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Tumoral acidic extracellular pH targeting of pH-responsive MPEG-poly (β-amino ester) block copolymer micelles for cancer therapy

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Abstract

The main objective of this study was to develop and characterize a pH-responsive and biodegradable polymeric micelle as a tumor-targeting drug delivery system. The pH-responsive block copolymer was synthesized by a Michael-type step polymerization of hydrophilic methyl ether poly (ethylene glycol) (MPEG) and pH-responsive and biodegradable poly(β-amino ester), resulting in an amphiphilic MPEG-poly(β-amino ester) block copolymer. This copolymer, which formed nano-sized self-assembled micelles under aqueous conditions, could be efficiently (74.5%) loaded with doxorubicin (DOX) using a solvent evaporation method. In an *in vitro* drug release study, these DOX-loaded polymeric micelles showed noticeable pH-dependent micellization–demicellization behavior, with rapid release of DOX from the micelles in weakly acidic environments (pH 6.4) but very slow release under physiological conditions (pH 7.4). Moreover, due to demicellization, the tumor cell uptake of DOX released from polymeric micelles was much higher at pH 6.4 than at pH 7.4. When *in vivo* anti-tumor activity of pH-responsive polymeric micelles was evaluated by injecting the DOX-loaded polymeric micelles into B16F10 tumor-bearing mice, these micelles notably suppressed tumor growth and also prolonged survival of the tumor-bearing mice, compared with mice treated with free DOX.

Keywords: pH-responsive; Polymeric micelles; Doxorubicin; Drug delivery system

1. Introduction

Many therapeutic anticancer drugs, while pharmacologically effective in cancer treatment, are limited in their clinical applications by serious toxicities. To overcome these obstacles, researchers have focused on the development of nano-sized anti-cancer drug carriers, which can improve therapeutic efficacy while also reducing unwanted side effects [1–3]. Among these carriers are polymeric micelles, typically 20 to 100 nm in diameter, which are formed by amphiphilic block copolymers under aqueous conditions [4–6]. Intravenously injected polymeric micelles exhibit prolonged circulation times by avoiding rapid renal clearance and unwanted uptake by the reticuloendothelial system (RES) [7,8], resulting in enhanced permeability and retention (EPR) of tumor tissue, in which disorganized visualization and defective vascular architecture have developed [9,10].

Stimuli-responsive polymeric micelles as nano-sized drug carriers have been considered for the controlled release of drug into tumor tissue, with temperature, ultrasound, and pH used to trigger drug release from polymeric micelles [11–15]. Among these stimuli, change of pH at tumor tissue is useful, because tumor tissues have a more acidic environment, due to lactic acid produced by hypoxia and by acidic intracellular organelles [16–18]. Therefore, various pH-responsive polymeric

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micelles have been used for rapid, acidic pH-triggered rapid anticancer drug release at tumor sites, with demicellization leading to drug release.

We have described the preparation of pH-responsive MPEG-poly(β-amino ester) polymeric micelles, which are composed of a hydrophilic MPEG shell and a hydrophobic pH-responsive poly(β-amino ester) core [19,20]. These MPEG-poly(β-amino ester) block copolymers were synthesized by a Michael-type step polymerization and their pH-responsive physicochemical characteristics (micellization–demicellization behavior, critical micelle concentration, average micelle size, etc.) were modulated by controlling the molar ratio of the hydrophilic MPEG and pH-responsive poly(β-amino ester) moieties, because the latter is pH-responsive due to its tertiary amine, with a pK_b of about 6.5 [21,22]. In particular, these pH-responsive polymeric micelles showed sharp pH-dependent micellization–demicellization transitions at the acidic extracellular pH of tumor cells (pH 6.8–7.2). To test the usefulness of these micelles in anti-tumor treatment, we prepared doxorubicin (DOX)-loaded micelles and tested their pH responsiveness and *in vivo* anti-tumor activity in B16F10 tumor-bearing mice.

2. Materials and methods

2.1. Materials

Analytical grade methyl ether poly(ethylene glycol) (MPEG, Mn=4,850, determined by GPC), acryloyl chloride, hexane-1,6-diol diacrylate (HDD), 4,4′-trimethylene dipiperidine (TDP), triethylamine, anhydrous chloroform, anhydrous dichloromethane (DCM), and DOX were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification.

