Pharmaceutical Research, Vol. 4, No. 4, 1987

Research Article

Coupling of Naltrexone to Biodegradable Poly(α-Amino Acids)

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Received January 21, 1987; accepted April 20, 1987

The narcotic antagonist naltrexone (I) was modified at the 3 and 14 hydroxyl positions and covalently coupled to a biodegradable poly(α-amino acid) backbone through a labile bond. Selective acetylation of I with acetic anhydride gave naltrexone-3-acetate (II), which was subsequently succinoylated to naltrexone-3-acetate-14-hemisuccinate (III) with succinic anhydride. The polymeric backbone chosen for initial coupling experiments was poly-N^5-(3-hydroxypropyl)-L-glutamine (PHPG). The side-chain hydroxyl functionality permitted covalent bonding of III through an ester linkage. Hydrolysis of covalently bound drug to give naltrexone or its derivatives (II and III) should be much slower than diffusion of drug through the polymer matrix. While hydrolysis of naltrexone from the polymer side chain is first order, release of drug from the matrix can be zero order due to the geometry of the device and the physical and chemical interactions between naltrexone and the polymer matrix. *In vitro* studies of PHPG–naltrexone conjugate in disk form did not show constant release because of the hydrophilic nature of the polymer backbone and the changing local chemical environment upon hydrolysis of drug–polymer linkages. The conjugated system was made more hydrophobic by coupling drug to copolymers of hydroxypropyl-L-glutamine (HPG) and L-leucine. Conjugates of III coupled with copoly(HPG-70/Leu-30) demonstrated a nearly constant, but slightly declining release rate of naltrexone and its derivatives for 28 days *in vitro*.

KEY WORDS: naltrexone; narcotic antagonist; biodegradable; poly(α-amino acid); poly-N^5-(3-hydroxypropyl)-L-glutamine (PHPG); copoly(hydroxypropyl-L-glutamine/L-leucine).

INTRODUCTION

Systems for the prolonged release of the narcotic antagonist naltrexone (I) from polymer matrices have been investigated for the long-term treatment of opiate addiction (1–5). Biodegradable polymer drug delivery systems have generally involved dispersion of the drug in a polymer matrix (6). Release of the drug in these systems is by either diffusion through the matrix, erosion of the matrix, or a combination of both. A new approach in the release of naltrexone from polymeric systems is the use of covalently bound polymer–drug compounds that are biodegradable and do not require removal after implantation.

Two physicochemical processes may influence drug release from the designed systems. First, drug is released from the backbone polymer by hydrolysis (enzyme and/or acid–base catalyzed). Second, free drug diffuses through the polymer matrix. In general, the rate of hydrolysis will be slower than the rate of diffusion and the release rate is expected to be governed by the rate of hydrolysis. This rate-determining process occurs at the boundary between unaffected nonswollen polymer and previously swollen, degraded, or permeated material. To obtain true zero-order release from this type of device, a stringent geometric requirement is imposed. Constant delivery rates are provided only by slab-shaped devices. Delivery rates that are seen to decrease with time will result from eroding cylinders and spheres, although the rate-determining kinetic process is, in fact, zero order (7).

There are five major parameters that can be varied to control the rate of drug release from the system: (i) the hydrophilic character and molecular weight of the backbone polymer, (ii) the length of the spacer group, (iii) the lability of the covalent bond to the drug, (iv) the initial drug loading, and (v) the particle size or geometry of the device. It has been shown (8,9) that near-zero-order release of steroids from such polymer–drug conjugates can be achieved.

Poly(hydroxyalkyl)-L-glutamines have been studied by several investigators (10,11). These polymers are prepared through the base-catalyzed polymerization of γ-benzyl-L-glutamate *N*-carboxyanhydride, giving poly(γ-benzyl-L-glutamate), followed by the displacement of the benzyl group with the desired hydroxyalkylamines. This study involves the use of the hydroxypropylamine to give poly-N^5-(3-hydroxypropyl)-L-glutamine (PHPG). The polymer is water soluble but becomes insoluble upon substitution with hydrophobic drugs (12). To investigate the effects of increased backbone hydrophobicity, hydroxypropyl-L-glutamine (HPG) was copolymerized with L-leucine using a molar feed ratio of 70/30 (HPG/Leu).

