Research Article

Novel Lansoprazole-Loaded Nanoparticles for the Treatment of Gastric Acid Secretion-Related Ulcers: *In Vitro* and *In Vivo* Pharmacokinetic Pharmacodynamic Evaluation

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Abstract. The objective of this study is to combine nanoparticle design and enteric coating technique to sustain the delivery of an acid-labile drug, lansoprazole (LPZ), in the treatment of acid reflux disorders. Lansoprazole-loaded Eudragit® RS100 nanoparticles (ERSNP-LPZ) as well as poly(lactic-co-glycolic acid) (PLGA) nanoparticles (PLGANP-LPZ) were prepared using a solvent evaporation/extraction method. The effects of nanoparticle charge and permeation enhancers on lansoprazole uptake was assessed in Caco-2 cells. The confocal microscopic images revealed the successful localization of nanoparticles in the cytoplasm of Caco-2 cells. The cellular uptake of positively charged Eudragit nanoparticles was significantly higher than that of negatively charged PLGA nanoparticles, which were enhanced by sodium caprate via the transcellular pathway. Both types of nanoparticles exhibited sustained drug release behavior *in vitro*. The oral administration of enteric-coated capsules filled with nanoparticles sustained and prolonged the LPZ concentration up to 24 h in ulcer-induced Wistar rats, and 92.4% and 89.2% of gastric ulcers healed after a 7-day treatment with either EC-ERSNP1010-Na caprate or EC-PLGANP1005-Na caprate, respectively.

KEY WORDS: Caco-2 cell; lansoprazole; nanoparticles; pharmacodynamic study; pharmacokinetic study.

INTRODUCTION

Acid-related disorders, such as peptic ulcers and gastro-esophageal reflux disease (GERD), frequently occur in older people and are associated with morbidity (1). Patients with GERD experience the reflux of stomach contents into the esophagus, which causes troublesome symptoms or mucosal injury in the esophagus (2,3). It is associated with sleep disturbance and decreases in work productivity (4,5). Acid suppression is currently the most effective therapeutic approach in the treatment of GERD.

Lansoprazole (LPZ) is a proton pump inhibitor that induces maximum antisecretory activity by inhibiting hydrogen/potassium ATPase pumps in gastric parietal cells to increase intragastric pH (6). It is effective in treating acid-reflux-related diseases, including gastric and duodenal ulcers, gastro-esophageal reflux disease (GERD), and erosive esophagitis (7). The once-daily administration of LPZ is popularly used to control gastric acid secretion in the clinic. However, some patients on LPZ therapy experience a nocturnal acid breakthrough, where the secretory activity of proton pumps returns (1,8). The once-daily oral administration of an extended-release formulation of LPZ (Prevacid capsule, TAP Pharmaceuticals Inc., IL, USA)

does not effectively control gastro-esophageal symptoms over the entire day (9,10). This results in nocturnal acid breakthrough in GERD patients during LPZ therapy. Thus, it is desirable to provide a form of LPZ that achieves sufficiently prolonged and steady LPZ concentrations to continuously inhibit proton pump activity, especially in the middle of the night.

Eudragit®RS100 and poly(lactic-co-glycolic acid) (PLGA) polymers have been used as drug carriers. Eudragit® RS100 is insoluble but swellable at physiological pH. It contains 4.5–6.8% quaternary ammonium groups, which impart a positive surface charge to the polymer and are responsible for the bioadhesive characteristics of this polymer (11). Eudragit®RS100 has been used to prepare sustained-release nanoparticulate dosage forms. Adibkia *et al.* developed the Eudragit nanoparticles to sustain delivery of naproxen for ocular and intra-joint administrations (12). Devarajan and Sonawane reported that the Eudragit nanoparticles achieved a sustained-release profile suitable for oral administration of gliclazide (13), whereas Dai *et al.* used the Eudragit nanoparticles as a carrier to enhance oral bioavailability of cyclosporine (14). PLGA is a biocompatible and biodegradable copolymer. It contains carboxylic groups that impart a negative charge to the polymer. PLGA is commonly applied in controlled release dosage forms for oral as well as subcutaneous administration. PLGA has been approved by the food and drug administration of the USA as a biomedical material and is widely used in controlled release dosage forms (15,16). The emulsification/solvent evaporation method is popularly applied to prepare micro- and nanoparticles, which are widely applicable to control delivery of drugs (11,17,18).

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The delivery of drugs by polymeric nanoparticles is one of the approaches used to modify the pharmacokinetics and biodistribution of drugs (19–25). Nanoparticles offer numerous advantages as a drug delivery system, including improving the bioavailability of active molecules, extending the half-life of the drug in the body, facilitating permeation of the drug across biological barriers and accumulating at the tumor sites. To date, no nanoparticulate delivery system for LPZ has been developed. It was reported that the surface charge played an important role in achieving the efficient uptake of nanoparticles in the ulcerated region of stomach tissue (26,27). The objective of the present work was to develop LPZ-loaded sustained release nanoparticles using positively charged Eudragit® RS100 and negatively charged poly(lactic-co-glycolic acid). The emulsion–solvent evaporation/extraction method was applied to prepare polymeric nanoparticles. Enteric coated capsules filled with LPZ-loaded nanoparticles were prepared using a dip coating method. The effects of nanoparticle surface charge and permeation enhancer, sodium caprate, on the cellular uptake and localization of nanoparticles was investigated in a human colon adenocarcinoma (Caco-2) cell monolayer. Sodium caprate is widely used as a permeation enhancer to improve the permeability of poorly absorbable drugs, and the Caco-2 cell monolayer is popularly used as an *in vitro* model to predict the oral absorption of drugs across the human small intestinal mucosa. Finally, the pharmacokinetic performance and ulcer healing response were evaluated in ulcer-induced Wistar rats after the oral administration of the enteric coated capsule filled with LPZ-loaded nanoparticles.

