ELSEVIER

International Journal of Pharmaceutics 136 (1996) 31–36

international journal of pharmaceutics

Macromolecular prodrugs. VII. Polymer-dopamine conjugates

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Received 24 January 1996; accepted 6 February 1996

Abstract

Dopamine (3,4-dihydroxyphenetylamine, DOP) was covalently linked to poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)] (PHEA) and styrene-maleic anhydride copolymer (SMA) in order to prepare polymeric prodrugs as a potentially more stable form of dopamine. Release of active substance from polymer drug conjugates was studied in different buffer solutions at 37 ± 0.1°C. The following rate constants for PHEA-DOP were obtained: $k = 2.50 \times 10^{-2}$ min^{-1} (pH = 1.2); $k = 4.09 \times 10^{-3}$ min^{-1} (pH = 7.4), and $k = 1.71 \times 10^{-2}$ min^{-1} (pH = 9.2). The rate constants for SMA-DOP were: $k = 2.27 \times 10^{-3}$ min^{-1} (pH = 7.4) and $k = 7.98 \times 10^{-3}$ min^{-1} (pH = 9.2).

Keywords: Dopamine; Poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]; Styrene-maleic anhydride copolymer; Polymeric prodrug; Polymer-drug conjugate; Sustained drug release

1. Introduction

A promising approach to improve drug delivery is chemical transformation of the active drug substances into inactive derivatives (prodrugs) that convert to the parent compounds within the body system (Bundgaard, 1985). One way to obtain such prodrugs is to link an active drug as a side substituent to a polymeric or oligomeric structure by means of cleavable bond. The aim of this procedure is to improve the therapeutic treatment. Pharmacological agents are mainly low molecular mass compounds which readily penetrate into all cell types and are often rapidly excreted from the body. Large and repeated doses must be given in order to maintain a therapeutic effect and this consequently increases adverse and side effects (Dumitriu et al., 1989a,b). The use of macromolecular prodrugs in which the drugs are covalently linked to a polymeric matrix could diminish these bad effects. One can also prolong drug release, decrease the required dose and increase drug solubility (Kopeček, 1982; Duncan and Kopeček, 1984; Kopeček and Duncan, 1987). Using targetable polymer-drug conjugates, it is possible to accumulate the drug at the site of the pathological process and to minimize its toxicity on normal tissue.

In our previous papers, the use of poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)] (PHEA) as a drug carrier for carboxylic and amino acid drugs has been described (Zorc et al., 1993a; Zorc et al.,

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PII S0378-5173(96)04484-H

1993b; Zorc and Butula, 1994; Zorc et al., 1995). The drugs were linked to PHEA via different types of bonds (ester, carbonate, phosphodiester) which readily underwent chemical hydrolysis, releasing the active compounds.

Styrene-maleic anhydride copolymer (SMA) belongs to a group of vinylic polymers. It has no teratogenic and no acute or chronic toxic effects (Muratov et al., 1975; Winek and Burgun, 1977). In spite of its immunogenic properties (Wieczorek et al., 1975), SMA has been used in preparation of chemically and diffusionally controlled polymeric prodrugs (Shima et al., 1970a,b,c; Iwai et al., 1984; Maeda et al., 1985; Triner, 1986) and for immobilization of enzyme (α-chimotripsin) (Lai and Cheng, 1978).

In this paper, we describe the attachment of an adrenergic agent dopamine (3,4-dihydroxyphenetylamine, DOP) to PHEA and SMA by an amide type bond. We also report kinetic studies of drug release from the polymer-dopamine conjugates in vitro.

2. Materials and methods

2.1. Materials

IR spectra were recorded on a Perkin-Elmer 457 and UV-spectra on a Pye Unicam SP-100 and Hewlett Packard 8452 A diode array spectrophotometers. For thin-layer chromatography silica gel sheets (Kieselgel 60 F_{254}, Merck) were used. The solvent system was *n*-butanol/AcOH/water 4:1:1. Dopamine hydrochloride was purchased from Merck (Darmstadt), styrene and maleic anhydride from Aldrich (Milwaukee). All solvents were of analytical grade quality and were dried and distilled prior to use. The following buffer solutions were used: KCl/HCl (pH = 1.2), Tris/HCl (pH = 7.4) and Na-borate/HCl buffer (pH = 9.2). The polymer solution was dialyzed against several changes of deionized water using Visking Dialysis Tubing (18/22 inch; Serva) with a molecular weight cut-off 12000–14000.