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0724-8741/87/0800-0305$05.00/0

In order to couple I covalently to the PHPG backbone, the phenolic hydroxyl group at the 3 position was protected by acetylation, and the 14 aliphatic hydroxyl group was succinoylated to provide a spacer between drug and polymer. We wish to report a new derivative of naltrexone which is appropriate for attachment to biodegradable poly(α-amino acids) and *in vitro* release data of naltrexone–polymer conjugates. The *in vitro* release studies utilized the polymer–drug conjugate in disk form. Our ultimate aim is to develop a system that can be injected subcutaneously as a suspension of fine particles which will deliver therapeutically effective levels of naltrexone for periods of 1 month to 1 year.

MATERIALS AND METHODS

Chemicals and Reagents. Naltrexone hydrochloride salt was obtained from Research Triangle Institute, Research Triangle Park, N.C. All chemicals were reagent or spectrometric grade. Melting points were determined using a Thomas Hoover capillary melting-point apparatus and are uncorrected. Thin-layer chromatography (tlc) [silica gel 60 F_{254}-precoated aluminum sheets, chloroform:methanol (4:1), Dragendorff's reagent for visualization] was used to monitor all reactions.

High-performance liquid chromatography (HPLC) conditions consisted of a mobile phase of 60% methanol, 40% aqueous (0.25% triethylamine, 0.01% sodium octyl sulfate, pH adjusted to 6.7 with H_3PO_4) passed through a LiChrosorb RP-18 (5-μm) column (E. Merck, Darmstadt, West Germany) and a Spheri-5 RP-18 (5-μm) precolumn (Brownlee Labs, Santa Clara, Calif.) at a flow rate of 1.0 ml/min. Samples (30 μl) were injected, the UV absorbance at 254 nm was recorded, and peak heights were determined and compared to standard curves.

Spectroscopic Analysis. Infrared (IR) spectra were recorded on a Beckman microlab 620 MX computing spectrometer (Beckman Instruments, Inc., Fullerton, Calif.). Samples were dissolved in acetone and aliquots were allowed to dry on sodium chloride disks. The ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ at 25°C using a JEOL HNM-FX 270 Fourier Transform NMR spectrometer. Chemical shifts were referenced to internal tetramethylsilane (TMS).

Preparation of Naltrexone Free Base (I). Naltrexone HCl (5.56 g, 14.72 mmol) was dissolved in double-distilled water (100 ml) and an equal volume of 2% $NaHCO_3$ was added in portions. The resulting solution was extracted with ether (150 ml) twice. Evaporation of the ether gave a semicrystalline solid which was dissolved in toluene (300 ml). Water was removed by azeotropic distillation, the solution was chilled, and I was recovered in a 91.4% yield (4.59 g, 13.45 mmol) (HPLC, R_t = 5.18 min; tlc, R_f = 0.78).

Preparation of Naltrexone-3-Acetate (II). Compound I (2.58 g, 7.56 mmol) was dissolved in tetrahydrofuran (THF; 25 ml), then acetic anhydride (0.93 ml, 9.83 mmol) and triethylamine (TEA; 3.16 ml, 22.7 mmol) were added and the solution was stirred for 24 hr in an ice-water bath (0–5°C) (Fig. 1). TEA and THF were removed *in vacuo* and the resulting sticky semisolid was dissolved in 10 ml 2.0% methanol/chloroform. The sample was then purified by flash chromatography (13) using 2.0% methanol/chloroform as eluent. Appropriate fractions were pooled, concentrated, and dried *in vacuo* to a constant weight. The product was recrystallized from hexane (1.74 g, 4.54 mmol; mp, 103–105°C) in a 60.0% yield (HPLC, R_t = 7.98 min; tlc, R_f = 0.83).

