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Paclitaxel-incorporated nanoparticles of hydrophobized polysaccharide and their antitumor activity

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ABSTRACT

The aim of this study was to characterize paclitaxel-incorporated polysaccharide nanoparticles and evaluate their antitumor activity *in vitro* and *in vivo*. Pullulan was hydrophobically modified using acetic anhydride to make the paclitaxel-incorporated nanoparticles. Pullulan acetate (PA) was used to encapsulate paclitaxel using the nanoprecipitation method. The particles had spherical shapes under electron microscopy with sizes <100 nm. The sizes of paclitaxel-incorporated nanoparticles increased to >100 nm, and higher drug feeding induced higher particle size and drug content. Initial drug burst release was observed until 2 days and then the drug was continuously released over 1 week. Intrinsic cytotoxicity of empty PA nanoparticles was tested with RAW264.7 macrophage cells for biocompatibilty. The viability of RAW264.7 cells was >93% at all concentrations of empty PA nanoparticles, indicating that the PA nanoparticles are not acutely cytotoxic to normal human cells. The nanoparticles showed lower antitumor activity *in vitro* against HCT116 human colon carcinoma cells than that of paclitaxel itself, indicating the sustained release properties of nanoparticles. An *in vivo* study using HCT116 human colon carcinoma-bearing mice showed that paclitaxel-incorporated PA nanoparticles reduced tumor growth more than that of paclitaxel itself. These results indicate that PA paclitaxel-incorporated nanoparticles are a promising candidate for antitumor drug delivery.

1. Introduction

Paclitaxel has been extensively used as a cancer chemotherapeutic agent and is currently used for chemotherapy of patients with lung, ovarian, breast, head and neck cancer, and advanced forms of Kaposi's sarcoma (Crown and O'Leary, 2000; Marchetti et al., 2002; Marupudi et al., 2007; Mekhail and Markman, 2002). However, despite its successful application to cancer chemotherapy, a disadvantage of paclitaxel is its limited clinical application, *i.e.* myelosuppression, neuropathy, myalgia, fatigue, alopecia, diarrhea, mucosal toxicity, and skin and nail changes have been reported (Rowinsky and Donehower, 1995). Furthermore, an ethanol–surfactant mixture is normally used to dissolve paclitaxel due to its extremely poor aqueous solubility, and this surfactant–ethanol mixture may induce unwanted side effects to patients (Gogate et al., 2009; Marupudi et al., 2007; ten Tije et al., 2003). Thus, various formulations to enhance the aqueous solubility of paclitaxel have been developed such as albumin nanoparticles (Kratz, 2008; Zhao et al., 2010), lipid nanoparticles (Chen et al., 2001; Huynh et al., 2009), polypeptide-conjugates (Van et al., 2010), polymeric micelles (Kim et al., 2001), polysaccharide conjugates (Sugahara et al., 2002), and nanoparticles (Jeong et al., 2005).

Nano-sized vehicles such as nanoparticles, core–shell type polymeric micelles, and macromolecular self-aggregates have been extensively investigated in the biomedical field (Chen et al., 2001; Jung et al., 2003; Jeong et al., 2006; Kwon et al., 1994; Na et al., 2010). Due to their small particle size, nanoparticles are suitable vehicles for intravenous injection of anticancer drugs and imaging agents (Jain et al., 2008; Jeong et al., 2009; Na et al., 2010). Furthermore, nanoparticles or self-associated macromolecules are regarded as a superb carrier for solubilizing lipophilic anticancer drugs with active or passive drug delivery to the site of action (Ayame et al., 2008; Jeong et al., 2006; Kwon et al., 1994; Yokoyama et al., 1990). Furthermore, advantages of self-assembled nanoparticles are extended blood circulation time of drug, avoidance of the reticuloendothelial system (RES), and a decreased number of side effects (Kwon et al., 1994; Yokoyama et al., 1990).

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In this study, we prepared paclitaxel-incorporated nanoparticles using a hydrophobized polysaccharide, pullulan acetate (PA). Pullulan is a non-ionic polysaccharide that has several advantages such as excellent water-solubility, non-toxicity, and no immunogenicity (Yuen, 1974; Jeanes, 1977). Due to its biocompatibility, pullulan has been used as a biomaterial and drug delivery vehicle in various fields (Abed et al., 2011; Li et al., 2011; Sizovs et al., 2010). Furthermore, multiple hydroxyl groups in the main pullulan chain can be used for chemical modifications. To make the nanoparticles, pullulan was hydrophobically modified using acetic anhydride, and paclitaxel was loaded into the hydrophobically modified pullulan derivatives. Paclitaxel-incorporated nanoparticles were characterized *in vitro* and their anticancer effect was evaluated using HCT116 human colon cancer cells *in vivo*.

2. Materials and methods

2.1. Materials

Dialysis tubing (molecular weight [MW] cut-off: 12,000 g/mol), thiazolyl blue tetrazolium bromide (MTT), Cremophor® EL, formamide, pyridine, methanol, ethanol, dimethylformamide (DMF), dimethylacetamide (DMAc), dimethysulfoxide (DMSO), and acetone were purchased from Sigma Co. Ltd. (St. Louis, MO, USA). Pullulan (average MW = 50,000–100,000 g/mol) was purchased from Wako Pure Chemical (Osaka, Japan). The MW of pullulan was measured by gel permeation chromatography as described previously (Jeong et al., 2006). Weight average MW, number average MW, and polydispersity of pullulan was 67,500, 62,400, and 1.081 ± 0.019, respectively. Paclitaxel was purchased from Samyang Pharmaceuticals (Daejeon, Korea).