2.2. Chemistry

2.2.1. Poly-DL-(2,5-dioxo-1,3-pyrrolidinediyl) (PSI)

PSI was prepared by thermal polycondensation of L-aspartic acid (L-Asp) in the presence of phosphoric acid under reduced pressure, at 160°C, for 3 h (Neri et al., 1973; Zorc et al., 1993b).

2.2.2. Styrene-maleic anhydride copolymer (SMA)

SMA was prepared following the procedure described by Braun et al. (1972) and Kovač-Filipović et al. (1989).

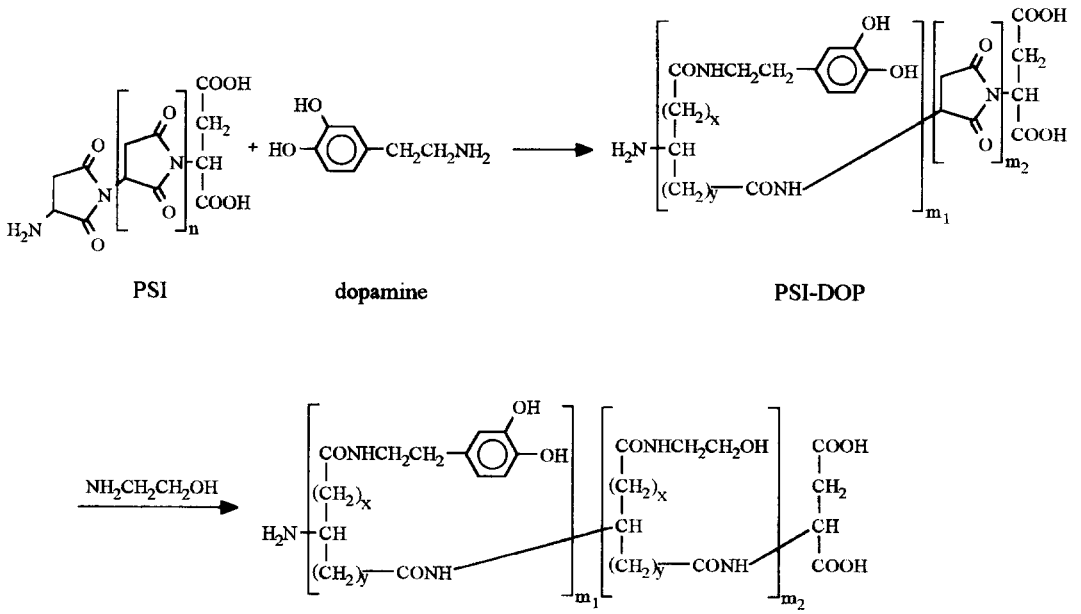
2.2.3. PHEA-DOP conjugate (1)

To a suspension of 0.46 g (0.0048 mol[1]) PSI in 10 mL *N,N*-dimethylformamide (DMF), 0.30 g (0.0016 mol) dopamine hydrochloride and 0.40 g (0.0048 mol) triethylamine (TEA) were added. Reaction was run under a nitrogen atmosphere in the presence of 0.02 g sodium dithionite. The reaction mixture was stirred for 20 h at 80°C, and then cooled to room temperature. 0.72 g (0.0117 mol) 2-aminoethanol was added dropwise. The reaction mixture was stirred at room temperature for 2 h. Glacial acetic acid was added to pH = 4. The solvent was evaporated under reduced pressure to a small volume and the polymeric product precipitated by addition of acetone. The product was filtered off, dissolved in water and precipitated after addition of acetone. The procedure was repeated three times. Product was filtered off, dissolved in water, dialyzed for 3.5 days and lyophilized. Yield: 0.22 g (24%). The amount of dopamine in PHEA-DOP conjugate (*1*) was 14.3%. IR (KBr): ν_{max} 3700–2700, 1725, 1660, 1530 cm^{-1}. UV: λ_{max} 280 nm (A = 0.509; γ = 274 $\mu g\ mL^{-1}$, H_2O).

