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Adriamycin-incorporated nanoparticles composed of deoxycholic acid-conjugated dextran : its antitumor activity against CT26 cancer cell

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아드리아마이신이 담지된 나노입자의 제조 및 CT26 대장암세포주에 대한 항암활성

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

국문 초록

Adriamycin이 담지된 nanoparticle의 CT26 암세포주에 대한 항암활성

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연구목적 : 본 연구에서 아드리아마이신이 담지된 나노입자를 deoxycholic acid (DA)와 dextran의 공유결합체(DexDA)를 이용하여 만들었으며 *in vitro* 및 *in vivo* 에서 항암성능을 평가하고자 한다.

실험방법 : DexDA 공유결합체의 나노입자를 투석하는 방법에 의하여 만들었다. 암세포에 대한 독성 실험은 CT26 암세포를 세포배양하여 *in vitro* 독성을 평가하고 쥐에 고형암을 유발시킨 뒤 각각 생존율과 종양부피를 측정하여 *in vivo* 독성을 평가하였다.

결 과 : 아드리아마이신이 담지된 나노입자는 투과전자현미경으로 관찰한 결과 구형의 모양을 가지고 있었고 대략 50-200 nm의 평균직경을 가지고 있는 것으로 조사되었다. 또한 deoxycholic acid (DA)의 치환도가 높을수록, 약물의 봉입양이 많을수록 입자의 크기는 커지고 zeta potential의 수치는 높아지는 것으로 나타났다. 또한 DA의 치환도가 높을수록, 약물의 봉입량이 많을수록 약물의 방출속도가 느려지는 것으로 나타났으며 알칼리성 pH보다 산성 pH에서 약물의 방출속도가 더 빨라지는 것으로 나타났다. CT26 암세포주를 이용한 *in vitro* 독성시험결과 나노입자가 아드리아마이신보다 더 높은 세포독성을 보였으며 나노입자가 세포내로 더 용이하게 도입되는 것으로

사료된다. *In vivo* 동물 암모델에서 나노입자는 아드리아마이신보다 종양성장의 억제에는 효과가 더 낮았지만 동물의 생존율을 훨씬 더 늘리는 것으로 나타났다.

결 론 : 아드리아마이신을 담지한 DexDA 나노입자는 항암전달체로서 우수한 특징을 가진 것으로 사료된다.

1. INTRODUCTION

Nanoparticles-based drug delivery system has been extensively investigated due to their potential of drug targeting to desired site of action [1]. Since nanoparticles have small sizes below 1000 nm, they can be primarily considered as a device for parenteral injection [2]. Nanoparticles have various advantages such as site-specific drug delivery *via* active or passive targeting mechanism, reduction in the amount of drug administered, minimizing of irritation at injection site, and minimizing side effects. For parenteral injection of drug, various carriers have been developed such as liposomes [3], polymeric micelles [4,5], and core-shell type nanoparticles [6,7] have been reported. Especially, polymeric micelles [4,8], core-shell type nanoparticles [6,7], and hydrophobized polysaccharides [9] have received great attention due to their self-assembling characteristics in aqueous environment. Self-assembled nano-carriers are generally characterized of a hydrophobic core and a hydrophilic shell, i.e. hydrophobic core acts as a drug incorporation part and outershell acts as a safe-guard from the attack of reticuloendothelial system. Therefore, they are considered as a superior drug carriers and developed by several researchers [4-11].

Dextran is a polysaccharide consisting of glucose molecules, mainly through the 1,6-glucosidic linkages and partly through the 1,3-glucosidic linkages. Dextrans are characterized to a colloidal, hydrophilic and water-solubility. Since dextrans are inert in biological systems and do not affect cell viability, it has been extensively used as a drug carrier system, including for antidiabetics, antibiotics, anticancer

drugs, peptides and enzymes [9,12-15].

Previously, Jeong *et al.* [10] reported that nanoparticles of poly (DL-lactide-co-glycolide)(PLGA)-grafted dextran copolymer are formed from self-assembling process and this nanoparticles can used as an antitumor drug-carrying vehicles [10]. Dextran part in copolymer would form the hydrophilic outer-shell, due to its aqueous solubility, while PLGA has formed the inner core of the self-assembly, due to its hydrophobic properties.

In this study, the author synthesized deoxycholic acid-conjugated dextran (abbreviated as DexDA) to make antitumor drug carrying vehicles. The author prepared nanoparticles using DexDA conjugates and characterized its physicochemical properties. The author evaluated its antitumor effect against CT 26 cancer cells i*n vitro* and *in vivo*.

2. MATERIALS AND METHODS

2.1. Materials

Dextran from *Leuconostoc mesenteroides* (average molecular weights (M.W.) : 38,900), triethylamine (TEA), adriamycin (ADR), deoxycholic acid (DA) and thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma Chem. Co. (St. Louis, USA). N,N′-dicyclohexyl carbodiimide (DCC) and 4-(N,N-dimethylamino)pyridine (DMAP) were purchased from Aldrich Chemical Co. USA. The dialysis membranes with a molecular weight cut-off (MWCO) of 8,000 g/mol were purchased from Spectra/Pro™ Membranes. Dichloromethane (DCM) and dimethyl sulfoxide (DMSO) were of HPLC grade or extra-pure grade.

2.2. Synthesis of dextran-deoxycholic acid (DexDA) conjugates

DexDA conjugates were synthesized as follows ; Dextran and deoxycholic acid were separately dissolved in anhydrous DMSO. 1.5 equivalent amount of DCC in DMSO solution were added to the DA/DMSO solution, which was then stirred for 3 hour to activate the carboxyl group of the deoxycholic acid. The resulting solution was added to the dextran/DMSO solution containing DMAP, and the reaction was allowed to continue at room temperature for 2 days. The reaction mixture was filtered off to remove the byproducts and then exhaustively dialyzed against deionized water for 3 days. Following this, dialyzed solution was lyophilized for another 3 days and then solid product was precipitated three times in DCM to remove unreacted DA and dried in a vacuum oven for 2 days.

The DS of deoxycholic acid to dextran was evaulated from the ^{1}H nuclear magnetic resonance (NMR) spectra and expressed as mol % versus 100 glucose unit.

2.3. Preparation of nanoparticles and drug incorporation

The adriamycin-incorporated nanoparticles of DexDA were prepared by precipitation-dialysis method [10]. 40 mg of DexDA was dissolved in 5 ml of DMSO. 5-10 mg adriamycin was separately dissolved in 2 ml DMSO with 2 equivalent molar ratio of TEA. This solution was added to DexDA/DMSO solution and stirred magnetically for 1 hour. Mixed solution was dropped into 15 ml of deionized water for 10 min to form nanoparticles. Solvent was removed by dialysis method, i.e. nanoparticle solution was introduced into dialysis membrane and dialyzed against distilled water for at least 1 days. During dialysis, distilled water was exchanged every 1-2 hour intervals. After that, resulting solution was used for analysis or lyophilized.

