP-1 at an MOI of 500. Media samples were collected 72 h postinfection and assayed for IR-GLP-1. GLP-1 production from transduced IEC-6 and 3T3-L1 cells fell below the level of detection of the total GLP-1 RIA. In addition, no increase in GLP-1 production was observed in transduced INS-1 cells (Fig. 3F).

Glucose-mediated regulation of GLP-1 secretion from transduced hepatocyte cell lines. The liver-type pyruvate kinase promoter contains elements that confer regulation by glucose. These elements are located within the region of the promoter included in the AdLPK-GLP-1 construct; therefore, hepatocytes expressing LPK-GLP-1 are expected to exhibit regulation by glucose. To test this, the WRL-68, Huh7, and HEPA 1-6 cell lines were transduced with AdLPK-GLP-1 at an MOI of 500. Twenty-four hours following infection, cells were incubated in 2 or 20 mmol/l glucose for 72 h, and media samples were assayed for IR-GLP-1. Interestingly, neither the WRL-68 nor the HEPA 1-6 cells exhibited glucose regulation of GLP-1 secretion (Fig. 4A and data not shown). In contrast, the Huh7 cell line secreted ~3.5-fold higher levels of GLP-1 at 20 mmol/l glucose than at 2 mmol/l glucose (78.75 ± 7.37 vs. 20.73 ± 3.11 pmol/l IR-GLP-1; Fig. 4B). This indicates that the construct itself contains the elements required to confer glucose regulation to GLP-1 production but that the WRL-68 and HEPA 1-6 cell lines may not contain the necessary components to mediate this regulation.

Development of a GLP-1 bioassay. The GLP-1 peptide produced by AdLPK-GLP-1 is modified to reduce susceptibility to cleavage by DPP-IV. The NH2-terminal modification to the GLP-1 peptide eliminates the epitope for the antibody that is used in the active GLP-1 ELISA that is commercially available from Linco (Fig. 5A). Although the commercially available total GLP-1 RIA available from Linco is sensitive enough to measure picomolar concentrations of GLP-1, cross-reactivity of the antibody with GLP-1(7–37), GLP-1(7–36), and...
GLP-1(9–36) precludes distinction between active and nonactive forms of the peptide. Therefore, we developed a GLP-1 bioassay to determine whether the immunoreactive GLP-1 secreted from AdLPK-GLP-1-transduced hepatocytes was biologically active. A construct containing the luciferase gene driven by the herpes simplex virus thymidine kinase promoter coupled to cAMP-response elements (pHTS-CRE) was introduced into HEK 293 cells engineered to stably express the human GLP-1 receptor. GLP-1 receptor activation in these cells therefore leads to cAMP production and subsequent luciferase gene expression, which can be quantified. The developed bioassay is capable of detecting picomolar concentrations of both native and NH2-terminally modified GLP-1 peptides including the Gly 8-modified peptide produced by AdLPK-GLP-1 (Fig. 5B). EC50 values for wild-type ([Ala8]), [Ser8], and [Gly8]GLP-1(7–36 amide) were 8.3, 18.2, and 28.4 pmol/l, respectively. Utilizing this bioassay, we determined that HEPA 1-6 cells transduced with AdLPK-GLP-1 are capable of producing significant levels of bioactive GLP-1 (Fig. 5C). Our results indicate that, of the GLP-1 peptide secreted from the transduced hepatocytes, ~7% remained biologically active (170.84 ± 24.05 vs. 2,483.23 ± 436.32 pmol/l at an MOI of 500) following a 48-h static incubation.

Using our bioassay, we quantified active GLP-1 levels in the plasma of mice following intraperitoneal injection of recombinant wild-type or [Gly8]GLP-1. As shown in Fig. 5D, injection of [Gly8]GLP-1 resulted in a peak active plasma GLP-1 level of 1.12 ± 0.30 nmol/l compared with 0.25 ± 0.09 nmol/l in animals injected with wild-type GLP-1. The increase in active GLP-1 in the plasma of mice receiving [Gly8]GLP-1 resulted in a significantly lower total glucose excursion as measured by area under the curve compared with glucose alone and a trend toward lower total glucose excursion compared with mice receiving wild-type GLP-1, with a P value of 0.059 (Fig. 5, E and F).