2.2. Synthesis of MPEG-poly(β-amino ester) block copolymers

MPEG-poly(β-amino ester) block copolymer was synthesized as described previously [23,24]. Briefly, MPEG (1 equiv.), dissolved in anhydrous DCM and protected from moisture with $CaCl_2$, was mixed with triethylamine (2 equiv.) and cooled to 0 °C. Following the dropwise addition of acryloyl chloride (1.5 equiv.), the mixture was stirred at 0 °C for 2 h, allowed to warm to room temperature, and stirred for 1 day. The product was extracted with dilute HCl and precipitated with hexane to obtain poly (ethylene glycol) methyl ether acrylate (monoacrylated MPEG). MPEG-poly(β-amino ester) block copolymer was synthesized via a Michael-type step polymerization using monoacrylated MPEG as the monocrylate, HDD as the diacrylate ester and TDP as the diamine. HDD (1 equiv.) and TDP (1.1 equiv.), each dissolved in chloroform, were mixed, and the resulting solution was added to monoacrylated MPEG (0.1 equiv.) and allowed to react for 2 days at 50 °C. The final product, designated MPEG-poly(β-amino ester) block copolymer, was precipitated in diethyl ether and vacuum dried. The average molecular weight of the synthesized polymer was measured by gel permeation chromatography (GPC), using KF-803L and KF-802.5 (Shodex) columns in series, with tetrahydrofuran (THF) as the eluent at a flow rate of 1 ml/min. The chemical structure of MPEG-poly(β-amino ester) block copolymer was further characterized by ^{1}H NMR spectroscopy. The ^{1}H NMR spectra were recorded using a 500 MHz FT-NMR (Utility Inova 500 MB, Varian) spectrometer with $CDCl_3$ as the solvent.

The pH-dependent micellization and demicellization behavior and critical micelle concentration (cmc) of MPEG-poly(β-amino ester) block copolymer were determined by a fluorescence technique using pyrene (1×10^{-7} mol/l) as a hydrophobic fluorescence probe, as described previously [23]. All fluorescence measurements were performed using an ISS K2 multi-frequency phase and modulation fluorometer (ISS, Champaign, IL). For measurements of intensity ratios for the first and the third transitions (I_1/I_3) in the excitation spectra for pyrene, the slit openings for excitation and emission were set at 2 and 0.5 mm, respectively. The excitation (λ_{ex}) and emission (λ_{em}) wavelengths were 334 and 337 nm, respectively.

2.3. Preparation of DOX-loaded polymeric micelles

DOX-loaded micelles of MPEG-poly(β-amino ester) block copolymer were prepared by a solvent casting method [24]. Briefly, DOX (10 mg) dissolved in 1 ml of chloroform/methanol (1:1, v/v) was stirred with 1.5 equiv of triethylamine, and the solution was added dropwise to a stirred solution containing 100 mg of MPEG-poly(β-amino ester) block copolymer in chloroform/methanol (1:1, v/v). After evaporating the co-solvent using a rotary evaporator, the polymer/drug thin film was dispersed by adding 10 ml of distilled water (pH 7.4) under gentle shaking for 3 h, followed by sonication using a probe-type sonifier (Sigma Ultrasonic Processor, GEX-600) at 90 W for 2 min, with the pulse turned off for 1 s after 5 s sonication to prevent heat build-up. The dispersed DOX-loaded polymeric micelles were passed through syringe filters (0.45 μm, Millipore) and free DOX and byproducts were further removed by dialysis (molecular weight cutoff=8000, Spectrum®), and the DOX-loaded micelles were lyophilized to give a red powder. The drug loading efficiency of DOX-loaded polymeric micelles dissolved in chloroform/methanol (1:1, v/v) was quantified by absorbance using UV–vis spectroscopy (Lambda Vis 7 spectrophotometer, Perkin-Elmer, CT) at 479 nm.

The particle sizes of unloaded and DOX-loaded polymeric micelles were measured by dynamic light scattering using a helium ion laser system (Spectra Physics Laser Model 127-35) at 633 nm at 25 °C. The polydispersity factor, represented as μ_2/Γ^2, was evaluated by the cumulant method [25]. The morphology of unloaded and DOX-loaded micelles was also measured by transmission electron microscopy (TEM), using a Philips CM 200 transmission electron microscope, operated at an acceleration voltage of 80 kV, after negatively staining the micelles with 2% (wt./vol.) uranyl acetate.