Empty nanoparticles of DexDA conjugates were prepared by same procedure described above without addition of adriamycin.

For evaluation of drug contents and loading efficiency, 5 mg of adriamycin-incorporated nanoparticles were dissolved in 10 ml of DMSO and diluted it with DMSO. Adriamycin concentration was evaluated using an UV-spectrophotometer (UV spectrophotometer 1201, Shimadzu Co., Osaka, Japan) at 479 nm. Empty nanoparticles of DexDA were used as a blank test.

Drug contents = [(drug weight in the nanoparticles)/(weight of nanoparticles)]×100

Loading efficiency = [(residual drug in the nanoparticle)/(initial feeding amount of drug)]×100

2.4. Analysis of nanoparticles

The characterization DexDA conjugates was performed in DMSO or D_2O using 400 MHz ^1H NMR spectroscopy (Varian 400 MHz NMR).

The morphology of the polymeric nanoparticles was observed using a transmission electron microscope (TEM, JEOL JEM-2000 FX Ⅱ, Tokyo, Japan). A drop of nanoparticle suspension containing phospho-tungstic acid (0.05 % (w/w)) was placed onto a carbon film coated on a copper grid for TEM. Observation was done at 80 kV.

Particle size and zeta potential of nanoparticles were measured with a dynamic laser scattering (DLS-7000, Otsuka Electonics Co., Osaka, Japan). A sample solution prepared by dialysis method was used for particle size measurement (concentration: 0.1 wt.-%).

2.5. Drug release study *in vitro*

The release experiment was carried out *in vitro* as follows: 3 mg of adriamycin-incorporated nanoparticles was distributed into 5 ml of phosphate-buffered saline and this solution was introduced into dialysis membrane. After that, dialysis membrane was placed in 200 mL bottle with 95 ml of PBS, and the media stirred at 100 rpm and 37 °C. At specific time intervals, the medium was taken for analysis of drug concentration. After that whole media was replaced with fresh PBS to prevent drug saturation. The concentration of the adriamycin released into PBS was determined using an UV-spectro-

photometer (UV spectrophotometer 1201, Shimadzu Co. Japan) at 479 nm.

2.6. Cell cytotoxicity test *in vitro*

CT26 cancer cells maintained in DMEM (10% fetal bovine serum, 5% CO_2 at 37 °C) was used to evaluate the cytotoxicity of nanoparticles by MTT cell proliferation assay. Cells were seeded at a density of 5×10^3 per well in 96-well plates with 100 microliter medium before addition of nanoparticles. After that, adriamycin, adriamycin-incorporated nanoparticles, and empty nanoparticles were added into 96 well plates at volume of 100 microliter. Control was treated with 0.5% v/v of DMSO. After 1 and 2 days of incubation, 30 microliter of MTT (5 mg/ml) was added to 96 well plates 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. U.S.A.). Each measurement of the drug concentration was obtained as the mean value of 8 wells. The amount of formazan present is proportional to the number of viable cells, as only living cells will reduce MTT to blue formazan. The results were expressed as a percentage of the absorbance present in the drug-treated cells compared to that in the control cells.

2.7. Animal tumor model using CT26 cancer cells

Animal experiment was performed according to the Animal Experiment Guidance of Chosun university. To make animal tumor model, BALB/c mice were used (5 weeks old, average body weight 20 g).

CT 26 colon carcinoma cells were grown at DMEM supplemented 10% FBS in 5% CO_2 incubator at 37 °C. 1×10^5 cells of CT26 at exponential growth phase were implanted subcutaneously (s.c.) into the back of mice. After that, mice freely fed with water and feed. About 2 weeks later, tumor xenograft were observed at the back of the mice and solid tumor was reached to approximately 3 mm × 3 mm (approximately after 10 days).

2.8. *In vivo* antitumor activity of nanoparticles

Antitumor activity of adriamycin-incorporated nanoparticles was evaluated with tumor xenograft model of CT 26 colon carcinoma. 10 days after tumor cell implantation, drug or nanoparticles were started to administer intravenously (IV). The animals were divided into treatment and control groups. Each group consisted of 10 tumor-bearing mice. The IV administration of drugs or nanoparticles began on day 7 and first drug injection was determined to beginning of the test, i.e. day 0. Each drug was administered at a volume of 0.1 ml/10g of mice for 4 times for 15 days at a intervals of 3 days. The control group was received the PBS (pH 7.4, 0.1 M). The mortality was monitored daily and tumor growth was measured at two or three day intervals by caliper measurement. Tumor volume was calculated using the following formula: Tumor volume (mm^3) = (Length × $width^2$)/2 [11].

3. RESULTS

3.1. Characterization of DexDA conjugates

DexDA conjugates were synthesized as illustrated in Figure 1. DA was conjugated to the hydroxyl group of dextran to increase hydrophobicity of dextran. ^{1}H NMR spectra were shown in Figure 2. As shown in Figure 2(b), specific peaks of dextran were shown at 3.0-3.8 ppm and 4.5-5.0 ppm while specific peaks of DA were shown at 0.5-2.4 ppm and 3.0-4.5 ppm at Figure 2(a). Figure 2(c) showed specific peaks both of dextran and DA between 0.5 and 5.0 ppm, i.e. dextran at 3.0-5.0 ppm and DA at 0.5-2.4 ppm were characterized each specific peaks. The DS of DA was estimated from the proton peaks ratio between 0.5-2.0 ppm of DA and 3.7 ppm of dextran (Table 1). DS value of DA was elevated by increase of DA feed ratio but DS value was lower than feeding mol % of DA (Table 1). This result might be due to that small amount of moisture remained in the solvent affected to the synthesis procedure.

3.2. Preparation and characterization of nanoparticles

Whether or not DexDA conjugates can form self-aggregates as a nanoparticle, DexDA conjugates dissolved in DMSO were distributed in water. Particle size was measured after solvent removed. DexDA conjugates were able to form nanoparticles (Table 1). Their sizes were ranged from 70 nm to 150 nm according to the DS value of DA. The higher DS value of DA induced incresed particle size (Table 1).

To make drug-incorporated nanoparticles, DexDA conjugates and adriamycin were dissolved in DMSO and then this solution was dropped into water slowly. From this process, nanoparticles were readily formed through self-aggregation phenomena. Solvent and free drug was removed by dialysis method. These results were summarized in Table 2. As shown in Table 2, drug contents was 12-15 % (w/w) and loading efficiency was 55-72 % (w/w) according to the series of DexDA and feeding ratio of DexDA/adriamycin. The higher DS value of DA induced increased drug contents and loading efficiency. Furthermore, the higher the drug feeding ratio induced increased loading efficiency.