MATERIALS AND METHODS

Materials

Lansoprazole (Alcon Biosciences Private Ltd., Mumbai, India) was used as a model drug. The polymers used in this study included Eudragit® RS100 (Degussa, Darmstadt, Germany), poly(lactic-co-glycolic acid) (copolymer ratio 50:50, Boehringer Ingelheim, Ingelheim, Germany), and hydroxyl propyl methyl cellulose phthalate HP55 (HPMCP; ShinEtsu, Tokyo, Japan). Polyvinyl alcohol (PVA, MW 22,000 Da, 88% hydrolyzed, Fisher Scientific Co. Inc., Leicestershire, United Kingdom) was used as a dispersing agent. Sodium caprate (Tokyo Chemical Industries, Tokyo, Japan) was used as an enhancer. RICH® capsule (Nang Kuang Pharmaceutical Co. Ltd., Tainan, Taiwan) is a commercial product that is bioequivalent to Prevacid (TAP Pharmaceuticals Inc., IL, USA). The human colon adenocarcinoma cell line (Caco-2 cells) was a gift from Dr. Li-Juan

Shen (National Taiwan University, Taipei, Taiwan) and originated from the American Type Culture Collection (Manassas, USA). Dulbecco modified eagle's medium (with 4.5 g/L D-glucose, with L-glutamine, without sodium pyruvate and sodium bicarbonate), non-essential amino acids, and fetal bovine serum were purchased from Biological Industries (Beit-Haemek, Israel). Hank's balanced salt solution (HBSS), propidium iodide (PI), and ribonuclease A (RNase A) were purchased from Invitrogen Corporation (Carlsbad, USA).

Preparation of LPZ-Loaded Eudragit® RS100 Nanoparticles (ERSNP-LPZ)

ERSNP-LPZ was prepared using an oil-in-water (o/w) emulsion solvent evaporation/extraction method. LPZ (200 mg) and various amounts of Eudragit® RS100 (20, 40, 100, or 200 mg) were previously dissolved in a mixture of dichloromethane/methanol (5/5 v/v). The organic solution was then added into the aqueous PVA solution (0.25% w/v, pH 9.0) under sonication using an ultrasonic probe (Sonics and materials Inc., Newtown, USA) at 4°C for 20 min. The emulsion was magnetically stirred at room temperature for 3 h followed by evaporation under reduced pressure at 35°C for 5 min. The nanoparticles were collected after centrifugation at 17,000 rpm for 30 min (Avanti J26 XP centrifuge, Beckman Coulter, Miami, USA). The collected nanoparticles were washed with deionized water three times, followed by freeze drying using sucrose (5% w/v) as a cryoprotectant.

Preparation of LPZ-Loaded PLGA Nanoparticles (PLGANP-LPZ)

PLGANP-LPZ was prepared using water-in-oil-in-water (w/o/w) double emulsion solvent evaporation/extraction technique. LPZ 200 mg and various amounts of PLGA (20, 40, and 100 mg) were previously dissolved in a mixture of dichloromethane/acetone (5/5 v/v). An aqueous solution of NaHCO₃ (0.2% w/v) was added to the organic solution and emulsified using a probe sonicator at 4°C for 2 min. The primary emulsion was then added to the aqueous PVA solution (0.25% w/v, pH 9.0) and emulsified. The following preparation procedures were the same as described for ERSNP-LPZ preparation.

Characterization of Nanoparticles

The freeze-dried nanoparticles were weighed, and the yield was calculated using Eq. 1.

$$\text{Yield}(\%) = \frac{\text{total amount of nanoparticles obtained}}{\text{total amount of drug and polymer added initially}} \times 100\% \quad (1)$$

The particle size and zeta potential of nanoparticles were determined using a Zetasizer (Nano ZS, Malvern Co. Ltd., Worcestershire, UK). The morphology of nanoparticles was

examined using a transmission electron microscope (TEM, H7100, Hitachi High-Technologies Corporation, Tokyo, Japan) where the freeze-dried nanoparticles were dispersed in deionized

water and stained with 2% phosphotungstic acid for 30 s followed by placement on copper grids for observation. For LPZ content determination, 5 mg of ERSNP-LPZ or PLGANP-LPZ was dissolved in 5 mL methanol or acetonitrile, respectively. Each sample was centrifuged at 14,000 rpm for 10 min, and a 20- μ L aliquot of the supernatant was injected into the HPLC system (Jasco International Company Ltd., Tokyo, Japan), which consisted of a pump (PU-2089) and a photodiode array detector (PDA, MD-2010) at 285 nm. A reversed phase silica column (C-18, 4.6 $\times$ 250 mm, 5 μ m, Phenomenex Inc., USA) was used. The mobile phase comprised water, acetonitrile, and triethylamine in a volume ratio of 50:50:0.1 (pH 7) at a flow rate of 1 mL/min.

