Synthesis of Poly(Hydroxypropylglutamine-Prazosin Carbamate) and Release Studies

Xiaoling Li,[1] Nathan W. Adams,[1] David B. Bennett,[1] Jan Feijen,[2] and Sung Wan Kim[1,3]

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Prazosin, an antihypertensive drug with postsynaptic α_1-adrenergic blocking activity, has been coupled to poly-N^5-(3-hydroxypropyl-L-glutamine) (PHPG) via a carbamate linkage. PHPG was activated by *p*-nitrophenyl chloroformate and then reacted with prazosin to form p(HPG-prazosin carbamate) conjugate. Drug loading was 23.9% (w/w). Activated polymer and conjugates were characterized by infrared spectroscopy and differential scanning calorimetry. *In vitro* studies proceeded in pH 7.4 isotonic phosphate-buffered saline solution. Prazosin was released at a rate of 0.92 mg/day/100 mg conjugate from p(HPG-prazosin carbamate) particles. *In vivo* studies were performed with New Zealand White rabbits. P(HPG-prazosin carbamate) conjugate particles (100 mg) were suspended in 2 ml saline and injected subcutaneously into both flanks of rabbits. P(HPG-prazosin carbamate) conjugates, following an initial burst, demonstrated a nearly constant plasma prazosin concentration profile above 2 ng/ml, which was maintained for 10 days.

KEY WORDS: polymeric prodrug; antihypertensive drug delivery system; prazosin; poly-N^5-(3-hydroxypropyl-L-glutamine); polymer.

INTRODUCTION

Prazosin, a selective postsynaptic α_1-adrenergic blocking agent, has been used in the treatment of hypertension and congestive heart failure. As an antihypertensive drug, prazosin lacks major symptomatic side effects, but it has a relatively low oral bioavailability of 43.5 to 69.3% and a short half-life of 2 to 3 hr (1), which constrain hypertensive patients to take the drug more than once a day. Such dosage regimens can result in poor patient compliance. The polymeric prodrug concept may be a good approach to the development of a long-term controlled-release delivery system for prazosin to improve patient compliance and eliminate fluctuations in drug plasma levels.

Efforts have been made to achieve the prolonged administration of prazosin by transdermal delivery (2,3), sustained-release granules (4), slow-release tablets (5), suppositories (6), and an osmotic pump (7). The objective of this study is to design a long-term release polymeric prodrug which can release drug from a water-insoluble polymeric prodrug by the hydrolysis of a labile bond between prazosin and a polymer backbone *in vivo*. A polymeric α-amino acid or its derivative is used as the backbone for this biodegradable delivery system and the drug is coupled to the backbone via a covalent bond (8,9). Since a biodegradable polymer is utilized, the system need not be removed after depletion of the drug.

Generally, two physicochemical processes are involved in drug release: (i) hydrolysis of a labile bond between the drug and the polymer backbone and (ii) diffusion of a free drug through the polymeric matrix. The latter is usually rapid and the former is considered to be the rate-limiting step. The drug is considered to have constant chemical activity in the bound (solid) state (10). Therefore, zero-order release of drug can be approached when the polymeric prodrug is insoluble in the release medium.

Our choice of a polymer backbone, poly-N^5-(3-hydroxypropyl-L-glutamine) (PHPG), which can be degraded into nontoxic amino acids, has been studied by several investigators (11–13) and its biodegradability has been shown. The biodegradable polymer–drug conjugate was synthesized by covalently coupling prazosin via its primary amino group to PHPG. An injectable system was made by grinding the conjugate into a size (20.8 ± 5.6 μm) small enough to allow passage through a syringe needle. The release of prazosin occurred via hydrolysis of the carbamate linkage between prazosin and PHPG and showed a zero-order pattern.