Figure 3(a) showed typical particle size distribution of DexDA-2 (Dex-2-1 in Table 2). Adriamycin-incorporated nanoparticles of DexDA conjugates showed monomodal distributions and size particle size was less than 500 nm with average size about 150 nm. Figure 3(b) showed TEM photograph of DexDA-2 nanoparticles. Nanoparticles of DexDA-2 showed spherical shapes and their sizes was approximately ranged from 100 nm to 300 nm. These results were quite similar to the result of particle size measurment at Figure 3(a).

To approve core-shell structure of this nanoparticles, empty nanoparticles were evaluated by ^{1}H NMR. Specific peaks both of dextran and DA were appeared at DMSO (Figure 4(a)), while specific peaks of DA was disappeared at D_2O (Figure 4(b)). These results indicated that hydrophobic moiety (DA) is associated by hydrophobic interaction at aqueous environment as an inner-core of nanoparticles while dextran forms outershell of the nanoparticles.

Figure 5 showed particle size and zeta potential changes according

to the changes of preparation conditions. As shown in figure 5(a), the higher DS value of DA and drug feeding ratio induced increased particle size. Figure 5(b) showed zeta potential of nanoparticles. The higher DS value of DA and drug feeding ratio induced the higher zeta potential of nanoparticles.

3.3. *In vitro* drug release

Figure 6 showed changes of drug release profiles according to the preparation conditions. As shown in Figure 6(a), the higher the DS of DA resulted in decreased release rate of drug. These results might be due to that increse of hydrophobicity at higher DS of DA may delayed the release of hydrophobic drug, adriamycin, by increased hydrophobic interaction between DA moiety and adriamycin (Figure 6(a)). Figure 6(b) showed the effect of polymer/drug feeding ratio on the release rate of drug from nanoparticles. The higher the drug contents induced the delayed release of drug. Figure 6(c) showed the effect of media pH on the release rate of drug from nanoparticles. Release rate of drug from nanoparticles were increased at acidic pH rather than alkaline pH. Drug release rate was highest at pH 6.8 and order of drug release rate was 6.8>6.0>7.4.

3.4. *In vitro* cell cytotoxicity

To examine the antitumor activity of adiamycin-incorporated DexDA nanoparticles, cytotoxicity of free drug, adriamycin-incorporated DexDA nanoparticles, and empty DexDA nanoparticles were tested against CT-26 colon carcinoma cells. Figure 7(a), (b) and (c) showed the effect of pH on the cytotoxicity of adriamycin-incorporated

DexDA nanoparticles with comparison of free adriamycin against CT 26 cells *in vitro*. To compare free adriamycin and nanoparticles at same condition, adriamycin and nanoparticles were treated with equivalent concentration of adriamycin. As shown in Figure 7, free adriamycin showed inherent cytotoxicity against CT26 cells in a dose-dependent manner. Adriamycin-incorporated nanoparticles at pH 7.4 and 6.8 were slightly more toxic than free adriamycin. Empty nanoparticles were treated similar concentration of adriamycin-incorporated nanoparticles (Figure 7(d)). The survived cells with treatment of empty nanoparticles were higher than 80 % at 1000 μg/ml, indicating that empty nanoparticles did not significantly affected to the survivability of tumor cells.

Enhanced antitumor activity of nanoparticles at *in vitro* might be due to the enhanced delivering capacity of nanoparticles into the cells. This assumption can be approved by fluorescence microscopic observation of adriamycin and nanoparticle-treated cells as shown in Figure 8. Nanoparticle-treated cells at pH 7.4, 6.8 and 6.0, respectively, revealed stronger red fluorescence compared to adriamycin treatment, indicating that nanoparticles were entered into cells easier and stayed longer in cells than adriamycin itself. Especially, fluorescence intensity at pH 7.4 between adriamycin and nanoparticles was distinctly changed. Furthermore, fluorescnece intensity at alkaline pH was higher than at acidic pH. These results might be due to that drug release at alkaline pH was slower than acidic pH and then remained drug in the nanoparticles at this pH was higher than acidic pH. Therefore, changes of fluorescence intensity at alkaline pH might be higher than acidic pH.

3.5. *In vivo* antitumor activity

To investigate the potential of *in vivo* antitumor activity, CT-26 colon carcinoma cells were injected subcutaneously (s.c) in the back of mice. Approximately 10 days of tumor implantation into back of the mice, tumor xenografts were well induced, and incidence of tumor approximately 2-3 mm in diameter was observed. Mice were divided 3 groups, i.e. PBS for control group, free adriamycin, and adriamycin-incorporated nanoparticles. As shown in Figure 9(a), survivability of mice was checked every day. Survived mice of control group were gradually decreased according to the time course. Especially, survived mice treated with free adriamycin were rapidly decreased than control group, indicating that free ADR might be highly cytotoxic to animal at this dose, while adriamycin-incorporated nanoparticles showed increased survivability of mice. As shown in Figure 9(b), tumor volume of control group was rapidly increased, while tumor volume with free adriamycin-treated mice was not significantly increased. At nanoparticles treated mice, tumor growth was lower than that of control group even though free adriamycin showed higher suppression for tumor growth. However, survivability of adriamycin treatment group was lower than that of nanoparticle treated group (Figure 9(a)). Therefore, these results indicated that nanoparticles are more effective to inhibit tumor growth and to increase survivability of mice.

4. DISCUSSION

Dextran is extensively investigated as biomaterials and drug carrying materials due to their water solubility, good biocompatibility, immunoneutrality, and easy of modification for antitumor drug [12,13]. For these kinds of application, dextran is required to be modified hydrophobic [10,11,14-17]. However, dextran is fully soluble in aqueous solution. Therefore, it should be modified hydrophobically to make nanoparticles. The author rendered dextran using DA as a hydrophobic moiety since DA is well-known biocompatible surfactant in the pharmaceutical industry, i.e. carboxylic acid group of DA was conjugated to the hydroxyl group of dextran. DexDA conjugates has amphiphilic properties because of dextran is hydrophilic domain and DA is regarded as hydrophobic moieties. Therefore, core-shell type nanoparticles can be prepared using DexDA conjugates. DA may form inner-core of the nanoparticles and dextran main chain act as a hydrophilic outershell of the nanopaticles. Especially, innercore composed of DA may act as a drug incorporation site through hydrophobic interaction between DA and hydrophobic drug such as hydrophobic anticancer agent, adriamycin.

The nanoparticles of DexDA copolymer were prepared by precipitation-dialysis method, i.e. DexDA dissolved in DMSO were dropped into water to form nanoparticles, and then organic solvent, DMSO, was removed by dialysis procedure at distilled water. Organic solvent can be completely removed by exchange of water. The formation of nanoparticles using DexDA showed in Figures 3. Furthermore, Figure 4 approved core-shell structure of DexDA, i.e. DexDA nanoparticles

at water did not reveal the specific peaks of DA because of DA forms solid inner-core of the nanoparticles while dextran is remained in the surface of the nanoparticles while peaks both of dextran and DA were appeared at DMSO. These results indicated that DexDA has surfactant properties in the aqueous solution. Due to the surfactant properties, DexDA is considered as a drug carrying vehicles.