2.2.4. SMA-DOP conjugate (2)

A solution of 0.31 g (0.0016 mol) dopamine hydrochloride and 0.17 g (0.0016 mol) triethylamine in 10 mL of DMF was added in solution of 0.30 g (0.0015 mol[1]) SMA in 15 mL of DMF. The reaction mixture was stirred for 20 h at 50°C

[1] moles of succinimide or styrene-maleic anhydride (1:1) units, respectively



Scheme 1. PSI, Poly-DL-(2,5-dioxo-1,3-pyrrolidinediyl); PSI-DOP, Poly-DL-(2,5-dioxo-1,3-pyrrolidinediyl)-dopamine conjugate; PHEA-DOP, Poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-dopamine conjugate.

in nitrogen atmosphere in the presence of 0.02 g sodium dithionite. The solvent was evaporated under reduced pressure. The obtained crude product was washed with water, HCl solution (pH = 3) and water again in order to remove the free drug. The product was dissolved in water-acetone 1:4 mixture. The solution was filtered and the solvent was evaporated under reduced pressure. Product *2* was washed several times with water and dried for 20 h over P_2O_5 under reduced pressure. Yield: 0.22 g (50%). The amount of dopamine in SMA-DOP conjugate (*2*) was 42.8%. IR (KBr): ν_{max} 3680–2700, 1705, 1600, 1515, 1445, 810, 785, 765, 710 cm^{-1}. UV: λ_{max} 282 nm (A = 0.851; γ = 153 $\mu g\ mL^{-1}$, EtOH).

2.2.5. Release of dopamine from PHEA-DOP and SMA-DOP conjugates

A solution of PHEA-DOP (*1*) (γ = 250 $\mu g\ mL^{-1}$) or SMA-DOP (*2*) (γ = 70 $\mu g\ mL^{-1}$) in an appropriate buffer solution was thermostated at 37 ± 0.1°C. The drug release was followed by UV-spectrometry at 280 nm and 282 nm, respectively, at suitable time intervals. Rate constants were computed using a nonlinear square fitting program.

3. Results and discussion

In the present report, synthesis of two polymer-

drug conjugates PHEA-DOP (*1*) and SMA-DOP (*2*) is described. The conjugate *1* was prepared by aminolysis of poly-DL-(2,5-dioxo-1,3-pyrrolidinediyl) (polysuccinimide; PSI) with dopamine and 2-aminoethanol. PSI is a linear polyimide which readily reacts with various amines and gives rise corresponding polyaspartamides (Kovach et al., 1961; Neri et al., 1973; Vlasak et al., 1979). In our experiments, PSI with an average relative molecular mass 24 000 was used. In order to achieve only partial aminolysis, one mole of dopamine per three succinimide units was employed. At room temperature dopamine did not react to a measurable degree with PSI dissolved in DMF (3 days, 20°C). A successful reaction was carried out at 80°C. The resulting PSI-DOP copolymer with partially opened succinimide rings was subjected to further aminolysis by 2-aminoethanol. In this way a water soluble polymer-drug conjugate PHEA-DOP (*1*) was prepared (see Scheme 1).

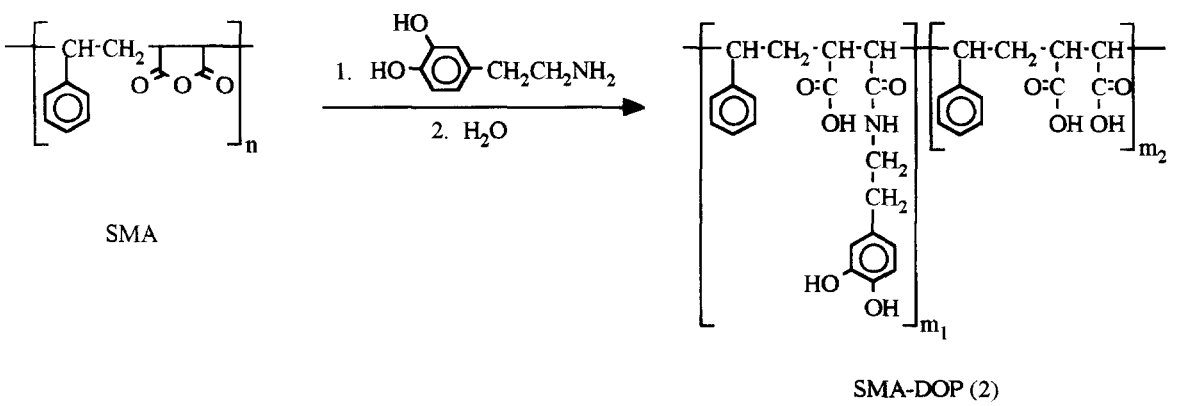
Synthesis of SMA-DOP conjugate is presented in Scheme 2. The starting SMA copolymer was produced by copolymerization of styrene and maleic anhydride (Braun et al., 1972; Kovač-Filipović et al., 1989). Very reactive anhydride rings in SMA could be modified in different ways. For example, SMA with various amines and water gave amidic acids (Song and Baker, 1992). We have used dopamine as aminolysing agent. Although one mole of dopamine per one mole of anhydride rings was used, aminolysis was only partial. The remaining anhydride rings were opened in subsequent reaction with water so the resulting product *2* had amide and carboxylic functional groups. Due to the last ones, product *2* was soluble in alkaline and pH 7.4 buffers.