2.4. In vitro DOX release from pH-sensitive polymeric micelles

A dispersed DOX-loaded polymeric micelles (1 mg/ml) was added to cellulose ester membrane tubes (molecular weight cutoff=8000, Spectrum®), and the dialysis tubes were incubated

in 30 ml phosphate buffer saline (PBS, pH 6.4 or 7.4) at 37 °C in a water bath at 80 rpm. At given times, the solution was removed and the pH-dependant DOX release profiles were determined by measuring the UV–vis absorbance at 479 nm.

2.5. Cellular uptake of DOX from pH-sensitive polymeric micelles

B16F10 melanoma cells, obtained from the American Type Culture Collection (Manassas, VA), were cultured in RPMI 1640 medium (Gibco, Grand Island, NY) containing 10% FBS (Gibco) and 1% penicillin–streptomycin (Gibco) at 37 °C in a humidified 5% CO_2–95% air atmosphere, and 5×10^5 cells were grown on 60φ culture dishes. After 1 day, the medium was replaced by 1 ml of fresh medium at pH 6.4 or 7.4, and free DOX or DOX-loaded polymeric micelles (10 μg/ml DOX equiv.) were added. After 1, 5, 10, and 30 min, the culture dishes were washed with PBS (pH 7.4) 3 times, fixed with 4% (wt./vol.) formaldehyde in PBS (pH 7.4) for 30 min at 4 °C, and cellular uptake of free DOX at pH 7.4, and DOX in polymeric micelles at pH 6.4 or 7.4 was visualized with an Axioskope-2 imaging microscope (Carl Zeiss, Oberkochem, Germany) equipped with a fluorescence filter set for TRITC (Omega Optical) and an Axiocam black and white CCD camera (Carl Zeiss Vision, Hallbergmoos, Germany). Image acquisition and analysis were driven by KS400 software (Carl Zeiss Vision).

2.6. Anti-tumor efficacy of DOX-loaded polymeric micelles

Subcutaneous tumors were established by inoculating 1.0×10^6 B16F10 cells into the backs of male C57BL/6 mice (7-weeks old, Institute of Medical Science, Tokyo) and allowing them to grow for 7 days. When the tumor volume reached 50 to 100 mm^3, tumor-bearing mice (5 per group) were injected in the tail veins with saline, polymeric micelles alone, free DOX (1 mg/kg and 2 mg/kg), or DOX-loaded polymeric micelles (1 mg DOX/kg and 2 mg DOX/kg) once every 3 days for 18 days. The survival rate and the body weight of the mice were recorded, and tumor volume was calculated as $a\times b^2/2$, where a was the largest and b the smallest diameter. Apoptotic and non-apoptotic cells in tumor tissues were histologically evaluated using the 4,6-diamidino-2-phenylindole (DAPI) and terminal deoxynucleotidyl transferase-mediated nick and labeling (TUNEL) assays with a commercial apoptosis detection kit (Promega Corp., WI) as described previously [26].

3. Results and discussion

3.1. Preparation of pH-responsive MPEG-poly(β-amino ester) block copolymer

To construct an appropriate pH-responsive block copolymer to form nano-sized polymeric micelles, we combined hydrophilic MPEG with poly(β-amino ester), since the latter is pH-sensitive due to its tertiary diamines, which have a pK_b of about 6.5 [21,22]. The pH-responsive MPEG-poly(β-amino ester) block copolymer was prepared by a Michael-type step polymerization of monoacrylated MPEG (0.1 equiv.), hexane-1,6-diol, acrylate (HDD, 1 equiv.), and 4,4′-trimethylene dipiperidine (TDP, 1.1 equiv.), in which monoacrylated MPEG acted as a hydrophilic moiety and HDD/TDP acted as a hydrophobic/pH-responsive moiety. The chemical structure of the pH-responsive MPEG-poly(β-amino ester) block copolymer is presented in Fig. 1a and the characteristics of the polymers used in this study are summarized in Table 1.

