International Journal of Pharmaceutics 434 (2012) 488–493

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Cell-penetrating peptide mimicking polymer-based combined delivery of paclitaxel and siRNA for enhanced tumor growth suppression

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ARTICLE INFO

Article history:
Received 27 January 2012
Received in revised form 23 April 2012
Accepted 30 April 2012
Available online 14 May 2012

Keywords:
Biomimetic polymer
Combined delivery
siRNA
Anticancer drug
Combination cancer therapy

ABSTRACT

Cancer chemotherapy is often limited, since more than one molecule is usually involved with the cancer pathogenesis. A combination of therapeutic drugs would be a promising approach to overcome the complexity of tumors. In this study, a conjugate (DA3) of deoxycholic acid and low molecular weight polyethylenimine (PEI 1.8 kDa), which has a property that mimics that of cell penetrating peptides (CPPs), was used for simultaneous delivery of an anticancer drug and siRNA. When complexed with siRNA, DA3 showed significantly higher target gene silencing efficiency than PEI 25 kDa. The gene silencing efficiency of DA3 was maintained even in the presence of endocytosis inhibitors, suggesting that the polymeric carrier can mediate an endocytosis-independent macromolecular transduction similar to CPPs. The capability of forming a micelle-like core–shell structure enables the conjugates to encapsulate and dissolve paclitaxel (PTX), a water-insoluble drug. The drug-loaded cationic micelles can then interact with siRNA to form stable complexes (PTX/DA3/siRNA). The PTX/DA3/siRNA showed significantly enhanced inhibition of cancer cell growth. When administered into tumor-bearing animals, the PTX/DA3/siRNA demonstrated significant suppression of tumor growth, providing potential usefulness in clinical settings.

1. Introduction

Although various strategies for cancer treatment have been developed for last decades, cancer is still a leading cause of death. This may be due to the inherent complexity of cancer pathogenesis, in which more than one causes are usually involved. Among the approaches, chemotherapy is the most widely used approaches for cancer treatment. Majority of anticancer drugs depend mainly on the induction of apoptotic cell death. However, chemotherapy often turns out to be suboptimal at a given dose due to the elevated expression of anti-apoptotic proteins such as inhibitors of apoptosis families (IAPs) and Bcl-2 in many types of cancers (Altieri, 2003; LaCasse et al., 1998).

RNA interference (RNAi) is a powerful endogenous gene silencing phenomenon that induces sequence-specific suppression of target gene expression (Caplen, 2004; Hannon, 2002; Tuschl, 2001). RNAi-based technology has been widely utilized, not only as an experimental tool, but also as a potential therapeutic to treat hard-to-cure diseases, including cancer, genetic disorders, and infectious diseases (Kim and Kim, 2009; Leung and Whittaker, 2005; Stevenson, 2004). Small interfering RNA (siRNA), an RNAi-inducing short double-stranded RNA composed of 19–23 base pairs, has been studied as a potential candidate for cancer treatment by suppressing expression of angiogenic factors such as VEGF and FGF that are closely related to tumor growth or by inducing apoptosis by silencing anti-apoptotic genes such as a survivin (Kim et al., 2006a,b, 2007, 2008; Urban-Klein et al., 2005). However, in addition to its inherent problems with poor intracellular uptake efficiency and a short in vivo half-life, suppression of single gene expression by siRNA seems not to be enough to achieve a desirable therapeutic effect (Sjoblom et al., 2006). Therefore, approaches to simultaneous treatment of the tumor site with at least two active anti-cancer therapies were introduced and showed improved therapeutic effect in various cancers (Lake and Robinson, 2005; Rose et al., 1999; Walsh et al., 1996). Recently, the systems designed to deliver an anticancer drug that induces apoptosis in tumor cells and siRNA or an antisense oligonucleotide that interferes with the expression of either drug efflux protein or anti-apoptotic proteins at a molecular level demonstrated promising results for cancer therapy (Kaneshiro and Lu, 2009; Wang et al., 2006; Zhu et al., 2010).

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http://dx.doi.org/10.1016/j.ijpharm.2012.04.083

In our previous study, we introduced polymeric conjugates of an amphiphilic bile acid and low-molecular-weighted polyethylenimine (PEI), which mimic macromolecular transduction phenomenon of cell penetrating peptides (CPPs). The polymeric conjugate forms cationic micelles in an aqueous medium and mediates highly efficient gene delivery via a characteristic endocytosis-independent mechanism (Chae et al., 2011). In this study, we used the micelle-forming property of the conjugate (deoxycholic acid-PEI, DA3) as a platform for the combined delivery of a water-insoluble anticancer drug and siRNA in a single vehicle. The anti-cancer effect of the combined delivery system was evaluated.