The HPLC analytical method was validated prior to sample analysis. It was linear over the concentration range of 0.25–25 μ g/mL, and the coefficient of determination (R^2) was ≥ 0.9999 . The accuracy was in the range of 95.5–102.3%, and the precision was in the range of 0.26–4.22%. The drug loading (DL) and encapsulation efficiency (EE) were calculated using Eqs. 2 and 3.

$$DL(\%) = \frac{\text{determined amount of drug in nanoparticles}}{\text{total amount of nanoparticles prepared}} \times 100\% \quad (2)$$

$$EE(\%) = \frac{\text{determined amount of drug in nanoparticles}}{\text{total amount of drug used for nanoparticles preparation}} \times 100\% \quad (3)$$

Preparation of Enteric Coated Capsules

The enteric coated capsules filled with ERSNP1010 or PLGANP1005 were prepared using a dip coating method. Each hard gelatin capsule was manually filled with LPZ nanoparticles. The capsule was immersed in a HPMCP acetone solution (10% w/v) followed by drying at room temperature using an air-blower. This procedure was repeated three times. The capsules before and after enteric coating were weighed, and the amount of HPMCP coated on each capsule was calculated. For the acid resistance study, the enteric coated capsule was suspended in 100 mL of 0.1 N HCl at $37 \pm 0.5^\circ\text{C}$ in a shaker bath at 75 rpm for 1 h. The capsule was then washed with deionized water, and the nanoparticles were removed from the capsule. The LPZ content in the nanoparticles was determined using the HPLC method described in “Characterization of Nanoparticles”

In Vitro Drug Release

LPZ-loaded nanoparticles equivalent to 1 mg LPZ were suspended in 5 mL pH 7.4 phosphate-buffered solution in a dialysis bag (MWCO 6,000–8,000 Da) that was immersed in 100 mL of the same release medium at $37 \pm 0.5^\circ\text{C}$ in a shaker bath at 75 rpm. Samples (1 mL) were collected at time intervals of 0.5, 1, 2, 4, 6, 8, 12, and 24 h, and the same volume of the fresh release medium was replaced. The amount of LPZ in each released sample was analyzed by HPLC. The release kinetics of LPZ from nanoparticle formulations was analyzed using various kinetic models, including zero-order, first-order, and Higuchi's square root models (28). In addition, the similarity factor (f_2) was used to compare all release profiles. It was computed using Eq. 4, where n is the number of time points, R_t and T_t are the mean percentages of drug released at each time point (t) from the reference and test release profiles, respectively. Generally, the f_2 value in the range of 50–100 ensures

similarity between the two comparative release profiles (27,29).

$$f_2 = 50 \times \log \left\{ \left[1 + (1/n) \sum_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \right\} \times 100 \quad (4)$$

Preparation of Coumarin-6-Loaded Fluorescent Nanoparticles

Coumarin-6-loaded fluorescent nanoparticles were prepared to investigate the cellular uptake of positively charged Eudragit® RS100 nanoparticles (ERSNP-C6) and negatively charged PLGA nanoparticles (PLGANP-C6) in Caco-2 cells. The nanoparticles were prepared using the o/w solvent evaporation method. Eudragit® RS100 or PLGA (200 mg) and coumarin-6 (1 mg) were dissolved in a 10-mL dichloromethane/acetone mixture (5/5 v/v). The following preparation procedures were the same as described previously. The amounts of coumarin-6 trapped in ERSNP-C6 and PLGANP-C6 were determined using fluorescence spectrophotometer (F4500, Hitachi Ltd., Tokyo, Japan), where the nanoparticles were dissolved in acetone for fluorescence measurement at an excitation wavelength of 430 nm and an emission wavelength of 490 nm. In addition, the leaching of coumarin-6 from the nanoparticles was evaluated. ERSNP-C6 and PLGANP-C6 (10 mg) were dispersed in 1 mL HBSS, pH 7.4, in a dialysis bag (MWCO 6,000–8,000 Da) that was immersed in 10 mL HBSS at $37 \pm 0.5^\circ\text{C}$ in a mechanical shaker at 50 rpm for 24 h. The sample was collected, and the fluorescence was measured.

