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Ocular Permeability of Pirenzepine Hydrochloride Enhanced by Methoxy poly(ethylene glycol)–poly (D,L-lactide) Block Copolymer

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Methoxy poly(ethylene glycol)–poly(D,L-lactide) block copolymer was tested as an ocular permeation enhancer for pirenzepine hydrochloride. The block copolymers with the methoxy poly(ethylene glycol) to poly(D,L-lactide) weight ratio of 80/20, 50/50, 40/60 were synthesized by a ring-opening polymerization procedure. In vitro transcorneal experiments demonstrated that the block copolymer 80/20 significantly enhanced the transcorneal permeation of pirenzepine at the mass ratio of 1/1.4 (pirenzepine hydrochloride/copolymer). Interaction between pirenzepine and copolymer was identified by infrared spectroscopy analysis and dialysis experiments. Ocular pharmacokinetics of pirenzepine/copolymer preparation by in vivo instillation experiments confirmed that block copolymer could enhance the ocular penetration of pirenzepine. Ocular chronic toxicity experiments of block copolymer and pirenzepine/copolymer preparation were studied on rabbits, and no significant toxicity in both groups was observed within 9 months. It could conclude that pirenzepine/copolymer preparation is effective and safe in ocular delivery of pirenzepine.

Keywords methoxy poly(ethylene glycol)–poly(D,L-lactide) block copolymer; pirenzepine hydrochloride; transcorneal permeation; ocular pharmacokinetics

INTRODUCTION

Pirenzepine hydrochloride (PRZ, Figure 1) is a relatively selective muscarinic (M_1) antagonist and is able to reduce the axial elongation and myopia in visually impaired chick eye (Truong et al., 2002). Recently, 2% PRZ ophthalmic gel developed by Valley Forge Pharmaceuticals was researched as an anti-myopia drug. A parallel, placebo-controlled, randomized, double-masked clinical study showed that PRZ gel (twice daily) was effective and relatively safe in slowing the progression of myopia over a 1-year treatment period (Tan et al., 2005).

As a hydrophilic compound, PRZ has very low transcorneal permeability and poor ocular bioavailability, which will affect its anti-myopia effect (Dai & Chu, 2002). Therefore, improvement of ocular permeability of PRZ is necessary.

Recently, polymeric micelles composed by amphiphilic block co-polymers were widely researched as novel drug carriers. Methoxy poly(ethylene glycol)-polylactide copolymer (mPEG-PDLLA) is a amphiphilic block polymer which is known to self-assemble into polymeric micelles with a mesoscopic size range about 20–50 nm in an aqueous medium (Torchilin, 2001). These micelles have a fairly narrow size distribution and are characterized by their unique core-shell architecture, where hydrophobic segments are segregated from the aqueous exterior to form an inner core surrounded by a palisade of hydrophilic segments. Due to anisotrophy, such micelles demonstrate a polarity gradient from highly hydrated surface (corona) to the hydrophobic core. As a result, the spatial position of a certain solubilized substance (drug) within a micelle will be depended on its polarity. In aqueous systems, nonpolar molecules will be entrapped in the core, polar molecules will be adsorbed on the surface, and substances with intermediate polarity will be distributed in certain intermediate positions.

Polymeric micelles (PM) were widely researched as drug carriers for targeting delivery. And PM was proven to be able to facilitate the internalization process (Hubbell, 2003; Savic & Luo, 2003). The mechanism of the enhancement was explained as endocytosis and endosomal permeation. Recently, PM ophthalmic delivery systems were reported. Ophthalmic polymeric micelles of ketorolac was prepared and evaluated by Gupta (Gupta & Madan, 2000). The ketorolac polymeric micelles showed two-fold increase in ocular availability with no corneal damage as compared with an aqueous suspension.

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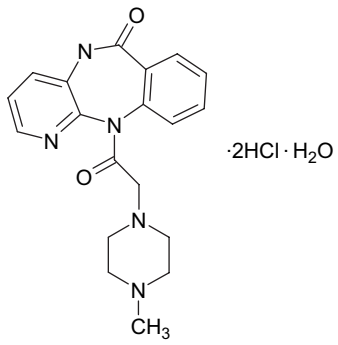

FIGURE 1. Chemical structure of pirenzepine hydrochloride.

In this paper, we aimed to investigate the interaction between mPEG-PDLLA and PRZ, the effects of mPEG-PDLLA on the ocular permeability of pirenzepine hydrochloride (PRZ).

MATERIALS AND METHODS

Materials

Pirenzepine dihydrochloride (purity > 99.5%) was purchased from Wanlian Pharmaceutical Co. (Ningbo, China); D,L-lactide was purchased from Purac America (Lincolnshire, Illinois). Stannous octoate and Methoxy poly(ethylene glycol) (mPEG) with molecular weights of 2000 were obtained from Sigma. Hydroxypropyl methylcellulose (HPMC) (Methocel K100M) was a gift from Colorcon (Shanghai, China). Polystyrene standards of narrow molecular weight distributions and various molecular weights were purchased from Polysciences (Warrington, Pennsylvania). Acetonitrile were HPLC grade (Fisher Scientific). All other chemicals used were analytical grade.

both male and female albino rabbits (purchased from Qinglongshan Experimental Animal Center, Nanjing, China) weighing 2~2.5 kg were used throughout. They were raised at a temperature of 25 ± 5°C and relative humidity 55 ± 10%, with 12h artificial lighting (from 08:00 to 20:00 h). They were fed a commercial pellet diet of 100 g per day per rabbit and had free access to tap water.

