Pharmacokinetics of Porcine Glucagon-Like Peptide-2 and Its Long-Acting Derivative in Rats (Postprint)

Authors: Jiajia Lü, Wu Jie, Qi Keke, Xu Ziwei

Date: 2017-10-11T00:00:00+00:00

Abstract

This study aimed to investigate the pharmacokinetic profiles of porcine glucagon-like peptide-2 (p[Gly2]GLP-2), polyethylene glycol-modified porcine glucagon-like peptide-2 (PEG-p[Gly2]GLP-2), and p[Gly2]GLP-2 microspheres in rats, to provide a reference for utilizing p[Gly2]GLP-2 to repair intestinal injury in weaned piglets. Eighteen male SD rats weighing approximately 280 g were randomly divided into three groups and received a single subcutaneous injection of p[Gly2]GLP-2 (5.64 nmol/kg), PEG-p[Gly2]GLP-2 (5.64 nmol/kg), or p[Gly2]GLP-2 microspheres (15 mg/animal), respectively. Blood samples were collected at designated time points, and plasma concentrations of glucagon-like peptide-2 (GLP-2) were determined by enzyme-linked immunosorbent assay (ELISA). The results showed that: 1) The half-life (t1/2) of PEG-p[Gly2]GLP-2 was 4-fold that of p[Gly2]GLP-2, the area under the concentration-time curve (AUC0-t) and mean residence time (MRT0-t) were 3-fold those of p[Gly2]GLP-2, and the clearance (CL) was half that of p[Gly2]GLP-2. The peak concentrations (Cmax) of the two formulations were comparable, while the time to peak concentration (Tmax) of PEG-p[Gly2]GLP-2 was delayed relative to p[Gly2]GLP-2. 2) The time to peak concentration of p[Gly2]GLP-2 microspheres was not substantially different from that of p[Gly2]GLP-2 or PEG-p[Gly2]GLP-2; however, its half-life was (72.20±6.02) h, and the mean residence time was (90.66±7.41) h. These results suggest that polyethylene glycol (PEG) modification substantially alters the pharmacokinetic behavior of p[Gly2]GLP-2, characterized by prolonged half-life, delayed Tmax, extended mean residence time, slower plasma clearance, and higher bioavailability. The p[Gly2]GLP-2 microspheres exhibited an even longer half-life with sustained and stable release, and offered operational convenience.

Full Text

Pharmacokinetic Studies of Porcine Glucagon-like Peptide-2 and Its Long-acting Forms in Rats

Jiajia Lü[1], Jie Wu[1], Keke Qi[1], Ziwei Xu[2]*

[1]College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, China
[2]Institute of Animal Science, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China

*Corresponding author: xzwfyz@sina.com

Abstract

This study investigated the pharmacokinetic profiles of porcine glucagon-like peptide-2 [Gly²] (p[Gly²]GLP-2), polyethylene glycol-modified p[Gly²]GLP-2 (PEG-p[Gly²]GLP-2), and p[Gly²]GLP-2 microspheres in rats to provide reference data for using p[Gly²]GLP-2 to repair intestinal injury in weaned piglets. Eighteen male Sprague-Dawley rats weighing approximately 280 g were randomly divided into three groups and received single subcutaneous injections of p[Gly²]GLP-2 (5.64 nmol/kg), PEG-p[Gly²]GLP-2 (5.64 nmol/kg), or p[Gly²]GLP-2 microspheres (15 mg per rat). Blood samples were collected at designated time points, and plasma GLP-2 concentrations were measured by enzyme-linked immunosorbent assay (ELISA). The results showed: (1) PEG-p[Gly²]GLP-2 exhibited a half-life (t /) four times longer than p[Gly²]GLP-2, with area under the curve (AUC) and mean residence time (MRT) three times greater, while its clearance rate (CL) was reduced by half. The peak concentrations (C) were similar between the two formulations, but the time to peak concentration (T) for PEG-p[Gly²]GLP-2 was delayed compared to p[Gly²]GLP-2. (2) The p[Gly²]GLP-2 microspheres showed similar T values to p[Gly²]GLP-2 and PEG-p[Gly²]GLP-2, but demonstrated a substantially prolonged half-life of (72.20±6.02) h and MRT of (90.66±7.41) h. These findings indicate that PEGylation significantly altered the pharmacokinetic behavior of p[Gly²]GLP-2, extending its half-life, delaying T , prolonging MRT, slowing plasma clearance, and improving bioavailability. The p[Gly²]GLP-2 microspheres offer an even longer half-life with sustained and stable release characteristics, suggesting convenient administration.