2.2. Synthesis of polymers

PA, hydrophobized pullulan, was synthesized as described previously (Jung et al., 2003). Pullulan (2 g) was suspended in 20 ml of formamide and dissolved by vigorous stirring at 54 °C.

Pyridine (6 ml) and 5 ml of acetic anhydride were then added, and the mixture was stirred at 54 °C for 48 h. A dark-brown precipitate was obtained and purified by reprecipitation with 1000 ml of distilled water and 500 ml of methanol. The solid material was vacuum-dried for 3 days, and a white powder was obtained.

2.3. Analysis of polymers

^{1}H nuclear magnetic resonance (NMR) spectra were employed to characterize synthesized PA 500 MHz NMR spectroscopy (500 MHz superconducting Fourier transform-NMR spectrometer, Varian Unity Inova 500 MHz NB High Resolution FT-NMR; Varian Inc., Santa Clara, CA, USA) at 25 °C. The particle sizes of empty and paclitaxel-incorporated nanoparticles were measured by dynamic light scattering (DLS-7000, Otsuka Electronics Company, Osaka, Japan).

X-ray powder diffractograms (XRDs) were constructed to analyze the crystallinity of the drug and drug-loaded nanoparticles using a Rigaku D/Max-1200 instrument (Rigaku, Tokyo, Japan) equipped with Ni filtered Cu Kα radiation (40 kV, 20 mA). The conditions of the powder XRD measurements were as follows:

- Data type = binary; goniometer = 1; attachment = 1; scan mode = continuous.
- Mode 2 (R/T) = reflection; and scan axis = 2-theta/theta.
- Start angle = 10,000; stop angle = 80,000; scan speed = 5000; sampling interval = 0.050; theta angle = 5000; 2 theta angle = 10,000; fixed time = 0.01; full scale = 1000; counting unit = CPS; and target = Cu.
- Wavelength, Ka1 = 1.540510; wave length, Ka2 = 1.544330; wave length, Ka = 1.541780; and wave length, Kb = 1.392170.
- 40.0 kV and 20.0 mA.

Paclitaxel, empty PA nanoparticles, and paclitaxel-incorporated PA nanoparticles were used to measure powder XRD. Empty PA nanoparticles (90 mg) were mixed with 10 mg of paclitaxel to prepare the physical mixture of PA and paclitaxel.

Nanoparticle morphology was observed with a field emission scanning electron microscope (S-4700; Hitachi Co., Ltd., Tokyo, Japan). The nanoparticles were placed on double-sided tape attached to a graphite surface. Each sample was coated with gold/palladium using an ion sputter (JEOL JFC-1100; Tokyo, Japan). Coating was performed at 20 mA for 4 min. Observations were conducted at 15 kV.

2.4. Preparation of paclitaxel-incorporated PA nanoparticles

Paclitaxel-incorporated PA nanoparticles were prepared as follows: briefly, PA and paclitaxel were completely dissolved in 5 ml of organic solvent, and this solution was slowly dropped into 20 ml of deionized water (pluronic F68, 0.1%, w/w) with magnetic stirring for 10 min. Nanoparticles were harvested by ultracentrifugation at $100{,}000 \times g$. The recovered nanoparticles were washed with deionized water and recovered again with ultracentrifugation ($100{,}000 \times g$) to remove the remaining solvent and surfactants. This process was repeated three times. The reconstituted nanoparticle solution in deionized water was analyzed or lyophilized (in the presence of mannitol, 0.5 g) for 2 days. Drug content was measured using high performance liquid chromatography (HPLC). Drug content = [amount of drug in the nanoparticles/weight of nanoparticles] × 100.

Empty nanoparticles were prepared using the same procedure without paclitaxel.

The HPLC measurement conditions were as follows: the Flexar HPLC system (PerkinElmer, Waltham, MA, USA) consisted of a Solvent Manager 5-CH Degasser, an autosampler, a quaternary LC pump, a column oven and an UV/Vis detector. Chromatography was performed using a guard column (SecurityGuard Guard Cartridge Kit, Phenomenex, Torrance, CA, USA) and a C18 column (Brownlee C18, 5 μm, 150 × 4.6, PerkinElmer) at 37 °C. Paclitaxel was eluted isocratically with a mobile phase (acetonitrile:methanol:1% acetic acid, 35:38:27) at a flow rate of 1 ml/min and monitored at 273 nm. The chromatograms were recorded and integrated with system software (Chromera 2.1, PerkinElmer)

The drug release test was conducted as follows: paclitaxel-incorporated nanoparticles (5 mg) were redistributed in 5 ml of phosphate-buffered saline (0.1 M, pH 7.4) and this solution was introduced into dialysis tubing. The dialysis tubing was added to a 100 ml bottle containing 95 ml PBS (0.01 M, pH 7.4, 0.1% (w/v) Tween80). At predetermined time intervals, all media were exchanged to prevent drug saturation. The concentration of released drug was measured with the HPLC system.