MATERIALS AND METHODS

Chemicals and Reagents. Prazosin was purchased from the U.S. Pharmacopoeial Convention (Rockville, MD). PHPG (MW 40,000) was purchased from Sigma Chemical (St. Louis, MO). *p*-Nitrophenyl chloroformate was purchased from Aldrich Chemical (Milwaukee, WI). Trimazosin was obtained from Pfizer Laboratories (Groton, CT) as a gift. LyphoMed (LyphoMed, Inc., Rosemont, IL) heparin sodium injection (10,000 USP U/ml) was used. Spectra/Por dialysis tubing (MW cutoff, 3500) was purchased from Spectrum Medical Industries, Inc. (Los Angeles, CA). All other chemicals used were of reagent, spectrometric, or HPLC grade. Reagent-grade dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were distilled under reduced pressure from appropriate desiccants (phosphorous pentoxide and calcium hydride, respectively) immediately before use. Reagent-grade triethylamine was distilled from calcium hydride and stored over sodium hydroxide until used. All other chemicals were used as received. New Zealand White male rabbits were housed in individual cages.

Spectroscopic Analysis. Infrared (IR) analysis was performed on a Beckman Microlab 620 MX computing spectrometer (Beckman Instruments, Inc., Fullerton, CA). Polymers were ground in an agate mortar and pestle with potassium bromide and pressed in a die to form pellets.

Differential Scanning Calorimetry (DSC). DSC thermograms were recorded on a Perkin–Elmer DSC-4 (Norwalk, CT) with a System 4 Thermal Analysis Microprocessor Controller, a Thermal Analysis Data Station, a TADS-1 Interface, and a Graphics Plotter 2. Aliquots of samples (2 to 5 mg) were used for measurements and calorimetric changes were recorded. PHPG, prazosin hydrochloride, a mixture of prazosin hydrochloride and p(HPG-*p*-nitrophenyl carbonate), and p(HPG-CO-prazosin) were each analyzed by DSC.

[1] Department of Pharmaceutics and Center for Controlled Chemical Delivery, University of Utah, Salt Lake City, Utah 84108.
[2] Department of Chemical Technology, University of Twente, Enschede, The Netherlands.
[3] To whom correspondence should be addressed.

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The thermograms were compared to observe the endo- or exothermal changes.

High-Performance Liquid Chromatographic (HPLC) Analysis. For *in vivo* studies, a Waters HPLC system (two Waters 501 pumps, automated gradient controller, 712 WISP, and 745 Data module) (Milford, MA) with a Shimadzu RF-535 fluorescence detector (Columbia, MD) and a 0.5-μm filter and a Rainin (Woburn, MA) 30 × 4.6-mm Spheri-5 RP-18 guard column followed by a Waters 30 cm × 3.9-cm i.d. (10-μm particle size) μ-Bondapak C_{18} column were used. The mobile phase consisted of a 45% aqueous solution, composed of 0.25% triethylamine, 0.9% phosphoric acid, and 0.01% sodium octyl sulfate, and of 55% methanol. The flow rate was 1.0 ml/min. Prazosin was measured at 384 nm after excitation at 340 nm (14).

Prazosin hydrochloride was dissolved in methanol and then diluted with distilled water. Plasma (200 μl) from New Zealand White rabbits was spiked with 10 μl of prazosin hydrochloride standard (11–704 ng/ml) giving concentrations between 0.478 and 30.610 ng of free base/ml of plasma. Trimazosin hydrochloride (26.6 ng) was added as an internal standard. A saturated sodium chloride solution (400 μl) was added to improve recovery (15) and the total volume was adjusted to 1.0 ml with distilled water. Sodium hydroxide solution (2 *N*, 200 μl) was then added, immediately followed by the addition of ether, and the sample was vortexed for 3 min. After centrifuging (3000 rpm) for 10 min, the aqueous phase was frozen in an acetone–dry ice bath. The ether layer was decanted and blown dry with nitrogen. The residue was dissolved in 200 μl of mobile phase and transferred to an HPLC microinsert. A 100-μl aliquot was injected onto the column. The ratio of the internal standard peak area to prazosin peak area was used to construct the standard curve. A typical chromatogram is shown in Fig. 1.

For *in vitro* release studies, the same system and mobile phase were used, but prazosin was detected with a Waters 484 UV detector at 254 nm.

