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Original article

Preparation and characterization of polyester- and poly (ester-carbonate)-paclitaxel conjugates

Marcin Sobczak*, Agnieszka Korzeniowska, Piotr Goś, Waclaw L. Kolodziejski

Medical University of Warsaw, Faculty of Pharmacy, Department of Inorganic and Analytical Chemistry, ul. Banacha 1, 02-097 Warsaw, Poland

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ABSTRACT

The polyester- and poly(ester-carbonate)-paclitaxel conjugates with low molecular weight were synthesized using dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) as catalysts. Polymeric matrices were obtained by ring-opening polymerization of ε-caprolactone (CL), *rac*-lactide (*rac*-LA), L-lactide (LLA) and trimethylene carbonate (TMC). The macromolecular conjugates were characterized by using spectroscopic techniques, such as ^{1}H, ^{13}C NMR and FTIR. The degree of degradation of polyester- and poly(ester-carbonate)-paclitaxel conjugates was tested in vitro under different conditions. The preliminary results of drug release were discussed.

1. Introduction

Paclitaxel (PACL) is a natural product with antitumor activity (Fig. 1). Taxol (paclitaxel) is obtained via a semi-synthetic process from *Taxus baccata*. PACL is one of the most common anticancer drugs used for chemotherapy. It is antimicrotubule agent that promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions.

PACL is usually used to treat patients with lung, ovarian, breast, head and neck cancer. However some problems such as allergic reactions, heart and blood vessel effects, infections due to low white blood cell count, hair loss, joint and muscle pain, irritation at the injection site, low red blood cell count, mouth or lip sores, numbness, tingling, or burning in the hands and/or feet, stomach upset and diarrhea, decrease in urine output and/or swelling of the hands, face, or feet, have limited its use [1–3]. Therefore, polymeric conjugates have been extensively studied and proved as promising delivery systems to augment therapeutic efficacy of chemotherapeutic agents in the treatment of cancer. Macromolecular conjugates of paclitaxel, such as poly(ethylene glycol) PEG [4], poly(L-glutamic acid) (PG) [5], monomethoxy-poly(ethylene glycol)-b-poly(lactide) (MPEG-PLA) [6], recently attracted more and more attention.

Poly(lactide) (PLA), poly(D,L-lactide-co-glycolide) (PLGA), and poly(caprolactone) (PCL) are biodegradable polymers, which are used most often in the literature of drug delivery [7–15]. Aliphatic polyesters are attractive, because they undergo hydrolysis to produce compounds which can be metabolized in vivo and in the environment [16]. Aliphatic polyesters are commonly prepared by two different routes: polycondensation and the ring-opening polymerization (ROP). The ROP of cyclic esters is initiated/catalyzed by metal complexes, organic compounds, or enzymes, to yield high molecular weight in excellent conversion and purity [16–21]. The most common catalysts are metal (Sn, Zn and Al) coordinating compounds, which are useful due to their selectivity and efficacy. On the other hand, for pharmaceutical applications metal residues are undesirable considering their toxicity.

Recently, we found that natural amino acids and creatinine are satisfactory initiators for ROP of cyclic esters [13,22].

The aim of the present study is to synthesize polyester-paclitaxel and poly(ester-carbonate)-paclitaxel conjugates of different molecular weights and to characterize their physicochemical properties. We hope that the obtained macromolecular conjugates are good potential candidates as implant drug delivery systems.

* Corresponding author. Tel.: +48 22 572 07 55; fax: +48 22 572 07 84.
E-mail addresses: marcin.sobczak@wp.pl, marcin.sobczak@wum.edu.pl (M. Sobczak).

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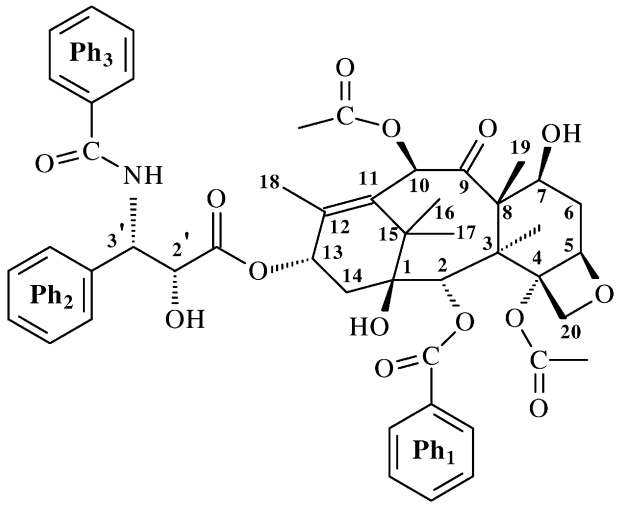

Fig. 1. Structure of paclitaxel.

