Synthesis of Ester-Linked Paclitaxel–Glycoside Conjugate and Its Drug Delivery System Using Hybrid-Bio-Nanocapsules Targeted With Trastuzumab

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Abstract
Synthesis of the ester-linked glucoside conjugate of paclitaxel, 7-propionylpaclitaxel 3-O-β-D-glucopyranoside, was carried out by chemoenzymatic procedures. The encapsulation efficiency (EE) values for hybrid-bio-nanocapsules of the compound were much improved in comparison with those of paclitaxel. The hybrid-bio-nanocapsules targeted with trastuzumab, which contained 7-propionylpaclitaxel 3-O-β-D-glucopyranoside, showed high anticancer activity.

Keywords
paclitaxel, prodrug, β-glucoside, bio-nanocapsules, trastuzumab, anticancer activity

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Paclitaxel is one of the most potent anticancer agents used in the treatment of breast and ovarian cancers. It presents disadvantages such as low water solubility and toxicity toward normal tissues. To date, efforts have been made to modify paclitaxel chemically in order to create a more soluble and more easily delivered drug.1 A drug delivery system is a useful method to deliver paclitaxel to tumor cells using a drug carrier. However, a drug carrier can incorporate only water-soluble compounds. Paclitaxel prodrugs that incorporate acids have attracted much attention, because an ester linkage improves the solubility of paclitaxel and can be enzymatically hydrolyzed to release paclitaxel.1

Glycosylation of bioactive compounds can enhance their water solubility, physicochemical stability, and biological half-life.2–7 Glycosides have been considered to be useful as prodrugs, which are hydrolyzed in living cells to release the active drug. In plant cells, a glycosylation reaction has diverse functions such as activation of biosynthetic intermediates and detoxification of toxic compounds generated from the environment. Many secondary metabolites, such as saponins and anthocyanins, which are accumulated in the form of glycosides in plants, have specific physiological activities and have been widely used in folk medicines. Plant glycosyltransferases can be used as biocatalysts in organic synthesis to produce glycosides of medicines as prodrugs.

We report here the chemoenzymatic synthesis of a highly water-soluble ester-linked glycoside conjugate of paclitaxel, ie, 7-propionylpaclitaxel 3-O-β-D-glucopyranoside, and its application to a drug delivery system using hybrid-bio-nanocapsules targeted with trastuzumab.

As shown in Figure 1, glycosylation of 3-hydroxypropionic acid (0.2 mol) was catalyzed by plant glucosyltransferase (100 U) from Phytolacca americana expressed in Escherichia coli to give carboxyethyl β-D-glucopyranoside (1) (35%). Compound 1 was benzylated with BnBr/NaH in N,N-dimethylformamide (DMF) at room temperature (RT) for 12 hours, followed by stirring with KOH (1.5 equiv.) to give 2 (90%). The 2-TES ester of paclitaxel (Wako Pure Chemicals, Tokyo, Japan, 0.15 mol) was treated with 2

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to provide 3 (92%), and subsequent desilylation with Pd black in HOAc–H2O (9:1, v/v) afforded 7-propionylpaclitaxel 3″-O-β-D-glucopyranoside (4) (0.13 mol, 95%).

The encapsulation efficiency (EE) value of paclitaxel itself for trastuzumab-targeting hybrid-bio-nanocapsules was 0.1, suggesting that little paclitaxel was incorporated in the hybrid-bio-nanocapsules. On the other hand, that of 7-propionylpaclitaxel 3″-O-β-D-glucopyranoside for trastuzumab-targeting hybrid-bio-nanocapsules was 9, indicating that 7-propionylpaclitaxel 3″-O-β-D-glucopyranoside was incorporated in the hybrid-bio-nanocapsules. The EE value of paclitaxel was improved by modification with an ester moiety having a β-glucosyl group.

Next, 7-propionylpaclitaxel 3″-O-β-D-glucopyranoside was applied to a drug delivery system using hybrid-bio-nanocapsules targeted with trastuzumab. Although the half-maximal inhibitory concentration (IC50) value of paclitaxel contained in trastuzumab-targeting immunoliposomes against HT-29 cells was 35 nM, that of 7-propionylpaclitaxel 3″-O-β-D-glucopyranoside contained in 7-propionylpaclitaxel 3″-O-β-D-glucopyranoside encapsulated in trastuzumab-targeting hybrid-bio-nanocapsules were 21 and 10 nM. These results indicate that 7-propionylpaclitaxel 3″-O-β-D-glucopyranoside was delivered to HT-29 cells successfully, using hybrid-bio-nanocapsules as the drug carrier.

Thus, the ester-linked glycoside conjugate of paclitaxel, 7-propionylpaclitaxel 3″-O-β-D-glucopyranoside, was synthesized by a chemoenzymatic method using glucosyltransferase as a biocatalyst. The EE value of 7-propionylpaclitaxel 3″-O-β-D-glucopyranoside for hybrid-bio-nanocapsules was improved by glucosylation modification. 7-Propionylpaclitaxel 3″-O-β-D-glucopyranoside incorporated in hybrid-bio-nanocapsules targeted with trastuzumab showed high anticancer activity toward HT-29 cells as judged by comparison of its IC50 value with that of paclitaxel.