AdLPK-GLP-1-transduced hepatocytes regulate plasma GLP-1 and glucose levels. To examine the potential therapeutic benefit of GLP-1-producing hepatocytes, we transplanted...
AdLPK-GLP-1-transduced GLP-1-producing HEPA 1-6 cells into the peritoneum of normal CD1 mice. The cells were first encapsulated in alginate to reduce the likelihood of immune-mediated destruction. To assess the effect of encapsulation on GLP-1 production, IR-GLP-1 levels were measured from cells prior to and following the encapsulation procedure. Significant levels of GLP-1 were detected in both unencapsulated and encapsulated cells up to 5 days postinfection (data not shown). We therefore tested the ability of encapsulated GLP-1-producing hepatocytes to increase plasma GLP-1 levels following intraperitoneal implantation. Twenty-four hours after infection with AdLPK-GLP-1, ∼3.8 × 10⁶ HEPA 1-6 cells were encapsulated and transplanted into the peritoneum of each mouse. Blood glucose was tracked for 2 days, with plasma samples being collected on day 2 posttransplantation. Circulating levels of GLP-1 were significantly higher in mice receiving a transplant of AdLPK-GLP-1-infected HEPA 1-6 cells vs. those transplanted with uninfected, encapsulated HEPA 1-6 cells (38.77 ± 6.02 vs. 18.93 ± 3.86 pmol/l; Fig. 6A). Commensurate with increased GLP-1 levels, fasting blood glucose levels decreased significantly from 9.52 ± 0.68 to 7.55 ± 0.28 mmol/l in these mice at 2 days posttransplantation (Fig. 6B). Capsules were harvested and examined for morphological differences, and the cells were tested for GLP-1 production. The capsules remained intact and appeared similar to capsules at the time of implantation (Fig. 6C and D). Significant levels of secreted GLP-1 could be detected from retrieved capsules at both 4 and 5 days postinfection (data not shown).

DISCUSSION

The therapeutic actions of GLP-1 are well documented, with continuous infusion of GLP-1 in subjects with type 2 diabetes resulting in the normalization of their blood glucose levels (29) and continuous delivery of GLP-1 into prediabetic nonobese diabetic (NOD) mice via miniosmotic pumps delaying the onset of diabetes (39). Given the rapid degradation and clearance of GLP-1, therapeutics with longer half-lives as well as alternative methods of delivering GLP-1 are highly sought after. Advances in the development of GLP-1 analogs with longer half-lives are being made through such means as conjugation of GLP-1 analogs with serum albumin (13, 14); however, these compounds still require routine injection to maintain therapeutic efficacy. In this study, we demonstrate the effectiveness of a gene therapy approach designed to express DPP-IV-resistant GLP-1 under the control of the glucose-regulated LPK promoter (AdLPK-GLP-1) to direct the expression of GLP-1 in hepatocytes.