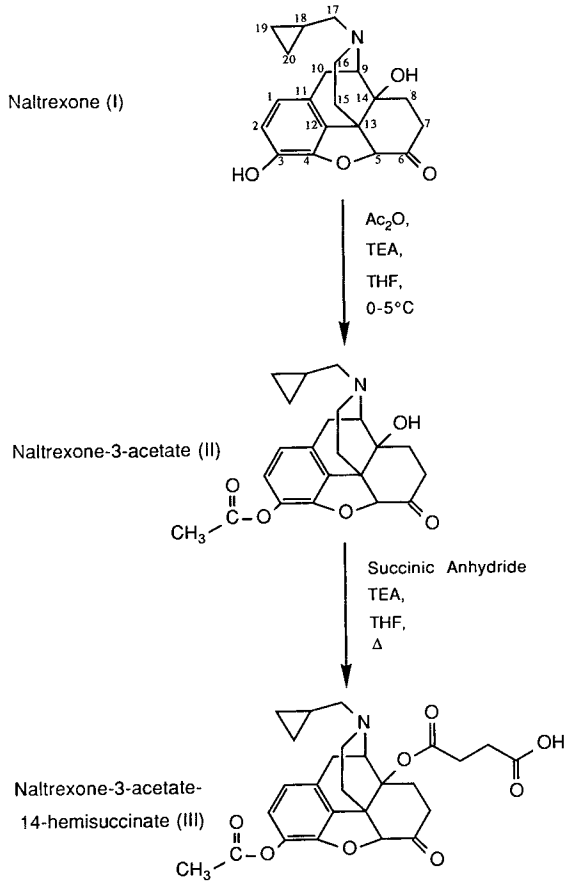

Fig. 1. Naltrexone modifications.

Preparation of Naltrexone-3-Acetate-14-Hemisuccinate (III). Compound II (1.61 g, 4.21 mmol) was dissolved in THF (25 ml), succinic anhydride (1.69 g, 16.8 mmol) and TEA (4.11 ml, 29.5 mmol) were added, and the solution was refluxed at 70°C for 72 hr (Fig. 1). The solution was then concentrated *in vacuo,* dissolved in a minimum volume of 14% methanol/chloroform, and subjected to flash chromatography. Appropriate fractions were pooled and concentrated, and the residue was dried to a constant weight. Compound III (0.92 g, 1.90 mmol; mp, 127–130°C) was recrystallized from diisopropyl ether in a 45.2% yield (HPLC, R_t = 2.52 min; tlc, R_f = 0.45).

Preparation of Poly-N^5-(3-Hydroxypropyl)-L-glutamine (PHPG). The biodegradable backbone polymer was synthesized as described previously (14). Viscosity measurements of the polymer dissolved in water (25.0 + 0.1°C) were made using an Ubbelohde viscometer. The molecular weight (MW = 40,000) was estimated from the [η] vs MW plots of Lupu-Lotan *et al.* (14) for PHPG in water.

Preparation of Hydroxypropyl-L-Glutamine/L-Leucine (70/30) Copolymer. Random copolymers of hydroxypropyl-L-glutamine (HPG) and L-leucine with monomer molar feed ratios of 70/30 were prepared according to the procedures of

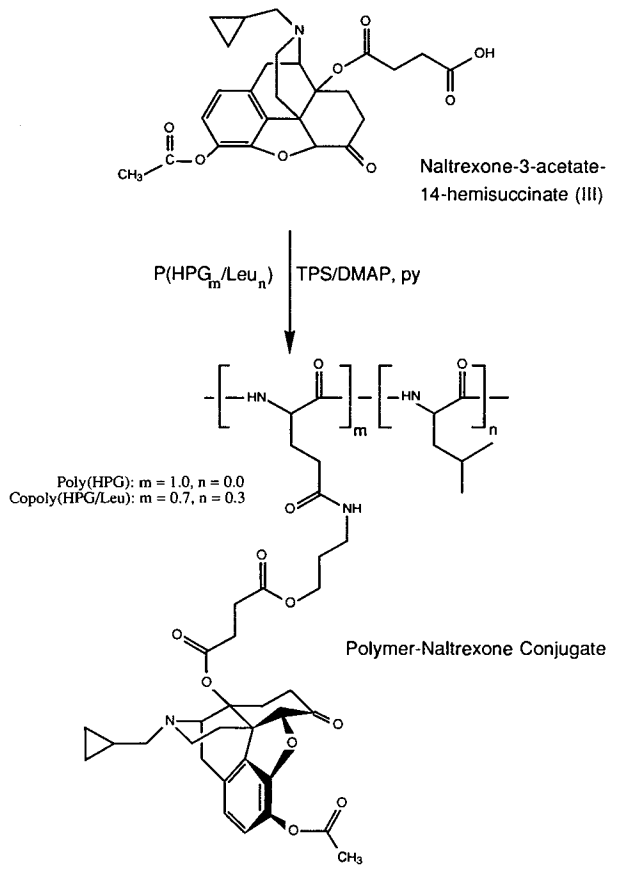

Fig. 2. Coupling of naltrexone to polymer.