2. Experimental

2.1. Chemicals

(3*S*)-*cis*-3,6-Dimethyl-1,4-dioxane-2,5-dione (L-lactide, 98%, LLA) was purchased from Aldrich and recrystallized from ethyl acetate for several times. 3,6-Dimethyl-1,4-dioxane-2,5-dione, (*rac*-lactide, 98%, *rac*-LA) (Aldrich) was crystallized from a mixture of dry toluene with hexane and dried at room temperature under vacuum. ε-Caprolactone (2-Oxepanone, CL, 99%) was purchased from Aldrich. Before use, it was dried and distilled over CaH_2 at reduced pressure. Paclitaxel (97%), from semi-synthetic (from *Taxus* sp., PACL) (Aldrich), creatinine (anhydrous, 99%, CE) (Aldrich), diethyl carbonate (DEC, 98%) (Aldrich), propane-1,3-diol (PD, 98%) (Aldrich), stannous octoate ($SnOct_2$, tin (II) 2-ethylhexanoate, 95%) (Aldrich), dicyclohexylcarbodiimide (DDC) (Aldrich 99%), dimethylaminopyridine (DMAP) (Aldrich 99%), ethanol (99.8%) (Aldrich), dichloromethane (POCh Poland) and methanol (POCh), were used as received.

2.2. Synthesis of trimethylene carbonate

Trimethylene carbonate (TMC) was synthesized in the reaction of equimolar quantities of DEC and PD in the presence of $SnOct_2$ as catalyst at 160 °C during 8 h. Then, ethanol and unreacted substances were evaporated under reduced pressure. TMC was produce by depolymerization of the polycarbonate. Then the monomer was crystallized from a mixture of dry benzene/tetrahydrofuran (1:4) and dried at 30 °C under vacuum [23].

2.3. Synthesis of polyesters and poly(ester-carbonate)s

Homo- and copolymerization of cyclic esters and TMC were carried out in the same way. Monomers (CL, *rac*-LA, LLA, TMC) and CE were placed in 10 mL glass ampoules under an argon atmosphere. The reaction vessels were then left standing at the required temperature in a thermostated oil bath for the appropriate time (Table 1). After desired time the ampoule was opened and methylene chloride was added in order to dissolve the products. Then, the obtained solutions were washed with methanol and dilute hydrochloric acid (5% aqueous solution) under vigorous stirring. The latter operation was repeated three times. The isolated polymer was dried in vacuum for 3 days.

PCL: ^{1}H NMR ($CDCl_3$, δ, ppm): 1.38 (2H, m, J = 8.0, 7.2 Hz, $-OCH_2CH_2C\mathbf{H_2}CH_2CH_2C(O)O-$), 1.63 (4H, m, $-OCH_2C\mathbf{H_2}CH_2C\mathbf{H_2}CH_2C(O)O-$), 2.29 (2H, t, J = 7.3 Hz, $-OCH_2CH_2CH_2CH_2C\mathbf{H_2}C(O)O-$), 3.73 (2H, t, J = 3.1 Hz, $-C\mathbf{H_2}OH$, end group), 4.04 (2H, t, J = 6.7 Hz, $-OC\mathbf{H_2}CH_2CH_2CH_2CH_2C(O)O-$); ^{13}C NMR ($CDCl_3$, δ, ppm): 24.9 ($-OCH_2CH_2\mathbf{C}H_2CH_2CH_2C(O)O-$), 25.8 ($-OCH_2CH_2CH_2\mathbf{C}H_2CH_2C(O)O-$), 28.4 ($-OCH_2\mathbf{C}H_2CH_2CH_2CH_2C(O)O-$), 33.8 ($-OCH_2CH_2CH_2CH_2\mathbf{C}H_2C(O)O-$), 63.9 ($-\mathbf{C}H_2C(O)OH$, end group), 64.5 ($-O\mathbf{C}H_2CH_2CH_2CH_2CH_2C(O)O-$), 173.8 ($-OCH_2CH_2CH_2CH_2CH_2\mathbf{C}(O)O-$); FTIR (KBr, cm^{-1}): 2944 ($\nu_{as}CH_2$), 2867 ($\nu_{as}CH_3$), 1722 (νC=O), 1244 (νC–O).