Cellular Uptake Study

Caco-2 cell monolayers were used for the cellular uptake study. Before the experiments, the monolayers were washed with HBSS (pH 7.4) twice, followed by preincubation with

HBSS at 37°C for 30 min. The HBSS (control), free coumarin-6 solution, and ERSNP-C6 as well as a PLGANP-C6 suspension in HBSS equivalent to 200 ng/mL of coumarin-6 were added and incubated at 37°C for 0.5 and 1 h in an atmosphere of 5% CO₂ and 90% relative humidity. The Caco-2 cells were then washed with phosphate-buffered saline (PBS, pH 7.4) three times, followed by trypsinization for 5 min (0.25% trypsin EDTA). Cold PBS was added, and the cells were centrifuged at 1,000 rpm for 5 min followed by resuspension in 2 mL PBS and analyzed using a fluorescence-activated flow cytometer (BD FACSCalibur) and BD CellQuest software (BD Biosciences, NJ, USA). This experiment was performed in triplicate. For confocal microscopic observation, the cell monolayer was incubated at 37°C for 0.5 h and then washed with PBS three times, followed by fixation with a 3.7% paraformaldehyde solution in PBS for 30 min. The cells were washed again followed by treatment with RNase solution for 30 min, and the nuclei were stained with PI for 30 min. The cell monolayer was mounted on a glass slide using FluoromountTM (Sigma-Aldrich, St. Louis, USA) as a mounting medium. The images were captured using a Leica confocal laser scanning microscopy imaging system (TCS SP5, Leica, Wetzlar, Germany).

In Vivo Pharmacokinetic Study

Male Wistar rats (250–300 g) were used in this study. They were obtained from the National Laboratory Animal Center, Taipei, Taiwan. All animal experiments were carried out in accordance with the regulations of the Institutional Animal Care and Use Committee (IACUC) (College of Medicine and College of Public Health, National Taiwan University, Taipei, Taiwan) and the “Guide for the Care and Use of Laboratory Animals” published by the National Institutes of Health. Rats were fasted but allowed free access to water overnight. Gastric ulcers were induced through the oral administration of absolute ethanol (5 mL/kg) (30). The ulcer-induced rats were divided into four groups, and each group consisted of four rats. The enteric coated hard gelatin capsules (#9, Torpac Inc., NJ, USA) filled with (1) ERSNP1010 (EC-ERSNP1010), (2) ERSNP1010 and sodium caprate (20 mg/kg) (EC-ERSNP1010-Na caprate), (3) PLGANP1005 (EC-PLGANP1005), or (4) PLGANP1005 and sodium caprate (20 mg/kg) (EC-PLGANP1005-Na caprate) equivalent to 5 mg LPZ/kg were orally administered to ulcer-induced Wistar rats. Blood samples were collected from the tail veins of the rats prior to drug administration and at time intervals of 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h after dosing. The blood samples were centrifuged at 12,000 rpm for 10 min at 4°C, and the supernatant was collected. The LPZ in each serum

Fig. 1. TEM images (50K X) and particle size distributions of ERSNP-LPZ (a and b) and PLGANP-LPZ (c and d)

sample was extracted using acetonitrile (27,31). The mixture was vortex and then centrifuged. The supernatant was collected, air dried, and reconstituted with mobile phase for HPLC analysis. The HPLC analytical method was validated prior to sample analysis. It was linear over a concentration range of 10–1,000 ng/mL, and the coefficient of determination (R^2) was ≥ 0.9979 . The lowest limit of quantification was 10 ng/mL. The accuracy was in the range of 97.70–100.34%, and the precision was in the range of 0.57–6.73%. The recovery of LPZ from serum samples was in the range of 97.81–98.79%. The pharmacokinetic parameters were obtained from the serum LPZ concentration-time data based on a noncompartmental pharmacokinetic analysis model (WinNonlin software, version 5.3, Pharsight Corporation, CA, USA).

Pharmacodynamic Study

The ulcer-induced Wistar rats (250–300 g) were used in this study. All procedures were approved by the IACUC, as mentioned previously. The ulcer-induced rats were divided into 12 groups, and each group consisted of four rats. Each group received (1) saline solution (control) (1 mL), (2) EC-ERSNP1010, (3) EC-ERSNP1010-Na caprate, (4) EC-PLGANP1010, (5) EC-PLGANP1010-Na caprate, or (6) RICH® capsule at a dose equivalent to 5 mg LPZ/kg once daily for 7 days. For the RICH® capsule, the commercial capsule was opened, and the enteric coated LPZ granules were weighed equivalent to the administration dose (5 mg/kg LPZ) for each rat. The weighed granules were added to the hard gelatin

capsule (#9, Torpac Inc., NJ, USA) and administered to the rats using a dosing needle. The rats were sacrificed 24 h after the last administered dose. The stomachs were cut along the greatest curvature, and the stomach mucosal surface was washed with saline solution. Photographic images (Nikon E5000, Nikon Corporation, Tokyo, Japan) of the stomach mucosal surface were taken, and the total ulcerated area and the total mucosal area were measured using Axio Vision software (version 4.8, Carl Zeiss International, NY, USA). The ulcer index (UI) was calculated using Eq. 5 (31).

$$\text{Ulcer index (UI)} = \frac{\text{total ulcerated area} \times 100}{\text{total mucosal area}} \quad (5)$$

Statistical Analysis

The data from the two groups were analyzed using a one-tailed student *t* test with SigmaStat software (Systat Software Inc., California, USA). A difference of $p < 0.05$ was considered to have statistical significance.