In order to avoid oxidative processes, all reactions were run in nitrogen atmosphere in the presence of sodium dithionite. Triethylamine was used for transformation of dopamine hydrochloride to dopamine base.

The drug loading in polymer-dopamine conjugates was estimated by UV-spectroscopy using the molar absorption coefficient for dopamine hydrochloride $\epsilon_{280} = 2650$ (in water, $c = 1.05 \times 10^{-4}$ mol L^{-1}). The percentage of dopamine in PHEA-DOP was 14.3% and in SMA-DOP was 42.8%.

In both polymer-drug conjugates dopamine was linked by an amide type bond. This bond could be hydrolytically or enzymatically cleaved. We have studied the kinetics of dopamine release from conjugates *1* and *2* in aqueous buffer solutions in the pH range 1.2–9.2 at 37 ± 0.1°C. The results are presented in Figs. 1 and 2.

The conjugates were dissolved in following buffer solutions: KCl/HCl (pH = 1.2), Tris/HCl (pH = 7.4) and Na borate/HCl buffer (pH =



Scheme 2. SMA, styrene-maleic anhydride copolymer; SMA-DOP, conjugate of styrene maleic anhydride copolymer and dopamine.

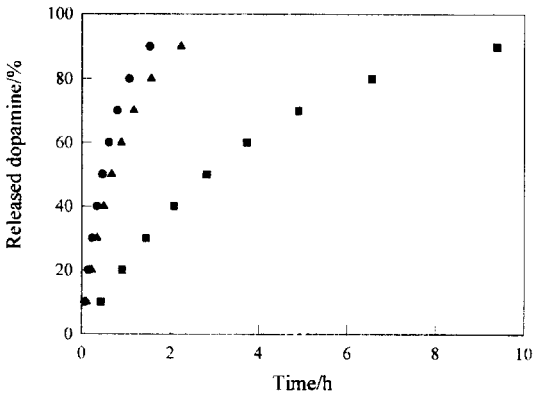


Fig. 1. Release of dopamine from PHEA-DOP conjugate (*1*) at pH = 1.2 (●), 7.4 (■) and 9.2 (▲).

9.2). The drug release was followed by UV-spectrometry at 280 nm (for PHEA-DOP) and at 282 nm (for SMA-DOP), at suitable time intervals. Rate constants were computed using a nonlinear square fitting program. The following rate constants for PHEA-DOP were obtained: $k = 2.50 \times 10^{-2}$ min^{-1} (pH = 1.2); $k = 4.09 \times 10^{-3}$ min^{-1} (pH = 7.4) and $k = 1.71 \times 10^{-2}$ min^{-1} (pH = 9.2).

Since SMA-DOP conjugate is insoluble in acidic medium, kinetic studies were performed only in neutral (pH = 7.4) and in basic medium (pH = 9.2). The rate constants $k = 2.27 \times 10^{-3}$ min^{-1} (pH = 7.4) and $k = 7.98 \times 10^{-3}$ min^{-1} (pH = 9.2) were obtained. The data fit

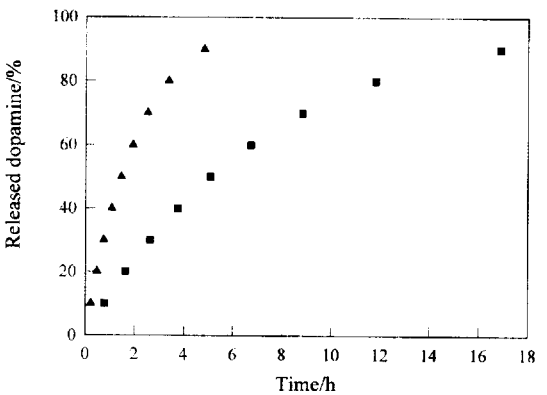


Fig. 2. Release of dopamine from SMA-DOP conjugate (*2*) at pH = 7.4 (■) and 9.2 (▲).

pseudofirst-order kinetics. In all experiments $r = 0.994–0.999$.

Preliminary kinetic studies showed that dopamine could be released from the prepared polymer-dopamine conjugates in prolonged time periods, but in vivo experiments would be crucial for complete evaluation.

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