The chemical structure and molecular weight of MPEG-poly(β-amino ester) block copolymer were confirmed using ^{1}H-NMR and GPC, respectively. From the ^{1}H-NMR spectrum, the typical signals for the oxyethylene (3.6 ppm) of MPEG, the methylene (2.63 and 2.84 ppm) of TDP, and the methylene (2.5 ppm) of HDD were used to determine the detailed composition of MPEG-poly(β-amino ester) block copolymer (data not shown). From the GPC measurements, the average molecular weight of MPEG-poly(β-amino ester) block copolymer was determined to be 17.4 kDa and its polydispersity about 1.3, indicating a narrow molecular weight distribution. Based on these ^{1}H-NMR and GPC measurements, we calculated that 2.6 molar equivalents of poly(β-amino ester) are copolymerized with 1 molar equivalent of MPEG in the block copolymer.

The pH-dependent micellization–demicellization behavior of MPEG-poly(β-amino ester) block copolymer was assessed using a fluorometer in the presence of pyrene, because pyrene has been widely used as a hydrophobic fluorescence probe for micelle formation [23]. In particular, the ratio I_{337}/I_{334}, defined as the intensity of the first relative to the third vibrational band, can be used as an index of micelle hydrophobicity. As expected, when the pH of the block copolymer solution was less than 6.8, the I_{337}/I_{334} band ratio did not change, because the tertiary diamine moieties of poly(β-amine ester) are fully ionized (Fig. 2a), indicating that the pH-responsive block copolymers are completely dissolved and did not form polymeric micelle structures. As the pH increased over 6.9, the I_{337}/I_{334} ratio increased, indicating that the pH-responsive block copolymer

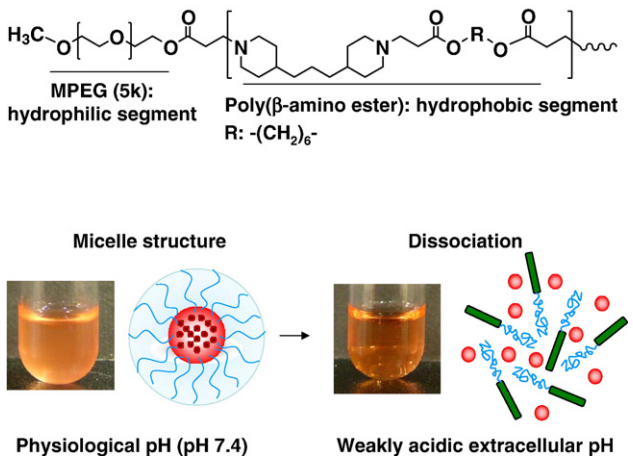

Fig. 1. Structure of MPEG-HPAE block copolymer. DOX-loaded MPEG-HAPE micelles under physiological pH were completely dissociated, and rapidly released DOX at weakly acidic pH environments.

Table 1
Physicochemical characteristics of MPEG-poly(β-amino ester) block copolymer

Sample	Feed mole ratio (MPEG:TDP: HDD)	Mn of MPEG-poly(β-amino ester) [a]	Mn of MPEG [a]	Mn of poly (β-amino ester)	PD [b]
MPEG-poly (β-amino ester)	0.1: 1.1: 1	17,430	4850	12,580	1.3

[a] As measured by gel permeation chromatography (GPC).
[b] Polydispersity (ratio of Mw/Mn).

starts to form polymeric micelle structures, due to the deionization of tertiary diamine moieties at higher pH. At physiological pH (7.4), the pH-responsive block copolymer formed micelles at critical micelle concentrations (cmc) ≥0.016 mg/ml (Fig. 2b), a threshold concentration much lower than that of low molecular weight surfactants (e.g., 2.3 mg/ml for sodium dodecyl sulfate in water) [27], indicating that thermodynamically stable polymeric micelles had formed under physiological conditions.

3.2. Preparation and characterizations of DOX-loaded polymeric micelles

DOX was loaded into the pH-responsive polymeric micelles by a solvent casting method [24]. Briefly, after dissolving free DOX and the block copolymer in an organic solvent, the solvent was evaporated and the dried thin film was dispersed by adding