Synthesis of mPEG-PDLLA

mPEG-PDLLAs with different mPEG/PDLLA weight ratio of 80:20, 50:50, and 40:60, respectively, were synthesized by a ring opening polymerization procedure. To make sure mPEG-PDLLA biocompatible, reaction glassware was washed and rinsed with sterile water, dried at 105°C, followed by depyrogenation at 250°C for 1 h. Appropriate quantities of D,L-lactide, mPEG, and 0.5% stannous octoate were added to a 250 mL, three-neck glass flask and the reactants were stirred using JB90-D electric stirrer (Changzhou Experimental Instrument Company, Jiangsu, China) under nitrogen stream. Heated the flask to 120–140°C until the reactants melted. Elevated the temperature to 160–180°C and kept for 6 h. The raw product was purified three times by dissolving the product in dichloromethane and precipitating in petroleum ether (0°C). The purified product was dried under vacuum.

Preparation of PRZ/ mPEG-PDLLA Ophthalmic Gel

To evaluate the effect of mPEG-PDLLA on ocular permeability of pirenzepine, 2% PRZ/ mPEG-PDLLA ophthalmic gel (with mPEG-PDLLA) was prepared as follow: Part 1: 4 g of HPMC were added into 100 mL water at 80–90°C and mixed until it was uniformly dispersed. The pH was adjusted to 5.0 ± 1.0 with sodium hydroxide. The mixture was sterilized at 121°C for 30 min. A homogeneous gel was obtained by cooling the mixture to room temperature.

Part 2: PRZ, mPEG-PDLLA were dissolved in acetate buffer solution (pH 5.0) to give 4% PRZ (based on free base) solution. Filtrate the solution through a 0.22 μm microporous membrane (Xinya Pharmaceutical Membrane Company, Shanghai, China).

100 mL PRZ solution of Part 2 was aseptically mixed with 100 mL gel of Part 1. A 2% PRZ/ mPEG-PDLLA ophthalmic gel was resulted and was filled into pre-sterilized ophthalmic container.

2% PRZ ophthalmic gel (without mPEG-PDLLA) was prepared as the similar processes except adding of mPEG-PDLLA.

Characterization of mPEG-PDLLA

The compositions and the number-average molecular weights of mPEG-PDLLA in $CDCl_3$ solution were determined by 500 MHz ^{1}H-NMR (Bruker AMX-500).

The molecular weights of the mPEG-PDLLA were also determined by gel permeation chromatography (GPC) at 45°C using a Shimadzu LC-10A HPLC pump and a Shimadzu RID-10A refractive index detector coupled to a Shodex GPC column (KF-800, 4.6×300 mm, Shodex). The mobile phase was tetrahydrofuran (THF) with a flow rate of 1.0 mL/min. the injection volume of the sample was 20 μL at a concentration of 0.1% (w/v) in THF. The molecular weights of mPEG-PDLLAs were calculated relative to polystyrene standards.

Fluorescence measurement was carried out to determine the critical micelle concentration (CMC) of mPEG-PDLLA block copolymers using pyrene as a fluorescencent probe (Soga & Nostrum, 2004). Briefly, mPEG-PDLLA aqueous solutions with polymer concentrations ranging from 5×10^{-4} to 5 mg/mL were prepared by dissolving mPEG-PDLLA and diluting with water. 15 μL of pyrene dissolved in acetone were added to 5mL of mPEG-PDLLA aqueous solution to provide final pyrene concentration of 6×10^{-6}M. The polymer

solutions with pyrene were incubated for 24 h at room temperature in the dark to allow evaporation of acetone. Fluorescence excitation spectra of pyrene were obtained as a function of the polymer concentration using Model RF-5301PC spectrofluorophotometer (Simadzu, Japan). The excitation spectra were recorded at 37°C from 300 to 360 nm with the emission wavelength at 390 nm.The excitation and emission band slits were 4 and 2 nm, respectively. The intensity ratio of I338/I333 was plotted against polymer concentration to determine the CMC.

Average sizes and size distributions of polymeric micelles in 3% mPEG-PDLLA aqueous solution with or without PRZ in mass ratio 1:1 were determined by dynamic light scattering (DLS) using an argon ion laser (Zetasizer 3000 HS_A, Malvern Instruments, UK).

Infrared Spectra (IR)

PRZ, mPEG-PDLLA 80/20, and PRZ/mPEG-PDLLA 80/20 (1/1.4, m/m) were dissolved in DI water, respectively. Lyophilization was conducted to obtain PRZ, mPEG-PDLLA 80/20, and polymeric micelles of PRZ (1/1.4, m/m) powders. Physical mixture of PRZ/mPEG-PDLLA 80/20 (1/1.4, m/m) was prepared by physically mixing lyophilized PRZ, mPEG-PDLLA 80/20 powders directly.

Infrared spectra (IR) of PRZ, mPEG-PDLLA, and polymeric micelles of PRZ, physical mixture of PRZ/mPEG-PDLLA (1/1.4, m/m) were determined by the KBr method using a NICOLET Impact 400 IR spectrophotometer (Nicolet Instrument Technologies Inc., WI, USA).