2.5. Reconstitution of paclitaxel-incorporated PA nanoparticles

The lyophilized paclitaxel-incorporated PA nanoparticles were reconstituted as follows: mannitol (500 mg) was added to the nanoparticle solution (approximately 50 mg of nanoparticles) prior to lyophilization. Paclitaxel-incorporated PA nanoparticles (drug content, 7.8% [w/w]) were reconstituted in PBS (0.01 M, pH 7.4).

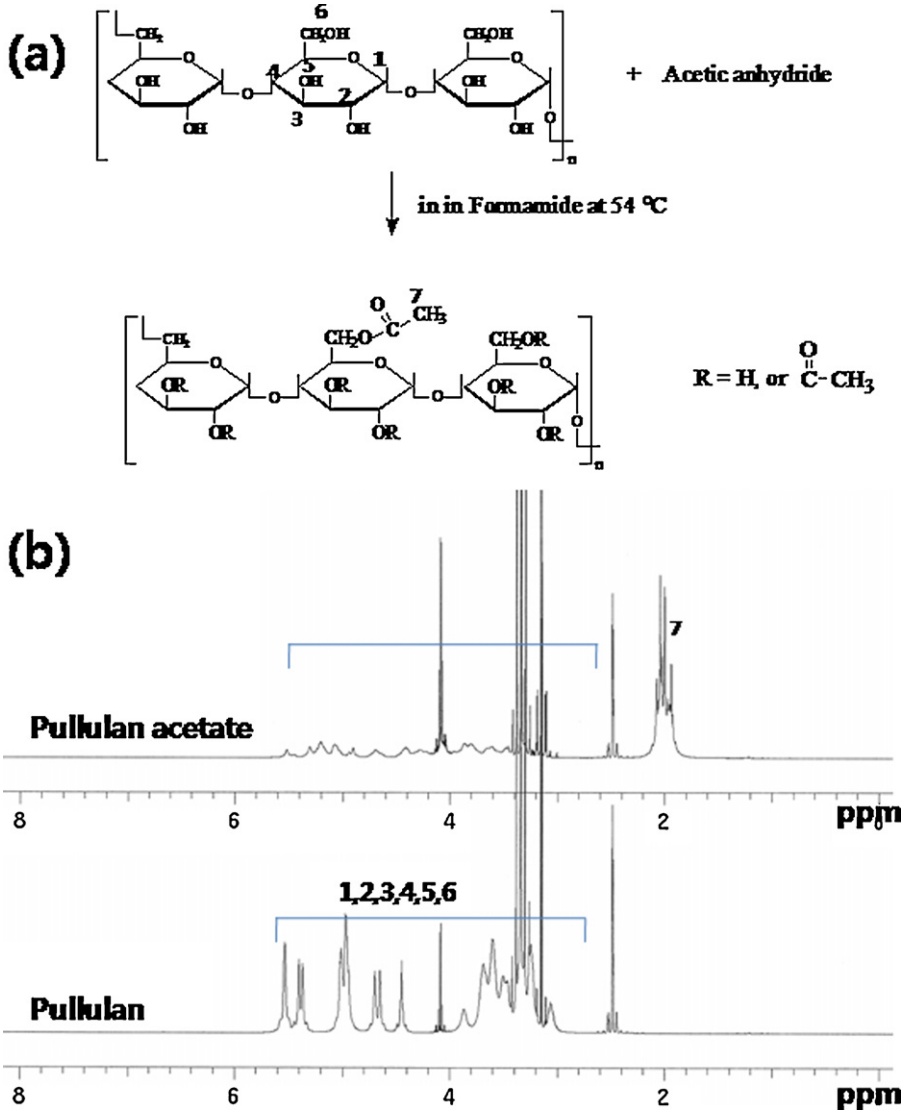

Fig. 1. Synthesis scheme (a) and ^{1}H NMR spectra of pullulan acetate (PA) (b).

2.6. Cytotoxicity of PA nanoparticles to RAW264.7 cells

The intrinsic cytotoxicity of the PA nanoparticles was tested using mouse macrophage RAW264.7 cells. RAW264.7 cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) in a 5% CO_2 incubator (37 °C). RAW264.7 cells were seeded in 96-well plates at a density of 3×10^4 cells/well and incubated overnight in a 5% CO_2 incubator at 37 °C. Empty PA nanoparticles were reconstituted in DMEM medium without FBS and added to wells. Cell viability was assayed 1–3 days later using the MTT colorimetric assay. After the incubation, 30 μl of MTT (5 mg/ml in PBS, pH 7.4) was added to each well and incubated for 4 h. The formazan crystals were then solubilized with DMSO and the absorbance (560 nm-test/630 nm reference) was determined using an automated computer-linked microplate reader (Molecular Device Co., Sunnyvale, CA, USA). Each experimental value was the mean of eight wells.

