Synthesis, Characteristics, and Pharmaceutical Properties of Ibuprofen-Cyclodextrin-PEG Conjugate

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The NSAIDs ibuprofen was chemically conjugated to the PEG-graft-β-CyD with an ester bond and its aqueous solubility was clearly improved. The preliminary release profile of ibuprofen in rat gastrointestinal tract contents was performed at 37°C within 12 hours. The polymeric conjugate almost did not release ibuprofen in the contents of stomach, released ibuprofen only 7.4% in the contents of small intestine, and evidently released ibuprofen up to 58.7% in the contents of colon, respectively. These results demonstrated that the polymeric conjugate was site-specifically biodegraded in the rat colonic contents. On the other hand, the xylene-induced ear swelling technique, the hot plate test, and the brewer’s yeast-induced hyperthermia model in mice were performed for evaluating the anti-inflammatory, analgesic, and antipyretic activities of the polymeric conjugate, respectively. The results revealed that the polymeric conjugate maintained a long and stable pharmacodynamic efficiency over a period of 24 hours. Hence, the present polymeric ibuprofen-cyclodextrin-PEG conjugate may be of value as an orally administered long-acting prodrug of ibuprofen through colon-targeting delivery.

1. Introduction

Ibuprofen, a nonsteroidal anti-inflammatory drug (Scheme 1), is widely prescribed to reduce fever and treat pain in clinic [1, 2]. However, oral administration of ibuprofen causes some side effects, such as gastric irritation and gastric bleeding [3–7]. Therefore, a colon-targeting delivery of ibuprofen is expected to be a novel promising formulation for reducing adverse effects of upper gastrointestinal tracts [8–11]. Several methodologies have been developed and applied for colon-targeting delivery of ibuprofen [12–16]. Kalala et al. synthesized poly(ether-ester) azopolymers, which were used to coat capsules of ibuprofen for colon drug delivery. Yashaswini and Swamy developed a multi-particulate system combining pH sensitive property and specific biodegradability for colon delivery of ibuprofen. Ofokansi and Kenechukwu prepared colon-targeted ibuprofen tablets based on Eudragit RL 100-chitosan interpolyelectrolyte complexes for colon drug delivery. The abovementioned coated capsules and tablets of ibuprofen for colon drug delivery are difficult to be prepared through traditional pharmaceutical technologies because the processes are complexly operated.

β-Cyclodextrin (β-CyD) is a kind of macrocyclic oligosaccharides composed of seven α-1, 4-linked D-glucopyranose subunits (Scheme 1), which contains three kinds of hydrophilic hydroxyl groups, i.e., C-6 hydroxyl groups on the narrow side, C-2, and C-3 hydroxyl groups on the other open side [17]. β-CyD and its derivatives are broadly used as hydrophilic excipients in the medicine industry because of outstanding inclusion abilities for a wide range of small molecule drugs [18, 19]. However, the inclusion complexes
of β-CyD are in dynamic balance between host molecules and guest molecules. When the inclusion complexes are taken orally, it would partially dissociate in the gastrointestinal contents, mainly depending on the stability constants of the complexes. Thus, the inclusion complexes of β-CyD are unfit for colon-targeting delivery drugs [20, 21]. One of the methods to avoid the drug dissociation is to link a drug chemically to β-CyD, which produces a new macromolecule named the conjugate [22]. Therefore, the pharmacodynamic and pharmacokinetic profiles of parent drugs can be altered through chemical modifications with β-CyD. In recent years, there are growing interests in developing the chemical β-CyD-based conjugates. Some NSAIDs-CyDs conjugates have been synthesized and their pharmaceutical properties have been evaluated [23]. Dhaneeshwar et al. synthesized and evaluated the primary conjugate of ibuprofen with β-CyD [24] and found that the primary conjugate was a good strategy of covering up the –COOH groups, which reduced the ulcerogenicity. To our knowledge, the solubility of the primary conjugate of β-CyD was poor, probably owing to the strong intramolecular hydrogen bond between the secondary hydroxyl groups. Polyethylene glycol (PEG) is widely used in medicine applications to increase the water solubility, chemical stability, and half-life of drugs [25]. In the present study, aiming at developing the application of ibuprofen/β-CyD conjugate for NSAIDs, we synthesized a novel ibuprofen-β-CyD-PEG conjugate, in vitro studied the release profiles of the polymeric conjugate to evaluate as a colon-targeting delivery prodrug (Scheme 2) and in vivo assessed the polymeric conjugate for the anti-inflammatory, analgesic, and antipyretic activities in mice.