2. Materials and methods

2.1. Materials

Paclitaxel (PTX) was obtained from Samyang Genex (Seoul, Korea). A human XIAP siRNA (sense: 5′-AAGUGGUAGUCCUGUUUCAGC-3′, antisense: 5′-GCUGAAACAGGACUACCAC-UU-3′) was synthesized from Bioneer (Daejeon, Korea). Polyethylenimine (Mw 1800, PEI 1.8 kDa) was purchased from Polysciences (Warrington, PA). Deoxycholic acid (DA), dicyclohexylcarbodiimide (DCC), and N-hydroxysuccinimide (NHS) were from Sigma (St. Louis, MO). Fetal bovine serum (FBS), Dulbecco's phosphate buffered saline (PBS), and Dulbecco's Modified Eagle's Medium (DMEM) were from Invitrogen (Carlsbad, CA).

2.2. Synthesis of polymer conjugate

A conjugate of DA and PEI was synthesized as previously described (Chae et al., 2011). Briefly, the carboxyl group of DA dissolved was activated in methylenchloride by DCC/NHS chemistry. The reaction stoichiometry was 1:3:3. The activated DA was purified by precipitating it in hexane and dried under vacuum. The DA–PEI conjugate was synthesized by slowly adding the activated DA to PEI in dichloromethane. The molar feed ratio or DA to PEI was 3:1. The product was dried, re-hydrated in 0.1 M HCl, and precipitated in cold acetone. The precipitate was dried under reduced pressure, dissolved in water, filtered, and freeze dried. The degree of substitution of the resulting conjugate (DA3) was 2.5, as determined by ^{1}H NMR.

2.3. Preparation of drug-loaded micelles

PTX-loaded DA3 micelles (PTX/DA3) were prepared by slowly adding the mixture of DA3 and PTX dissolved in methanol to deionized water while sonicated. The resulting solution was dialyzed against deionized water (MWCO 10,000), filtered through 0.45 μm filter unit to remove unincorporated paclitaxel, and lyophilized. The drug loading content and loading efficiency were 15.6% and 78%, respectively, as determined by HPLC.

2.4. Cell culture and drug delivery

Human colorectal cancer cells (HCT-116) were cultured in DMEM supplemented with 15% FBS and maintained at 37 °C in a humidified 5% CO_2 atmosphere. For transfection, the cells (5×10^4) were plated in a 35 mm culture dish. The cells were plated in 6-well plates at a density of 5.0×10^5 cells/well and incubated for 24 h at 37 °C. To prepare the PTX/DA3/siRNA complex, the PTX/DA3 was dissolved in deionized water, and siRNA were separately diluted in Opti-MEM (Invitrogen, Carlsbad, CA). The solutions were then mixed at desired polymer/siRNA weight ratios and stored at ambient temperature for 20 min before transfection. The cells were treated with the pre-determined formulations and incubated for 4 h, after which the medium was removed and supplemented with fresh DMEM containing 10% FBS. After additional incubation for 24 h, the protein expression profiles were observed by Western blot analysis using a desired antibodies, including anti-XIAP (BD-Biosciences, San Jose, CA), anti-caspase-3 (Cell signaling, Beverly, MA), and anti-β-actin (Sigma, St. Louis, MO).

2.5. MTT assays

The cytotoxicity effect of PTX/DA3/siRNA on cancer cells was evaluated in HCT-116 cells. HCT116 cells were plated in a 96 well plate at a seeding density of 5×10^3/well in a growth medium consisting of DMEM with 10% FBS and grown for 24 h at 37 °C. Desired formulations diluted in Opti-MEM were added to the cells and incubated for 5 h, after which the medium was replaced with fresh DMEM containing 10% FBS. After an additional 48 h incubation, 100 μl of fresh growth medium containing 50 μg MTT were added to each well, and the cells were incubated at 37 °C for 2 h. The insoluble formazan crystal was dissolved in DMSO. Absorbance was measured at 450 nm in a microplate reader (EL 808, Bio-tek instrument, VT). Survival percentage was calculated as compared to mock-treated cells (100% survival).

2.6. Animal experiment

Male nude mice (BALB/c *nu/nu*) were purchased from Japan SLC (Hamamatsu, Japan) and used at seven weeks of age. The animal tumor model was generated by subcutaneous injection of HCT-116 cells (1.5×10^6 cells) into one of the flank regions of the mouse. When the volume of the tumor xenograft reached 100 mm^3, the indicated formulations were intratumorally administered on days 0, 3, 7, and 10. The dose amounts for PTX and XIAP siRNA were 400 μg/kg and 100 nmole/kg, respectively. The tumor growth was monitored by measuring perpendicular diameters using a caliper. The tumor volume was calculated using the following equation: tumor volume (mm^3) = [length × (width)^2]/2.