2.7. Antitumor activity in vitro

HCT116 human colon carcinoma cells were obtained from the Korean Cell Line Bank (Seoul, Korea). The medium for cell culture and the proliferation inhibition assay was RPMI1640 supplemented with 10% FBS. The cells were maintained in this medium in a 5% CO_2 incubator at 37 °C. To study antitumor activity *in vitro*, paclitaxel-incorporated PA nanoparticles were prepared and inhibition of tumor cell growth was determined using the MTT cell proliferation assay. HCT116 cells were seeded in 96-well plates at a density of 5×10^3 cells/well and incubated overnight in a 5% CO_2 incubator at 37 °C. Control cells were treated with media containing 0.5% DMSO. Paclitaxel dissolved in DMSO was diluted with medium, and the final DMSO concentration was adjusted to <0.5%. The paclitaxel-incorporated nanoparticle solution, prepared as described above, was reconstituted in medium. Cell viability was assayed 1–2 days later using the MTT colorimetric assay.

2.8. Antitumor activity in vivo

Antitumor activity of paclitaxel-incorporated PA nanoparticles *in vivo* was assessed using athymic nude BALB/c male mice (20 g). HCT116 cells (5×10^6) were implanted subcutaneously in the backs of mice, and the animals were divided into three groups 1 week later: control, paclitaxel treatment, and paclitaxel-incorporated treatment. PBS (0.01 M, pH 7.4) was used for the control. For the paclitaxel treatment, paclitaxel was dissolved in Cremophor® EL/ethyl alcohol (5:5, [v/v]) solution and diluted ten

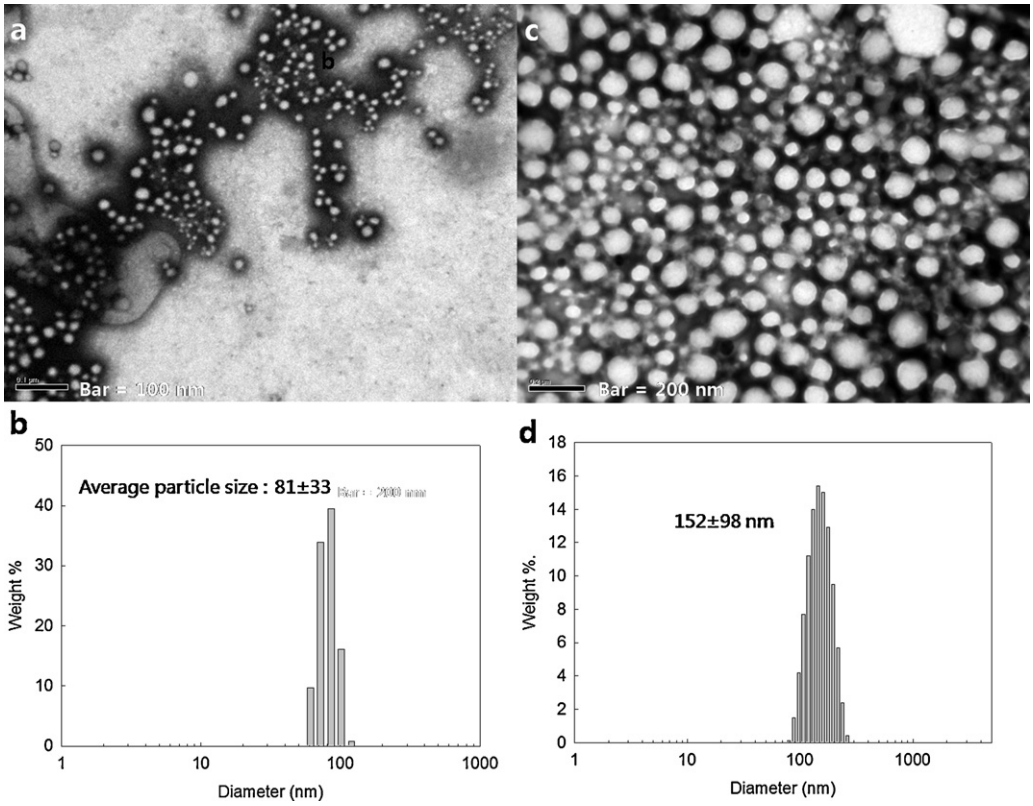

Fig. 2. Morphological observation of pullulan acetate (PA) nanoparticles using transmission electron microscopy (TEM) (a and c) and a typical particle size distribution (b and d). Empty PA nanoparticles (a and b) and reconstituted paclitaxel-incorporated PA nanoparticles (c and d). Reconstitution process is described in Section 2.

times with PBS. Lyophilized paclitaxel-incorporated PA nanoparticles were reconstituted in deionized water for the nanoparticle treatment. Each treatment group was comprised of five tumor-bearing mice. PBS, the paclitaxel solution, or the nanoparticle solution (100–200 μl) was administered intravenously *via* the tail vein. Tumor growth was measured at 2-day intervals using calipers. Tumor volume was calculated from the following equation: tumor volume (mm^3) = (length × $width^2$)/2. All experiments using mice followed the guidelines approved by the Committee for the Care and Use of Laboratory Animals at Chonnam National University.

2.9. Statistical analysis

Results are expressed as average ± standard deviation, and statistical significance between experimental groups was assessed using the Student's *t*-test. A *p*-value < 0.05 was considered significant.