Keywords: p[Gly²]GLP-2; PEG-p[Gly²]GLP-2; p[Gly²]GLP-2 microspheres; pharmacokinetics; rats

---

Introduction

Glucagon-like peptide-2 (GLP-2) promotes the restoration of damaged intestinal mucosal structure and improves absorptive and barrier functions by specifically

stimulating intestinal epithelial cell proliferation, inhibiting epithelial cell apoptosis [1], increasing intestinal blood supply [2], reducing intestinal permeability [3], and suppressing gastric acid secretion [4]. However, porcine GLP-2 (pGLP-2) is rapidly degraded by dipeptidyl peptidase-IV (DPP-IV) in blood [5], with an in vivo half-life of only 8.4 minutes, necessitating frequent administration.

Teduglutide, which is already in clinical use [6], extended recombinant polypeptide (XTEN) modification currently under investigation [7], and polyethylene glycol (PEG) modification [8-9] have all effectively prolonged the release time of pGLP-2 in vivo. Our research group previously used monomethoxy polyethylene glycol succinimidyl propionate (mPEG-SPA) to modify pGLP-2, and successfully isolated mPEG-Lys30-p[Gly2]GLP-2 using a one-step cation exchange chromatography method. This PEGylated form exhibited a 16-fold longer in vitro half-life compared to p[Gly2]GLP-2 (where the alanine residue at position 2 of the pGLP-2 N-terminus was replaced with a glycine residue) and effectively alleviated colonic lesions in mice [9]. Wu et al. [10] prepared p[Gly2]GLP-2 microspheres that, after a single injection, significantly inhibited weight loss and colon shortening in mice with colitis, improved mucosal integrity, reduced intestinal inflammation, and promoted small intestine growth. While these studies demonstrate the feasibility of pGLP-2 delivery approaches, the distribution and elimination patterns of these formulations in animal bodies remain unclear.

This study aimed to investigate the pharmacokinetic characteristics of p[Gly2]GLP-2, PEG-modified p[Gly2]GLP-2 (PEG-p[Gly2]GLP-2), and p[Gly2]GLP-2 microspheres in rats, to provide reference data for using PEG-p[Gly2]GLP-2 to treat intestinal injury and p[Gly2]GLP-2 microspheres to prevent intestinal injury.

---

1. Materials and Methods

1.1 Materials and Instruments The following materials were used: mPEG-SPA (molecular weight [MW] 5 kDa, Beijing Kaiming Biological Technology Co.); p[Gly2]GLP-2 (MW 3,990.1, peptide sequence: HGDGSFSDEMNTVLDNLATRDFINWLLHTKITDSL, Hangzhou Zhongtai Biochemical Co.); CM Sepharose Fast Flow resin (GE Healthcare Bio-Sciences, Switzerland); ultrafiltration centrifuge tubes (MW cutoff 3,000, Millipore, USA); enzyme-linked immunosorbent assay (ELISA) kit (Millipore, USA); dimethyl sulfoxide (DMSO, Shanghai Bioengineering Co.); poly(lactic-co-glycolic acid) (PLGA, lactic/glycolic acid ratio 50:50, MW 14,000-17,000, Sigma); polyvinyl alcohol (PVA, MW 65,000-75,000, Shanghai Bioengineering Co.); PEG (MW 6,000, Shanghai Bioengineering Co.). Equipment included: IKA RH basic 1 magnetic stirrer (IKA, Germany); PRO200 homogenizer (PRO Science, USA); and EPOCH microplate reader (BioTek, USA).