*Preparation of p(HPG-*p*-nitrophenyl Carbonate).* PHPG (1.000 g, 5.370 mEq OH), anhydrous triethylamine (1.12 ml, 8.04 m*M*), and dimethylaminopyridine (DMAP; 65.6 mg, 0.537 m*M*) were dissolved in 45 ml of freshly distilled DMF and the solution was cooled to 4°C. *p*-Nitrophenyl chloroformate (1.624 g, 8.057 m*M*) was added and the reaction mixture stirred for 72 hr. The mixture was then added dropwise, with vigorous stirring, to 1 liter of ether and stirred overnight. The suspension was filtered and the residue was Soxhlet-extracted with absolute ethanol overnight. The residue was then dried under vacuum to give 1.147 g of poly(hydroxypropylglutamine-*p*-nitrophenyl carbonate) as an off-white powder. IR (cm^{-1}): 3450, 3340, 3250, 3020, 2950, 2860, 1750, 1650, 1590, 1570, 1490, 1440, 1390, 1280, 1250, 1220, 1190, 1020, 850, 790.

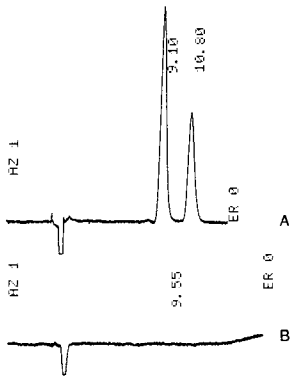

Fig. 1. (A) Chromatogram of extracted plasma containing prazosin (9.10 min) and the internal standard trimazosin (10.80 min). (B) Chromatogram of extracted blank plasma.

Preparation of p(HPG-Prazosin Carbamate). p(HPG-*p*-nitrophenyl carbonate) (1.000 g, 2.846 mEq active ester) was suspended in anhydrous DMSO and sonicated for 20 min. Prazosin hydrochloride (1.434 g, 3.416 m*M*) and triethylamine (0.873 ml, 6.26 m*M*) were added and the mixture stirred at room temperature with the exclusion of light for 72 hr. The mixture was then poured into 1 liter of ether and stirred overnight. The suspension was filtered and the residue was Soxhlet-extracted with absolute ethanol for 3 days. The residue was then dried under vacuum to give 1.491 g of product as an off-white powder. The IR spectrum was then measured.

In Vitro *Release Studies.* Twenty milligrams of p(HPG-prazosin carbamate) particles, 20.8 ± 5.6 μm, were packed in a cellulose acetate dialysis bag with 200 μl of pH 7.4 isotonic phosphate-buffered saline (PBS). The dialysis bags were suspended in bottles filled with 200 ml of PBS. The bottles were placed in a shaking water bath (37°C) at 50 strokes/min. Upon sampling, the entire release medium was replaced with fresh PBS to maintain sink conditions. Prazosin concentrations in release samples were determined by HPLC. Release rate was normalized to 100 mg of conjugate. Drug loading was calculated by accumulating released prazosin.

In Vivo *Studies.* P(HPG-prazosin carbamate) particles were sterilized by washing with 75% ethanol. Exactly 100 mg of the sterilized particles was suspended in 2 ml of sterile saline, and 1 ml was injected subcutaneously into each flank of a 2-kg New Zealand White male rabbit. Three rabbits were used in the *in vivo* study. Approximately 0.5 to 1.0 ml of blood was collected from ear veins, with a syringe which was wetted with heparin sodium, after the first 6 hr and every 24 hr thereafter. The blood samples were collected until 30 days after p(HPG-prazosin carbamate) was initially given. Blood samples were then centrifuged and plasma frozen for later analysis. Aliquots (200 μl) of plasma were worked up using the same protocol as the standards. Prazosin plasma levels were determined by comparison with a standard curve.

RESULTS AND DISCUSSION

From earlier studies it has been shown that the drug release rate from a polymeric prodrug was governed by (i) the hydrophobicity and/or solubility of the polymeric prodrug, (ii) the length of the spacer group between drug and polymer backbone, (iii) the lability of the covalent bond which links the drug to the polymer, (iv) the initial drug loading, and (v) the particle size or geometry of the device (9). The type of linkage between the polymer backbone and drugs, i.e., ester, amide, or carbamate, plays an important role in determining the drug release rate. It has been shown