Previous efforts aimed at increasing plasma GLP-1 levels via gene delivery have generally made use of ubiquitous promoters such as chicken β-actin (26) or CMV (15, 17, 33) to express the biologically active GLP-1(7–37) peptide. In a study by Lee et al. (17), a single intravenous injection of an adeno-virus encoding a CMV promoter-driven GLP-1 construct resulted in increased plasma GLP-1 levels for up to 4 wk in ob/db mice. This was associated with improved glucose tolerance and insulin sensitivity. Separate studies have also demonstrated a reduced susceptibility to streptozotocin-induced diabetes in CD-1 mice and improved fasting blood glucose levels in db/db mice receiving intramuscular injections of a GLP-1/IgG-Fc fusion construct (15, 33). Although these studies demonstrated an increase in the plasma levels of GLP-1, the site of production within the body remains unclear. Oh et al. (26) demonstrated GLP-1 gene expression in the livers of rats following intravenous injection of a polyethyleneimine/GLP-1 complex but did not report whether the transgene was expressed in other tissues. The ability of this transgene to express in virtually any tissue may present safety concerns in regard to the development of a novel therapeutic. Along similar lines, Parsons et al. (27) developed a GLP-1 construct containing a furin-cleavable exendin-4 leader sequence, driven by the ubiquitin promoter. In their study, adeno-viral delivery of this construct to db/db mice and ZDF rats resulted in a significant but transient reduction in random-fed blood glucose levels, consistent with observations that adeno-viral expression of transgenes is limited in duration. In addition, the use of an unregulated promoter such as CMV will result in constitutive expression of the GLP-1 transgene, thereby maintaining chronically elevated levels of GLP-1. Although chronic elevation of systemic GLP-1 levels is not yet associated with a decrease in GLP-1 receptor signaling, and continuous infusion of GLP-1 is effective in reducing hyperglycemia in diabetic individuals, we
sought to develop a therapeutic strategy that more closely mimics the endogenous secretion pattern of GLP-1. In addition to providing liver-specific expression of our GLP-1 transgene, LPK promoter activity can be upregulated in the presence of elevated glucose, such as occurs following meal ingestion. Given the insulinotropic properties of GLP-1, it may be advantageous for GLP-1 levels to be increased following ingestion of a meal. Mimicking physiological secretion of GLP-1 may additionally act to reduce side effects associated with chronic increases in serum GLP-1 levels. We observed glucose regulation of GLP-1 production in the Huh7 cell line, suggesting that LPK-GLP-1 does contain the necessary elements to confer glucose regulation on promoter activity. In addition, a lack of increase in GLP-1 secretion from the non-liver-type cell lines 3T3-L1, INS-1, and IEC-6, suggests that LPK activity is liver specific.

Activation of the LPK promoter in hepatocytes requires the activity of hepatocyte nuclear factor (HNF)-1 and HNF-4 (31, 35). mRNA levels for these transcription factors are low in most nonliver cells (2, 22), likely accounting for the lack of GLP-1 production from nonhepatocytes transduced with AdLPK-GLP-1. A correlation between HNF-1α mRNA levels and LPK activity has been previously reported (18), and several studies have demonstrated that HNF-1 and/or HNF-4 mRNA levels are variable in different liver cell lines (2, 10, 18). It is therefore possible that variable expression of these transcription factors may account for the differing amounts of GLP-1 produced from each of the liver cell lines used in this study by resulting in variable activation of the LPK promoter. Given the similar expression levels of β-galactosidase in these cell lines following transduction with AdCMV-βGal (Fig. 2), differing transduction efficiencies by adenovirus do not appear to account for the observed variability in GLP-1 secretion.

The active GLP-1 peptides GLP-1(7–37) and GLP-1(7–36 amide) are highly sensitive to cleavage by the ubiquitous DPP-IV enzyme. To prevent rapid degradation of our LPK-GLP-1 product, we engineered a single nucleotide substitution in the DNA sequence resulting in a substitution of glycine for alanine at position 8 relative to the NH2 terminus (7). This substituted form of GLP-1 cannot be measured effectively by the Linco active GLP-1 ELISA. Therefore, we developed a bioassay using HEK 293 cells engineered to stably express the human GLP-1 receptor. These cells were also engineered to express the luciferase gene in response to increases in cAMP generated
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via activation of the GLP-1 receptor (9, 37). As a result, the level of luminescence signal produced is proportional to the amount of biologically active GLP-1 that the cells are exposed to. As shown in Fig. 5B, NH2-terminally modified GLP-1 peptides, including the [Gly8]GLP-1 peptide utilized in the current study, are less potent at activating the human GLP-1 receptor (7, 38). Despite reduced potency, NH2-terminally modified GLP-1 peptides demonstrate similar efficacy and retain the ability to reduce both total glucose excursions and fasting blood glucose levels in vivo (Figs. 5 and 6). This is likely due to the significant increase in plasma half-life conferred by DPP-IV resistance, resulting in a significantly higher level of active GLP-1 in plasma (Fig. 5D) and an improved glycemic response to glucose challenge in vivo (Fig. 5, E and F, and Ref. 38).