3. Results and discussion

3.1. Characterization of hydrophobically modified polysaccharide

Because pullulan itself is fully soluble in water, it was hydrophobically modified using acetic anhydride. This hydrophobically modified pullulan is called PA, as described previously (Jung et al., 2003). Compared to pullulan, the advantages of PA are its enhanced processability and superior organic solubility against organic solvents such as acetone, DMF, DMSO, DMAc, 1,4-dioxane, and dichloromethane (Jung et al., 2003). PA was synthesized by attaching an acetyl group to the hydroxyl group of pullulan (Fig. 1). The PA acetylation degree was >81% by ^{1}H NMR spectroscopy. It was easy to fabricate the nanoparticles by nanoprecipitation into deionized water.

3.2. Characterization of paclitaxel-incorporated PA nanoparticles

Amphiphilic macromolecules such as block copolymers, graft copolymers, and hydrophobized polysaccharides form nanosized vehicles with a core–shell structure in an aqueous environment (Gref et al., 1994; Jeong et al., 2006; Kwon et al., 1994). These properties of amphiphilic macromolecules are suitable for entrapping hydrophobic anticancer drugs (Ayame et al., 2008; Jeong et al., 2006; Jung et al., 2003; Kwon et al., 1994). Jeong et al. (2005, 2006, 2009) previously reported that nano-vehicles based on a micellar structure or self-aggregates of hydrophobized polysaccharides are superior carriers for anticancer drug delivery and tumor targeting *in vitro* and *in vivo*.

PA and paclitaxel were dissolved in organic solvent and dropped into water to load paclitaxel. The paclitaxel-incorporated nanoparticles were prepared by the nanoprecipitation method as summarized in Table 1. The empty and paclitaxel-incorporated PA nanoparticles had a spherical morphology (Fig. 2). The average size of the empty nanoparticles was <100 nm, whereas the size of paclitaxel-incorporated nanoparticles was >100 nm. Small

Table 1
Characterization of paclitaxel-incorporated PA nanoparticles.

Used solvent	Paclitaxel/polymer weight ratio	Drug contents (%, w/w)		Particle size (nm)[a]
		Theoretical	Experimental	
Acetone	45/5	10	7.8	154.6 ± 50.2
Acetone	40/10	20	13.1	253.0 ± 65.5
DMAc	45/5	10	7.2	127.6 ± 30.7
DMSO	45/5	10	6.7	140.5 ± 20.8
DMF	45/5	10	8.1	132.6 ± 28.7

[a] Weight average.

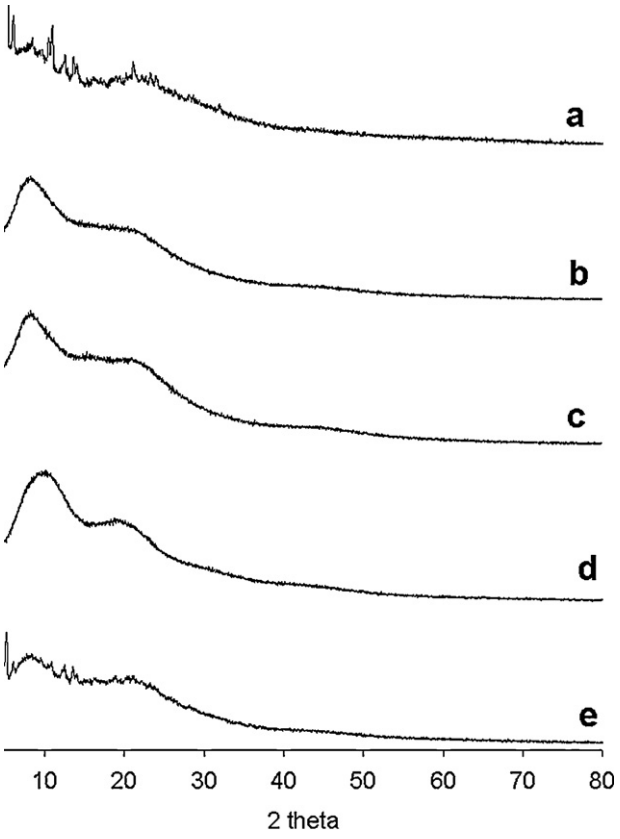

Fig. 3. X-ray powder diffraction patterns of the paclitaxel-loaded nanoparticles. Paclitaxel (a); paclitaxel-loaded nanoparticles (drug content: 7.8% [w/w]) (b); paclitaxel-loaded nanoparticles (drug content: 13.1% [w/w]) (c); empty pullulan acetate (PA) (d); and empty nanoparticle/paclitaxel physical mixture (weight ratio of empty nanoparticle/paclitaxel = 9:1) (e).