Dialysis of PRZ/mPEG-PDLLA Ophthalmic Gel

PRZ release from PRZ/mPEG-PDLLA ophthalmic gel was evaluated over 6 h by a dialysis system, consisting of a dialysis sac (cut-off: 1000 Da, Shanghai Bao Company, China) loaded with 2 mL of 2% PRZ/ mPEG-PDLLA ophthalmic gel and soaked in DI water (receptor) at room temperature and under slow magnetic stirring. At determinate intervals, aliquots of 5 mL of the receptor solution were withdrawn and immediately restored with the same volume of fresh DI water. The amount of PRZ released was determined by HPLC.

Dialysis of 2% PRZ ophthalmic gel against water was conducted as control.

In Vitro Transcorneal Experiments

Receptor Buffer

Receptor solution was glutathione bicarbonate Ringer's solution (GBR), which was composed of 0.092 g/L glutathione, 2.454 g/L sodium bicarbonate, 0.115 g/L calcium chloride dihydrate, 0.358 g/L potassium chloride, 0.159 g/L magnesium hydrochloride pentahydrate, 0.103 g/L sodium dihydrogenphosphate, 6.2 g/L sodium chloride, and 0.9 g/L glucose, and adjusted to pH 7.2.

Experiments

Rabbits were sacrificed by intravenous injection of excess sodium pentobarbital, and the whole eyes were enucleated. The corneas of both eyes were excised and then mounted on a diffusion chamber (Figure 2). 5.00 mL of receptor solution were added to the endothelial side, and 0.5 mL of PRZ ophthalmic gel were added to the epithelial side. The receptor solution was aerated with the mixture of 95% O_2 and 5% CO_2 to maintain oxygenation of cornea. The temperature in the diffusion chamber was maintained at 34 ± 0.5°C by a thermostatic water bath. Receptor buffer was taken out at predetermined time intervals 0.5, 1, 1.5, 2, 3, 4 h, and immediately replaced by fresh receptor buffer, which was aerated before use. Filter the solution through 0.45 μm microporous membrane, the filtrate was kept in 4°C until analyzed by HPLC.

Data Analysis

Cumulative amount of PRZ permeated through cornea (Q) was calculated as follows:

$$Q = V_0(C_n + \frac{V}{V_0}\sum_{i=1}^{n-1} C_i) = V_0 C_n + V\sum_{i=1}^{n-1} C_i$$

where C_n is the concentration of PRZ in receptor solution at *n*th sampling point, and C_i is the concentration of PRZ before *n*th sampling point. V_0 is the volume of receptor solution, and V is the sample volume.

The apparent permeation coefficient (Papp, cm/s) of PRZ was given by:

$$P_{app} = \frac{\Delta Q}{\Delta t \cdot C_0^D \cdot A \cdot 3600}$$

Where C_0^D is the initial concentration of PRZ in donor solution, A is the area of the cornea. $\Delta Q/\Delta t$ is the steady-state rate of drug permeation across the intact cornea.

The Steady-state flux was determined by:

$$Jss = C_0^D \; P_{app}$$

In Vivo Instillation Experiments

New Zealand healthy albino rabbits with body weight between 2.0–2.5 kg were used for in vivo study. 50 μL of PRZ ophthalmic

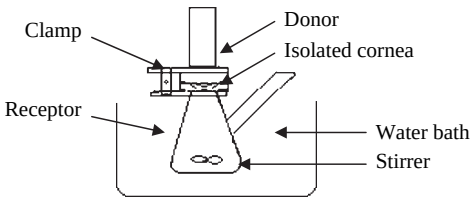

FIGURE 2. Schematic diagram of the corneal diffusion apparatus.

gel or PRZ/ mPEG-PDLLA ophthalmic gel were instilled into the conjunctival sac of each eye of the rabbits. The rabbits were anaesthetized by intravenous injection of 20% urethane (Shanghai Chemical Co., China) solution (1.0 g/kg) at predetermined times. 100 μL of aqueous humor were withdrawn and put into a 1.0 mL Eppendorf tube. 100 μL of methanol were added to precipitate proteins, mixed vigorously for 5 min and centrifuged at 5000 g for 15 min. 20 μL of the supernatant were injected into the HPLC system.

Values for PRZ pharmacokinetic parameters, including observed Cmax, Tmax, AUC_{0-t}, were calculated using standard noncompartmental methods.

HPLC Analysis of PRZ

Analysis of PRZ was conducted by the method reported previously (Tu & Li, 2005). A Luna RP18 5 mm 4.6×150 mm column (Phenomenex Sci-Tech Co. Ltd, CA) and a guard column (Huaiyin Hangbang Sci-Tech Co. Ltd, China.) were employed. The column temperature was kept at 35°C. The mobile phase was methanol/0.02 M KH2PO4/sodium 1-pentanesulfonate (350/650/1, v/v/m, pH was adjusted to 8.0 by adding 1 M NaOH). The flow rate was 1 mL/min and the UV detector was set at 280 nm. The injected volume was 20 μL.

Ocular Chronic Toxicity Study

To test ocular chronic toxicity of mPEG-PDLLA and PRZ/ mPEG-PDLLA ophthalmic gel, sterile mPEG-PDLLA ophthalmic gel at mPEG-PDLLA concentration of 15 and 3%, PRZ/ mPEG-PDLLA ophthalmic gel at PRZ concentration of 9.2, 4.5, and 2.6% were prepared as the procedures in section.