3. Results and discussion

Previously, conjugates of low molecular weight PEI (PEI 1.8 kDa) and bile acid (BA), having different degree of hydrophobicity, were synthesized (Chae et al., 2011). The hydrophobicity of the conjugates was varied by selecting different bile acid, cholic acid (CA), deoxycholic acid (DA), and lithocholic acid (LA) as a hydrophobic moiety and the degree of substitution. The conjugates are self-assembled to form cationic micelles, which can interact with a negatively charged nucleic acid drug, generating nano-sized polyelectrolyte complexes. Among the conjugates, one of the deoxycholic acid conjugates, DA3, could mediate highly efficient intracellular gene delivery via both endocytic and non-endocytic pathways. This interesting phenomenon, polymer-induced macromolecular transduction, may be due to the amphiphilic characteristics of the bile acid. When the conjugate formed the complexes with nucleic acid, the hydrophilic face of the deoxycholic acid moieties can be exposed on the surface of the complex, while hydrophobic steroid rings are oriented toward the core of the complex. The exposure of the bile acid moieties was evidenced by observing receptor-mediated endocytosis of the complexes in bile acid receptor-positive cells. Although the detailed mechanism is not clear, the bile acid moieties may interact with the plasma membrane to trigger the intracellular uptake in a way that can be observed with the macromolecular transduction phenomenon of cell penetrating peptides (CPPs). In this study, DA3 was used as a combined delivery platform for a water-insoluble anticancer drug, paclitaxel, and siRNA.

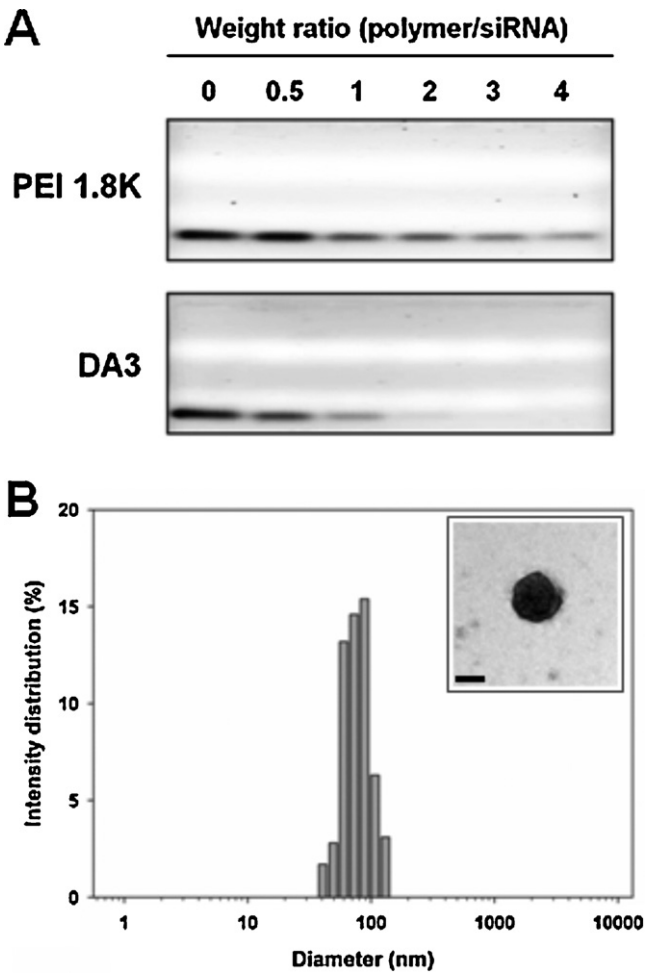

Fig. 1. (A) Formation of DA3/siRNA complexes. Polycation-mediated electrophoretic band mobility shift of siRNA (60 nM) in 8% acrylamide gel. (B) Hydrodynamic diameter and transmission electron microscope (TEM) image of DA3/siRNA complex (inset, scale bar = 50 nm).