2. Experimental Section

2.1. Chemistry. β-CyD was purified by recrystallization twice in distilled water and was dried under vacuum at 110°C for 24 hours before use. Pyridine (Py) was dried over CaH₂ for 24 hours, distilled, and stored in a brown bottle over 4 Å molecular sieves. All other reagents and chemicals were of commercial grade and used directly without further purification. The inclusion complex of ibuprofen with β-CyD was prepared by the freeze-drying method (the molar ratio of ibuprofen:β-CyD, 1:1:2). The NMR spectra of the polymeric conjugate were measured on a Bruker AM-600 spectrometer in DMSO-d₆ solutions referred to tetramethyldisilane (TMS) as the internal standard.

2.1.1. Synthesis of the Ibuprofen-β-CyD-PEG Conjugate. A solution of acid-terminated PEG (Mw = 600) (6.0 g, 10 mmol) and DCC (6.2 g, 30 mmol) in 150 cm³ dried pyridine was added to the solution of β-CyD (13.6 g, 12 mmol), dimethylaminopyridine (1.0 g, 8.2 mmol), and LiCl (0.7 g, 17 mmol) dissolved in 100 cm³ dried pyridine. Then, the reaction solution was stirred at 0°C for 12 hours and warmed to room temperature for another 48 hours. The precipitate was then filtered, and the solution was evaporated under vacuum until approximately 30 cm³. The obtained solution was precipitated through adding diethyl ether, filtered, and washed with diethyl ether. To the dried precipitant in dried pyridine (200 cm³) under an ice bath, ibuprofen acid chloride (1.2 equiv.) was added. The reaction mixture was stirred under ice bath for 8 hours and warmed to room temperature for another 24 hours. Finally, a small amount of water was added to quench the reaction, and the reaction solvent was evaporated under vacuum. The obtained residue was dissolved in a small amount of DMF and precipitated through adding 300 cm³ of acetone. The obtained precipitate was filtered, washed with acetone, and dried under vacuum. Thus, the ibuprofen-β-CyD-PEG conjugate was obtained in 46% yields. ESI-MS: m/z 1927 ([M + Na⁺]). 13C NMR (150 MHz, DMSO-d₆): δ = 19.0, 22.6, 30.1, 31.2, 44.1, 44.7, 60.4, 68.1, 69.2, 72.5, 72.8, 73.5, 81.3, 81.9, 98.3, 101.9, 102.4, 127.6, 129.4, 135.6, 139.9, 162.8, 174.6.

2.1.2. Hydrolysis of the Ibuprofen-β-CyD-PEG Conjugate. According to the published literature procedures [26], the hydrolysis profiles were performed at 37°C in the gastrointestinal tract fluids from male SD rats, i.e., male Kunming SD rats (200 ± 10 g) were anesthetized with diethyl ether and midline incisions were carried out. Fluids of stomach and intestine were separated, and accurately diluted to half concentration using a pH 4.4 acetate buffer for stomach contents and pH 6.8 phosphate buffer for intestine contents, respectively. Then the dispersions were filtered in a Buchner funnel with gauze. The polymeric conjugate solution (10.0 cm³, 5.0 × 10⁻⁴ mol/L) in 2.0% DMF-buffer) was mixed with the filtrate (10.0 cm³) in an air-tight glass vessel and incubated in the corresponding content at 37°C. The pH value of reaction solutions was regulated to 4.4 (for stomach content) or 6.8 (for intestine content) by the addition of 0.1 mol/L NaOH solution. At an interval of one or two hours, ibuprofen was extracted out from the reaction solution (0.8 cm³) with ethyl acetate. The organic phase was evaporated under vacuum, and the obtained residue was dissolved in analytical methanol (0.1 cm³). The concentration of ibuprofen was determined by HPLC-UV.

2.2. Pharmacological Evaluation. Male Kunming mice (25 ± 2.0 g) were used for the pharmacological studies. The animals were housed under controlled conditions of temperature (25 ± 1°C) and relative humidity (50 ± 5%) on 12 h light/dark cycle, and allowed free access to standard laboratory’s food and water. All the groups of animals chosen for the studies were fasted for 2 days before the treatment and were divided into parallel groups containing 8 animals each. All the animals were treated humanely in accordance with the guidelines by the Institutional Animal Ethics Committee (IAEC). Experiments were approved by the Institutional Animal Care and Use Committee of Ningxia Medical University.