von Dreele *et al.* (15). The molecular weight (MW = 36,000) was determined from the viscosity measurements of 2% polymer solutions in 0.2 *M* NaCl at 25.0°C (16).

Preparation of the Conjugate of III and PHPG. Compound III was covalently coupled to PHPG using the 2,4,6-triisopropylbenzenesulfonyl chloride/4-dimethylaminopyridine (TPS/DMAP) method (17,18) (Fig. 2). Compound III (1.23 g, 2.55 mmol), DMAP (0.41 g, 3.37 mmol), and TPS (1.29 g, 4.27 mmol) were dissolved in pyridine (20 ml) while stirring. PHPG (0.36 g) was added after 20 min and dissolved rapidly. After 72 hr the reaction mixture was filtered and the polymer precipitated by dropwise addition to ether (1.0 liter). The product was washed with ether (300 ml) and then with ethanol (400 ml) to remove any DMAP hydrochloride or arylsulfonic acid. The hygroscopic product was dried *in vacuo* to a constant weight (0.45 g). The percentage loading

Table I. IR Carbonyl Stretching Bands (cm^{-1}) of Esters of Naltrexone

Compound	IR frequency (cm^{-1})[a]
Naltrexone	1730 (6-keto),[s]
Naltrexone-3-acetate	1730 (6-keto),[s]
	1770 (3-acetate),[s]
Naltrexone-3-acetate-14-hemisuccinate	1730 (6-keto, 14-succinate,)[b]
	1770 (3-acetate)

[a] b, broad band; s, sharp band.

(w/w) of naltrexone onto the polymer was determined to be 21.5% as measured by HPLC for samples dissolved in 2% methanolic KOH.

Preparation of the Conjugate of III and Copoly(HPG/Leu). The synthetic conditions for the coupling of III to copoly(HPG-70/Leu-30) were similar to those used with the homopolymer. Loading of naltrexone onto the copolymer was 33.8% by weight.

In Vitro *Release Studies.* Disks of the polymer–drug conjugate were prepared by a combination of solvent casting and compression. The addition of a small amount of dimethylformamide to the conjugate and subsequent evaporation at 50°C under vacuum gave a brittle film. A known amount (100–150 mg) of the film was placed in a dye (diameter = 11.0 mm) and subjected to 100 kg pressure while heating (70°C) using a laboratory press with heating plates. Three disks were placed in separate vials and 10.0 ml phosphate-buffered saline (PBS; pH 7.4) was added to each. The vials were placed in a shaking water bath and agitated gently (50 strokes/min, 2.0 cm/stroke) at 37°C. Upon sampling, the entire release medium was replaced with fresh PBS to simulate sink conditions. Concentrations of naltrexone and its derivatives were determined by HPLC analysis.

RESULTS AND DISCUSSION

Acetate esters of naloxone have been prepared using acetic anhydride and pyridine at room temperature (19). Upon similar treatment of naltrexone it was found that a mixture of the 3-acetate and the 3,14-diacetate was produced. Lowering the reaction temperature to 0–5°C produced no change with regard to selectivity. By using the stronger base, triethylamine, and lowering the reaction temperature to 0–5°C, a completely selective acetylation of the phenolic hydroxyl group was achieved. Succinoylation

Table II. ^1H Chemical Shifts (δ) of the Esters of Naltrexone[a]

Compound	H position		
	1, 2 (A, B quartet)	5	9
Naltrexone	6.74, 6.70, 6.61, 6.57	4.72	3.00 (±0.5)[b]
Naltrexone-3-acetate	6.86, 6.82, 6.69, 6.65	4.70	3.00 (±0.5)[b]
Naltrexone-3-acetate-14-hemisuccinate	6.92, 6.88, 6.75, 6.71	4.82	4.88 (±0.5)[b]

[a] The δ value (ppm) from the TMS internal reference in $CDCl_3$.