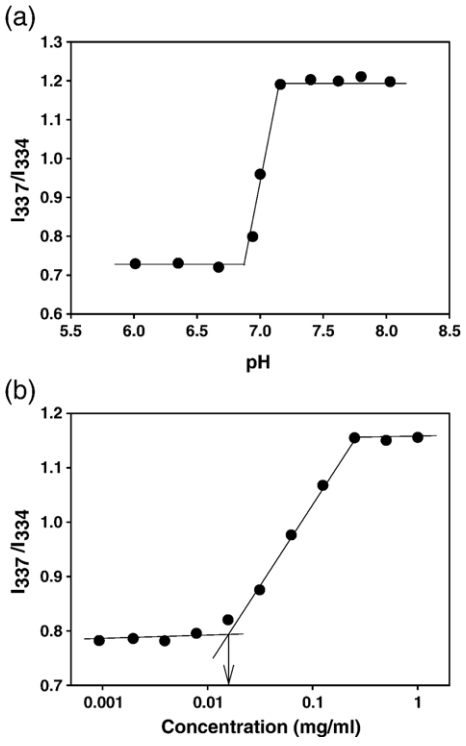

Fig. 2. The pH sensitivity and critical micelle concentration (cmc) of MPEG-HPAE polymer. Intensity ratio (I_{337}/I_{334}) from pyrene excitation spectra as (a) a function of pH and (b) as a function of MPEG-HPAE concentration in PBS buffer at pH 7.4.

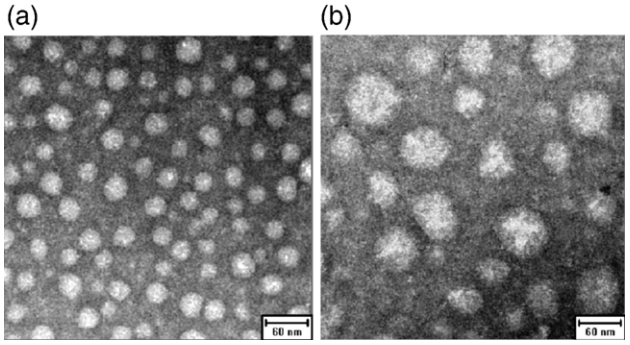

Fig. 3. Transmission electron microscopy images of (a) MPEG-HPAE and (b) DOX-MPEG-HPAE micelles.

distilled water (pH 7.4). The dispersed solution was vigorously stirred and sonicated, yielding DOX-loaded polymeric micelles with a high loading efficiency (74.5%). Based on light scattering experiments, the average size of the dispersed DOX-loaded polymer solution was about 62 nm, which is greater than that of the pH-sensitive MPEG-poly(β-amino ester) block copolymer (42 nm), indicating that DOX molecules are trapped in the hydrophobic inner cores and that these entrapped DOX molecules increase the average size of DOX-loaded polymeric micelles. Importantly, the polydispersity factors (μ_2/Γ^2) of DOX-loaded polymeric micelles, as estimated by the cumulant method, were fairly low (0.023), indicating a narrow size distribution [25]. TEM showed that these pH-responsive unloaded and DOX-loaded polymeric micelles were nearly spherical (Fig. 3), with average size and size distribution of the latter similar to those obtained by dynamic light scattering. These results indicated that the DOX-loaded polymeric micelles were well dispersed in aqueous media and formed homogeneous nano-sized micelle structures. The physicochemical characteristics of DOX-loaded polymeric micelles are summarized in Table 2.

The pH-dependent DOX release profile from the pH-responsive polymeric micelles was investigated by dialysis. We found that pH had a strong effect on DOX release from the polymeric micelles (Fig. 4). DOX was slowly released from the micelles at pH under pH 7.4. Beyond the initial burst (~17%), the release profile reached a plateau at pH 7.4 for 1 day, suggesting that DOX-loaded polymeric micelles maintained micellar structures under physiological conditions, resulting in a

Table 2
Physicochemical characteristics of MPEG-poly(β-amino ester) block copolymer

Samples	Efficiency [a](%)	Size (nm) [b]	Cmc (mg/ml) [c]	$\mu_2/\Gamma^{2,d}$
MPEG-poly(β-amino ester)	–	42	0.016	0.014
DOX-MPEG-poly(β-amino ester)	74.5±2.5	62	N.D.	0.023

[a] Percentage of loaded doxorubicin, based on fed amount.
[b] Mean diameter measured by dynamic light scattering.
[c] Critical micelle concentration determined from pyrene excitation spectra.
[d] Polydispersity factor for micelles.