PLA: ^{1}H NMR ($CDCl_3$, δ, ppm): 5.17 (1H, q, J = 6.7 Hz, $-\mathbf{C}H(CH_3)-$), 4.36 (1H, q, J = 7.3, 6.7 Hz, $-C\mathbf{H}(CH_3)OH$, end group), 1.58 (3H, d, $-C\mathbf{H_3}$); ^{13}C NMR ($CDCl_3$, δ, ppm): 169.9 ($-\mathbf{C}(O)O-$), 69.4 ($-\mathbf{C}H(CH_3)-$), 16.9 ($-\mathbf{C}H_3$); FTIR (KBr, cm^{-1}): 2999 ($\nu_{as}CH_3$), 2949 (ν_sCH_3), 2884 (νCH), 1761 (νC=O), 1454 ($\delta_{as}CH_3$), 1346–1389 (δ_sCH_3), 1365–1370 (δ_1CH + δ_sCH_3), 1315–1300 (δ_2CH), 1270 (δCH + νCOC), 1215–1185 (ν_{as}COC + $r_{as}CH_3$), 1131 ($r_{as}CH_3$), 1100–1090 (ν_sCOC), 1045 (νC–CH_3), 960–950 (rCH_3 + νCC), 875–860 (νC–COO), 760–740 (δC=O), 715–695 (γC=O), 515 (δ_1C–CH_3 + δCCO), 415 (δCCO), 350 (δ_2C–CH_3 + δCOC), 300–295 (δCOC + δ_2C–CH_3), 240 (τCC).

2.4. Synthesis of macromolecular conjugates of paclitaxel

A 0.1 g quantity of homopolymer or copolymer and 25 mg of paclitaxel were dissolved in 50 mL anhydrous methylene chloride.

Table 1
Synthesis of polyesters and poly(ester-carbonate)s.

Code	Monomer I	Monomer II	$M_I/M_{II}/I$	Yield (%)	M_n[a] (Da)	PD[a]	M_n[b] (Da)	PD[b]	TMC[c] (% mol)	η_{inh}[d] (dL/g)	η_{inh}[e] (dL/g)
PCL	ε-CL	–	50:1	81	4400	1.1	3200	1.1	–	0.06	0.05
PLA-1	LLA	–	50:1	62	4000	1.2	2800	1.2	–	0.12	0.07
PLA-2	*rac*-LA	–	50:1	57	3900	1.2	2700	1.1	–	0.15	0.09
COP-1	ε-CL	TMC	25:25:1	60	2900	1.2	–	–	36	0.06	0.06
COP-2	ε-CL	TMC	30:20:1	67	3200	1.2	–	–	26	0.08	0.07
COP-3	ε-CL	TMC	40:10:1	72	3700	1.2	–	–	18	0.09	0.07
COP-4	LLA	TMC	25:25:1	43	2400	1.1	–	–	44	0.11	0.10
COP-5	LLA	TMC	30:20:1	54	2900	1.2	–	–	38	0.15	0.14
COP-6	LLA	TMC	40:10:1	58	3600	1.2	–	–	28	0.18	0.16
COP-7	*rac*-LA	TMC	25:25:1	39	2300	1.1	–	–	39	0.12	0.10
COP-8	*rac*-LA	TMC	30:20:1	55	3200	1.2	–	–	31	0.20	0.18
COP-9	*rac*-LA	TMC	40:10:1	61	3400	1.2	–	–	25	0.22	0.19

I – initiator (CE).
Reaction conditions: time – 72 h, temp. – 140 °C.
[a] Determined by GPC.
[b] Determined by MALDI-TOF.
[c] TMC units content in copolymer chain.
[d] Determined by viscosity method (before degradation).
[e] Determined by viscosity method (after 8 weeks).