**Overcome the Doxorubicin Resistance by Multimodal Nanoparticles in Mice**


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**ABSTRACT**

The efficacy of many chemotherapeutic agents is reduced in cells that have developed multiple drug resistance (MDR). To address this important problem, a biodegradable polymer was coupled to the photosensitizer and the resulting photosensitizer-nanoparticles were loaded with the chemotherapeutic agent doxorubicin. The combination of photosensitizer and chemotherapeutic agent had a synergistic action on a doxorubicin-resistant breast cancer MCF-7 cells. To increase the effectiveness of this combination, D-alpha-tocopheryl poly(ethylene glycol) 1000 succinate (TPGS), an inhibitor of the multidrug transporter overproduced in these resistant cells, was added during the formation of the nanoparticles. Clearly, the tumor regrowth delay was observed after administration of doxorubicin-loaded photosensitizer-nanoparticles plus light. This combination of photodynamic activity in a powerful nanocarrier loaded with the chemotherapeutic agent doxorubicin can be used to deliver two types of cancer therapy simultaneously and can overcome the drug resistance in vivo. Therefore, this innovative delivery system can act as a potential nanomedicine for both drug-sensitive and drug-resistant cancer therapy.

**Keywords:** drug resistance, doxorubicin, p-glycoprotein, nanoparticles, in vivo

1 INTRODUCTION

Cancer is the leading cause of human death worldwide. Although there are many scientists make effort to fight this disease; however, the potential effects of many chemotherapeutic agents are undermined by the presence of multiple drug resistance (MDR). One well studied mechanism of MDR involves over-expression of efflux pumps, such as, the ABC-transporter family P-glycoprotein (P-gp), on the cell surface, which transport chemotherapeutic agents out of the cells [1] and prevent their intracellular accumulation and therefore decrease the efficacy [2, 3]. P-gp, which can transport a wide range of structurally and functionally unrelated cytotoxic drugs, e.g., doxorubicin (Dox), etoposide, paclitaxel, and vincristine out of tumor cells [4-6], is expressed in approximately 40% of breast cancer tumors; and such tumors are three times less likely to respond to chemotherapy than those that do not express P-gp [7].

Inhibition of ABC-transporters as a method to reverse MDR in cancer cells has also been studied extensively [8]. In addition to small molecule inhibitors, surfactants such as Tween 80, Cremophor EL, several Pluronics, and Vitamin E TPGS (TPGS 1000) are known to modulate efflux pump activity via possible mechanisms including competitive inhibition of substrate binding, alteration of membrane fluidity, and inhibition of efflux pump ATPase [9, 10]. Thus, using surfactant-coated nanoparticle-based drug delivery seems to provide a new strategy to overcome the MDR phenotype [11].

In doxorubicin (Dox)-resistant breast cancer cells, drug accumulates within acidic organelles [12-14]. This type of accumulation leads to increased drug sequestration in acidic vesicles, followed by transport to the outer cell wall and extrusion into the external medium. This sequence is the basis of the protonation, sequestration, and secretion (PSS) model that has been proposed to account for the resistance of MDR cells to weak base chemotherapeutic drugs [15].

Recently, nanotechnology has provided a platform for a functionalized drug delivery system. In applications of this technique, a number of nanomaterials have been investigated as potential carriers for hydrophobic drugs. Nanoparticles, one such nanomaterial, are submicron-sized polymeric colloidal particles[16, 17]. Because enhanced permeability and retention of these nanoparticles is seen in tumor tissue compared to normal tissue, nano-sized polymer-drug conjugates may preferentially accumulate in the tumor microenvironment over time [18-20]. By localizing in the tumor in this manner, drug-loaded nanoparticles (NPs) of biodegradable polymers also have the potential to reduce toxicity to normal tissues. Our group has developed several nano-sized delivery systems using polyamidoamine dendrimers and chitosan nanoparticles [21-24]. We have also developed bifunctionalized micelles including chemotherapeutic agents and photosensitizer for synergistic chemotherapy/photodynamic therapy combination [25, 26].

In the current report, two strategies, based on our previous efforts in drug delivery systems, were utilized to achieve the goal of overcoming Dox-resistance MCF-7 breast cancer cells. The first strategy was to use an inhibitor of P-gp, Vitamin E TPGS (Vitamin E TPGS (TPGS 1000), a water-soluble vitamin E derivative containing lipophilic...
nonpolar head and a hydrophilic tail that is a surfactant with high emulsification efficiency and also a P-gp inhibitor [27-29]. Its high emulsification efficiency enables it to reduce nanoparticle size and increase the amount of drug encapsulated in the nanoparticle. In addition, it may also increase cancer cell death by increasing the cellular uptake of nanoparticles [30]. The second strategy used to overcome drug resistance was photochemical internalization (PCI), a novel drug delivery method [31, 32] that increases the amount of drug internalized in multidrug resistant cells [33, 34]. An extension of photodynamic therapy (PDT), PCI is carried out by irradiating a photosensitizer that is localized on cellular membranes. The membranes rupture after irradiation with a specific wavelength, and a drug such as doxorubicin can then be released into the nucleus and bypass the mechanism causing the drug resistance, as shown in our previous results [33].