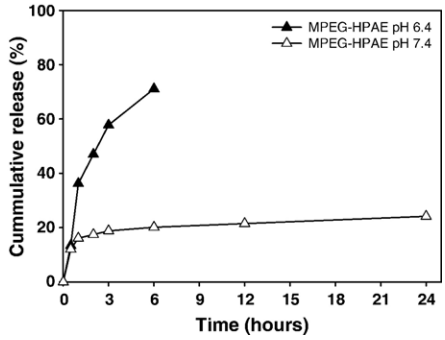

Fig. 4. Release profiles of DOX from DOX-MPEG-HPAE micelles at different pHs. Release profiles were measured by UV–vis spectrophotometry.

sustained drug release profile. However, the DOX release rate from micelles at lower pH (pH 6.4) was much faster, with >71% released within 6 h, indicating that DOX-loaded polymeric micelles were rapidly demicellized and released drug in weakly acidic environments.

3.3. In vitro cellular uptake of DOX

The intracellular localization of free DOX and DOX-loaded polymeric micelles was investigated using fluorescence microscopy (Fig. 5). When cells were incubated with free-DOX, cellular uptake of free DOX was same irrespective of different pH conditions such as pH 6.4 and pH 7.4. Free-DOX was distributed in the cytoplasm and nuclear region within 5 min incubation, and strong fluorescence was exhibited after 10 min incubation, particular in the nucleus, as reported previously [28,29]. When cells

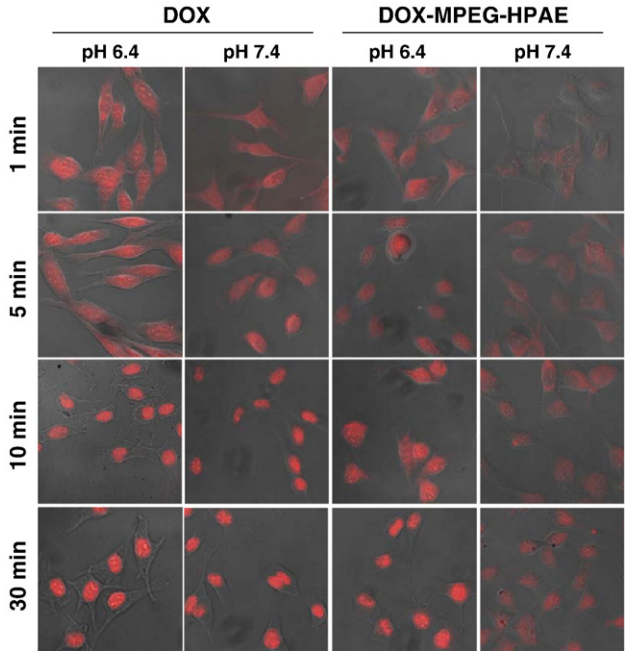

Fig. 5. Cellular uptake of DOX on B16F10 cells. Fluorescence cellular uptake images of B16F10 cells treated with DOX or DOX-MPEG-HPAE micelles in RPMI 1640 medium at pH 7.4 or 6.4 for 1, 5, 10, and 30 min.