[b] Superimposed on other proton absorption bands and therefore the exact resonance is not assignable.

Table III. ^{13}C NMR Chemical Shifts (δ) of Esters of Naltrexone[a]

Identification of carbon	I	II	III
1	119.87	119.36	119.66
2	117.67	122.91	123.94
3	138.71	132.52	133.09
4	143.49	147.69	147.86
14	70.32	70.02	81.33
3-$\underline{C}H_3CO$		20.85	20.74
3-$CH_3\underline{C}O$		168.54	168.33
14-$\underline{C}O(CH_2)_2COOH$			72.20
14-$C\underline{O}(CH_2)_2COOH$			177.14

[a] The δ value (ppm) from the TMS internal reference in $CDCl_3$.

proved to be more difficult and required large excesses of reactants as well as high temperatures.

The structures of the synthesized compounds (II and III) were characterized by IR, ^1H NMR, and ^{13}C NMR. The IR data in Table I, in which the carbonyl stretching absorption frequencies of relevant C=O groups are listed, confirm the chemical modifications of I. The C=O stretching of the 3-acetate group absorbs at a higher frequency (1770 cm^{-1}) than that of normal ester C=O stretching (1740 cm^{-1}) due to phenyl conjugation. The C=O stretching of 14-succinate on compound II appears as a broad band due to its overlapping with the 6-keto group.

The proton NMR data in Table II reveal the considerable effect of succinoylation of the 14-hydroxyl group on the chemical shift of the C-9 proton. This large downfield shift may be the result of the collective effects of the chemical