Experimental

General

High-performance liquid chromatography (HPLC; Shimazu, Tokyo, Japan) analyses were carried out with a Puresil C18 column (Waters) using MeOH:H2O (1:3, v/v) as eluent (detection, UV280; flow rate, 1 mL min−1).

Synthesis of 7-Propionylpaclitaxel 3″-O-β-D-Glucopyranoside

cDNA of glucosyltransferase from P. americana (PaGT) was cloned into pQE30, and the resulting plasmids were transformed into E. coli M15 cells. The expression and purification of PaGT were performed as described previously. The purified enzyme solution was dialyzed with 50 mM Tris-HCl (pH 7.2) containing 5 mM dithiothreitol, and stored at −80 °C. Glucosylation reactions were performed at 35 °C for 24 hours in 5 mL of 50 mM potassium phosphate buffer (pH 7.2) supplemented with 0.02 mol hydroxypropionic acid, 0.03 mol UDP-glucose, and 100 U enzyme. Large-scale synthesis of 1 was carried out by enlarging 10-fold. The incubation was stopped by adding 1.5% trifluoroacetic acid; the reaction mixture was analyzed by HPLC. The reaction mixture was extracted with n-BuOH. The n-BuOH fraction was concentrated by evaporation and the residue was dissolved in water. The water fraction was applied to Diaion HP-20, washed with water, and eluted with methanol. The methanol solution was subjected to preparative HPLC to afford carboxyethyl β-D-glucopyranoside (1). To a solution of BnBr/NaH in 5 mL of DMF was added carboxyethyl β-D-glucopyranoside (1). The mixture was stirred at RT for 12 hours, followed by stirring with aqueous KOH (1.5 equiv). The reaction mixture was quenched with saturated aqueous NaHCO3 and extracted with ethyl acetate (20 mL × 3). The ethyl acetate layer was concentrated in vacuo and purified by column chromatography to give carboxyethyl 2,3,4,6-tetra-O-benzyl-β-D-glucopyranoside (2). To a solution of paclitaxel and imidazole in dry DMF (4 mL) was added chlorotriethylsilane dropwise at RT. The reaction mixture was stirred at RT for 2 hours and diluted with ethyl acetate. The mixture was washed with water and brine, dried over MgSO4, and concentrated in vacuo. Column chromatography of the residue on silica gel gave the 2′-TES ester of paclitaxel. To a mixture of the 2′-TES ester of paclitaxel in the presence of EDCI/DMAP in CH2Cl2 (10 mL) was added 2 (1.2 equiv). The mixture was stirred at RT for 12 hours. The reaction mixture was extracted with ethyl acetate. The organic layer was concentrated in vacuo and purified...
by column chromatography on silica gel to give 3. To a solution of Pd black in HOAc-H2O (9:1, v/v) was added 3. The suspension was stirred at RT for 24 hours. Extraction of the reaction mixture with n-butanol followed by column chromatography on silica gel yielded 7-propionylpaclitaxel 3′′-O-β-D-glucopyranoside (4). Compound 4 was detected at 15 minutes by HPLC analysis (see Supplemental General).

**Anticancer Effects of Hybrid-Bio-Nanocapsules Containing 7-Propionylpaclitaxel 3′′-O-β-D-Glucopyranoside**

Paclitaxel prodrug, 7-propionylpaclitaxel 3′′-O-β-D-glucopyranoside, was encapsulated in hybrid-bio-nanocapsules by electroporation to give hybrid-bio-nanocapsules containing paclitaxel prodrug. EE was calculated as the ratio of the amount of drug encapsulated into hybrid-bio-nanocapsules to the initial amount of drug. The sensitivity of HT-29 cells to 7-propionylpaclitaxel 3′′-O-β-D-glucopyranoside electroporated into hybrid-bio-nanocapsules was determined as follows. Cells were diluted with culture medium to the seeding density (10⁵ cells/mL), suspended in 96-well tissue culture plates (100 μL/well), preincubated at 37 °C for 4 hours, and then treated for 24 hours with 7-propionylpaclitaxel 3′′-O-β-D-glucopyranoside electroporated into hybrid-bio-nanocapsules at various concentrations to obtain a dose–response curve for each compound. After incubation, 20 μL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, yellow tetrazole) solution (2.5 mg/mL) was added to each well and the plates were further incubated for 4 hours. Absorbance at 570 nm was measured with a microplate reader model 450 (BIO-RAD). Dose–response curves were plotted on a semi-log scale as percentage of the cell numbers in control cultures not exposed to test compounds to calculate IC₅₀ values.

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**Supplemental Material**

Supplemental material for this article is available online.

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