Particle size of DexDA nanoparticles was increased according to the increase of DA content in the cojugates and their sizes were ranged about 50 - 150 nm (Table 1). Their sizes are acceptable to administer intravenously. Furthermore, nanoparticles with smaller than 200 nm has benefit for drug targeting to the wanted site of action and is acceptable to prevent attack of reticuloendothelial system (RES) [1].

The author choose adriamycin as an anticancer agent and incorporated it to the nanoparticles of DexDA conjugates. The drug contents and loading efficiency were shown in Table 2. Drug contents and loading efficiency were increased according to the DA content in the conjugates. Increase of drug feeding induced higher drug contents and loading efficiency. Particle size was also increased according to the increase of drug contents and DA contents in the conjugates. Size of adriamycin-incorporated nanoparticles were increased compared to empty nanoparticles. Since size of nanoparticle less than 200 nm is more desirable for long blood circulation of drug, and then passive drug targeting, adriamycin-incorporated nanoparticles has acceptable features for drug targeting [18-20].

Drug was continuously released over one week. As shown in Figure 6, the higher the drug contents and the DS of DA induced delayed

release of drug. These results indicated that stronger hydrophobic interaction between higher drug contents and higher DA contents may induced and drug can be crystallized or aggregated at inner-core of the nanoparticles. Crystallized or aggregated drug in the nanoparticles may have reduced solubility against water and liberated from nanoparticles more slowly compared to molecular dispersion of drug. Especially, release rate of adriamycin was increased at acidic pH. These phenomena was due to that solubility of adriamycin is increased at acidic pH. Unexpectedly, adriamycin release rate was slightly decreased at pH 6.0 compared to 6.8 even if it was higher than 7.4. These results might be due to that dehydration of surface hydroxyl group of dextran can offset increased solubility of adriamycin at acidic pH and penetration of water into the core of nanoparticles also can be reduced.

To study the antitumor activity of adriamycin-incorporated nanoparticles of DexDA, cytotoxicity of adriamycin and nanoparticles were tested using CT 26 cancer model at *in vitro* and *in vivo*. At *in vitro* test, antitumor activity of nanoparticles was slightly higher than adriamycin itself. One of possible explanation of these results is that nanoparticles were effectively endocytosed into the cells and then they effectively inhibited cell growth. Microscopic observations of cells under red fluorescence can support these results as shown in Figures 8. Fluorescence intensity of nanoparticles were clearly higher than adriamycin itself (Figure 8). Since adriamycin express strong self fluorescence, this results indicated that adriamycin concentration in the cells was higher at nanoparticle treatment than adriamycin treatment. These results were similar at pH 6.8

and pH 6.0. These results might be due to the sustained release properties of nanoparticles.

In the case of *in vivo* environment, drug is rapidly removed from the body. Therefore, sustained release function has advantages in *in vivo* experiment. As shown in Figure 9, nanoparticles were effectively prolonged survivability of mice compared to control and adriamycin itself. Even though adriamycin showed excellent result for inhibition of tumor growth, most of mice were died during30 days due to the systemic cytotoxicity of adriamycin. These results indicated that DexDA nanoparticles were better candidate to reduce inherent cytotoxicity of adriamycin with and to increase survivability.

From these results, DexDA nanoparticles are considered as a good candidate for antitumor drug, adriamycin, and it can be used to drug targeting.

5. CONCLUSION

DexDA conjugates were synthesized to make nanoparticles for antitumor drug delivery. Adriamycin-incorporated nanoparticles were prepared using DexDA conjugates. DexDA nanoparticles showed monomodal size distribution and spherical shapes. Adriamycin-incorporated DexDA nanoparticles showed around 50 ~ 200 nm in particle sizes according to preparation conditions, i.e. the higher DS of DA and higher drug feeding ratio induced increased drug contents and loading efficiency of drug. Furthermore, the higher DS of DA and higher drug feeding ratio induced increased particle size and zeta potential. Drug release was delayed by increase of higher DS of DA and higher drug feeding ratio. Furthermore, acidic pH was accelarateed the drug release rate compared to alkaline pH. In *in vitro* cytotoxicity test using CT26 cancer cells, the nanoparticles showed slightly higher cytotoxicity than free adraimycin. These results were supported by microscopic observation of cells under fluorescence, i.e. tumor cells treated with nanoparticle showed higher red fluorescence intensity compared to adriamycin itself. These results indicated that nanoparticles are more easily entered into the cells. In *in vivo* animal tumor model using CT-26 cells, nanoparticles showed increased survivability of mice even if free adriamycin showed higher effectiveness for inhibition of tumor growth. These results suggested that adriamycin-incorporated DexDA nanoparticles are promising vehicles for antitumor drug delivery.

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Table 1. Characterization of dextran-deoxycholic acid (DexDA) conjugates

	Mol % of deoxycholic acid[a]	DS (%)[b]	Particle size (nm)[c]		
			Intensity ave.	Weight ave.	Number ave.
DexDA-1	5	4.1	90.7±22.0	82.1±20.4	75.6±16.8
DexDA-2	10	8.7	110.3±20.1	96.8±17.9	90.8±16.5
DexDA-3	20	15.6	120.5±27.8	105.6±20.8	98.6±20.1

[a] Mol % of DA against glucose unit of dextran
[b] DS of DA was expressed based on number of deoxycholic acid versus 100 glucose unit. DS % was calculated from ^{1}H NMR data.
[c] Particle size of empty nanoparticles

Table 2. Characterization of adriamycin-incorporated DexDA nanoparticles

	Weight ratio of polymer/drug (mg/mg)	Drug contents (%, w/w)	Loading efficiency (%, w/w)
DexDA-1	20/5	12.7	57.9
DexDA-2-1	20/5	13.5	62.6
DexDA-2-2	20/10	30.1	86.0
DexDA-3	20/5	15.3	72.0