2.2.1. Anti-Inflammatory Activity. Anti-inflammatory activity of the polymeric conjugate was examined by the xylene-induced ear swelling model at equimolar doses
equivalent to 35 mg/kg (ibuprofen) [27]. Mice were divided to groups of 8 animals each. One hour after oral administration of drugs, xylene (20 µL) was applied smoothly to the anterior and posterior surfaces of the right ear of mice. The left ear of mice worked as the control. 30 minutes after xylene application, all the mice were rapidly sacrificed by cervical dislocation, and both ears were surgically removed. Circular sections of the right and left ears were carefully made with a cork borer (diameter of 8 mm), weighed, and measured. The value of ear edema was calculated based on the weight of the testing group and the control group.

2.2.2. Analgesic Activity. Analgesic activity of the polymeric conjugate was examined by the hot plate test (55 ± 2°C) using the YLS-6B apparatus (Precision Instruments Co., Ltd., Shanghai) [28]. Before actually testing on the hot plate, the mice were in the habit of the testing apparatus for 1 min. Thirty minutes later, mice were put on the prepared hotplate to determine the value of baseline responsiveness. When the animal licked one of its fore paws or hind paws or jumped, the time was recorded (latency time, in seconds). To prevent tissue damage, a 50 s cut-off was set. The mice, presenting latency time less than 5 s or higher than 30 s, were excluded from the test. After drugs at equimolar doses equivalent to 35 mg/kg (ibuprofen) were orally administered, the hot plate tests were carried out successively after 0.5, 1, 4, 12, and 24 h.

2.2.3. Antipyretic Activity. Antipyretic activity of the polymeric conjugate was examined by monitoring the temperature variation of the rectum according to the published procedure [29]. To induce fever in mice, they were injected with 30% brewer’s yeast in normal saline (10 mg/mL) subcutaneously. Using a lubricated digital thermometer, initial rectal temperature was recorded by inserting it into the rectum of the mice at a depth of around 7 mm. To minimize the possible experimental error, the same thermometer was used for all the mice in each group. 7 h after brewer’s yeast injection, drugs at equimolar doses equivalent to 35 mg/kg (ibuprofen) were orally administered. The rectal temperature was monitored successively after 0.5, 1, 4, 12, and 24 h.

2.3. Statistical Analysis. All the values are mean of five independent determinations. Data are expressed as mean ± SEM and were tested by one-way analysis of variance (ANOVA, Dunnett’s test) for the possible significant identification between various groups. The P value of <0.05 or <0.01 was considered as statistically significant or highly significant, respectively. Statistical analysis was performed by software SPSS 13.0.

3. Results and Discussion

3.1. Chemistry

3.1.1. Synthesis and Characterization. The ibuprofen-β-CyD-PEG conjugate was prepared in a simple, two-step process. In the first step, the PEG-graft-β-CyD was synthesized by using the combination of β-CyD and PEG (MW 600) in the presence of dicyclohexylcarbodiimide (DCC). In the second step, ibuprofen (Ibu) was conjugated to PEG-graft-β-CyD through an ester bond.

The structure of ibuprofen-β-CyD-PEG conjugate was characterized by MS and NMR spectra. Its ESI-MS spectrum showed the molecular ion [M+Na]+ at m/z 1927. Because 1H NMR spectra of CyDs are rather complicated existing overlap, 13C NMR spectra are often used for the analysis of CyDs. As reported in detail by Breslow [30], arylation of a hydroxyl group of CyDs often leads to a downfield chemical shift of α-carbon, a small upfield chemical shift of β-carbon, and a little smaller shift of γ-carbon. As shown in Figure 1, 13C NMR of the polymeric conjugate demonstrated a chemical shift at δ 98.3 ppm (C-1′), which obviously indicates that the ibuprofen group was at the 2-position of β-CyD. On the other hand, 13C NMR of the polymeric conjugate also showed the peaks (Cβ 127.6, Cc 129.4, Ca 135.6, and Cd 139.9 ppm) corresponding to the aromatic ring part of ibuprofen, and the peaks (Ct 19.0 and Ce 22.6 ppm) corresponding to the methyl groups of ibuprofen.

The aqueous solubility of the polymeric conjugates was approximately 32.3 mg/cm² at 37°C, which was equivalent to 3.5 mg/cm² ibuprofen. Thus, the solubility of ibuprofen (about 0.077 mg/cm² at 37°C) was markedly increased 45 folds by formation of the polymeric ibuprofen-β-CyD-PEG conjugates.