The test solution (50 μL) was applied directly into the conjunctival sac of one eye of the rabbit two times per day for 9 months, the other was NaCl 0.9% solution as control. Each preparation was carried out on 12 rabbits.

For all studies, animals were observed for overt toxicity, mortality at least twice daily. Animal weights and food consumption were determined twice weekly. Ophthalmoscopic examinations on each rabbits were conducted prior to treatment and at study termination (Weeks 39). Blood samples were collected by cardiac puncture. Clinical chemistry and hematology were performed prior to treatment and at study termination (Weeks 39). Hematology parameters evaluated included cell morphology, corrected leukocyte count, erythrocyte count, hematocrit, hemoglobin, leukocyte count, leukocyte differential, and platelet count. Serum chemistry parameters investigated included alanine aminotransferase (ALT), albumin, aspartate aminotransferase (AST), blood urea nitrogen (BUN), lactate dehydrogenase (LDH), alkaline phosphatase, calcium, chloride, creatinine, globulin, glucose, inorganic phosphorus, potassium, sodium, total bilirubin, total cholesterol, total protein, triiodothyronine (T3), and thyroxine (T4). Urinalysis measurements included bilirubin, glucose, ketones, pH, protein, specific gravity, and urobilinogen.

All animals surviving to study termination (Week 39) were anesthetized by methoxyflurane, exsanguinated, and subjected to gross and microscopic examinations. A complete necropsy was performed on all animals. The following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, testes (with epididymides), thymus, and thyroid/parathyroids. The following tissues from each animal were preserved in 10% neutral-buffered Formalin: adrenals, aorta, bone marrow (sternum), brain with brain stem, cecum, esophagus, eyes (fixed in Bouin's and stored in 70% alcohol), femur, heart, kidneys, duodenum, jejunum, ileum, colon, rectum, lacrimal gland, liver, lung, mammary gland, mesenteric lymph nodes, ovaries, pancreas, pituitary, salivary gland, sciatic nerve, skeletal muscle, spinal cord (three levels), spleen, stomach, testes with epididymides, thymus, thyroid/parathyroids, trachea, urinary bladder, uterus, and any other tissues with gross lesions. Preserved tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

RESULTS AND DISCUSSION

Characteristics of mPEG–PDLLA and Polymeric Micelles of PRZ

Three types of diblock copolymers were synthesized, mPEG–PDLLA 80/20, mPEG–PDLLA 50/50, mPEG–PDLLA 40/60. The copolymer nomenclature is designated mPEG-PDLLA X/Y, where X and Y are the weight percentage of monomers mPEG and PDLLA, respectively.

The molecular weights (MW), critical micelle concentrations (CMCs) and the average sizes of micelles of the copolymers were listed in Table 1. The calculated molecular weights

TABLE 1
Molecular Weights, Critical Micelle Concentrations (CMC), and the Average Sizes of Micelles of mPEG-PLA

Polymer	Calculated MW by ^{1}HNMR[a]	Measured MW by GPC	CMC (μg/mL)	Average Size without PRZ (nm)	Average Size with PRZ (nm)
80/20	2560	2048	45.61	25.9	152.5
50/50	4250	5920	10.44	27.1	89.6
40/60	5080	13580	7.55	30.6	50.2

[a]Calculated by $MW=(1+W_{PDLLA}/W_{mPEG})\times 2000$.