1.2 Preparation of PEG-p[Gly2]GLP-2 Following the method of Qi et al. [9], p[Gly2]GLP-2 at 1.2 mg/mL was dissolved in 50 mmol/L Tris-HCl buffer (pH 8.5) and reacted with mPEG-SPA at a 1:6 molar ratio for 30 minutes at room temperature. The reaction was terminated with 1% trifluoroacetic acid (TFA). The product, mPEG-Lys30-p[Gly2]GLP-2 (MW 8,867), was purified by one-step cation exchange chromatography.

1.3 Preparation of Microspheres Based on the method of Wu et al. [10] with the drug loading adjusted to 2%, microspheres were prepared as follows: 1 mg of lyophilized p[Gly2]GLP-2 powder was dissolved in 12.5 µL DMSO and mixed with 500 µL of dichloromethane solution containing 50 mg PLGA and 5 mg PEG. The mixture was homogenized at high speed for 30 seconds to form a primary emulsion, which was quickly poured into 20 mL of aqueous solution containing 2% (w/v) PVA and 5% NaCl. After magnetic stirring at 2,000 r/min for 3 minutes to obtain a double emulsion, the emulsion was transferred to 100 mL distilled water and stirred at 400 r/min for 3 hours. Microspheres were collected by centrifugation, washed several times with distilled water, and lyophilized. The resulting microspheres had a particle size of (31.17±8.17) µm, encapsulation efficiency of 74.15%, burst release rate of 20.36%, actual drug loading of 0.74%, and cumulative release of 37% over 9 days in vitro.

1.4 Experimental Animals The experiment was conducted at the Laboratory Animal Center of Zhejiang University. Eighteen male Sprague-Dawley rats weighing approximately 280 g were randomly divided into three groups based on body weight.

1.5.1 ELISA Kit Assay Conditions The following conditions were established for the ELISA kit: (1) Blank controls were set up. (2) Quality control samples QC1 (GLP2-107) and QC2 (GLP2-207) were required to yield concentrations between 2.9-7.7 ng/mL and 13-28 ng/mL, respectively. (3) To eliminate background color interference, samples were measured at both 450 nm and 590 nm after binding with pGLP-2 detection antibody, with 450 nm as the detection wavelength and 590 nm used to correct for background error. (4) The measurement range of the ELISA kit was 1-64 ng/mL. Results were considered valid only when all these conditions were met.

1.5.2 Standard Curve The standard (75 ng/mL) was serially diluted to prepare standard samples at concentrations of 1.172, 2.344, 4.688, 9.375, 18.750, and 37.500 ng/mL. Following the kit protocol, the standard curve was fitted using a logistic equation (four-parameter model) with the OD values (after subtracting blank control and 590 nm background) as the y-axis and standard sample concentrations (ng/mL) as the x-axis.

1.6 Pharmacokinetic Studies in Rats Prior to the experiment, SD rats were fasted for 12 hours with free access to water. The p[Gly2]GLP-2 group

received a subcutaneous injection of 5.64 nmol/kg p[Gly2]GLP-2 solution, and blood samples (0.5 mL) were collected via the retro-orbital venous plexus at 4, 8, 12, 16, 30, 45, 60, 90, and 150 minutes post-injection. The PEG-p[Gly2]GLP-2 group received the same dose subcutaneously, with blood sampling at 5, 15, 20, 30, 45, 60, 120, 240, and 360 minutes. The p[Gly2]GLP-2 microsphere group was injected with 15 mg microspheres per rat, with blood collection at 10, 20, 30, 60, 120, 180, and 360 minutes, and on days 1, 2, 3, 4, 5, 6, 7, 8, and 9.

Because p[Gly2]GLP-2 is susceptible to degradation by DPP-IV enzymes in blood, each blood sample was immediately mixed with 5 µL of DPP-IV inhibitor. Samples were centrifuged at 3,000 r/min for 15 minutes at 4°C, and the serum supernatant was stored at -80°C until analysis.

Serum samples were assayed according to the ELISA kit protocol. Measured OD values were applied to the standard curve to determine plasma drug concentrations at each time point.