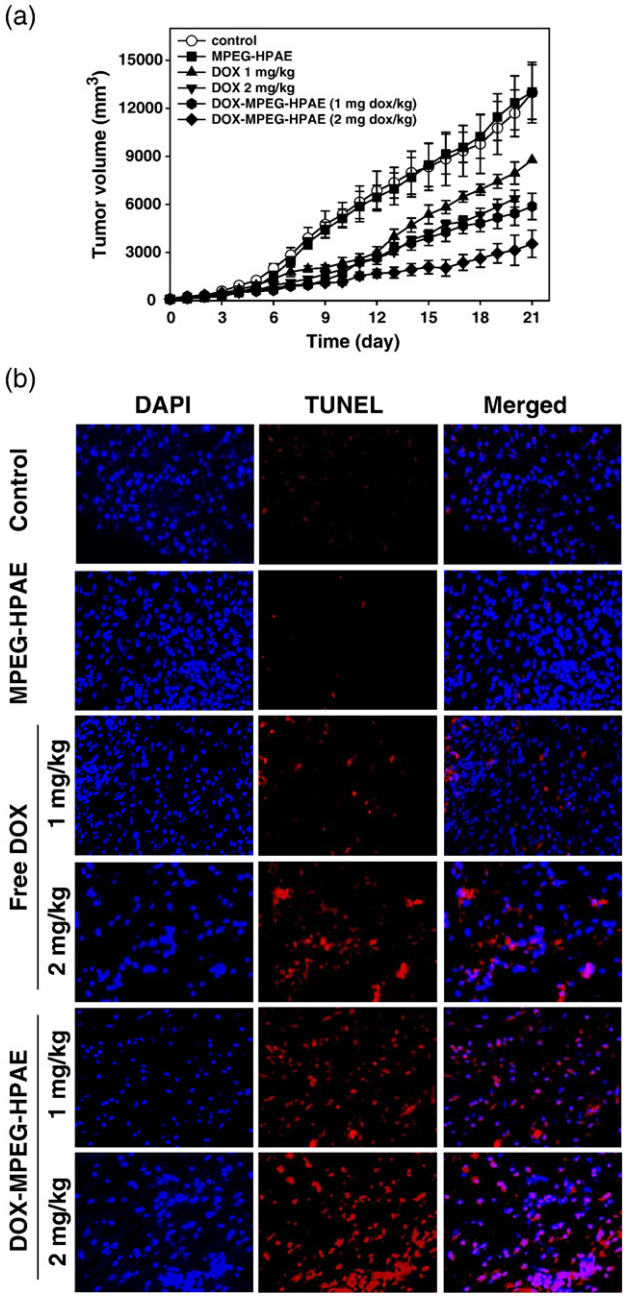

Fig. 6. Antitumor effects of MPEG-HPAE micelles, DOX, and DOX-MPEG-HPAE micelles. (a) Tumor growth of B16F10 tumor-bearing mice treated with MPEG-HPAE micelles, DOX, or DOX-MPEG-HPAE micelles. Mice were given s.c. injections of B16F10 cells (1×10^6 cells/mouse). When tumor volume reached 50 to 100 mm^3, mice were given i.v. injections of normal saline (○), MPEG-HPAE (■), DOX at 1 mg/kg (▲) and 2 mg/kg (▼), or DOX-MPEG-HPAE at 1 mg DOX/kg (●) and 2 mg DOX/kg (◆). Each treatment group contained 5 mice. Tumors were measured daily. (b) Immunohistological detection of apoptotic cells in B16F10 melanoma tumors growing in C57BL/6 mice treated with saline, MPEG-HPAE micelles, free DOX, and DOX-MPEG-HPAE micelles, respectively.

were incubated with DOX-loaded polymeric micelles under physiological pH (7.4), considerable fluorescence intensity was detected in the mainly cytoplasm, suggesting that free DOX molecules were slowly released from the pH-responsive polymeric micelles at pH 7.4, as expected from the drug released

profile of pH-responsive polymeric micelles at pH 7.4. At pH 6.4, however, cellular fluorescence was much higher, and, after 30 min, most of the DOX molecules were localized in the cell nuclei, with a distribution similar to that of free DOX. DOX localized in the cell nuclei is likely intercalated into DNA strands, thereby showing its toxicity against tumor cells. This pH-dependent cellular uptake profile of DOX molecules is likely due to the rapid demicellization of the DOX-loaded polymeric micelles under weakly acidic extracellular conditions, leading to the rapid release of DOX from polymeric micelles and subsequent internalization into cell nuclei. These findings suggested that DOX-loaded polymeric micelles may be suitable for targeting of the acidic extracellular environments of tumors.

3.4. In vivo anti-tumor efficacy of DOX-loaded polymeric micelles

The *in vivo* anti-tumor efficacy of free DOX, pH-sensitive polymeric micelles, and DOX-loaded/pH-responsive polymeric micelles was evaluated in B16F10 tumor-bearing mice. The tumor growth rates of mice treated with saline and polymeric micelles (without DOX) were similar, indicating that pH-responsive polymeric micelles alone had no effect on tumor growth (Fig. 6a). When the mice were treated with free DOX or DOX-loaded polymeric micelles, however, the tumor growth rate was much slower. After 21 days, free DOX at 1 and 2 mg/kg suppressed tumor volumes by 32.4% and 45.75%, respectively, compared with the control group. Importantly, DOX-loaded polymeric micelles at 1 and 2 mg DOX/kg significantly inhibited tumor sizes by 52.74% and 72.69%, respectively. An *in vivo* TUNEL assay confirmed the anti-tumor activity of DOX-loaded polymeric micelles against B16F10 tumor-bearing mice (Fig. 6b). Apoptotic cell nuclei were rarely detectable in the saline and polymer control groups, but were clearly detected in the tumors of mice treated with free DOX and DOX-loaded polymeric micelles. In particular, more apoptotic cells were found in tumor tissues treated with DOX-loaded polymeric micelles than in those treated with free DOX, reinforcing that DOX-loaded polymeric micelles have more effective anti-tumor activity than free DOX.