RESULTS AND DISCUSSION

Characterization of ERSNP-LPZ

A single emulsion–solvent evaporation/extraction method was employed to encapsulate LPZ using Eudragit®

Fig. 2. Effect of the polymer/drug ratio on **a** the yield (%), **b** particle size (nm), **c** drug loading (%), and **d** encapsulation efficiency (%) of ERSNP-LPZ. The values represent the mean $\pm$ SD ($n=3$). Asterisk indicates $p < 0.05$, and NS indicates $p > 0.05$ by Student's *t* test

Fig. 3. Effect of the polymer/drug ratio on **a** the yield (%), **b** particle size (nm), **c** drug loading (%), and **d** encapsulation efficiency (%) of PLGANP-LPZ. The values represent the mean $\pm$ SD ($n=3$). Asterisk indicates $p < 0.05$, and NS indicates $p > 0.05$ by Student's t test

RS100. The aqueous PVA solution was used as an external phase, and its pH was adjusted to 9 to avoid the degradation of acid-labile LPZ during nanoparticle preparation (32).

Fig. 4. The LPZ release profiles of white triangle ERSNP1010, white square PLGANP1005, black triangle EC-ERSNP1010, and black square EC-PLGANP1005 in pH 7.4 phosphate-buffered solution. The values represent the mean $\pm$ SD ($n=3$)

Figure 1a and b illustrate the TEM images and the particle size distributions of ERSNP-LPZ. The ERSNP-LPZ exhibited a spherical morphology and was in the nano-sized range with a narrow size distribution ($PDI < 0.2$). The effect of the polymer/drug ratio (e.g., 10/1, 10/2, 10/5, and 10/10) on the yield, particle size, drug loading, and encapsulation efficiency of ERSNP-LPZ was investigated, and the results are shown in Fig. 2. The yield of ERSNP-LPZ was in the range of 72.7 ± 4.1 – $80.6 \pm 3.1\%$ (Fig. 2a), and the mean particle size was 196.6 ± 11.3 – 204.1 ± 4.9 nm (Fig. 2b). The decrease of the polymer/drug ratio from 10/1 to 10/10 did not affect the yield or particle size of ERSNP-LPZ ($p > 0.05$). ERSNPs-LPZ exhibited a positive zeta potential in the range of $+36.8 \pm 0.6$ – $+39.6 \pm 0.2$ mV due to the quaternary ammonium groups of Eudragit® RS100 (12,33–35). ERSNP-LPZ prepared in different polymer/drug ratios presented a significant difference in the drug loading (Fig. 2c), which increased from 4.9 ± 0.2 to $41.4 \pm 1.9\%$ as the polymer/drug ratio decreased from 10/1 to 10/10 ($p < 0.05$). Similarly, the encapsulation efficiency significantly increased from 58.5 ± 2.5 to $76.4 \pm 1.6\%$ as the polymer/drug ratio decreased from 10/2 to 10/10 ($p < 0.05$), and there was no significant difference between 10/1 and 10/2 ($p > 0.05$; Fig. 2d). The limited solubility of LPZ in the external aqueous phase prevented drug loss during the encapsulation process, which led to an increase in the drug loading and encapsulation efficiency as the polymer/drug ratio decreased.

Fig. 5. **a** Flow cytometry images and **b** cellular uptake efficiency (%) of **a** HBSS (control), **b** coumarin-6, **c** ERSNP-C6, **d** PLGANP-C6, **e** ERSNP-C6-Na caprate, and **f** PLGANP-C6-Na caprate in Caco-2 cells after a 0.5-h incubation. The values represent the mean $\pm$ SD ($n=3$). Asterisk indicates $p<0.05$, and NS indicates $p>0.05$ by Student's t test

Characterization of PLGANPs-LPZ

The double emulsion solvent evaporation/extraction method was employed to encapsulate LPZ with PLGA. Because the acid end groups of the PLGA copolymer may affect LPZ stability, an alkaline stabilizer, sodium bicarbonate, was added to maintain LPZ stability in PLGA nanoparticles (36). Figure 1c and d show a TEM image and the particle size distribution of PLGANP-LPZ. PLGANP-LPZ had a spherical morphology and was in the nano-sized range, with a narrow size distribution similar to ERSNP-LPZ. The effect of the polymer/drug ratio (e.g., 10/1, 10/2, and 10/5) on the yield, particle size, drug loading, and encapsulation efficiency of PLGANP-LPZ nanoparticles was investigated,

and the results are shown in Fig. 3. The yield of PLGANP-LPZ was in the range of 72.6 ± 4.6 – $73.3\pm1.7\%$ (Fig. 3a), and the mean particle size was in the range of 235 ± 11 – 217 ± 2 nm (Fig. 3b). There was no change in the yield or particle size of PLGANP-LPZ at various polymer/drug ratios ($p>0.05$). PLGANPs-LPZ possessed a negative zeta potential in the range of -26.0 ± 2.6 to -29.1 ± 2.5 mV due to the carboxyl groups of PLGA. PLGANP-LPZ prepared in different polymer/drug ratios presented significant alterations to drug loading and encapsulation efficiency, similar to ERSNP-LPZ. The drug loading and encapsulation efficiency of PLGANP-LPZ increased from 0.87 ± 0.01 to $27.6\pm2.6\%$ (Fig. 3c) and from 9.8 ± 0.5 to 78.5 ± 8.2 (Fig. 3d), respectively, as the polymer/drug ratio decreased from 10/1 to 10/5 ($p<0.05$).