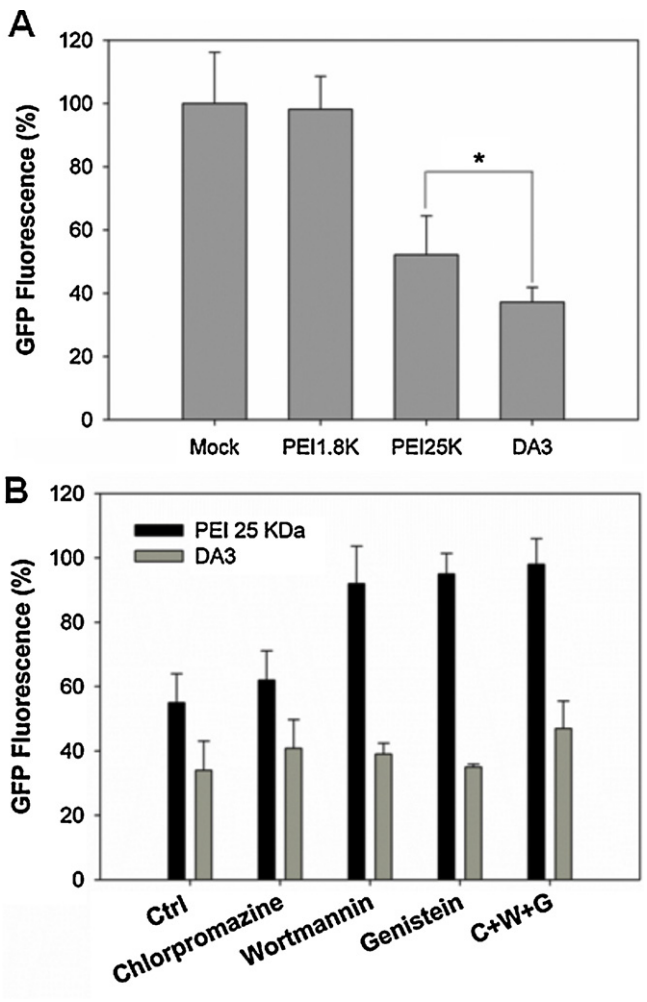

Fig. 2. (A) Gene silencing efficiency of DA3/siRNA complexes in GFP-expressing MDA-MB-435-GFP cells (*$P < 0.01$). (B) Effects of endocytosis inhibitors (10 μg/ml chlorpromazine, 100 nM wortmannin, 200 μM genistein and a combination of the three endocytosis inhibitors (C + W + G)) on cellular transfection of PEI 25 kDa and DA3 (w/w = 2). Note that gene silencing efficiency was expressed as percent of remaining GFP fluorescence (GFP fluorescence of mock-treated cells = 100%). Values are given as the mean ± SD of triplicates.

The capability of DA3 to condense siRNA was first observed. Although the low molecular weight PEI (Mw 1.8 kDa) was used as a backbone for the conjugates, DA3 successfully condensed siRNA at a weight ratio of higher than 1 (Fig. 1A). It should be noticed that the unmodified PEI 1.8 kDa failed to condense siRNA even at high weight ratios. Due to its structural rigidity, double-stranded siRNA may not easily form a compact polyelectrolyte complex with the short polycation. This result agrees well with a previous observation (Lee et al., 2010). The complexes showed 88.4 ± 16 nm in diameter and a spherical morphology (Fig. 1B). The transfection efficiency of DA3 was evaluated in green fluorescence protein (GFP)-expressing breast cancer cells, MDA-MB-435-GFP, using siRNA targeting GFP. As shown in Fig. 2A, DA3 exhibited significantly higher GFP-gene silencing activity, compared to PEI 25 kDa. The polymer maintained its gene silencing activity even in the presence of several endocytosis inhibitors, including 10 μg/ml chlorpromazine (inhibitor of clathrin-mediated endocytosis), 100 nM wortmannin (inhibitor of macropinocytosis) and 200 μM genistein (inhibitor of caveolae-mediated endocytosis) (Fig. 2B). In addition, combination of the three endocytosis inhibitors did not seriously affect transfection ability of DA3, suggesting that DA3 may be able to use an alternative cellular uptake pathway that is endocytosis-independent, suggesting that the polymer can mimic the endocytosis-independent macromolecular transduction phenomenon of CPP. Similar phenomenon was also observed in our previous report with plasmid DNA (Chae et al., 2011).

Since DA3 forms cationic micelles in an aqueous medium, DA3 was used as a vehicle for a hydrophobic anticancer drug, paclitaxel, and siRNA targeting XIAP gene. Paclitaxel belongs to a class of mitotic inhibitors that inhibit tubulin polymerization and microtubule formation, leading to apoptotic cell death. Paclitaxel exhibits cytotoxicity, antimetastatic, and antiagiogenic activities in a dose dependent manner. The X-linked inhibitor of apoptosis (XIAP), one of the members of inhibitors of apoptosis (IAP), is known as the most potent molecule that inhibits caspase 3, 7, and 9. Elevated level of XIAP expression was detected in clinical samples from colorectal, prostate, and lung cancers (Krajewska et al., 2003, 2005; Tamm et al., 2000). In addition, most of the National Cancer Institute-60 (NCI-60), a panel of 60 diverse human cancer cell lines, showed higher XIAP expression than normal cells, suggesting its significant role in neoplastic activities (Holcik et al., 2001; Schimmer et al., 2004; Zhang et al., 2007). Therefore, XIAP would be a reasonable target for siRNA/paclitaxel combination therapy to inhibit tumor progression as well as increase drug sensitivity.

The intracellular amount of XIAP was measured after transfection of the complexes to human colorectal cancer cells (HCT-116)

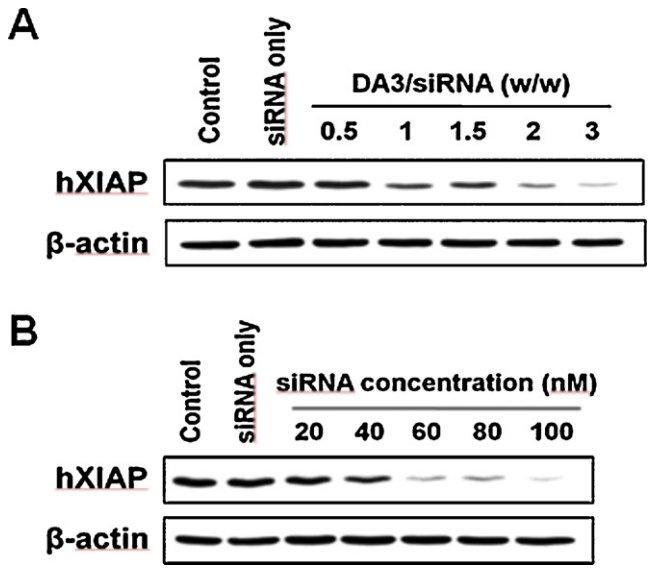

Fig. 3. Effect of the amount of polymer (A) and XIAP siRNA on the suppression of human XIAP expression at a fixed amount of siRNA (60 nM) and DA3/siRNA weight ratio (w/w = 2) (B).

using Western blot analysis. The DA3-based formulation was further optimized by varying the amount of polymer to form the complexes and siRNA concentration. As shown in Fig. 3A, an appropriate suppression level of XIAP expression was achieved at the weight ratio of higher than 2, at which the formulation also demonstrated dose-dependent gene silencing according to siRNA concentration (Fig. 3B).