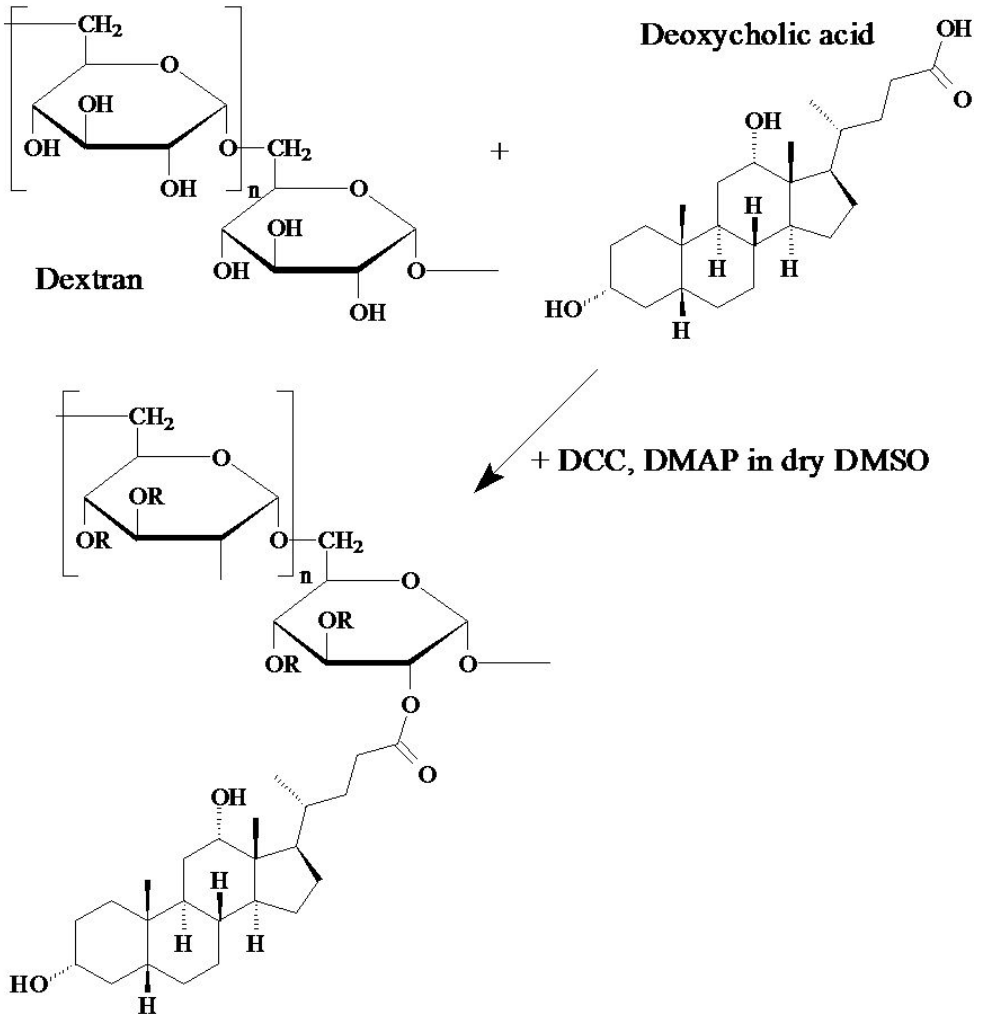

Figure 1. Synthesis scheme of deoxycholic-acid conjugated dextran

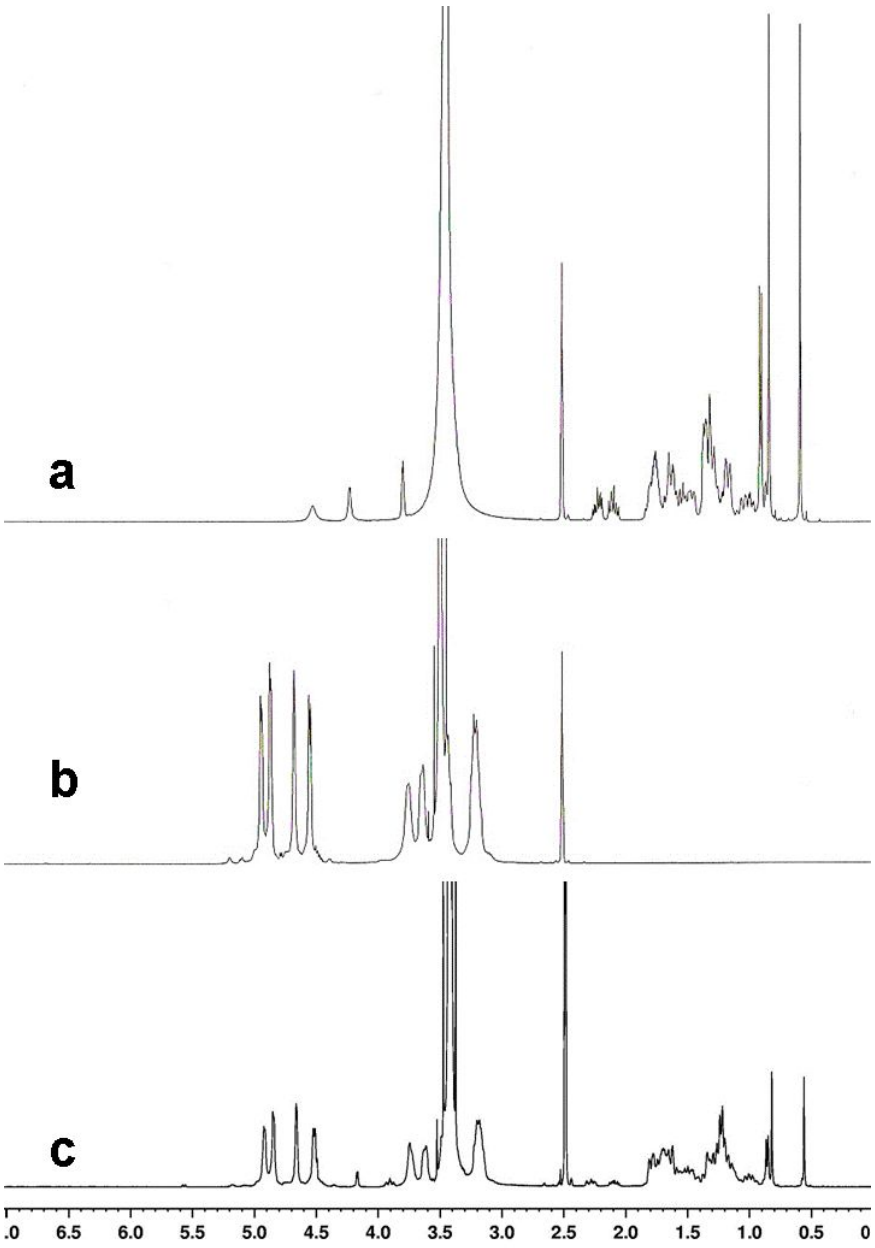

Figure 2. ^{1}H NMR spectra of deoxycholic acid (a), dextran (b), and dextran-deoxycholic acid (DexDA) conjugates (c).