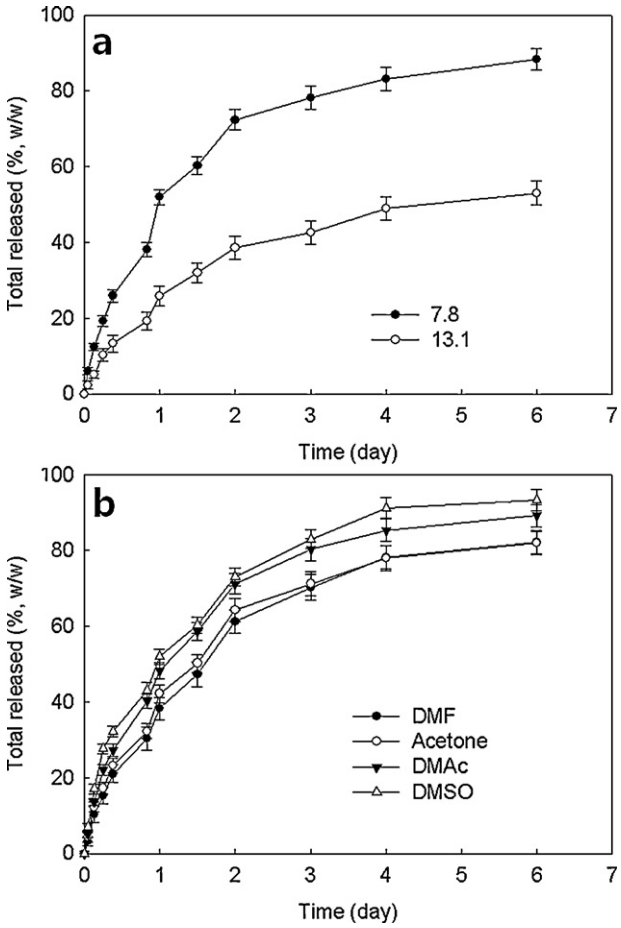

Fig. 4. Paclitaxel release from pullulan acetate (PA) nanoparticles. The effect of drug content (a) and the various solvents used (b).

particles of <200 nm are advantageous because reduced particle size nanoparticles can increase blood circulation of an anticancer drug, avoid the RES, and passively target the anticancer drug (Kwon et al., 1994; Yokoyama et al., 1990). Hydrophilic pullulan did not form nanoparticles, and particle size was not measurable (data not shown). As shown in Table 1, particle size increased according to drug content in the nanoparticles, *i.e.*, higher drug feeding induced greater particle size and higher drug content. Among the various solvents used, DMAc resulted in the smallest particle size, whereas DMF resulted in higher drug content at the same polymer:drug feeding ratio (Table 1). Although nanoparticles with a 40:10 polymer:drug ratio had increased size, no precipitation was found. Significant secondary aggregation and some precipitation were observed at polymer:drug ratios > 40:10. Fig. 3 shows the XRD patterns of paclitaxel and the paclitaxel-incorporated PA nanoparticles. Paclitaxel itself had intrinsic crystalline peaks, whereas broad peaks were shown for the empty PA nanoparticles. In particular, the paclitaxel-incorporated PA nanoparticles showed a similar broad spectrum compared to that of the empty nanoparticles, whereas the physical mixture showed crystalline peaks of paclitaxel and broad peaks of empty PA nanoparticles, indicating that paclitaxel was molecularly dispersed in the nanoparticle core with 7.8% (w/w) and 13.1% (w/w) drug content. This result was similar to other reports (Gref et al., 1994; Jeong et al., 2006) showing that hydrophobic crystalline drug is molecularly dispersed in the solid core of nanoparticles and that molecular dispersion of the drug occurs easily in aqueous solution.

The drug release properties are shown in Fig. 4. Paclitaxel was released over 1 week. An initial burst release was observed for 2 days and then the drug was released continuously over 1 week. As shown in Fig. 4a, the higher the drug content, the slower the release rate. This phenomenon has been reported previously (Gref et al., 1994; Jeong et al., 2006), *i.e.*, a hydrophobic drug with higher drug loading is normally aggregated in the nanoparticle core resulting in a slower release than a molecularly dispersed drug at lower drug loading. Fig. 4b shows drug release based on the solvent used. Release with DMSO tended to be faster than that of other solvents, but no significant difference was observed among solvents. This result might be due to lower drug loading on the DMSO nanoparticles.

The characteristics of the reconstituted nanoparticles were studied prior to the anticancer activity experiment (Fig. 5). Paclitaxel-incorporated PA nanoparticles were clearly redistributed in aqueous solution even though the particle size distribution was broader and drug content decreased slightly to 7.2% (w/w). The spherical morphology of the nanoparticles was observed by transmission electron microscopy (Fig. 5). These results indicate that the reconstitution process did not significantly change the properties of the nanoparticles.

3.3. Cytotoxicity of empty PA nanoparticles to RAW264.7 human macrophage cells

The cytotoxicity of the empty PA nanoparticles was tested with RAW264.7 mouse macrophage cells. As shown in Fig. 5, RAW264.7 cell viability was >93% at the highest concentration of empty PA nanoparticles, indicating that PA nanoparticles are not acutely cytotoxic. Furthermore, cell viability was >97% at a concentration of 10 μg/ml empty PA nanoparticles, which was the same polymer