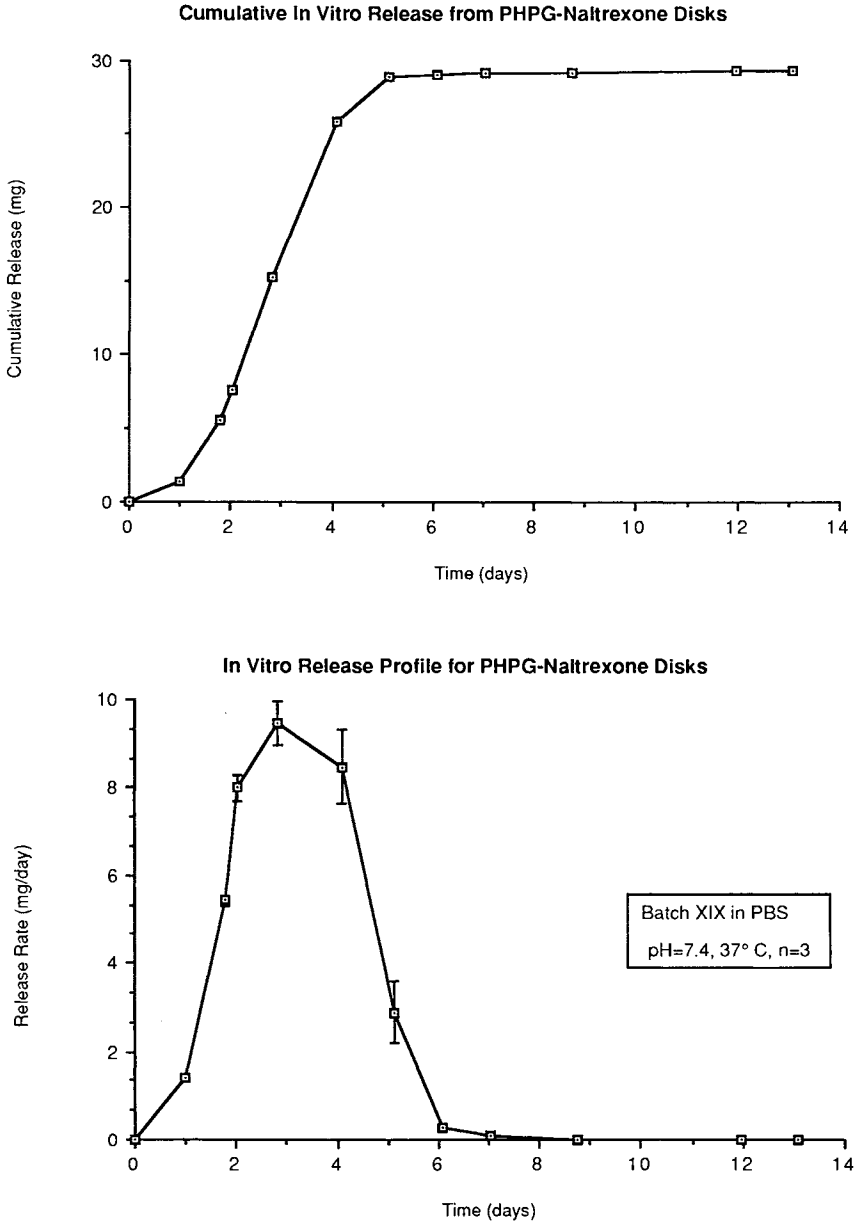

Fig. 3. *In vitro* release studies of PHPG–naltrexone conjugate.

changes of the 14-hydroxyl moiety and the resultant changes in its interaction with the piperidine nitrogen.

In Table III, the assignment of the carbon resonances of compounds in carbon-13 NMR was accomplished by comparison with literature values (20). On changing a C-3 hydroxyl group (I) to a C-3 acetoxyl group (II and III), substituent constants for methine carbons in substituted benzenes predict that C-3 resonance should be shifted upfield by ca. 3–4 ppm because of a smaller α effect of the C-3 acetoxyl group. The C-2 and C-4 resonance should be shifted downfield by ca. 6 ppm owing to the larger ortho effect of the C-3 acetoxyl substituent (21). In addition, the C-14 resonance in compound III was downfield because of the expected substituent effect of C-14 succinoylation (21).

In vitro release studies of the polymer–naltrexone conjugates demonstrated that the released species include not only the parent drug (I), but also compounds II and III as well as another species, presumably naltrexone-14-hemisuccinate (HPLC, R_t = 2.00 min). On those occasions in which HPLC analyses could be performed immediately after sampling, it was found that free drug (I) comprised the majority (>75%) of the total species released. Approximately equal amounts (10%) of II and III were found to contribute to the species released *in vitro*. The remainder (5%) was presumed to be the deacetylated hemisuccinate compound. Cleavage of the labile ester linkage between drug and polymer should be much faster than cleavage of the peptide bonds of the polymer backbone; however, it is possible that a small fraction of the release may include drug coupled to an HPG residue or larger polymer fragment.

Release studies using the PHPG–naltrexone conjugate showed a widely fluctuating release rate of drug which does not approach zero order (Fig. 3). Complete release of the loaded naltrexone was achieved within 7 days. At 24 hr the

