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Preparation and characterization of novel poly(ethylene glycol) paclitaxel derivatives

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ABSTRACT

Paclitaxel has been found to be very effective against several human cancers; one of the major problems with its use is its poor solubility, which makes necessary its solubilization with excipients that can determine allergic reactions often severe. The aim of this study is to develop highly water-soluble prodrugs of paclitaxel. For this purpose we prepared a series of new paclitaxel–poly(ethylene glycol) (PEG) conjugates that were characterized and evaluated for their *in vitro* stability and cytotoxicity. In particular, in order to modulate the release of paclitaxel from prodrugs, we prepared different compounds introducing PEG in the drug C2′ and/or C7 positions *via* ester or carbamate linkage. The conjugates were obtained in high purity and good yield. The carbamate prodrugs were highly stable in different media, while the compounds obtained linking PEG at C2′ position through an ester bond showed lower stability. Finally, the cytotoxic activity of the conjugates was evaluated on two cancer cell lines and the results showed that all the derivatives had a reduced cytotoxicity compared to that of paclitaxel.

1. Introduction

Paclitaxel (PTX) is a natural product isolated from the bark of *Taxus breviflolia* (Pacific yew tree) and is today considered to be one of the most important drugs in cancer chemotherapy for the clinical treatment of many types of cancers (Kingston and Newman, 2007; Rowinsky, 1997). It is active against a number of cancer types including breast, lung, prostate, ovarian and some leukaemias (Bonomi et al., 1997; Bookman et al., 1996; Nabholtz et al., 1996; Wani et al., 1971). At molecular level, PTX exerts its antitumor activity by interacting with tubulin (Schiff et al., 1979). In contrast to other anti-mitotic agents, such as Vinca alkaloids, which act to inhibit microtubule formation, PTX promotes tubulin polymerization and stabilizes the microtubules. Therefore, cell division is blocked in the late G2 mitotic phase of cell cycle (Kumar, 1981; Manfredi and Horwitz, 1984). However, limited response rates and significant side effects are the major obstacles for more effective cancer therapy. Additionally, PTX's very low water solubility is a real problem in intravenous administration; PTX is currently administered in a vehicle containing Cremophor EL® (polyethoxylated castor oil) and ethanol. Significant side effects associated with hypersensitivity to Cremophor EL® have been observed (Dorr, 1994; Fjallskog et al., 1993), and premedication with corticosteroids and antihistamines is often required (Weiss et al., 1990). In order to overcome these problems, new aqueous-based formulations for PTX, that do not require solubilization by Cremophor EL®, have been developed (Marupudi et al., 2007; Skwarczynski et al., 2006). The prodrug strategy is a promising way in terms of improving the drug solubility and keeping the pharmacological functions unaltered (Stella and Nti-Addae, 2007). Several reports of water-soluble prodrugs of PTX have been reported that are considered to improve water solubility of the parent drug and to avoid the use of toxic detergents during administration (Vyas and Vittorio, 1995).

One of the most used polymers for prodrug delivery is poly(ethylene glycol) (PEG) (Greenwald et al., 2003). PEG is an amphiphilic polymer that is soluble in organic solvents as well as in water, non-toxic and is eliminated from the body by a combination of renal and hepatic pathways; thus, this molecule is ideal to be employed in pharmaceutical applications; moreover, PEG has been approved by the FDA for human intravenous, oral and dermal applications (Hooftman et al., 1996). Some papers describe the different synthetic approaches adopted to covalently attach PEG to PTX: PEG of various molecular weights was linked to the C2′ and/or C7 positions of PTX through different bonds either directly or through suitable spacers (*i.e.* amino acids) or linkers (Feng et al., 2002; Greenwald et al., 1994, 1996; Li et al., 1996; Schoenmakers et al., 2004).

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However, there are still some problems related to PEG–PTX prodrugs described in literature till now, such as for example poor stability or low improvement of solubility that lead to a limitation in their clinical use (Skwarczynski et al., 2006). Thus, a very attractive challenge in this field is still to obtain new stable water-soluble PTX conjugates with improved activity.

To reach this goal, the aim of this study was to develop highly water-soluble prodrugs of PTX. For this purpose, we prepared a series of new PEG–PTX conjugates that were characterized and evaluated for their *in vitro* stability and cytotoxicity. In particular, in order to modulate the release of paclitaxel from prodrugs, we prepared several conjugates introducing PEG in the drug C2′ and/or C7 positions using different synthetic routes and the properties of these derivatives are discussed, together with a preliminary examination of their *in vitro* antitumor activity.

2. Materials and methods

2.1. Materials and instruments

Unless stated otherwise, all reagents and solvents were obtained from commercial sources and were used without further purification. Paclitaxel was a gift from Indena (Milan, Italy). PEG derivatives (alpha-methoxy-omega-amino poly(ethylene glycol), m-PEG-NH_2 5 and 20 kDa) were purchased from IRIS Biotech GmbH (Marktredwitz, Germany).