The body weight of mice treated with saline or pH-sensitive micelles without drug continuously increased due to probably their non-toxic effects as well as the rapid growth of tumor. In free DOX and DOX-loaded polymeric micelle treated groups at 2 mg DOX/kg, the body weight of mice did not significantly increase because it might be due to their anti-tumor efficacies. On the other hand, free DOX at 1 mg/kg appears to have weight gain, resulting from the less anti-tumor efficacy compared to those of other treated groups (Fig. 7a). The *in vivo* survival rates of free DOX and DOX-loaded polymeric micelles were also monitored (Fig. 7b). After injection of saline or polymeric micelles, 40% of the mice died within 3 weeks. The survival rates of mice treated with 1 and 2 mg/kg of free DOX were 20% and 0% at 3 weeks, respectively. When mice were injected with DOX-loaded polymeric micelles at 1 and 2 mg DOX/kg, however, 60% and 40%, respectively, were alive at 3 weeks. It is considered that the increased survival time of DOX-loaded polymeric micelle treated groups is probably due to their better anti-tumor efficacies compared to free DOX treated groups.

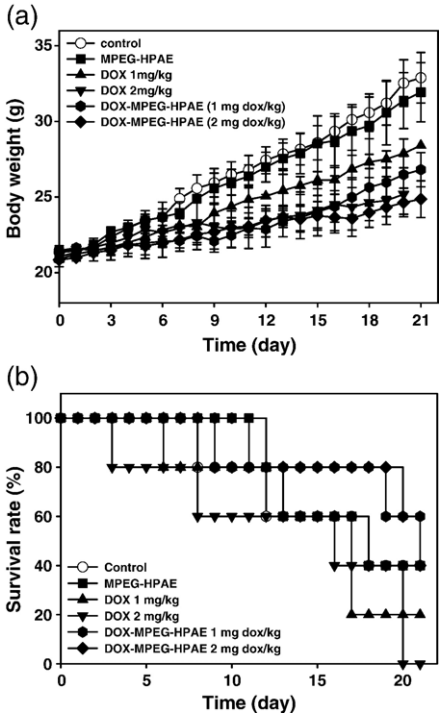

Fig. 7. Change in body weight and the survival rates of mice. (a) Body weight changes of mice treated with normal saline (○), MPEG-HPAE (■), DOX at 1 mg/kg (▲) and 2 mg/kg (▼), and DOX-MPEG-HPAE at 1 mg DOX/kg (●) and 2 mg DOX/kg (◆), (b) Survival rates of tumor-bearing mice treated with saline (○), MPEG-HPAE (■), DOX at 1 mg/kg (▲) and 2 mg/kg (▼), and DOX-MPEG-HPAE at 1 mg DOX/kg (●) and 2 mg DOX/kg (◆).

Other pH-sensitive approaches to the delivery of anti-tumor drugs have included the pH-triggered drug release caused by cleavage of chemical bonds, including the acid labile hydrazone linkage [30,31] and acetal groups in polymeric micelles or particles [32,33]. These approaches, however, are not suitable for drug release triggered by extracellular pH because these bonds are cleavable at much lower pH, such as intracellular endosomal pH (below pH 6.0). More effective drug delivery requires a more prompt response to a small pH change, as in the weakly acidic extracellular pH environments of tumor tissues. Our pH-responsive polymeric micelles containing DOX showed fast drug release at weakly acidic pH within 6 h, indicating that these micelles are more suitable for anticancer drug release at tumor sites. Also, the pH-responsive polymeric micelles were stable under physiological conditions, with drug release minimized, indicating that this micelle-based drug delivery system can reduce unwanted side effects of anticancer drugs during cancer therapy.

4. Conclusion

The anticancer drug DOX was efficiently loaded into pH-responsive polymeric micelles of MPEG-poly(β-amino ester) block copolymer. DOX molecules were rapidly released from

these pH-responsive polymeric micelles and localized in the nuclei of cells under acidic conditions, indicating that these micelles have tumor targeting ability. In mice, these DOX-loaded, pH-responsive polymeric micelles showed noticeable antitumor efficacy, and enhanced survival rates, compared with free DOX. These results suggest that pH-responsive polymeric micelles may be an effective anticancer drug delivery system for cancer chemotherapy.

Acknowledgments

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