Fig. 6. Confocal microscopy images of Caco-2 cells after incubation with ERSNP-C6, ERSNP-C6-Na caprate, PLGANP-C6, or PLGANP-C6-Na caprate at 37°C for 0.5 h. *Green* denotes nanoparticles, and *red* denotes nuclei. *XY1*, *XY2*, and *XY3* represent images with the FITC filter, RITC filter, and FITC-RITC filter overlay. The *green* images in *YZ* projections illustrate the internalized nanoparticles

Fig. 7. The serum LPZ concentration *versus* time profiles in ulcer-induced Wistar rats after the oral administration of *white triangle* EC-ERSNP1010, *white square* EC-PLGANP1005, *black triangle* EC-ERSNP1010-Na caprate, or *black square* EC-PLGANP1005-Na caprate (5 mg LPZ/kg). The values represent the mean $\pm$ SD ($n=4$)

Characterization of Enteric Coated Capsules

The enteric coated capsules filled with ERSNP1010 or PLGANP1005 were prepared in HPMCP acetone solution using a dip coating method. The mean weights of EC-ERSNP1010 and EC-PLGANP1005 were 65.5 ± 2.3 and 66.6 ± 1.7 mg, and the average amount of HPMCP coated on each capsule was 25.2 ± 1.5 mg and 25.7 ± 1.4 mg, respectively. The enteric coated capsules were evaluated for their acid resistance, and $95.1 \pm 2.5\%$ and $96.8 \pm 1.8\%$ of the LPZ remained stable in the nanoparticle-filled enteric coated capsules, EC-ERSNP1010 and EC-PLGANP1005, respectively, after acid treatment for 1 h. This result confirmed that the HPMCP enteric coated capsule successfully protected LPZ from degradation in an acidic medium (36).

In Vitro LPZ Release

Figure 4 illustrates the release profiles of two enteric coated nanoparticle formulations (e.g., ERSNP1010 and PLGANP1005) and two nanoparticle-filled, enteric coated capsules (e.g., EC-ERSNP1010 and EC-PLGANP1005) in a pH 7.4 release medium.

Table I. Pharmacokinetic Parameters of LPZ After the Oral Administration of EC-ERSNP1010, EC-PLGANP1005, EC-ERSNP1010-Na Caprate, or EC-PLGANP1005-Na Caprate in Ulcer-Induced Wistar Rats at a Dose of 5 mg LPZ/kg ($n=4$)

PK parameters	EC-ERSNP1010	EC-PLGANP1005	EC-ERSNP1010-Na caprate	EC-PLGANP1005-Na caprate
$AUC_{0-\infty}$ ($\mu\text{g h/mL}$) ^{a,b,c}	3.98±0.39	2.00±0.24	3.91±0.37	2.93±0.08
C_{max} ($\mu\text{g/mL}$) ^{a,b,c}	0.44±0.10	0.27±0.04	0.42±0.04	0.33±0.04
T_{max} (h)	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
k (h^{-1})	0.19±0.02	0.14±0.02	0.16±0.01	0.15±0.02
$t_{1/2}$ (h)	3.71±0.31	5.02±0.71	4.23±0.40	4.79±0.55
V_d/F (L/kg) ^{a,b,c}	6.79±1.09	18.4±3.4	7.84±1.07	11.2±0.9
CL/F (L/h/kg) ^{a,b,c}	1.27±0.12	2.53±0.31	1.29±0.12	1.61±0.06
MRT (h)	8.52±0.56	9.68±0.76	8.93±0.23	9.57±0.89

$AUC_{0-\infty}$ area under the serum concentration vs. time curve from zero to infinity, C_{max} maximum drug concentration, T_{max} time to reach the C_{max} , k elimination rate constant, $t_{1/2}$ elimination half life, V_d/F apparent volume of distribution, CL/F clearance, MRT mean residence time

^a Indicates the statistical significance between EC-ERSNP1010 and EC-PLGANP1005 ($p<0.05$)

^b Indicates the statistical significance between EC-PLGANP1005-Na caprate and EC-PLGANP1005 ($p<0.05$)

^c Indicates the statistical significance between EC-ERSNP1010-Na caprate and EC-PLGANP1005-Na caprate ($p<0.05$)

All nanoparticles exhibited sustained release behavior. Eudragit® RS100 and PLGA are water-insoluble polymers that behave as rate-limiting membranes to control and sustain drug release from

the nanoparticles for up to 24 h (37). There was negligible drug release from enteric coated EC-ERSNP1010 ($0.55\pm0.34\%$) and EC-PLGANP1005 ($0.33\pm0.17\%$) during the first 0.5 h compared