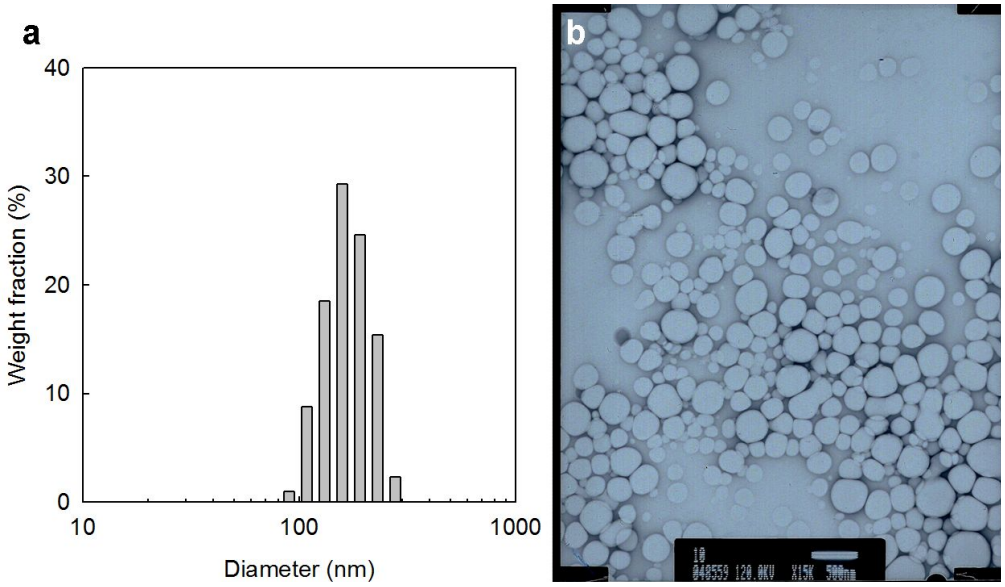

Figure 3. Typical particle size distribution (a) and morphology (b) of nanoparticles prepared from DexDA-2 conjugates. Parcle size was measured by dynamic light scattering morphology was observed with transmission electron microscope (TEM).

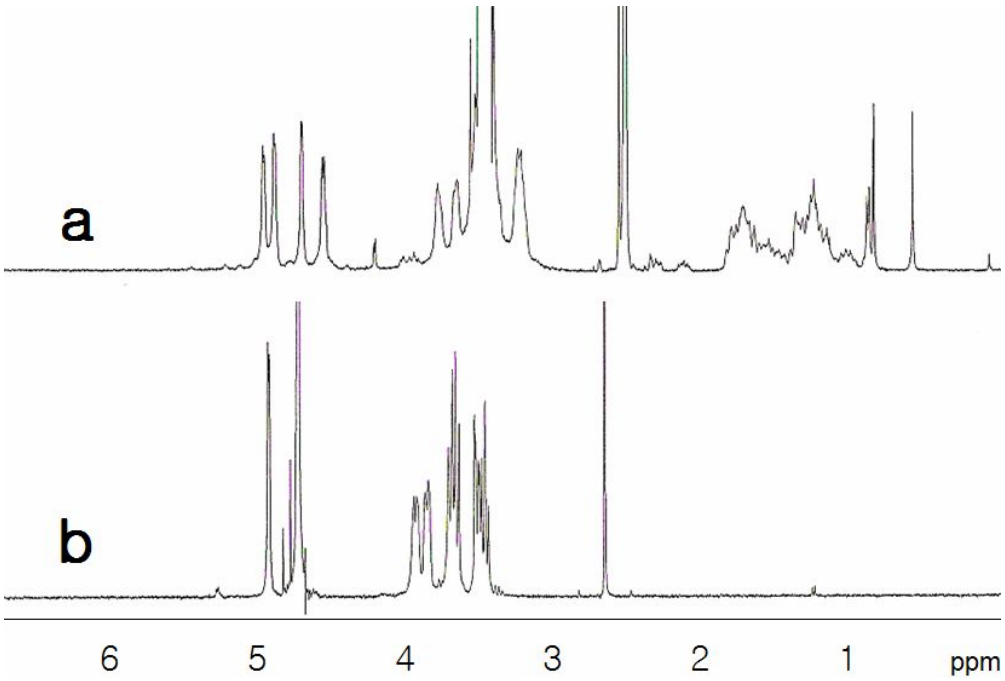

Figure 4. ^{1}H NMR spectra of DexDA-2 nanoparticles in DMSO(a) and D_2O (b).

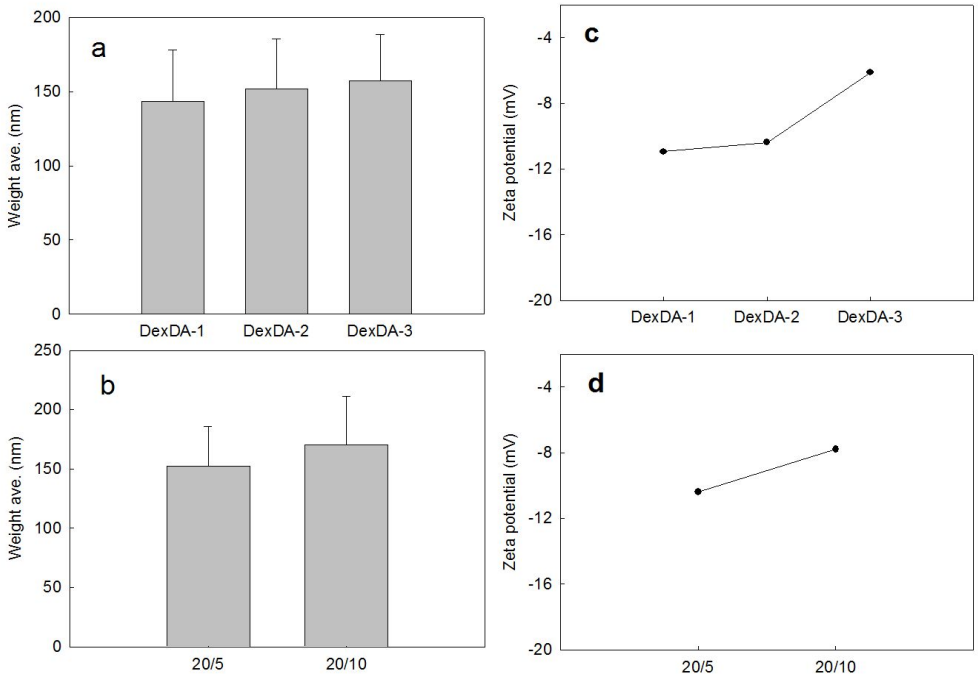

Figure 5. Effect of series of DexDA (a) and polymer/drug weight ratio (DexDA-2) (b) on the size changes of nanoparticles. Effect of series of DexDA (c) and polymer/drug weight ratio (DexDA-2) (d) on the changes of zeta potential of nanoparticles.