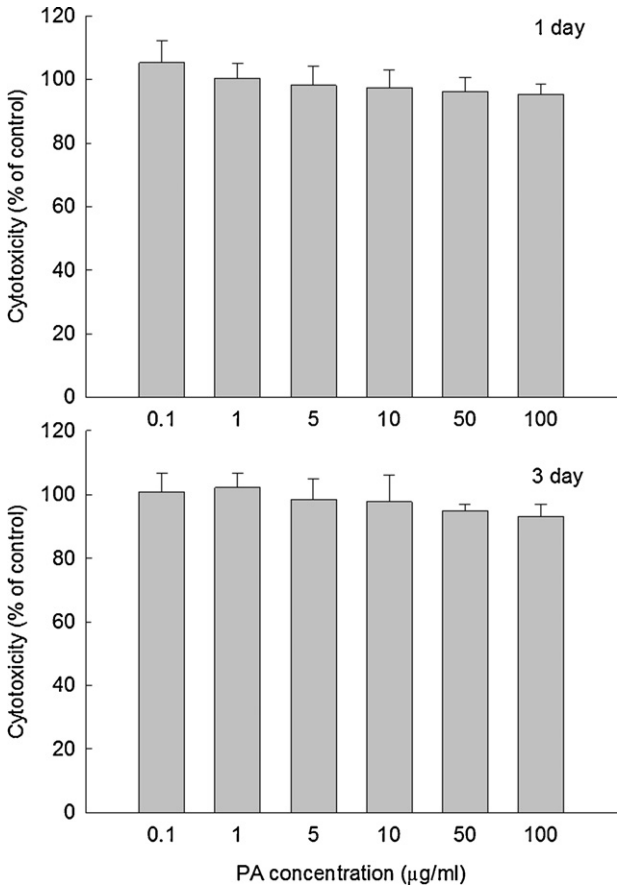

Fig. 5. Cytotoxicity of empty pullulan acetate (PA) nanoparticles against RAW264.7 mouse macrophage cells. A total of 3×10^4 cells were seeded onto 96 wells, empty PA nanoparticles were reconstituted in fetal bovine serum (FBS)-free DMEM medium, and RAW264.7 cell viability was evaluated with the MTT assay.

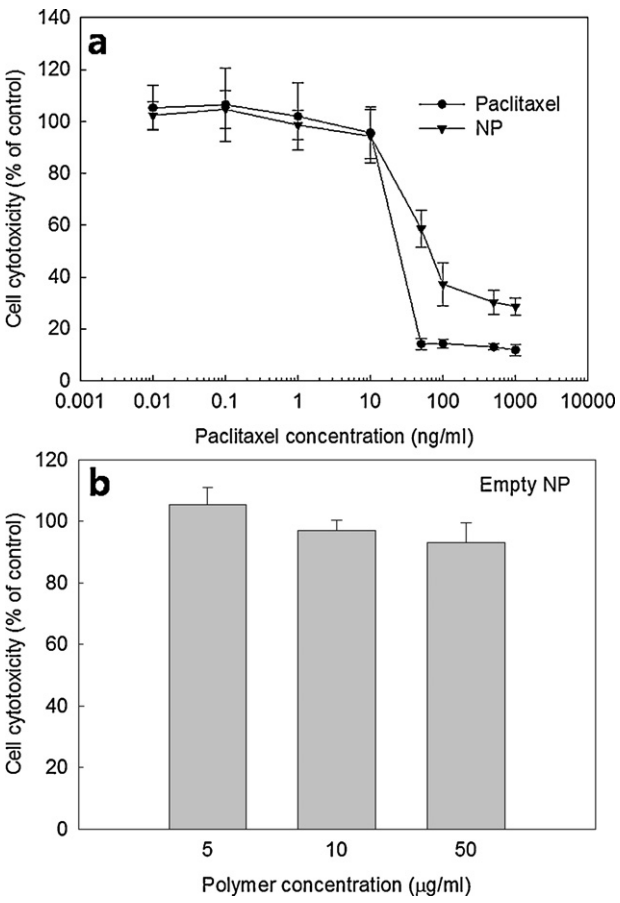

Fig. 6. Cytotoxicity of paclitaxel or paclitaxel-incorporated pullulan acetate (PA) nanoparticles (a) and empty nanoparticles of PA (b) against HCT 116 cells.

concentration used in the paclitaxel-incorporated nanoparticle treatment (Fig. 6). Due to its biocompatibility, pullulan and its derivatives have been extensively used as biomedical materials and drug delivery vehicles (Abed et al., 2011; Li et al., 2011; Rekha and Sharma, 2009; Sizovs et al., 2010; Tanaka et al., 2004). Furthermore, Marques et al. (2002) reported that acetylated derivatives of polysaccharides are biocompatible.

3.4. Anticancer activity of paclitaxel-incorporated PA nanoparticles against HCT116 human colon cells

HCT116 colon carcinoma cells were employed, and growth inhibition by paclitaxel itself and the nanoparticles were tested *in vitro* and *in vivo*. Cell viability was not significantly affected until a concentration of 10 ng paclitaxel/ml, and the density of surviving cells decreased at concentrations >50 ng/ml paclitaxel alone. Otherwise, the paclitaxel-incorporated nanoparticles showed similar cytotoxicity until 10 ng/ml, but the anticancer effect of the paclitaxel-incorporated nanoparticles was less than that of paclitaxel itself at concentrations >10 ng/ml. These results might be due to the sustained release properties of the nanoparticles, whereas the empty PA nanoparticles did not significantly affect cell viability. The IC_{50} of each compound is summarized in Table 2.