1.7 Data Analysis Pharmacokinetic modeling of plasma concentration-time data was performed using WinNonlin software version 5.2.1 (Pharsight, USA) to obtain plasma concentration-time curves and relevant pharmacokinetic parameters for each administration route.

---

2. Results

2.1 Accuracy of ELISA Measurements The standard curve for pGLP-2 is shown in Figure 1 [Figure 1: see original paper], described by the equation Y = (A-D)/[1+(X/C)^B] + D, with R^2 = 0.99992, where A = 3.48943, B = -2.06666, C = 12.81706, and D = 0.04483. The measured concentrations were 6.197 ng/mL for QC1 and 23.44 ng/mL for QC2, both within the valid range.

2.2 Plasma Concentration-Time Profiles and Pharmacokinetic Parameters of p[Gly2]GLP-2 and PEG-p[Gly2]GLP-2 Following subcutaneous injection of p[Gly2]GLP-2 and PEG-p[Gly2]GLP-2, the plasma concentration-time curves are shown in Figure 2 [Figure 2: see original paper], with key pharmacokinetic parameters presented in Table 1 . As shown in Figure 2, the p[Gly2]GLP-2 group exhibited a rapid increase in plasma concentration, peaking at approximately 16 minutes, followed by a rapid decline. In contrast, the PEG-p[Gly2]GLP-2 group showed a more gradual rise, reaching maximum concentration at 30-45 minutes, followed by a slow decrease. Pharmacokinetic analysis revealed that PEG-p[Gly2]GLP-2 had a 4-fold longer half-life (t /), 3-fold greater area under the curve (AUC) and mean residence time (MRT), and 50% lower clearance rate (CL) compared to p[Gly2]GLP-2. Additionally, the peak concentrations (C) were similar between the two formulations, while the time to peak concentration (T) for PEG-p[Gly2]GLP-2 was delayed relative to p[Gly2]GLP-2.

2.3 Plasma Concentration-Time Profile and Pharmacokinetic Parameters of p[Gly2]GLP-2 Microspheres After subcutaneous injection of the microsphere suspension, the plasma concentration-time curve is shown in Figure 3 [Figure 3: see original paper], with pharmacokinetic parameters listed in Table 2 . Analysis indicated that the p[Gly2]GLP-2 microspheres had a similar T to p[Gly2]GLP-2 and PEG-p[Gly2]GLP-2, but exhibited a substantially prolonged half-life of (72.20±6.02) h and mean residence time of (90.66±7.41) h, demonstrating excellent sustained-release properties.

---

3. Discussion

3.1 Pharmacokinetics of p[Gly2]GLP-2 and PEG-p[Gly2]GLP-2 Since Drucker et al. [11] first reported that GLP-2 specifically promotes intestinal mucosal growth and repair, numerous studies [1-4] have confirmed this finding and demonstrated that GLP-2 is more effective than other non-specific intestinal growth factors previously identified. However, like other protein and peptide drugs, pGLP-2 development has been limited by its susceptibility to degradation, poor water solubility, short circulating half-life, and low bioavailability. Particularly, protein peptides are readily recognized and cleared by the immune system, resulting in shortened plasma half-life. To maintain therapeutic efficacy, dosing must be increased and administered frequently, leading to cumbersome operations, higher costs, enhanced immunogenicity and toxicity, and increased stress on the organism. Boushey et al. [12] administered human [Gly2]GLP-2 to mice at 80 g/kg twice daily for 6 days; Drucker et al. [13] injected mice with 17.5 g/kg human [Gly2]GLP-2 twice daily for 10 days; and Alavi et al. [14] gave rats 50 g/kg GLP-2 intravenously daily for 14 days to alleviate inflammatory responses in animal models. Applying GLP-2 to piglets would be time-consuming, labor-intensive, and would exacerbate the stress of "post-weaning syndrome."