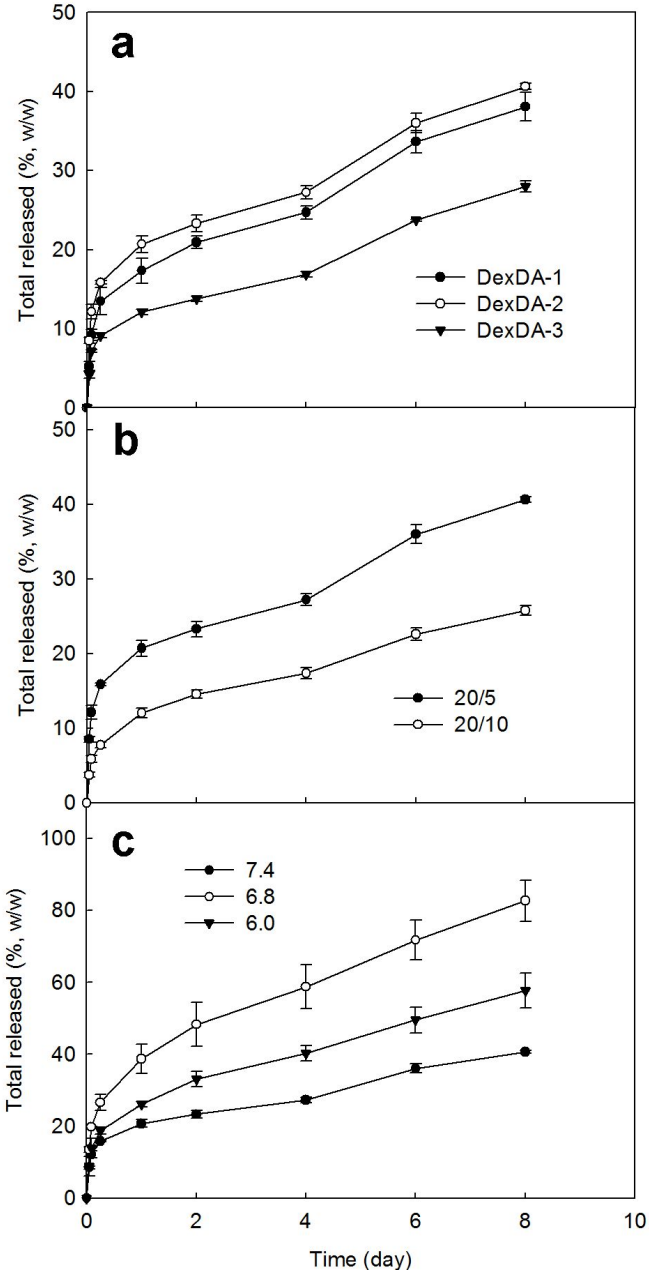

Figure 6. Drug release from DexDA nanoparticles. The effect of series of DexDA conjugates (a), drug feeding ratio (b) and media pH (c).

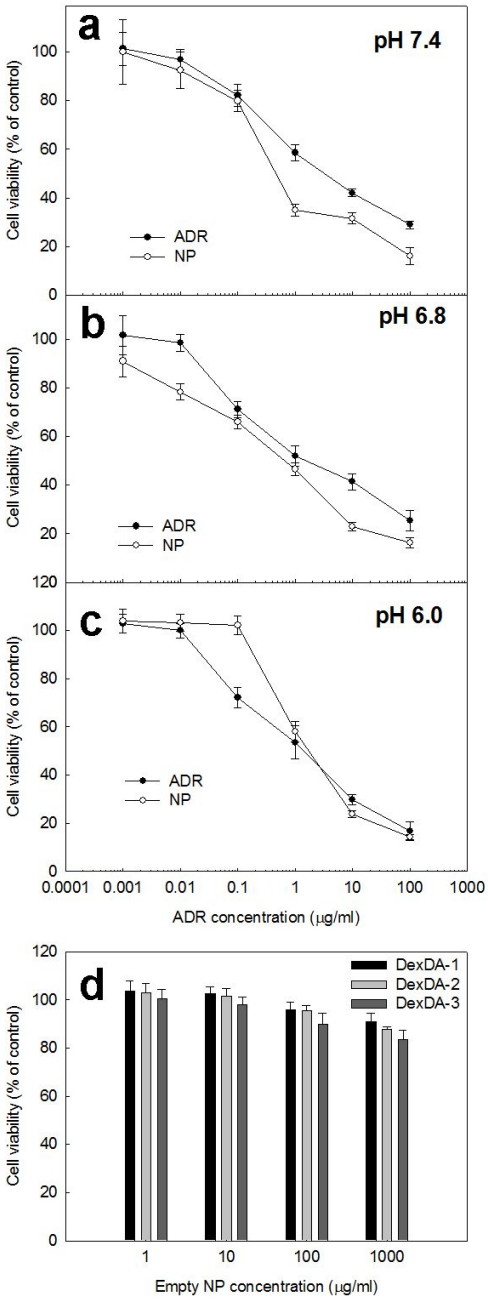

Figure 7. *In vitro* cell cytotoxicity of adriamycin-incorporated nano particles against CT26 cancer cells. The effect of pH 7.4 (a), pH 6.8 (b), pH 6.0 (c) media on the cell survivability. Empty nanoparticles (d) on the cell survivability.

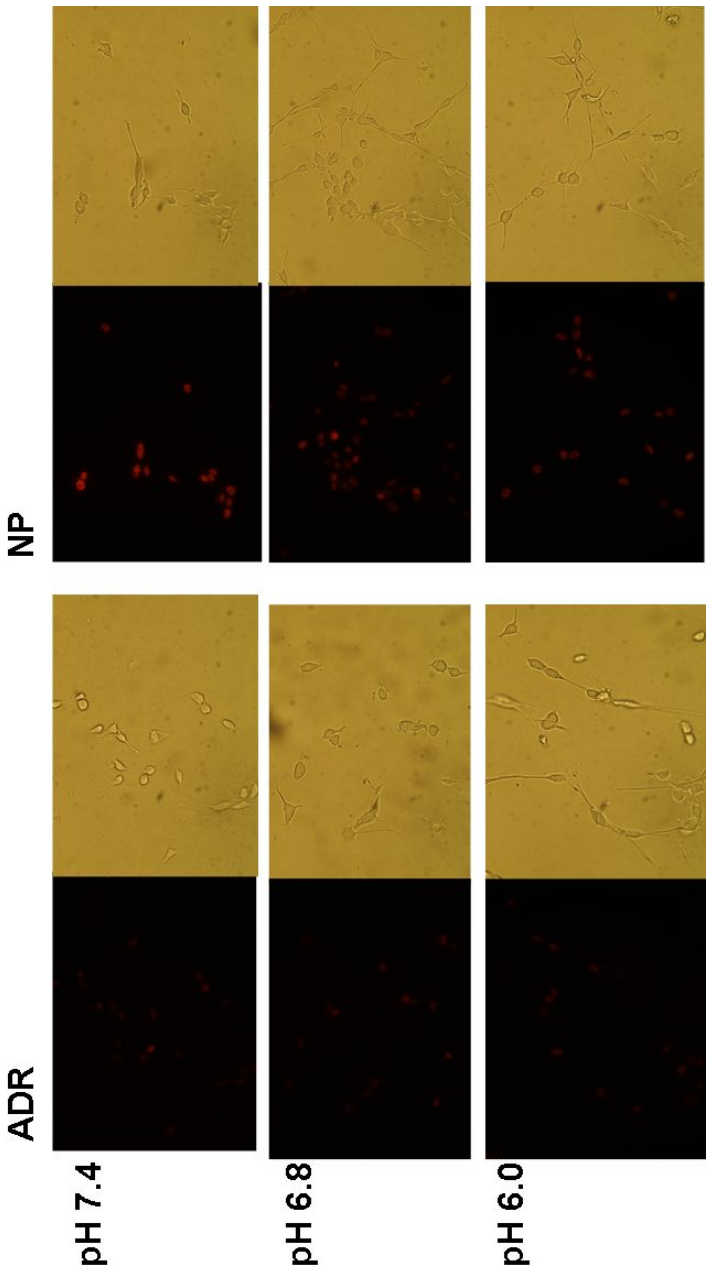

Figure 8. Fluorescence microscopic observation of tumor cells at pH 7.4 (a), pH 6.8 (b) and pH 6.0 (c). Tumor cells were treated with adriamycin or nanoparticles at 1 μg/ml of adriamycin for 24 h. Cells were observed under red fluorescence filter.