3.1.2. Hydrolysis in Rat Gastrointestinal Tract Fluids. The in vitro release profile of ibuprofen in rat gastrointestinal tract contents was studied at 37°C. As shown in Figure 2, the ibuprofen-β-CyD-PEG conjugate almost did not release ibuprofen in the contents of stomach, released merely 7.4% ibuprofen in the contents of small intestine, and released ibuprofen up to 58.7% in the contents of the colon after 12 h, respectively. These results demonstrated that the polymeric conjugate was site-specifically biodegraded in the rat colonic contents. The mechanisms of drug release from the polymeric conjugates are that the polymeric conjugates were biodegraded into small saccharide derivatives by the enzymes in the rat colon [26], and subsequently released ibuprofen. On the other hand, the release curves of the polymeric conjugates demonstrate that ibuprofen can be smoothly released in a long time and minimized the initial burst release.

3.2. Pharmacology

3.2.1. Anti-Inflammatory Activity. The anti-inflammatory effect of the polymeric conjugate was studied in vivo by the xylene-induced ear edema in the mice model. Figure 3 shows the anti-inflammatory profiles of ibuprofen from the polymeric conjugate, the complex of ibuprofen with β-CyD, and the parent drug ibuprofen. One hour after oral administration of drugs, the polymeric conjugate, the complex of ibuprofen with β-CyD, and the parent drug ibuprofen produced obvious reductions of edema (P < 0.01) with anti-inflammatory effects of about 45.7%, 59.3%, and 49.4%,
respectively. According to the reported literature [31], the complex of ibuprofen with β-CyD exhibited the best anti-inflammatory activity possibly due to the enhanced dissolution rate of ibuprofen, and the polymeric conjugate had the moderate anti-inflammatory activity probably due to a slow release of ibuprofen from the prodrug.

3.2.2. Analgesic Activity. The analgesic effect of the polymeric conjugate was studied in vivo by the hot plate test in the mice model. The hot plate tests were performed successively 0, 0.5, 1, 4, 12, and 24 hours after oral administration. In general, the polymeric conjugate, the complex of ibuprofen with β-CyD, and the parent drug ibuprofen was
quite effective in inducing antinociception in the hotplate tests at 30 minutes after their administration (Figure 4). Though the complex of ibuprofen with β-CyD was more effective than the parent drug ibuprofen in the hotplate tests, the similar tendency was found that the antinociceptive effect of the two groups had subsided 1 hour after their administration. However, the polymeric conjugate had a more prolonged antinociceptive effect in the hotplate test, and its maximum antinociception appeared at around 4 hours after its administration. On the other hand, it is evident that the polymeric conjugate maintained a long and
stable antinociceptive efficiency over a 24 h period, probably due to the ability of time-release of ibuprofen produced by the controlled hydrolysis of the polymeric conjugate.

3.2.3. Antipyretic Activity. The antipyretic effect of the polymeric conjugate was studied in vivo by means of 30% brewer’s yeast-induced hyperthermia in the mice model. Rectal temperature of mice was recorded successively 0, 0.5, 1, 4, 12, and 24 hours after oral administration. As shown in Figure 5, the polymeric conjugate, the complex of ibuprofen with β-CyD, and the parent drug ibuprofen displayed the potent antipyretic activity. The complex of ibuprofen with β-CyD showed the potent antipyretic tendency similar to that of the parent drug ibuprofen over a 24 h period, and their maximum antipyretic effects appeared at around 4 hours after their administrations, which are consistent with the instruction of ibuprofen orally taken every 4–6 h [2]. However, the polymeric conjugate maintained a long and stable antipyretic efficiency over a 24 h period, probably on account of the ability of time-release of ibuprofen produced by the controlled hydrolysis of the polymeric conjugate. The results were in agreement with those of their analgesic activity.

4. Conclusion

Colon-targeting drug delivery can be achieved with special carriers by designing conjugates of drugs, which can integrally survive passage through stomach and release active moiety by enzymes in colon. Ibuprofen was chemically linked to PEG-graft-β-CyD conjugate through an ester bond, and its aqueous solubility was greatly improved by 45-fold. The in vitro release profile of ibuprofen indicated that activation of the polymeric conjugate happened specifically in the rat colon. Moreover, the polymeric conjugate demonstrated a long and stable pharmacodynamic efficiency over a 24 h period in mice, including the anti-inflammatory, analgesic, and antipyretic activities. Therefore, the present polymeric ibuprofen-cyclodextrin-PEG conjugate may be of value as an orally administered long-acting prodrug of ibuprofen through colon-targeting delivery.

Data Availability

The NMR and MS data used to support the findings of this study are included within the article.

Conflicts of Interest

All the authors declare no conflicts of interest.

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