To overcome these limitations of protein peptides, various strategies have been employed, among which the most successful is PEGylation—conjugating water-soluble PEG polymers to therapeutic proteins [15-16]. PEGylated protein peptides acquire a protective barrier that renders them less susceptible to proteolytic degradation, reduces antigen-antibody binding to suppress immune responses, decreases glomerular filtration and renal clearance, enhances solubility of hydrophobic molecules, and prolongs drug half-life in vivo [17-18]. Currently approved PEGylated drugs include PEG-insulin, PEG-asparaginase, and PEG-interferon. Qi et al. [8-9] prepared mPEG-Lys30-p[Gly2]GLP-2 with a 16-fold longer in vitro half-life than p[Gly2]GLP-2, which effectively alleviated colonic lesions in mice and intestinal injury in weaned piglets. However, its distribution and elimination patterns in animals remain unclear and require further investigation.

Pharmacokinetics is the science of analyzing drug dynamics in vivo using math-

ematical approaches, holding significant theoretical and practical value. Lee et al. [19] intravenously administered 1 g/kg glucagon-like peptide-1 (GLP-1) and PEG2K-Lys-GLP-1 to rats, finding that PEG2K-Lys-GLP-1 had a 10-fold longer half-life and 16-fold greater mean residence time than GLP-1. After subcutaneous injection of 10 g/kg GLP-1 and PEG2K-Lys-GLP-1, PEG2K-Lys-GLP-1 showed 7.5-fold greater AUC, 2.5-fold longer half-life, and 3-fold delayed T compared to GLP-1. Our study found that PEG-p[Gly2]GLP-2 had a 4-fold longer half-life than p[Gly2]GLP-2, demonstrating that PEGylation extends the half-life of proteins and peptides, allowing PEG-p[Gly2]GLP-2 to remain in rat bodies longer with slower elimination, which is beneficial for therapeutic efficacy.

The mean residence time of PEG-p[Gly2]GLP-2 was three times that of p[Gly2]GLP-2, indicating prolonged retention in vivo and enhanced therapeutic effect. The AUC of PEG-p[Gly2]GLP-2 was three times greater than p[Gly2]GLP-2, suggesting greater absorption and higher bioavailability. While peak concentrations were similar, T was substantially delayed for PEG-p[Gly2]GLP-2. The clearance rate of PEG-p[Gly2]GLP-2 was half that of p[Gly2]GLP-2; p[Gly2]GLP-2 concentrations declined rapidly, whereas PEG-p[Gly2]GLP-2 maintained high plasma concentrations for an extended period. These results demonstrate that PEGylation significantly alters the pharmacokinetic behavior of p[Gly2]GLP-2: extended half-life, delayed T , prolonged MRT, slowed plasma clearance, and improved bioavailability. These improvements address major drawbacks of protein peptide drugs, including rapid clearance, short duration of effective plasma concentrations, and need for frequent dosing.

3.2 Pharmacokinetics of p[Gly2]GLP-2 Microspheres Microsphere drug delivery systems have gained popularity in recent years for their ability to alter drug distribution, reduce enzymatic degradation, prolong drug action, and decrease dosing frequency. The first peptide microsphere formulation approved by the U.S. Food and Drug Administration (FDA) for prostate cancer treatment —leuprolide acetate microspheres—provides sustained effects for 30 days in humans. Choi et al. [20] developed GLP-1 microspheres that released stably for two weeks in diabetic rats, maintaining normal blood glucose levels. Liu et al. [21] investigated the sustained-release properties of GLP-2 microspheres in vivo. Wu et al. [10] demonstrated that a single intraperitoneal injection of 10 mg p[Gly2]GLP-2 microspheres in colitis mice effectively inhibited weight loss and colon shortening, improved mucosal integrity, reduced intestinal inflammation, and promoted small intestine growth.