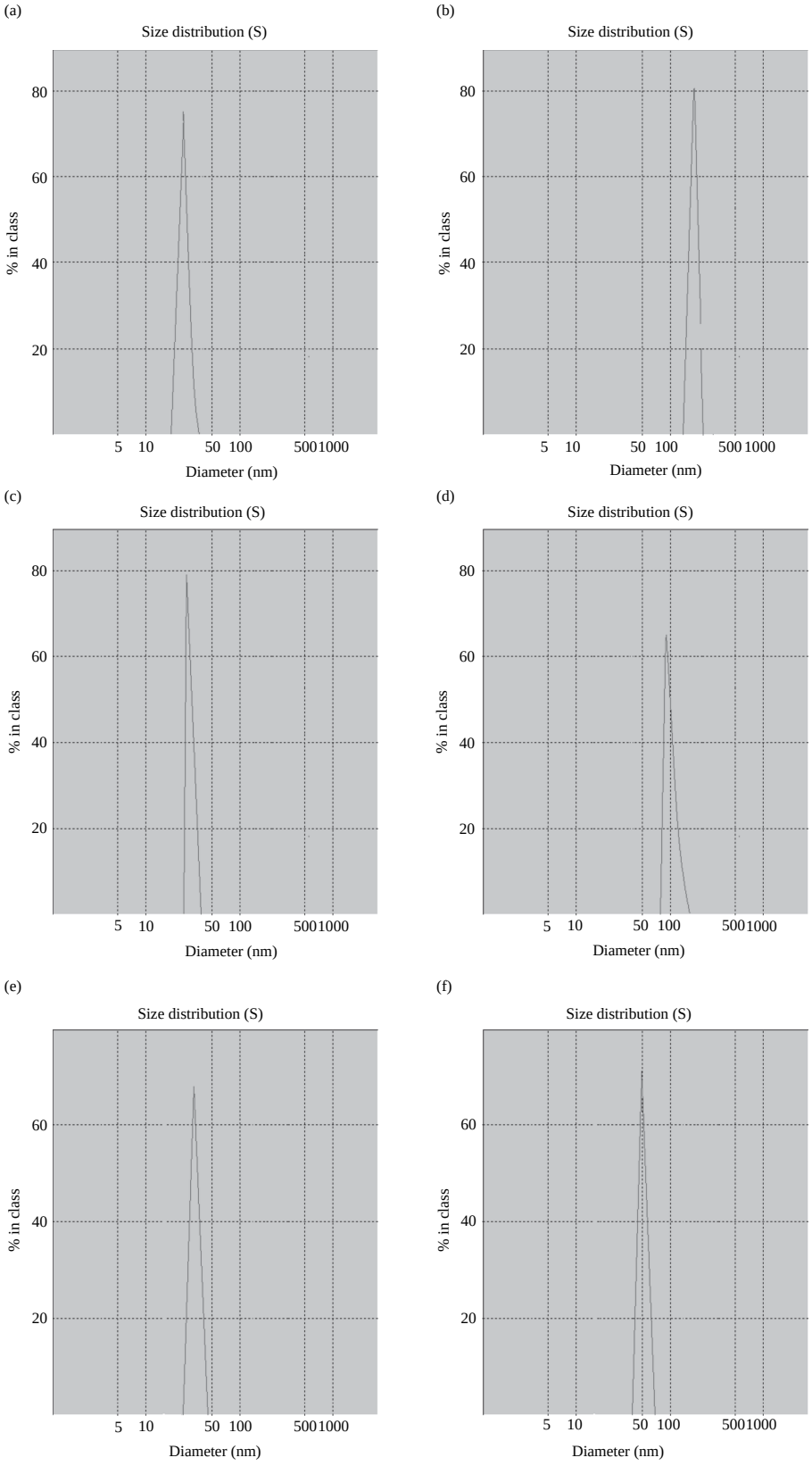

FIGURE 3. Particle size distribution profile of polymeric micelles of mPEG-PDLLA determined by dynamic light scattering (DLS): (a) 80/20 without PRZ, (b) 80/20 with PRZ, (c) 50/50 without PRZ, (d) 50/50 with PRZ, (e) 40/60 without PRZ, (f) 40/60 with PRZ.

of mPEG-PLA by ^{1}HNMR were closer to the theoretical molecular weights of the copolymers than the measured molecular weights by GPC. This can be attributed to that latter was based on polystyrene standards directly without calibration by Mark-Houwink parameters for the copolymers.

The determined CMC values of mPEG-PDLLAs were lower than 50 μg/mL and depend on the weight percentage of PDLLA in copolymer.

Particle size and distribution of polymeric micelles were analyzed by dynamic light scattering (DLS) (Table 1). For blank polymeric micelles of mPEG-PDLLA 80/20, 50/50, and 40/60, the average size was observed as 26, 27, and 30 nm, respectively. After incorporating of PRZ by the ratio of PRZ/ mPEG-PDLLA (1/1.4, m/m), the average size of polymeric micelles increased to be 153, 90, and 50 nm, respectively. The results (Figure 3) indicated that incorporating PRZ into the polymeric micelles resulted in increasing average size of polymeric micelles. According to the order of average size, the order of PRZ content in polymeric micelles could be supposed to be mPEG-PDLLA 80/20 > 50/50 > 40/60. This phenomenon could be explained as that PRZ is adsorbed within mPEG corona of polymeric micelles due to the interaction between hydrophilic PRZ and mPEG corona of polymeric micelles, and this kind of adsorption resulted in increasing of the size of the micelle. The amount of PRZ adsorbed or above-mentioned interaction depends on the weight percentage of mPEG. Higher percentage of mPEG in copolymer will result in higher PRZ incorporation, stronger interaction between PRZ and mPEG and larger particle size.

Because PRZ is water soluble, it is impossible to determine the exact PRZ loading in polymeric micelles. Dialysis experiments were set to investigate the interaction between PRZ and mPEG-PDLLA 80/20. The PRZ dialysis results from PRZ/ mPEG-PDLLA 80/20 and PRZ ophthalmic gel was shown in Figure 4. the release equilibrium reached within 2 h. Nearly complete release of PRZ from PRZ gel was observed, while that was only 80% for PRZ/ mPEG-PDLLA 80/20 gel. The results demonstrated that there is a weak interaction between PRZ and polymeric micelles.

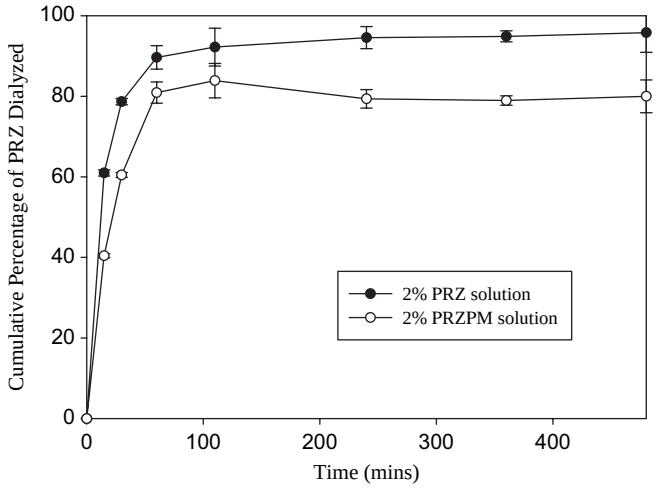

FIGURE 4. PRZ dialysis profiles from PRZPM and PRZ ophthalmic gel.