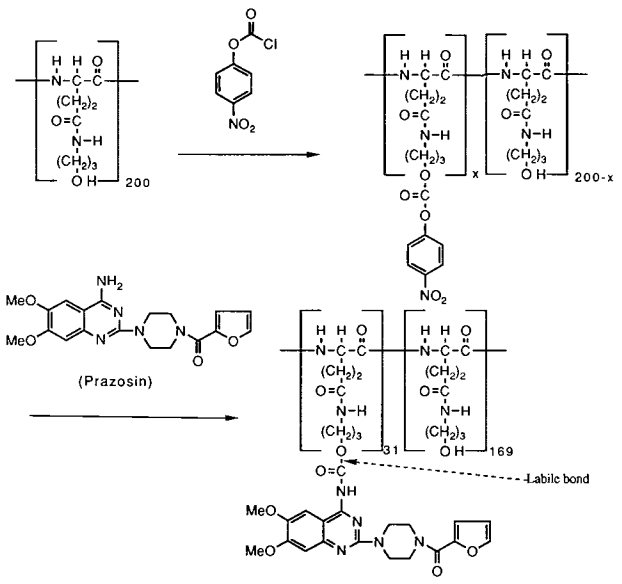

Fig. 2. Synthesis of p(HPG-prazosin carbamate).

that hydrolysis of carbamate is markedly slower than that of ester linkages (16), while amide linkages are more stable against hydrolysis than either ester or carbamate linkages. Our previous work (10) suggested that prazosin could be coupled to poly(glutamic acid) via an amide bond to its primary amino group, the only appropriate functional group for reaction (see Fig. 2). However, the hydrolysis of an amide bond to release prazosin would be too slow to deliver effective quantities of drug. Therefore, we designed a carbamate linkage between polymer and prazosin which is expected to be more stable than an ester linkage but less stable than an amide bond. The system should have an appropriate release rate for prazosin.

The exothermal and endothermal peaks for p(HPG-prazosin carbamate) and a prazosin + P(HPG-*p*-nitrophenyl carbonate) mixture were 314.56 and 286.23°C and 310.5 and 290.25°C, respectively. P(HPG-*p*-nitrophenyl carbonate) showed a T_g at 174.09°C and an exotherm at 316.31°C. The incorporation of prazosin was estimated as 25.1% (w/w) by endothermal peaks, which is consistent with results from *in vitro* release studies.

It has been shown that hydroxyl groups on polymer or solid surfaces can be activated by forming a carbonate ester (17–19). Subsequently, amines can react with carbonate ester and give a product with a carbamate linkage. *p*-Nitrophenyl chloroformate is one of the reagents used in the

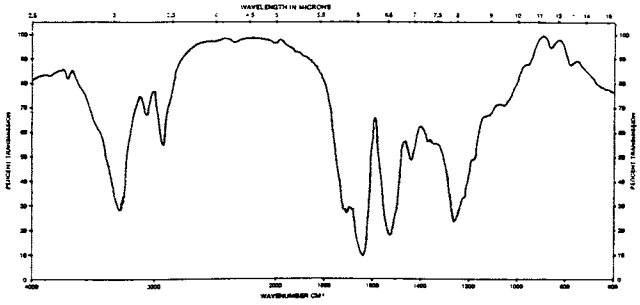

Fig. 3. IR spectrum for p(HPG-prazosin carbamate).

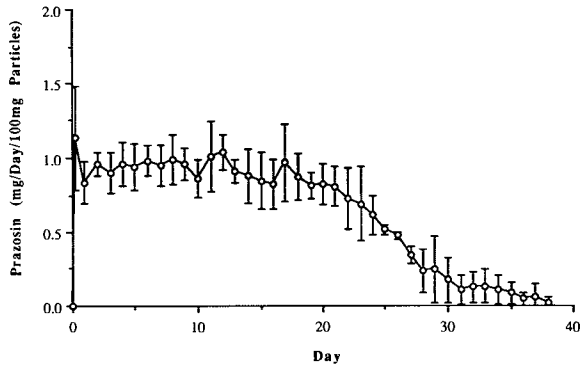

Fig. 4. *In vitro* release profile of p(HPG-prazosin carbamate); n = 3; drug loading, 23.9% (w/w).

activation of hydroxyl groups. PHPG was activated by acylating it with *p*-nitrophenyl chloroformate to give an activated intermediate which gave an IR absorbance at 1750 cm^{-1}, an indication of the formation of carbonate ester. The activated polymer was then reacted with the amino groups on prazosin to yield a conjugate which was insoluble in water and organic solvents. This prevented analysis by NMR. However, IR spectra showed strong absorption at 1712 and 1725 cm^{-1}, which suggests the presence of carbamate carbonyls (see Fig. 3). Nevertheless, the long-term *in vitro* and *in vivo* release observed was strong evidence for the covalent attachment of prazosin to PHPG, since these periods were much longer than would occur in diffusion from a mixture.