Given the convenient administration and sustained, stable release characteristics of p[Gly2]GLP-2 microspheres, they hold promise for preventing "post-weaning syndrome" in piglets, though their release mechanisms in animals remain unknown. Zhang et al. [22] found that oral thymic peptide microspheres in rats produced higher plasma concentrations and AUC values than thymic peptide

capsules, with extended half-life and significantly improved bioavailability. Lu [23] compared subcutaneous injection of interferon -2b-PLGA sustained-release microspheres versus interferon -2b injection in rats, reporting T values of 4 h and 1.5 h, C values of 5,490.228 and 25,315.32 pg/mL, MRT values of 6.855 and 0.099 days, and AUC values of 23,149.018 and 2,309.497 pg · day/mL, respectively. The microspheres exhibited an initial burst release to reach peak concentration, followed by slow decline and prolonged maintenance at low drug levels. Xiao et al. [24] observed significant sustained-release characteristics for both ivermectin PLA and PLGA microspheres in beagle dogs. Wu et al. [10] showed that p[Gly2]GLP-2 microspheres significantly inhibited weight loss and colon shortening, improved mucosal integrity, reduced intestinal inflammation, and promoted small intestine growth in colitis mice after a single injection.

However, the in vivo processes and mechanisms of microspheres remain poorly understood. Our study found that p[Gly2]GLP-2 microspheres had a half-life of (72.20±6.02) h and MRT of (90.66±7.41) h, substantially longer than both p[Gly2]GLP-2 and PEG-p[Gly2]GLP-2 groups, while T was similar. The plasma concentration-time curve showed a peak at approximately 20 minutes post-administration, attributed to burst release of p[Gly2]GLP-2 adsorbed on the microsphere surface combined with diffusion of small amounts of peptide from the microspheres. Concentrations then declined slowly until 6 hours, after which they remained relatively stable around 4.5 ng/mL for 9 days, indicating sustained release from the microspheres with continued slow drug release even during the elimination phase.

Due to manufacturing variability in microsphere production, accurate calculation of actual administered dose was not possible, precluding direct pharmacokinetic parameter comparisons with p[Gly2]GLP-2. Nevertheless, the unique plasma concentration-time profile, sustained and stable release pattern, and convenient administration method make p[Gly2]GLP-2 microspheres highly promising for preventing intestinal disease in weaned piglets.

Conclusions

1. PEGylation significantly altered the pharmacokinetic behavior of p[Gly2]GLP-2, extending its half-life, delaying time to peak concentration, prolonging mean residence time, slowing plasma clearance, and improving bioavailability. These changes substantially overcome the limitations of peptide drugs, including rapid clearance, short duration of effective plasma concentrations, and need for frequent administration.

2. p[Gly2]GLP-2 microspheres offer a long half-life with sustained and stable release, demonstrating excellent sustained-release characteristics. These properties enable convenient administration and reduce the potential for stress responses in animals.