The FT-IR spectra of PRZ, mPEG-PDLLA 80/20, polymeric micelles of PRZ and physical mixture of PRZ/mPEG-PDLLA 80/20 (1/1.4, m/m) were shown in Figure 5. Distinct absorption peaks of PRZ corresponding to N-H stretching vibration (3502.71, 3434.42 cm^{-1}), C=O stretching vibration (1704.49, 1664.07 cm^{-1}), aromatic C-H stretching vibration (3009.19, 2963.82 cm^{-1}), piperazinyl N-H stretching vibration (2670.04–2424.26 cm^{-1}) were observed with pure PRZ sample. Physical mixture of PRZ and mPEG-PDLLA showed the simple addition of the absorption peaks of PRZ and mPEG-PDLLA. The absoption peaks of piperazinyl N-H stretching vibration (2670.04–2424.26 cm^{-1}) diminished in the polymeric micelles of PRZ. The results confirmed the entrapment of PRZ in polymeric micelles.

In Vitro Transcorneal Experiments

Effect of Composition of Copolymer on Transcorneal Permeability of PRZ

to evaluate the effect of composition of copolymer on ocular permeation of PRZ, different mPEG-PDLLA was added into the PRZ ophthalmic gel in a polymer to PRZ mass ratio of 1.4:1. Figure 6 depicted the in vitro transcorneal permeation profiles of PRZ affected by different mPEG-PDLLA. The results suggested an order of mPEG-PDLLA 80/20 > 50/50 > 40/60 in enhancing the transcorneal permeation of PRZ, which was in reverse order of average size of PRZ/ mPEG-PDLLA micelles. It might due to the differences of the binding capacity between corneal epithelium and polymeric micelles.

Effects of the Mass Ratio of Polymer to Drug on Transcorneal Permeation of PRZ

To optimize the mass ratio of mPEG-PDLLA 80/20 to PRZ, ophthalmic gel of PRZ with the polymer to drug ratio of 0:1, 1.4:1, 2:1, respectively, was used as donor solution. As shown in Figure 7, cumulative amount of PRZ permeated, apparent permeation coefficient, steady-state flux from the ratio of 1.4:1 were greater than that from the other two ratios. The results demonstrated that drug content in polymeric micelles played an important role in PRZ transcorneal absorption.

In Vivo Study

In vivo studies were conducted to compare ocular permeability of 2% PRZ/mPEG-PDLLA ophthalmic gel with PRZ ophthalmic gel. PRZ concentrations in the aqueous humor were measured up to 24 h after instillation. The concentration-time profiles for the two formulations are shown in Figure 8. The PRZ/mPEG-PDLLA preparation yielded significantly higher aqueous humor PRZ concentrations after instillation