To access *in vivo* anticancer activity of the paclitaxel-incorporated PA nanoparticles, HCT116 cells were implanted in the backs of athymic mice. After 13 and 17 days, paclitaxel and paclitaxel-incorporated nanoparticles were administered intravenously *via* the tail vein. As shown in Fig. 7a, *in vivo* anticancer activity of paclitaxel-incorporated PA nanoparticles was evaluated by growth of solid tumors on the backs of mice. Weight changes of the mice were recorded (Fig. 7b). The body weights of control mice increased, whereas those treated with the paclitaxel-incorporated nanoparticles did not show a significant change of body weight. Interestingly, mice in the paclitaxel treatment showed that body weight did not significantly change until 27 days after tumor cell implantation but body weight increased after that, indicating that body weight changes at this time point might be reflecting tumor growth. Tumor growth in the paclitaxel treatment group decreased slightly compared to that in the control until 21 days after tumor cell implantation (Fig. 7a). However, tumor volume increased at a similar rate to that in the control after that. Otherwise, tumor volume decreased significantly compared to that in the control and paclitaxel treatment (Fig. 7a). These results suggest that paclitaxel-incorporated nanoparticles enhanced antitumor activity *in vivo*. As PA itself did not have an active targeting moiety, the reason for reduced tumor volume might be due to enhanced vascular permeability and retention (EPR) effect of nanoparticles as reported previously (Greish et al., 2005; Jeong et al., 2009; Maeda et al., 2000; Noguchi et al., 1998). Macromolecular conjugated drug carriers can accumulate in solid tumors, and higher MW copolymers have higher accumulation ratios in solid tumors

Table 2
IC_{50} of paclitaxel and paclitaxel-incorporated PA nanoparticles.

	IC_{50} (μg/ml)
Paclitaxel	0.035
Paclitaxel-incorporated PA nanoparticles	0.072
Empty PA nanoparticles	>100

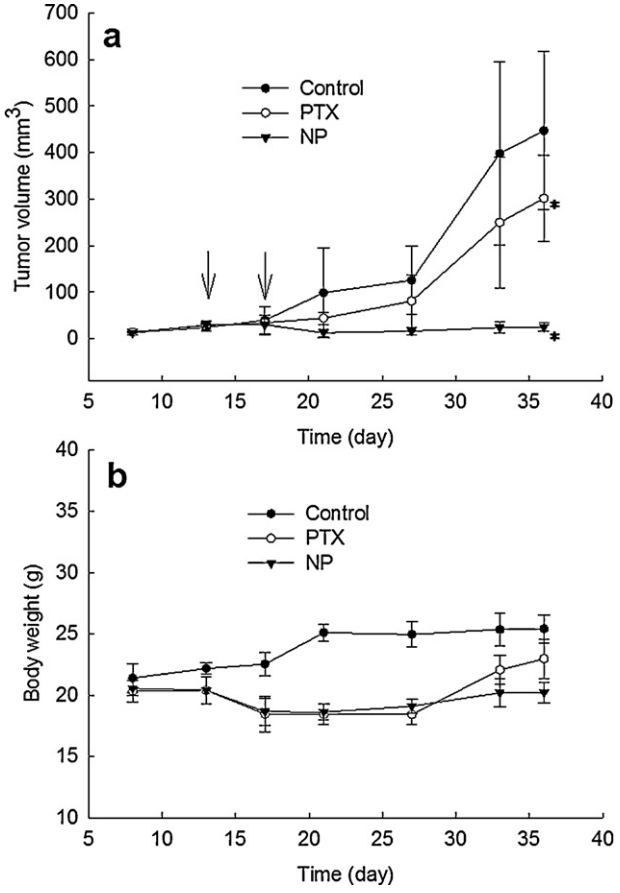

Fig. 7. Changes in tumor volume (a) and body weight (b). Tumor cells were inoculated subcutaneously into the backs of mice and then PTX or PTX-incorporated nanoparticles (equivalent dose of 5 mg PTX/kg mouse) was administered *via* the tail vein 13 and 17 days after tumor cell inoculation. PBS (pH 7.4) was used as a control. PTX, paclitaxel; and NP, paclitaxel-incorporated nanoparticles. $^{*}p < 0.01$.

(Noguchi et al., 1998). Furthermore, these properties of nanoparticulated macromolecular drug carriers show marked antitumor activity with decreased unwanted side effects (Greish et al., 2005; Yokoyama et al., 1990, 1991). Polymeric micellar anticancer agents show marked antitumor activity with significantly low cytotoxicity (Yokoyama et al., 1990, 1991). Our results suggest that efficient accumulation of nanoparticles in tumors must be due to the EPR effect and the passive targeting function, although the nanoparticles did not have an active targeting function. We suggest that the paclitaxel-incorporated PA nanoparticles are a superior candidate for antitumor drug delivery.

4. Conclusion

Paclitaxel-incorporated PA nanoparticles were prepared using a nanoprecipitation method. The particles had spherical shapes with sizes of about 80 nm. The sizes of the paclitaxel-incorporated nanoparticles increased to >100 nm and higher drug feeding induced higher particle size and drug content. The initial burst release of drug was observed until 2 days and then the drug was released continuously over 1 week. Nanoparticles showed lower antitumor activity *in vitro* than that of paclitaxel itself, indicating that the sustained release properties of the nanoparticles might be the reason for the results. However, paclitaxel-incorporated PA nanoparticles showed a higher reduction in tumor volume in an *in vivo* study using HCT116 human colon carcinoma cells.

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