To achieve combined delivery of paclitaxel and siRNA in a single vehicle, paclitaxel-loaded DA3 micelles were formed using a solvent diffusion method, dialyzed against deionized water, and freeze dried. The drug loading content was 15.6%, as determined by HPLC. The dried cationic micelles can be readily hydrated to form a clear solution upon the addition of water or PBS. The micelles were then used as a carrier for XIAP siRNA. The drug-loaded carrier can condense siRNA as efficiently as drug-free DA3 (data not shown). The resulting combined delivery vehicle (PTX/DA3/siRNA) demonstrated a significant level of XIAP-specific gene silencing, which was similar extent as that of DA3/siRNA complexes. The RNAi-mediated XIAP suppression effect was also confirmed by comparing the relative mRNA level with the protein expression level. Since RNAi is a post-transcriptional process that regulates the protein level by the specific cleavage of a target mRNA, it was expected that XIAP mRNA level would be consistent with XIAP protein suppression level. The quantitatively measured XIAP mRNA level was consistent with the XIAP protein expression level (Fig. 4A and B). The results indicate that XIAP siRNA could mediate gene-specific silencing of XIAP expression regardless of the presence of paclitaxel. The increased XIAP mRNA level after the paclitaxel treatment may be due to the activation of nuclear factor-kappa B (NFκB) induced by paclitaxel since NFκB regulates the expression of a series of antiapoptotic proteins such as XIAP, IAP1, IAP2, Bcl-2, and Bcl-xL (Aggarwal et al., 2005). The cytotoxic effect of the PTX/DA3/siRNA formulation was evaluated in human colorectal cancer cells (HCT-116). The PTX/DA3 exhibited a similar cytotoxicity profile to free PTX dissolved in DMSO at the concentration range from 0.01 to 1000 nM (data not shown). The combination of siRNA with the PTX/DA3 formulation demonstrated a significantly enhanced cytotoxic effect on the cancer cells compared to PTX (PTX only and PTX/DA3) and siRNA (DA3/siRNA) formulations (Fig. 4C). The cell viability was reduced by 47%, 54%, and 45% after the treatment of DA3/siRNA, 30 nM free PTX, and 30 nM PTX/DA3, respectively. In contrast, 71% reduction in

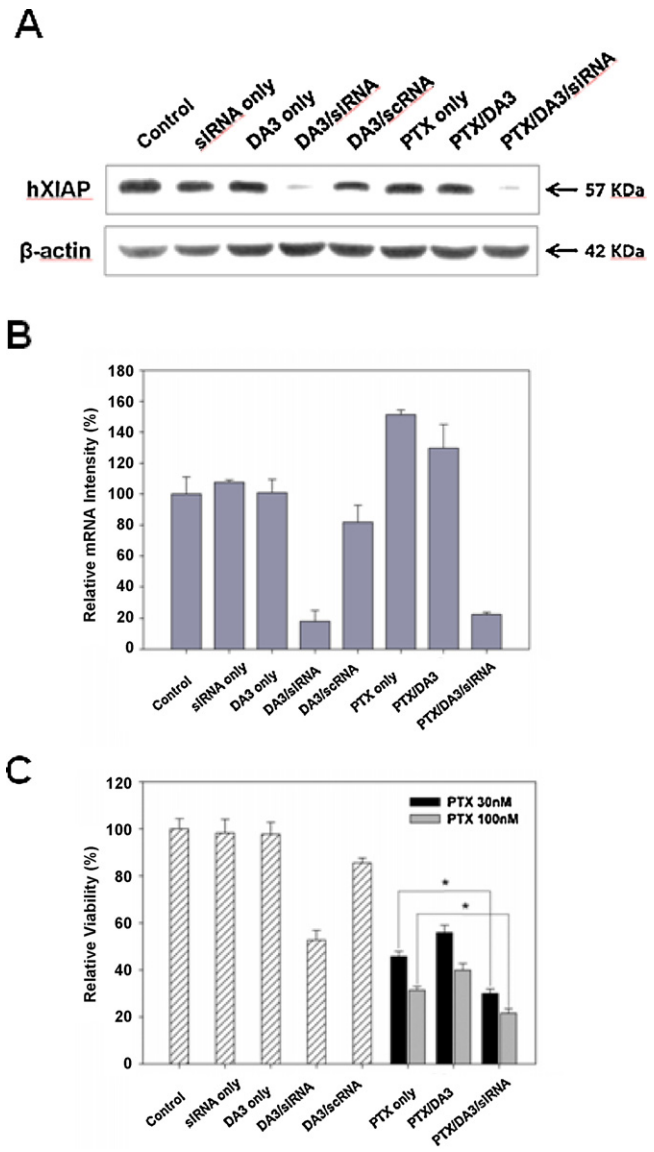

Fig. 4. Suppression of target gene-specific XIAP expression at protein level (A, Western blot) and XIAP mRNA level (B, quantitative RT-PCR) by combined formulation of PTX and XIAP siRNA. (C) Enhanced anticancer effect on HCT-116 by combined delivery of XIAP siRNA and paclitaxel (*$P < 0.05$). The data are presented as mean ± SD ($n = 8$/group).