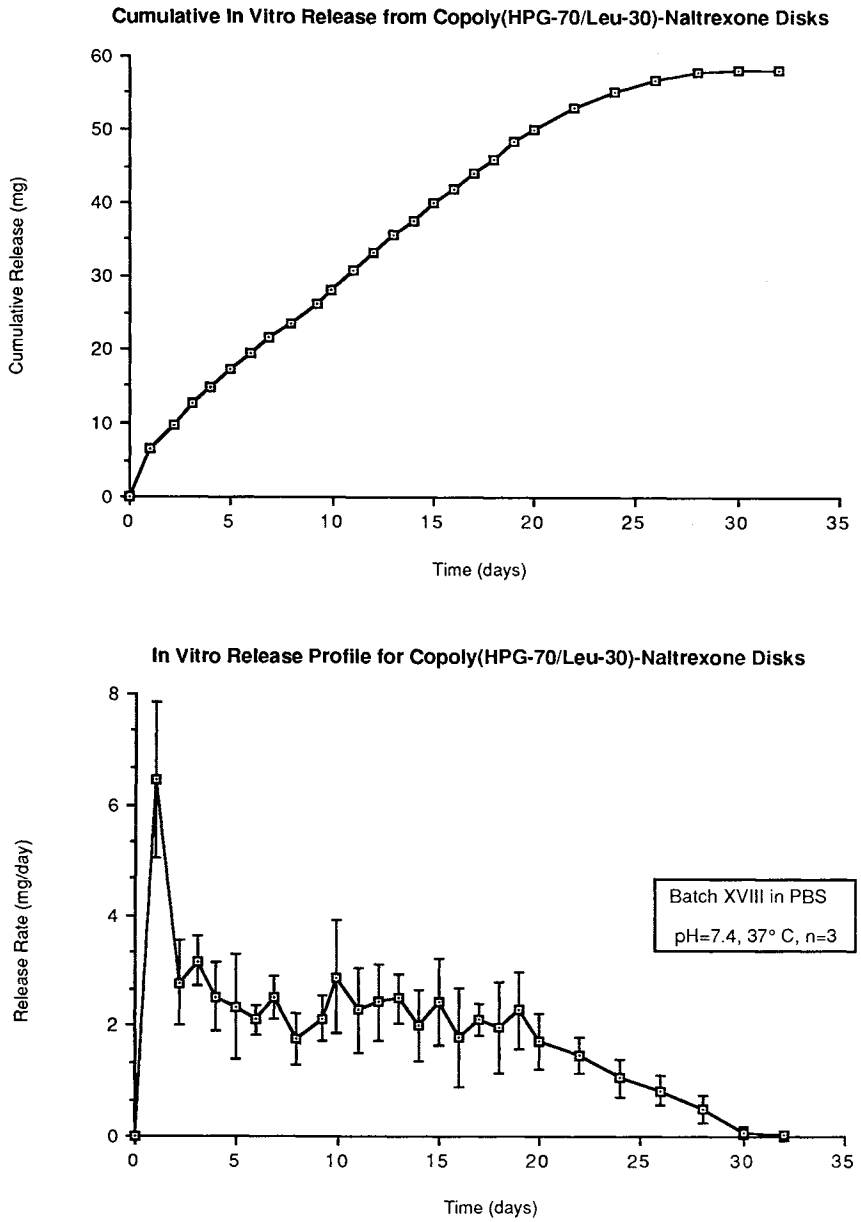

Fig. 4. *In vitro* release studies of P(HPG-70/Leu-30)–naltrexone conjugate.

disks were greatly swollen and at 14 days they were completely dissolved. The lag in release before 24 hr may be due to the time required for complete hydration of the disks. Fluctuations in rate after the lag period may be due to low molecular weight chains hydrolyzing before high molecular weight chains, increasing the hydrophilic environment within the disk after cleavage of the drug moieties, the number of sites available for cleavage, and the degradation of the device before depletion of the drug.

The relatively hydrophobic copolymer conjugate showed much more steady release characteristics than the homopolymer conjugate. Release of antagonist (including species I, II, and III) averaged 2.26 ± 1.09 mg/day (Fig. 4) for over 28 days. This rate falls within the commonly administered dosage rane of 1 to 80 mg subcutaneously (22). These disks remained intact for more than 40 days. No lag time was seen with these devices; rather they exhibited a burst effect, with the rate of release slightly declining until complete release was achieved. The burst effect may be due to several factors: low molecular weight conjugate chains hydrolyzing quickly, an excess of drug moieties oriented at the surface of the device, edge effects, and those random HPG/Leu copolymers with a higher HPG content becoming solvated faster and thus releasing drug more quickly.

These results show the considerable effect that increasing the hydrophobic nature of the conjugate has on the release rate. The rate of release for the copolymer conjugate was less than half that of the homopolymer conjugate. Naltrexone release was prolonged for nearly a month due to both increased hydrophobicity and drug loading of the copolymer conjugate.

Increasing the duration of this type of device is essential. This may be facilitated in a number of ways, including increasing the loading of the drug onto polymer, increasing the hydrophobic nature of the polymer backbone by using copolymers of glutamic acid and leucine with a greater leucine content, and increasing the molecular weight of the backbone polymer. Long *in vivo* duration of this biodegradable system may result in a reliable and convenient method of long-term antinarcotic treatment without the need for removal of the device.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Jun Mizutani for his assistance in development of the HPLC conditions, Mr. Jay Olsen for NMR spectral data, and Dr. Nathan Adams for his help in spectral interpretation and suggesting flash chromatography for purification of the intermediates. This work has been supported by NIDA Grant DA 02391.

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