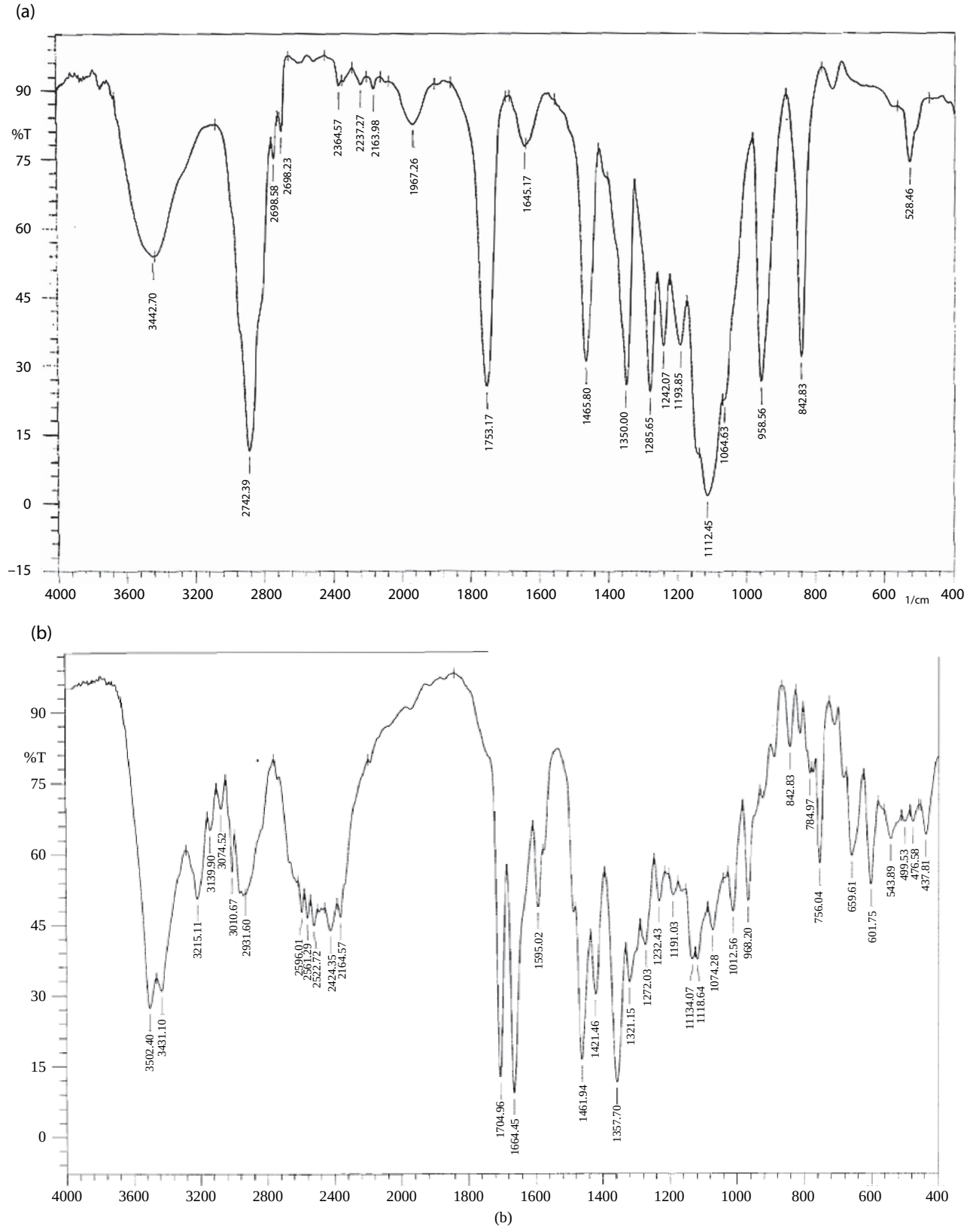

FIGURE 5. IR spectra of (a) mPEG-PDLLA 80/20, (b) PRZ, (c) physical mixture of PRZ/mPEG-PDLLA 80/20 (1/1.4, m/m) and (d) polymeric micelles of PRZ.