cancer cell viability was achieved by 30 nM PTX/DA3/siRNA, which was even higher than cell growth inhibition by 100 nM PTX. The results suggest that the suppression of XIAP is sufficient enough to sensitize the tumor cells to paclitaxel by removing the molecular block that inhibits the passage of apoptotic signals. This agrees with a previously published result, in which cancer cells having stable XIAP-knockout showed enhanced chemosensitization even in the presence of other anti-apoptotic proteins, including the members of the IAP and Bcl-2 families (Connolly et al., 2009).

The enhanced chemosensitization effect by the combined therapy (PTX/DA3/siRNA) was further tested in tumor bearing animals. The combined formulation or various other formulations were intratumorally injected into the mice with HCT-116 colorectal cancer. The injection was initiated when tumor size became 100 mm^3 (day 0) and repeated on days 3, 7, and 10. In accordance with the in vitro results, simultaneous delivery of PTX and siRNA in a single vehicle (PTX/DA3/siRNA) exhibited a significantly enhanced inhibitory effect on tumor growth in vivo

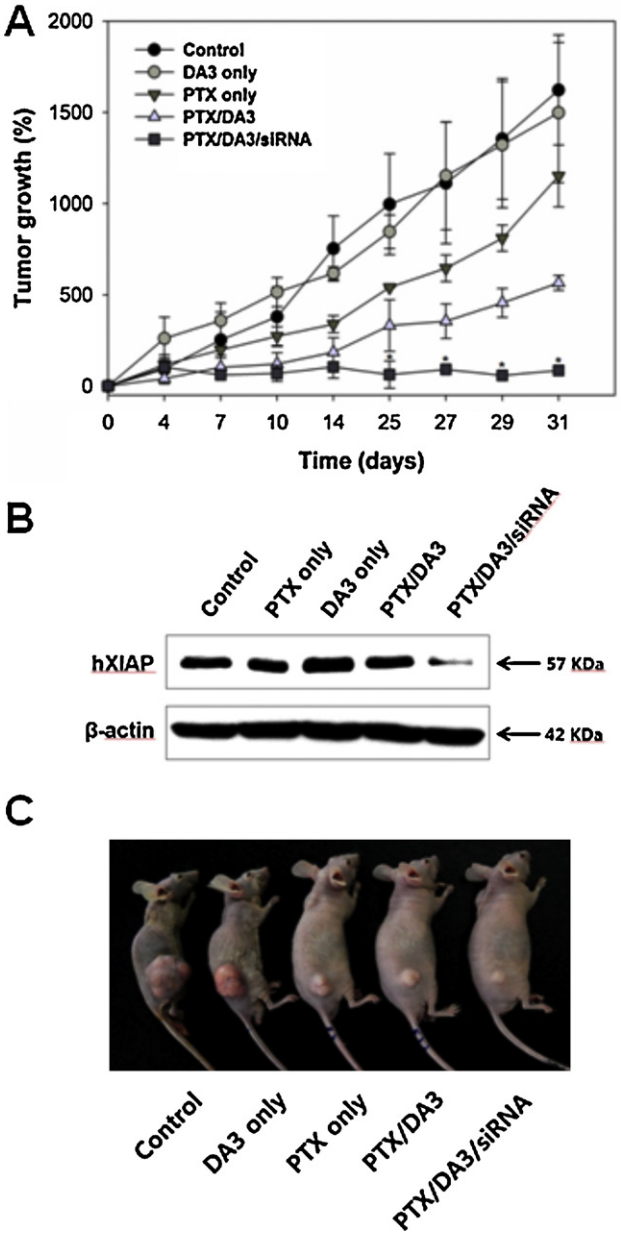

Fig. 5. (A) Inhibition of HCT-116 tumor growth by PTX/DA3/siRNA. Fifty microliters of the desired formulations were intratumorally administered to tumor bearing mice on days 3, 7, and 10. Tumor growth was monitored by measuring tumor volume as described in Materials and method section and expressed as percent of initial volume (100 mm^2). The dose amounts for PTX and XIAP siRNA were 400 μg/kg and 100 nmole/kg, respectively. The results represent mean ± SD (n = 6/group). $^*P < 0.01$ versus controls. (B) Amount of intratumoral XIAP in HCT-116 tumor xenograft. Tumor was removed one day after the final injection (day 11). (C) Representative images of HCT-116 tumors after 31 days.