All reactions requiring anhydrous conditions were performed under an Ar or N_2 atmosphere.

The reactions were monitored by thin-layer chromatography (TLC) on F_{254} silica gel pre-coated sheets (Merck, Milan, Italy); after development, the sheets were visualized by irradiation by UV light and/or by exposition to iodine vapor. Flash-column chromatography was performed on 230–400 mesh silica gel (Merck).

HPLC analyses were carried out using a LiChroCART C18 column (250 mm × 4 mm i.d., 5 μm particle size) equipped with a C18 column guard (Merck) on a Merck-Hitachi HPLC system. The column was eluted using two solvents: water with 0.05% trifluoroacetic acid (TFA) (solvent A) and acetonitrile with 0.05% TFA (solvent B). The flow rate was maintained at 1 mL/min using a gradient protocol as follows: solvent A 90% for 5 min, a linear gradient from A 90% to A 10% for 30 min, A 10% for 10 min, a linear gradient from A 10% to A 90% for 5 min. The eluting fractions were monitored at 227 nm using an L4000UV detector. Peak heights and areas were recorded and processed on a CBM-10A Shimadzu interface (Shimadzu, Milan, Italy).

The ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker 300 Ultrashield instrument (Karlsruhe, Germany) in $CDCl_3$ solution at room temperature, with $SiMe_4$ as internal standard. UV–vis spectra were obtained on a Beckman 730 spectrophotometer (Beckman Coulter, Milan, Italy).

2.2. Chemistry

2.2.1. Preparation of paclitaxel-2′-succinyl-NHS

2′-Succinyl-paclitaxel (**1**) was prepared as reported elsewhere with modifications (Deutsch et al., 1989). Briefly, PTX (33 mg, 0.0386 mmol) was dissolved in 500 μL of dry pyridine to which 7.7 mg of succinic anhydride (0.0772 mmol) and 0.5 mg (0.00386 mmol) of 4-dimethylaminopyridine were added. The resulting solution was stirred for 3 h at room temperature. The product was purified by flash chromatography with elution in chloroform/methanol (90/10, v/v) to give 34.6 mg of pure product, 94% yield. ^{1}H NMR ($CDCl_3$): δ 1.11 (s, 3H, C17-H), 1.19 (s, 3H, C16-H), 1.62 (s, 3H, C19-H), 1.76 (s, 3H, C18-H), 2.2 (m, 2H, C14-H), 2.23 (s, 3H, C10-OAc), 2.43 (s, 3H, C4-OAc), 2.6 (m, 4H, $COCH_2CH_2CO$), 3.34 (d, 1H, C3-H), 4.17 and 4.28 (d, 2H, C20-H), 4.48 (dd, 1H, C7-H), 4.96 (d, 1H, C5-H), 5.51 (d, 1H, C2′-H), 5.67 (d, 1H, C2-H), 5.80 (d, 1H, C3′-H), 6.21 (t, 1H, C13-H), 6.27 (s, 1H, C10-H), 7.07 (d, 1H, NH), 7.3 (m, 3′-Ph), 7.4 (m, 3′-NBz), 7.5 (m, 2-OBz), 7.73 (d, 3′-NBz), 8.1 (d, 2-OBz).

The carboxyl function of 2′-succinyl-paclitaxel (**1**) (20 mg, 0.0210 mmol) was activated in the corresponding *N*-hydroxysuccinimidil derivative (**2**) by reaction with *N*-hydroxysuccinimide (NHS) (3.2 mg, 0.0278 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) (5.6 mg, 0.0269 mmol) in dry dichloromethane. The reaction mixture was stirred for 6 h at room temperature. After filtration and evaporation the crude product was dissolved in dichloromethane and washed with brine and did not required any further purification step (19 mg, yield 85%). ^{1}H NMR ($CDCl_3$): δ 1.11 (s, 3H, C17-H), 1.19 (s, 3H, C16-H), 1.62 (s, 3H, C19-H), 1.76 (s, 3H, C18-H), 2.2 (m, 2H, C14-H), 2.23 (s, 3H, C10-OAc), 2.43 (s, 3H, C4-OAc), 2.63 (m, 2H, 2′-$OCOCH_2$), 2.66 (m, 4H, $NCOCH_2CH_2CO$), 2.92 (m, 2H, CH_2CON), 3.34 (d, 1H, C3-H), 4.17 and 4.28 (d, 2H, C20-H), 4.48 (dd, 1H, C7-H), 4.96 (d, 1H, C5-H), 5.51 (d, 1H, C2′-H), 5.67 (d, 1H, C2-H), 5.80 (d, 1H, C3′-H), 6.21 (t, 1H, C13-H), 6.27 (s, 1H, C10-H), 7.07 (d, 1H, NH), 7.3 (m, 3′-Ph), 7.4 (m, 3′-NBz), 7.5 (m, 2-OBz), 7.73 (d, 3′-NBz), 8.1 (d, 2-OBz).