References

[1] TSAI C H, HILL M, ASA S L, et al. Intestinal growth-promoting properties of glucagon-like peptide-2 in mice[J]. American Journal Physiology-Endocrinology Metabolism, 1997, 273(1): E77–E84.
[2] GUAN X F, STOLL B, LU X F, et al. GLP-2-mediated up-regulation of intestinal blood flow glucose uptake nitric oxide-dependent TPN-fed piglets[J]. Gastroenterology, 2003, 125(1): 136–147.
[3] BENJAMIN M A, MCKAY D M, YANG P C, et al. Glucagon-like peptide-2 enhances intestinal epithelial barrier function of both transcellular and paracellular pathways in the mouse[J]. Gut, 2000, 47(1): 112–119.
[4] WØJDEMANN M, WETTERGREN A, HARTMANN B, et al. Inhibition of sham feeding-stimulated human gastric acid secretion by glucagon-like peptide-2[J]. The Journal of Clinical Endocrinology & Metabolism, 1999, 84(7): 2513–2517.
[5] PEDERSEN N B, HJOLLUND K R, JOHNSEN A H, et al. Porcine glucagon-like peptide-2: structure, signaling, metabolism effects[J]. Regulatory Peptides, 2008, 146(1/2/3): 310–320.
[6] JEPPESEN P B, PERTKIEWICZ M, MESSING B, et al. Teduglutide reduces need for parenteral support among patients with short bowel syndrome with intestinal failure[J]. Gastroenterology, 2012, 143(6): 1473–1481.
[7] ALTERS S E, MCLAUGHLIN B, SPINK B, et al. GLP2-2G-XTEN: a pharmaceutical protein with improved serum half-life and efficacy in a rat Crohn' s disease model[J]. PLoS One, 2012, 7(11): 50630.
[8] QI K K, WU J, DENG B, et al. PEGylated porcine glucagon-like peptide-2 improved the intestinal digestive function and prevented inflammation of weaning piglets challenged with LPS[J]. Animal, 2015, 9(9): 1481–1489.
[9] QI K K, WU J, WAN J, et al. Purified PEGylated porcine glucagon-like peptide-2 reduces the severity colonic injury a murine model experimental colitis[J]. Peptide, 2014, 52: 11–18.
[10] WU J, QI K K, XU Z W, et al. Glucagon-like peptide-2-loaded microspheres as treatment for ulcerative colitis in the murine model[J]. Journal of Microencapsulation, 2015, 32(6): 598–607.
[11] DRUCKER D J, ERILICH P, ASA S L, et al. Induction of intestinal epithelial proliferation by glucagon-like peptide 2[J]. Proceedings of the National Academy of Sciences of the United States of America, 1996, 93(15): 7911–7916.
[12] BOUSHEY R P, YUSTA B, DRUCKER D J. Glucagon-like peptide 2 decreases mortality and reduces the severity of indomethacin-induced murine enteritis[J]. American Journal of Physiology-Endocrinology and Metabolism, 1999, 277(5): E937–E947.
[13] DRUCKER D J, YUSTA B, BOUSHEY R P, et al. Human [Gly2]GLP-2 reduces the severity of colonic injury in a murine model of experimental colitis[J]. American Journal of Physiology-Gastrointestinal and Live Physiology, 1999, 276(1): G79–G91.

[14] ALAVI K, SCHWARTZ M Z, PALAZZO J P, et al. Treatment of inflammatory bowel disease in a rodent model with the intestinal growth factor glucagon-like peptide-2[J]. Journal of Pediatric Surgery, 2000, 35(6): 847–851.
[15] BAILON P, WON C Y. PEG-modified biopharmaceuticals[J]. Expert Opinion on Drug Delivery, 2009, 6(1): 1–16.
[16] HARRIS J M, CHESS R B. Effect of pegylation on pharmaceuticals[J]. Nature Reviews Drug Discovery, 2003, 2(3): 214–221.
[17] VERONESE F M, PASUT G. PEGylation, successful approach to drug delivery[J]. Drug Discovery Today, 2005, 10(21): 1451–1458.
[18] FEE C J, ALSTINE J M V. PEG-proteins: reaction engineering and separation issues[J]. Chemical Engineering Science, 2006, 61(3): 924–939.
[19] LEE S H, LEE S, YOUN Y S, et al. Synthesis, characterization and pharmacokinetic studies of PEGylated glucagon-like peptide-1[J]. Bioconjugate Chemistry, 2005, 16(2): 377–382.
[20] CHOI S, BAUDYS M, KIM S W. Control of blood glucose by novel GLP-1 delivery using biodegradable triblock copolymer of PLGA-PEG-PLGA type 2 diabetic rats[J]. Pharmaceutical Research, 2004, 21(5): 827–831.
[21] 刘琳娜, 李欣, 张琰等. 胰高血糖素样肽-2/聚乳酸-羟基乙酸微球的制备及其体外释药性质研究 [J]. 中国药房, 2010(29): 2755–2757.
[22] 张海松, 刘卫红, 张基展, 等. 口服胸腺肽微球在大鼠体内的药代动力学和生物利用度 [J]. 中国生化药物杂志, 2000, 21(1): 15–17.
[23] 陆蕾. 干扰素 -2b PLGA 缓释微球药代动力学及药效学研究 [D]. 硕士学位论文. 上海: 第二军医大学, 2006: 18–29.
[24] 肖田安, 怀彬彬, 李帅鹏, 等. 伊维菌素 PLA 及 PLGA 微球混悬液在犬体内的药代动力学研究 [J]. 华南农业大学学报, 2014, 35(3): 8–12.

Note: Figure translations are in progress. See original paper for figures.