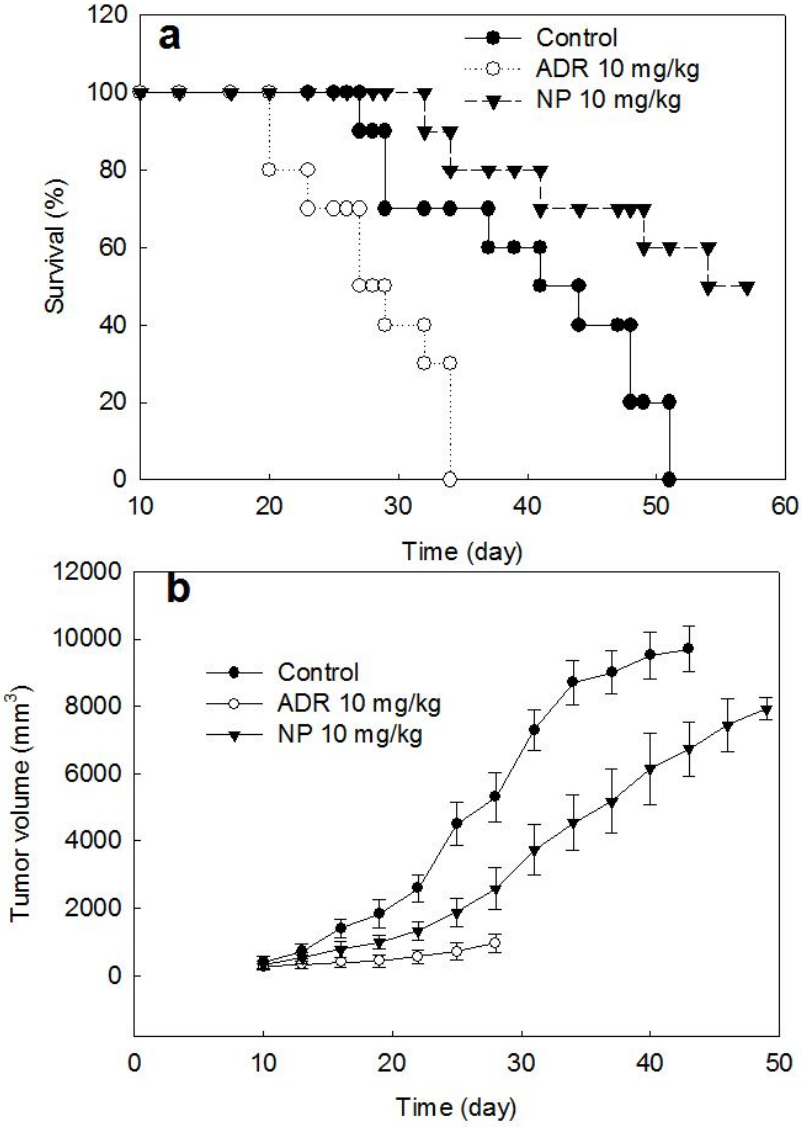

Figure 9. Survival ratio (a) and tumor volume changes (b) of adriamycin or nanoparticle treated mice. Adriamycin or nanoparticles were administered via teil vein at day 15. Drug injection was four times at intervals of 3 day. PBS was injected for control mice. Dose of adriamycin was 10 mg adriamycin/kg mouse. Calculated amount of nanoparticles were also injected. DexDA-2-1 was used for the treatment of nanoparticles. 10 mice were used for each group.

ABSTRACT

Adriamycin-incorporated nanoparticles composed of deoxycholic acid-conjugated dextran : its antitumor activity against CT26 cancer cell

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Objectives : In this study, adriamycin-incorporated nanoparticles were prepared using DexDA conjugates and evaluated its antitumor activity *in vitro* and *in vivo*.

Experimental : Adriamycin-incorporated nanoparticles were prepared using deoxycholic acid (DA)-conjugated dextran (DexDA) by dialysis method. Cytotoxicity of adriamycin-incoporated nanoparticles against CT26 tumor cells were examined by cell culture at *in vitro* and, solid tumor model using mouse for evaluating survivability of mice and tumor volume changes.

Results : Adriamycin-incorporated DexDA nanoparticles showed round shapes at observation of transmission electron microscope (TEM) with particle size around 50 - 200 nm. Particle sizes was chnaged according to preparation conditions, i.e. the higher degree of substitution (DS) of DA and higher drug feeding ratio induced increased drug contents and loading efficiency of drug. Furthermore, the higher DS of DA and higher drug feeding ratio

induced increased particle size and zeta potential. Drug release was delayed by increase of higher DS of DA and higher drug feeding ratio. Acidic pH accelerated the drug release rate compared to alkaline pH. In addition, *in vitro* cytotoxicity analysis showed that the nanoparticles show slightly higher cytotoxicity than free adraimycin. Microscopic observation of cells supported these results, i.e. tumor cells treated with nanoparticle showed higher red fluorescence intensity at all variations of pHs compared to adriamycin itself. These results indicated that nanoparticles are more easily entered into the cells compared to adriamycin itself and then showed higher cytotoxic effect. In CT26 xenograft model, treatment with adriamycin-incorporated DexDA nanoparticles showed increased survivability of mice even if free adriamycin showed higher effectiveness for inhibition of tumor growth.

Conclusion : These results suggested that adriamycin-incorporated DexDA nanoparticles are promising vehicles for anti-tumor drug delivery.

저작물 이용 허락서

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논문제목	한글 : 아드리아마이신이 담지된 나노입자의 제조 및 CT26 대장암세포주에 대한 항암활성				
	영어 : Adriamycin-incorporated nanoparticles of deoxycholic acid-conjugated dextran : Preparation of nanoparticles and its antitumor activity against CT26 colon carcinoma				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(○) 반대( )

2009 년 8 월 일

저작자: 박 광 범 (서명 또는 인)

조선대학교 총장 귀하