The engineered bioassay confirmed that biologically active GLP-1 was produced from AdLPK-GLP-1-transduced hepatocytes (Fig. 5C). The level of bioactive GLP-1 reflects ~7% of total immunoreactive GLP-1 as assessed by GLP-1 RIA. The lower percentage of bioactive GLP-1 compared with total GLP-1 is not surprising given that degradation of NH2-terminally modified GLP-1 can still occur. Although the GLP-1 sequence in AdLPK-GLP-1 was constructed to be DPP-IV resistant, other enzymes, including NEP 24.11, can be involved in GLP-1 degradation (28). Since the transduced cells are cultured in FBS-supplemented medium, it is possible that this enzyme, along with others, is also present to degrade GLP-1 to biologically inactive but still immunoreactive fragments. Furthermore, bioactive GLP-1 was measured following 3 days of culture, increasing the likelihood of GLP-1 degradation (28). Since the transduced cells are resistant, other enzymes, including NEP 24.11, can be involved in GLP-1 degradation (28).

A critical aspect of developing a GLP-1-based gene therapy approach to treat diabetes is elevating plasma GLP-1 levels. We show in this study that transplantation of ~3.8 × 10^6 GLP-1-producing hepatocytes into the peritoneal cavity of normal, healthy CD-1 mice was sufficient to raise plasma GLP-1 levels approximately twofold. This rise in GLP-1 levels was sufficient to reduce fasted plasma glucose levels in animals at 2 days posttransplantation. Although a twofold increase in plasma immunoreactive GLP-1 levels observed in this study appears relatively small, previous studies have demonstrated that increases in plasma total GLP-1 levels two- to fourfold are sufficient to significantly lower fasting blood glucose levels in subjects with type 2 diabetes (6, 19).

Strategies aimed at increasing the duration of GLP-1 production from hepatocytes as well as physiological regulation of its production and secretion will be required before such a gene therapy approach could be adapted for therapeutic use. Injection of primary hepatocytes with AdLPK-GLP-1 may improve the duration of expression as these cells are largely nondividing. Integrative viral vectors such as lentiviruses may allow for stable expression of GLP-1 in primary hepatocytes, thus further improving the likelihood of long-term transgene expression. The random integration of lentiviral-delivered genes may represent a significant disadvantage of this delivery method due to potential regulatory disruption of endogenous genes; however, in the case of ex vivo gene delivery, properly functioning cells could be selected for prior to transplantation. We are currently pursuing additional gene delivery technologies to increase duration and efficiency of GLP-1 production from the liver.

To improve physiological regulation of GLP-1 secretion, improvements can be made to the promoter driving GLP-1 gene expression. A recent study has shown that a chimeric promoter consisting of elements from the glucose-6-phosphatase and LPK promoters can enhance insulin gene expression in hepatocytes while conferring activation by glucose and inhibition by insulin (16). Such a promoter driving GLP-1 expression in liver would potentially result in increased GLP-1 production postmeal that would subsequently be reduced as insulin levels rose.
The current study demonstrates a strategy for ex vivo delivery of a GLP-1 construct driven by the liver-specific, glucagon-responsive LPK promoter that is capable of significantly increasing plasma GLP-1 levels following intraperitoneal transplantation. Given the well-established antidiabetic actions of GLP-1, the current strategy may represent an important step toward the development of novel treatment option for diabetes.

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