Fig. 8. **a** Photographs of the stomach opened along the greatest curvature and **b** the ulcer index values of ulcerated Wistar rats after the oral administration of saline solution (control), EC-ERSNP1010, EC-PLGANP1005, EC-ERSNP1010-Na caprate, EC-PLGANP1005-Na caprate, or RICH® (5 mg LPZ/kg/day) for 7 days. The arrows indicate the ulcerated regions. Asterisk indicates $p<0.05$, and NS indicates $p>0.05$

to non-enteric coated ERSNP1010 ($3.71 \pm 0.66\%$) and PLGANP1005 ($3.89 \pm 0.31\%$). This delay was referred to as the lag time required to completely dissolve the HPMCP enteric coating layer of the capsules to expose the nanoparticles. Burst releases of 12.1 ± 2.0 , 13.7 ± 6.2 , 6.7 ± 0.4 , and $8.0 \pm 0.5\%$ of the available LPZ from ERSNP1010, PLGANP1005, EC-ERSNP1010, and EC-PLGANP1005, respectively, were observed during the first 1 h due to diffusion of the drugs located at or near the surface of the nanoparticles. These release profiles were compared based on f_2 values, and there were no differences between ERSNP1010 and PLGANP1005 ($f_2=75$) or EC-ERSNP1010 and EC-PLGANP1005 ($f_2=77$). To further understand the release kinetics of drugs released from nanoparticle formulations, various kinetic models, including zero-order, first-order, and Higuchi's square root models, were applied to the *in vitro* release data. The results showed that the best fit ($R^2=0.94 \pm 0.01$ – 0.97 ± 0.01) was observed using Higuchi's square root model. It implied that the release of LPZ from these nanoparticle formulations was dominated by diffusion, and the release kinetics of LPZ from PLGANP1005 was similar to those of ERSNP1010.

Cellular Uptake of Coumarin-6-Loaded Nanoparticles

Coumarin-6 is popularly used as a fluorescence marker for confocal microscopic observation due to its high fluorescence intensity (38–40). Coumarin-6-loaded fluorescent nanoparticles were prepared to demonstrate the cellular uptake and biodistribution of the positively charged Eudragit nanoparticles and the negatively charged PLGA nanoparticles in Caco-2 cells, which were monitored using a flow cytometer and a confocal microscope. Figure 5a displays the fluorescence intensity of the cells incubated with HBSS (control group), free coumarin-6, and ERSNP-C6 or PLGANP-C6 nanoparticles in the presence or absence of sodium caprate as a penetration enhancer for 0.5 h, and the corresponding nanoparticle uptake efficiency is shown in Fig. 5b. The fluorescence intensity of cells incubated with free coumarin-6 was $2.56 \pm 2.53\%$, which was not significantly different from the control group ($0.98 \pm 0.02\%$). It was implied that free coumarin-6 is not accessible for uptake by Caco-2 cells. Similarly, Dong and Feng reported that raw coumarin-6 cannot be internalized by cells due to its poor aqueous solubility and irregular shape (41). There was a significant increase in cell fluorescence intensity after incubation with the fluorescent nanoparticles ERSNP-C6 and PLGANP-C6 for 0.5 h, and the corresponding cellular uptake efficiencies were $81.1 \pm 4.5\%$ and $44.8 \pm 4.6\%$, respectively, which was further increased to $98.8 \pm 2.3\%$ and $74.2 \pm 4.4\%$ after a 1-h incubation (data not shown in Fig. 5). The cellular uptake of positively charged ERSNP-C6 was significantly higher than that of negatively charged PLGANP-C6 ($p < 0.05$). The presence of sodium caprate further enhanced their cellular uptake to $94.1 \pm 4.2\%$ and $74.6 \pm 3.7\%$, respectively. Figure 6 shows confocal microscopic images of Caco-2 cells after incubation with ERSNP-C6 and PLGANP-C6 in the presence or absence of sodium caprate for 0.5 h. The strong green fluorescence indicated that the nanoparticles were internalized and localized in the cytoplasm of the Caco-2 cells, which was further confirmed using three-dimensional analysis via z-axis reconstruction of the confocal images.

Pharmacokinetic Study

In vivo pharmacokinetic characteristics of LPZ were evaluated in the ulcer-induced Wistar rats following the oral administration of enteric coated capsules filled with either positively charged ERSNP1010 or negatively charged PLGANP1005 nanoparticles in the presence or absence of sodium caprate. Figure 7 shows the serum LPZ concentration *versus* time profiles, and the corresponding pharmacokinetic parameters are listed in Table I. The LPZ concentration was prolonged up to 24 h following the oral administration of enteric coated capsules filled with nanoparticles. Both types of nanoparticles had similar $T_{1/2}$ and MRT. The C_{max} of EC-ERSNP1010 (0.44 ± 0.10 $\mu\text{g/mL}$) was significantly higher than that of EC-PLGANP1005 (0.27 ± 0.04 $\mu\text{g/mL}$; $p < 0.05$), and their corresponding T_{max} values were both 6 h. Similarly, the $AUC_{0-\infty}$ of EC-ERSNP1010 (3.98 ± 0.39 $\mu\text{g h/mL}$) was significantly higher than that of EC-PLGANP1005 (2.00 ± 0.24 $\mu\text{g h/mL}$; $p < 0.05$). EC-ERSNP1010 displayed a much higher $AUC_{0-\infty}$ and C_{max} than PLGANP1005 due to the efficient cellular uptake of EC-ERSNP1010, as shown in Fig. 5. The electrostatic interaction of positively charged ERSNP100 toward negatively charged