(Fig. 5A). It should be noticed that the tumor growth was almost completely impeded by the combined formulation. Significantly reduced expression of XIAP was also observed in the tumor tissues treated with PTX/DA3/siRNA formulation (Fig. 5B). Representative animal images from each group dramatically showed the therapeutic effect of the combination therapy (Fig. 5C). In contrast, only moderate inhibition of tumor growth could be observed with free PTX at the same dose. The PTX/DA3 exhibited higher inhibition of tumor growth than free PTX, which was similar to PTX/DA3 in terms of in vitro cell cytotoxicity (Fig. 4C). This result may be due to improved drug solubility and enhanced intracellular delivery of PTX by the carrier in the compact tumor tissue. In a previous study, DA3 could mediate enhanced intracellular delivery of plasmid DNA via polymer-induced macromolecular transduction, which mimics cellular transduction process by cell-penetrating peptides (CPPs). The hydrophilic face of the amphiphilic bile acid moieties of DA3, which is localized on the outside the PEI shell, may play an important role in the interactions between the micelles and the plasma membrane, resulting in improved cellular uptake.

4. Conclusions

In this study, we demonstrated a biomimetic polymer based carrier system that mediates simultaneous delivery of siRNA and a water-insoluble anticancer drug. The biomimetic micelle-forming PEI–bile acid conjugate, DA3, mediated efficient dissolution of paclitaxel and condensation of siRNA to form nano-sized complexes. The combined delivery of XIAP siRNA and paclitaxel greatly enhanced the extent of tumor growth inhibition. Since the recent advances in modern medical technology make it possible to visualize and access solid tumor in any location of the body using minimally invasive trans-catheters, the suggested combined delivery system could be considered as a potential candidate for clinical combination cancer therapy.

Acknowledgments

This research was supported by the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2011-0019775, 2009-0088722, 2010-0027955, and 2011-0093632) and by a grant of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs (A110879).

References

Aggarwal, B.B., Shishodia, S., Takada, Y., Banerjee, S., Newman, R.A., Bueso-Ramos, C.E., Price, J.E., 2005. Curcumin suppresses the paclitaxel-induced nuclear factor-kappaB pathway in breast cancer cells and inhibits lung metastasis of human breast cancer in nude mice. Clin. Cancer Res. 11, 7490–7498.

Altieri, D.C., 2003. Validating survivin as a cancer therapeutic target. Nat. Rev. Cancer 3, 46–54.

Caplen, N.J., 2004. Gene therapy progress and prospects. Downregulating gene expression: the impact of RNA interference. Gene Ther. 11, 1241–1248.

Chae, S.Y., Kim, H.J., Lee, M.S., Jang, Y.L., Lee, Y., Lee, S.H., Lee, K., Kim, S.H., Kim, H.T., Chi, S.C., Park, T.G., Jeong, J.H., 2011. Energy-independent intracellular gene delivery mediated by polymeric biomimetics of cell-penetrating peptides. Macromol. Biosci. 11, 1169–1174.

Connolly, K., Mitter, R., Muir, M., Jodrell, D., Guichard, S., 2009. Stable XIAP knockdown clones of HCT116 colon cancer cells are more sensitive to TRAIL, taxanes and irradiation in vitro. Cancer Chemother. Pharmacol. 64, 307–316.

Hannon, G.J., 2002. RNA interference. Nature 418, 244–251.

Holcik, M., Gibson, H., Korneluk, R.G., 2001. XIAP: apoptotic brake and promising therapeutic target. Apoptosis 6, 253–261.

Kaneshiro, T.L., Lu, Z.R., 2009. Targeted intracellular codelivery of chemotherapeutics and nucleic acid with a well-defined dendrimer-based nanoglobular carrier. Biomaterials 30, 5660–5666.

Kim, S.H., Jeong, J.H., Lee, S.H., Kim, S.W., Park, T.G., 2006a. PEG conjugated VEGF siRNA for anti-angiogenic gene therapy. J. Control. Release 116, 123–129.

Kim, S.H., Jeong, J.H., Lee, S.H., Kim, S.W., Park, T.G., 2008. Local and systemic delivery of VEGF siRNA using polyelectrolyte complex micelles for effective treatment of cancer. J. Control. Release 129, 107–116.

Kim, W.J., Chang, C.W., Lee, M., Kim, S.W., 2007. Efficient siRNA delivery using water soluble lipopolymer for anti-angiogenic gene therapy. J. Control. Release 118, 357–363.

Kim, W.J., Christensen, L.V., Jo, S., Yockman, J.W., Jeong, J.H., Kim, Y.H., Kim, S.W., 2006b. Cholesteryl oligoarginine delivering vascular endothelial growth factor siRNA effectively inhibits tumor growth in colon adenocarcinoma. Mol. Ther. 14, 343–350.

Kim, W.J., Kim, S.W., 2009. Efficient siRNA delivery with non-viral polymeric vehicles. Pharm. Res. 26, 657–666.