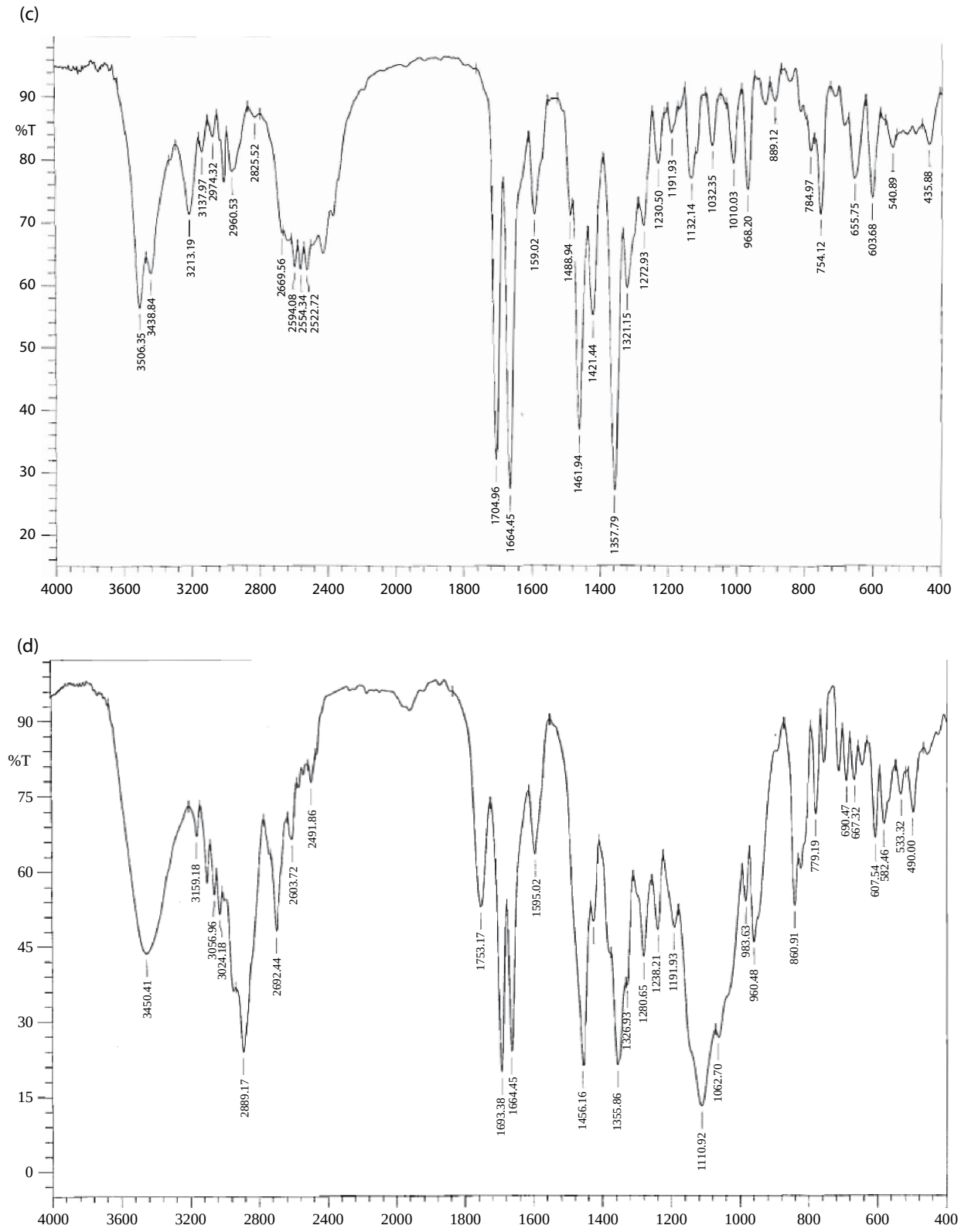

FIGURE 5. *(Continued).*

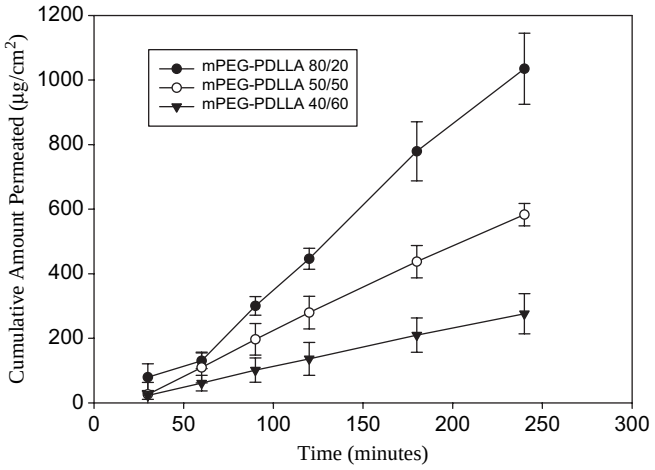

FIGURE 6. Effect of the composition of copolymer on in vitro transcorneal permeation of PRZ.

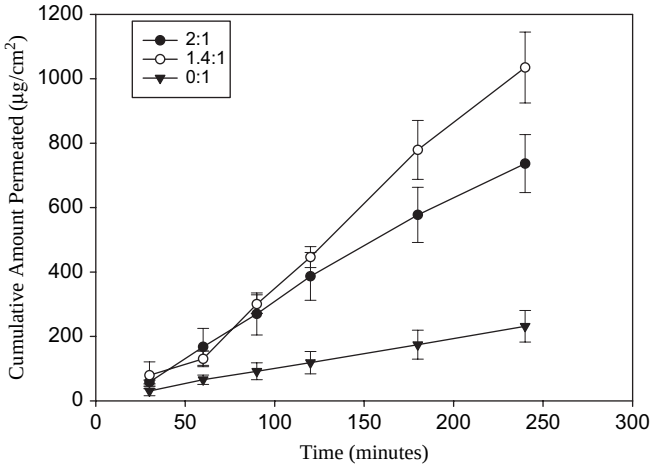

FIGURE 7. Effects of the mass ratio of polymer to PRZ.

than no mPEG-PDLLA preparation. Both formulations showed prolonged release characteristics. The Cmax and AUC_{0-24} of the PRZ/mPEG-PDLLA preparation were 2223.02 ng/mL, 7405.9 ng h/mL, while those of no mPEG-PDLLA preparation were 1035.88 ng/mL, 3069.2 ng h/mL. The Tmax of PRZ/ mPEG-PDLLA preparation was determined about 1 h, which is identical to that of no mPEG-PDLLLA preparations. The bioavailability of the PRZ/mPEG-PDLLA preparation is almost double of PRZ gel. The result is consistent to in vitro transcorneal permeation results.

Ocular Chronic Toxicity Study

Instillation of PRZ/mPEG-PDLLLA and mPEG-PDLLLA into rabbit eyes during a chronic toxicity study did not show any significant toxicity, indicating the PRZ/ mPEG-PDLLLA and mPEG-PDLLA were safe in ophthalmic application.

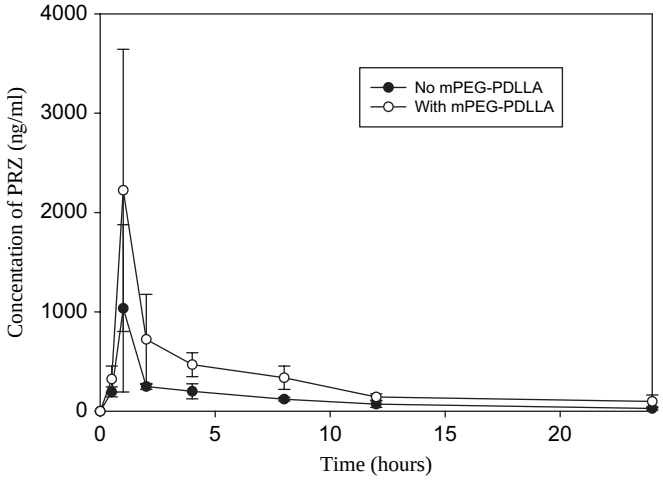

FIGURE 8. Concentration–time profiles of PRZ in the aqueous humor (n = 5).

CONCLUSIONS

Based on both in vitro and in vivo experiments, we could draw a conclusion that mPEG-PDLLA 80/20 is able to enhance the transcorneal permeation of pirenzepine hydrochloride. And the optimal mass ratio of polymer to PRZ was determined as 1.4:1. The mechanism of enhancement was believed to be incorporation of PRZ to polymeric micelles. Furthermore, the ocular chronic toxicity study showed that PRZPM and mPEG-PDLLA 80/20 are safe in ophthalmic application.

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