In vitro release studies of p(HPG-prazosin carbamate) gave constant release of prazosin for 3 weeks, with a rate of 0.92 mg/day/100 mg conjugate (see Fig. 4). A linear relationship for the cumulative release of prazosin from polymeric prodrug and release time indicated that the release rate was zero order for up to 22 days after release (see Fig. 5). Prazosin was the only species detected by HPLC in the release medium. *In vitro* studies showed a release rate that was promising for *in vivo* studies, which would require an antihypertensive dosage of 1 mg/day in humans (20). Prazosin loading, as determined by depletion of the system, was 23.9% (w/w), which was less than one-third of the theoretical loading. Morphology of the particles was observed with scanning electronic microscopy. After drug release, the par-

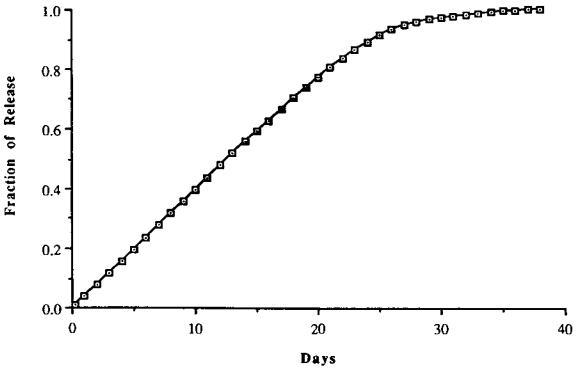

Fig. 5. Cumulative *in vitro* release of prazosin from p(HPG-prazosin carbamate); drug loading, 23.9% (w/w).

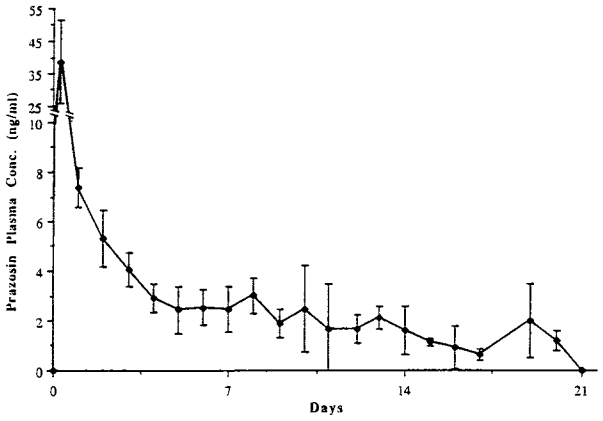

Fig. 6. *In vivo* study of p(HPG-prazosin carbamate); $n = 3$; drug loading, 23.9% (w/w).

ticles lost their dense nature, becoming more porous and smaller fragments.

Pharmacokinetic and pharmacodynamic studies for prazosin have been reported for rabbits (21,22). The present *in vivo* study for p(HPG-prazosin carbamate) showed that, following an initial burst, a nearly constant plasma level was maintained at ~2 ng/ml for over 10 days, although a gradual decrease was seen (see Fig. 6). The plasma level was slightly lower than the level which was predicted from the *in vitro* release studies using pharmacokinetic parameters from the literature (21,22). This may be accounted for by the formation of a fibrous capsule around the conjugate particles which could build up a barrier against drug release. After 3 weeks, prazosin levels fell below the detection limit. This was consistent with the *in vitro* release study in which the release rate dropped after 21 days. The burst effect may be due to local tissue inflammation. It would be expected that inflammation would decrease local tissue pH (23,24) and the release of hydrolytic enzymes could increase the hydrolysis of labile bonds, thereby increasing the prazosin release rate and, subsequently, increasing the prazosin plasma level.

The p(HPG-prazosin carbamate) prodrug used in this study may represent a new approach in the treatment of chronic hypertension. The long-term zero-order release of prazosin gave a constant plasma level, avoiding the sawtooth effect seen with most conventional dosage forms. Therapeutic efficiency and patient compliance may be improved with this new concept.

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