Krajewska, M., Kim, H., Kim, C., Kang, H., Welsh, K., Matsuzawa, S., Tsukamoto, M., Thomas, R.G., Assa-Munt, N., Piao, Z., Suzuki, K., Perucho, M., Krajewski, S., Reed, J.C., 2005. Analysis of apoptosis protein expression in early-stage colorectal cancer suggests opportunities for new prognostic biomarkers. Clin. Cancer Res. 11, 5451–5461.

Krajewska, M., Krajewski, S., Banares, S., Huang, X., Turner, B., Bubendorf, L., Kallioniemi, O.P., Shabaik, A., Vitiello, A., Peehl, D., Gao, G.J., Reed, J.C., 2003. Elevated expression of inhibitor of apoptosis proteins in prostate cancer. Clin. Cancer Res. 9, 4914–4925.

LaCasse, E.C., Baird, S., Korneluk, R.G., MacKenzie, A.E., 1998. The inhibitors of apoptosis (IAPs) and their emerging role in cancer. Oncogene 17, 3247–3259.

Lake, R.A., Robinson, B.W., 2005. Immunotherapy and chemotherapy—a practical partnership. Nat. Rev. Cancer 5, 397–405.

Lee, S.Y., Huh, M.S., Lee, S., Lee, S.J., Chung, H., Park, J.H., Oh, Y.K., Choi, K., Kim, K., Kwon, I.C., 2010. Stability and cellular uptake of polymerized siRNA (poly-siRNA)/polyethylenimine (PEI) complexes for efficient gene silencing. J. Control. Release 141, 339–346.

Leung, R.K., Whittaker, P.A., 2005. RNA interference: from gene silencing to gene-specific therapeutics. Pharmacol. Ther. 107, 222–239.

Rose, P.G., Bundy, B.N., Watkins, E.B., Thigpen, J.T., Deppe, G., Maiman, M.A., Clarke-Pearson, D.L., Insalaco, S., 1999. Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. N. Engl. J. Med. 340, 1144–1153.

Schimmer, A.D., Welsh, K., Pinilla, C., Wang, Z., Krajewska, M., Bonneau, M.J., Pedersen, I.M., Kitada, S., Scott, F.L., Bailly-Maitre, B., Glinsky, G., Scudiero, D., Sausville, E., Salvesen, G., Nefzi, A., Ostresh, J.M., Houghten, R.A., Reed, J.C., 2004. Small-molecule antagonists of apoptosis suppressor XIAP exhibit broad antitumor activity. Cancer Cell 5, 25–35.

Sjoblom, T., Jones, S., Wood, L.D., Parsons, D.W., Lin, J., Barber, T.D., Mandelker, D., Leary, R.J., Ptak, J., Silliman, N., Szabo, S., Buckhaults, P., Farrell, C., Meeh, P., Markowitz, S.D., Willis, J., Dawson, D., Willson, J.K., Gazdar, A.F., Hartigan, J., Wu, L., Liu, C., Parmigiani, G., Park, B.H., Bachman, K.E., Papadopoulos, N., Vogelstein, B., Kinzler, K.W., Velculescu, V.E., 2006. The consensus coding sequences of human breast and colorectal cancers. Science 314, 268–274.

Stevenson, M., 2004. Therapeutic potential of RNA interference. N. Engl. J. Med. 351, 1772–1777.

Tamm, I., Kornblau, S.M., Segall, H., Krajewski, S., Welsh, K., Kitada, S., Scudiero, D.A., Tudor, G., Qui, Y.H., Monks, A., Andreeff, M., Reed, J.C., 2000. Expression and prognostic significance of IAP-family genes in human cancers and myeloid leukemias. Clin. Cancer Res. 6, 1796–1803.

Tuschl, T., 2001. RNA interference and small interfering RNAs. Chembiochem 2, 239–245.

Urban-Klein, B., Werth, S., Abuharbeid, S., Czubayko, F., Aigner, A., 2005. RNAi-mediated gene-targeting through systemic application of polyethylenimine (PEI)-complexed siRNA in vivo. Gene Ther. 12, 461–466.

Walsh, T.N., Noonan, N., Hollywood, D., Kelly, A., Keeling, N., Hennessy, T.P., 1996. A comparison of multimodal therapy and surgery for esophageal adenocarcinoma. N. Engl. J. Med. 335, 462–467.

Wang, Y., Gao, S., Ye, W.H., Yoon, H.S., Yang, Y.Y., 2006. Co-delivery of drugs and DNA from cationic core–shell nanoparticles self-assembled from a biodegradable copolymer. Nat. Mater. 5, 791–796.

Zhang, S., Ding, F., Luo, A., Chen, A., Yu, Z., Ren, S., Liu, Z., Zhang, L., 2007. XIAP is highly expressed in esophageal cancer and its downregulation by RNAi sensitizes esophageal carcinoma cell lines to chemotherapeutics. Cancer Biol. Ther. 6, 973–980.

Zhu, C., Jung, S., Luo, S., Meng, F., Zhu, X., Park, T.G., Zhong, Z., 2010. Co-delivery of siRNA and paclitaxel into cancer cells by biodegradable cationic micelles based on PDMAEMA-PCL-PDMAEMA triblock copolymers. Biomaterials 31, 2408–2416.