Fig. 9. Histopathologic illustrations of healthy tissue and healing ulcer tissues after the oral administration of saline solution (control), RICH® capsule, EC-ERSNP1010, or EC-PLGANP1005 with or without sodium caprate for 7 days. The arrows indicate granulation tissue development during the ulcer healing process (magnification $\times 100$)

cell surfaces accounted for this result (42). Similar concentration–time profiles were observed between EC-ERSNP1010 and EC-ERSNP1010-Na caprate regardless of the presence of Na caprate. In contrast, the concentration profile of EC-PLGANP1005-Na caprate was much higher than that of EC-PLGANP1005, and the $AUC_{0-\infty}$ and C_{max} of the former (2.93 ± 0.08 $\mu\text{g h/mL}$ and 0.33 ± 0.04 $\mu\text{g/mL}$) were significantly higher than those of the latter (2.00 ± 0.24 $\mu\text{g h/mL}$ and 0.27 ± 0.04 $\mu\text{g/mL}$; $p < 0.05$). However, the T_{max} , $T_{1/2}$, and MRT of EC-PLGANP1005 were not affected by sodium caprate. All of these results implied that EC-ERSNP1010 and EC-PLGANP1005 nanoparticles possess the potential to treat acid-related disorders, especially to control nocturnal acid breakthrough due to their prolonged LPZ release.

Pharmacodynamic Study

The healing-promoting activity of EC-ERSNP1010 and EC-PLGANP1005 in the presence or absence of sodium caprate was further evaluated in ulcer-induced Wistar rats. Figure 8a displays photographic images of the stomach in ulcer-induced Wistar rats after the oral administration of saline solution, EC-ERSNP1010, EC-PLGANP1005, EC-ERSNP1010-Na caprate, EC-PLGANP1005-Na caprate, or the commercial product, RICH®, respectively, for 7 days. The corresponding gastric ulcer indexes were in the following order: EC-ERSNP1010-Na caprate (1.2 ± 0.3) < EC-ERSNP1010 (1.6 ± 0.4) < EC-PLGANP1005-Na caprate (1.7 ± 0.2) < RICH® (2.6 ± 0.6) < EC-PLGANP1005 (4.3 ± 0.8) < saline solution (15.8 ± 1.9) (Fig. 8b). This result revealed that the induced gastric ulcer was gradually healed within a week of the oral administration of the LPZ nanoparticle formulations, and 92.4% and 89.2% gastric ulcers healed using EC-ERSNP1010-Na caprate or EC-PLGANP1005-Na caprate, respectively. All nanoparticle formulations except EC-PLGANP1005 showed better ulcer-healing efficacy than the commercial RICH® capsules. The efficient ulcer-healing response of the nanoparticle formulations was due to their sustained release properties and prolonged *in vivo* absorption, which suppressed acid secretion continuously for 24 h.

Figure 9 illustrates the histopathology of the healing ulcer tissues after the oral administration of the above six formulations. In the control group, a large ulcer was present, with several dilated gastric glands where the gastric ulcer mucosal surface was damaged, and the ulcer deeply penetrated the muscularis mucus, causing vascular and microvascular injury. After the oral administration of the LPZ nanoparticle formulations and the commercial LPZ micro-pellets, granulation tissue developed. Granulation tissue is an important component during the ulcer-healing process, as it supplies connective tissue cells and microvessels for the restoration of the microvasculature within the ulcer scar (43). These histopathological images clearly illustrate the restoration of the mucosal architecture by the LPZ nanoparticle formulations, and the effective treatment of gastric ulcers was achieved.

CONCLUSION

Novel, LPZ-loaded, enteric capsule nanoparticles for the treatment of gastric acid secretion-related ulcers were demonstrated *in vitro* and *in vivo*. Two types of LPZ-loaded Eudragit® RS100 and PLGA nanoparticles were prepared using an

emulsion solvent evaporation/extraction method, and the size was approximately 200 nm, with spherical morphology. The *in vitro* release of LPZ confirmed the sustained-release character of these nanoparticles. The cellular uptake of LPZ nanoparticles in Caco-2 cells appeared charge specific; the positively charged Eudragit nanoparticles demonstrated significantly greater uptake than the negatively charged PLGA nanoparticles. Enhanced uptake of PLGA nanoparticles was further achieved using sodium caprate via the transcellular pathway. The Eudragit and PLGA nanoparticles sustained and prolonged the LPZ concentration up to 24 h in ulcer-induced Wistar rats. The oral administration of the LPZ-loaded, enteric capsule nanoparticles for 7 days resulting in varying abilities to heal the gastric ulcers: EC-ERSNP1010-Na caprate > EC-ERSNP1010 > EC-PLGANP1005-Na caprate > RICH® > EC-PLGANP1005. Thus, LPZ-loaded, enteric capsule nanoparticles may be an alternative oral dosage form for the treatment of acid-related disorders, especially to control night-time acid disorders.

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