2.2.2. Preparation of 4-nitrophenyl-carbonate paclitaxel derivatives

The different carbonate paclitaxel derivatives (**5**–**7**) were prepared following the method described by de Groot (de Groot et al., 2000) with minor modifications. The reactions were carried out under an argon atmosphere. PTX (50 mg, 0.0585 mmol) was dissolved in dry dichloromethane containing 4 drops of pyridine. For the preparation of 2′-(4-nitrophenyl carbonate)paclitaxel (**5**), 200 mg (1.06 mmol) of 4-nitrophenyl chloroformate in dry dichloromethane was added and the reaction proceeded for 5 h at −35 °C. In the case of the synthesis of 2′,7-(4-nitrophenyl biscarbonate)paclitaxel (**7**) PTX was reacted with 300 mg (1.59 mmol) of 4-nitrophenyl chloroformate for 24 h at room temperature. Then the reaction mixtures were washed with a solution of potassium bisulfate and dried with anhydrous magnesium sulfate. The solvent was then removed under reduced pressure and the crude products were purified by flash chromatography (hexane/ethyl acetate 55/45 (v/v) for **5**, 60/40 (v/v) for **6** and 70/30 (v/v) for **7**). 7-(4-Nitrophenyl carbonate)paclitaxel (**6**) was directly obtained starting from the crude 2′,7-(4-nitrophenyl biscarbonate)paclitaxel (**7**) that was left for one night in the column before purification.

Compound **5**, yield 65% (38 mg) ^{1}H NMR ($CDCl_3$): δ 1.11 (s, 3H, C17-H), 1.19 (s, 3H, C16-H), 1.62 (s, 3H, C19-H), 1.76 (s, 3H, C18-H), 2.2 (m, 2H, C14-H), 2.23 (s, 3H, C10-OAc), 2.43 (s, 3H, C4-OAc), 3.34 (d, 1H, C3-H), 4.17 and 4.28 (d, 2H, C20-H), 4.48 (dd, 1H, C7-H), 4.96 (d, 1H, C5-H), 5.51 (d, 1H, C2′-H), 5.67 (d, 1H, C2-H), 6.10 (dd, 1H, C3′-H), 6.21 (t, 1H, C13-H), 6.27 (s, 1H, C10-H), 7.07 (d, 1H, NH), 7.3 (m, 3′-Ph), 7.35 (d, nitrophenyl), 7.4 (m, 3′-NBz), 7.5 (m, 2-OBz), 7.73 (d, 3′-NBz), 8.1 (d, 2-OBz), 8.26 (d, nitrophenyl).

Compound **6** yield 74% (44 mg) ^{1}H NMR ($CDCl_3$): δ 1.11 (s, 3H, C17-H), 1.19 (s, 3H, C16-H), 1.62 (s, 3H, C19-H), 1.76 (s, 3H, C18-H), 2.2 (m, 2H, C14-H), 2.23 (s, 3H, C10-OAc), 2.43 (s, 3H, C4-OAc), 3.34 (d, 1H, C3-H), 4.17 and 4.28 (d, 2H, C20-H), 4.80 (d, 1H, C2′-H), 4.96 (d, 1H, C5-H), 5.26 (dd, 1H, C7-H), 5.67 (d, 1H, C2-H), 5.78 (d, 1H, C3′-H), 6.21 (t, 1H, C13-H), 6.27 (s, 1H, C10-H), 7.07 (d, 1H, NH), 7.3 (m, 3′-Ph), 7.35 (d, nitrophenyl), 7.4 (m, 3′-NBz), 7.5 (m, 2-OBz), 7.73 (d, 3′-NBz), 8.1 (d, 2-OBz), 8.26 (d, nitrophenyl).

Compound **7** yield 86% (59 mg) ^{1}H NMR ($CDCl_3$): δ 1.11 (s, 3H, C17-H), 1.19 (s, 3H, C16-H), 1.62 (s, 3H, C19-H), 1.76 (s, 3H, C18-H), 2.2 (m, 2H, C14-H), 2.23 (s, 3H, C10-OAc), 2.43 (s, 3H, C4-OAc), 3.34 (d, 1H, C3-H), 4.17 and 4.28 (d, 2H, C20-H), 4.96 (d, 1H, C5-H), 5.28 (dd, 1H, C7-H), 5.53 (d, 1H, C2′-H), 5.67 (d, 1H, C2-H), 6.15 (dd, 1H, C3′-H), 6.21 (t, 1H, C13-H), 6.27 (s, 1H, C10-H), 7.07 (d, 1H, NH),