Thus, a Dox-encapsulated branched star photosensitizer-polymeric nanoparticle (PPN), for combined chemotherapy and photodynamic therapy was prepared in this study.

2 MATERIALS AND METHODS

2.1 Materials

Photosensitizers: Chlorin e6, Chlorin e6-polyacaprolactone-formed nanoparticles (PPN), doxorubicin-loaded PPN. Chemotherapeutic agent: doxorubicin.

2.2 Anti-tumor efficacy of the PPN-mediated PDT plus doxorubicin

The in vivo experimental protocols were approved by the Institutional Animal Care and Use Committee of National Chung Hsing University (IACUC of NCHU). Female BALB/cAnN.Cg-Foxn1nu/CrlNarl mice (4-5 weeks old, 20±2 g) were purchased from the National Laboratory Animal Center, Taiwan. All mice were kept in an air-conditioned facility fitted with an artificial light-dark regime and provided standard food and filtered water. The mice were kept in this environment for at least 3 days. For the implantation of estrogen pellets, mice were first anesthetized and the 17-β-estradiol-sustained release pellets (0.72 mg, 60-day release, Innovative Research of America) were implanted subcutaneously into the right back between the ear and shoulder. After three days, mice were injected subcutaneously in the right and left hindquarters with 5 × 10⁶ MCF-7/ADR cells suspended in 50 μL serum-free Minimum Essential Medium mixed with 50 μL Matrigel (Geltrex™ Reduced Growth Factor Basement Membrane Matrix, Gibco). The tumor sizes and body weights were measured every 3 or 4 days for the duration of the experiment (21 days). Tumor volume was calculated as 1/2(4π/3)(L/2)(W/2)H, where L is the length, W is the width, and H is the height of the tumor. Treatments were started when the tumors reached a volume of 100 mm³, which was designated as day 0.

2.3 Necropsy and immunohistochemical analysis

After the mice were sacrificed, the tumors were excised and weighed. For the immunohistochemical and hematoxylin and eosin (H&E) staining procedures, the tumor tissue was fixed in formalin and embedded in paraffin. The paraffin-embedded 2 μm tumor sections were analyzed by immunohistochemistry for proliferating cell nuclear antigen (PCNA) expression. Briefly, sections were subjected to deparaffinization and rehydrated. The sections were incubated in 3% H₂O₂ to inhibit endogenous peroxidase activity and then diluted normal blocking serum was used to block nonspecific protein binding sites. The sections were incubated with primary antibody against PCNA (PC10, 1:300, Dako, Denmark). After being rinsed with d.d.H₂O, the tissue sections were incubated with biotinylated secondary antibodies for 30 min at room temperature. Then avidin–biotin complexes were visualized with the 3,30-diaminobenzidine tetrahydrochloride (DAB) chromogen. Sections were also counterstained with hematoxylin. Stained sections were monitored at low power (40×) and counted at high power magnifications (400×). Cells that were positively stained for PCNA were counted at 400× magnification in at least 5 different fields. Images of the stained sections were acquired using a light microscope (BX 50, OLMPUS) equipped with a digital camera (DP 20, OLMPUS). Positive rates (%) of PCNA stained cells were quantified with Image Pro Plus software (Media Cybernetics).

3 RESULTS AND DISCUSSIONS

3.1 In vivo PDT effects in drug-resistant MCF-7/ADR xenografted mice.

The antitumor efficacy of doxorubicin-loaded PPN-PDT was evaluated by measuring tumor growth rates (Fig. 1). In general, no antitumor effects was observed using doxorubicin in drug-resistant animal model. The relative tumor volumes in each treatment group were increased from day 3 to day 21 after the treatments; the relative tumor volumes were greatest in the PBS control, doxorubicin and Ce6-PDT groups. On day 21, the mean relative tumor volumes of PDT-untreated animals (PBS control and free Dox groups) reached almost 3–4 times the initial volumes. The relative tumor volumes in the PBS control, free Dox and Ce-PDT groups increased much more steeply than in the PPN-PDT and Dox-PPN-PDT groups. Obviously, Dox-PPN-PDT reveal greater antitumor efficacy in drug-resistant cells that means the PCI effect and P-glycoprotein inhibitor may work together to overcome the drug-resistance.
3.2 Effects of the combined treatments on the tumor host microenvironment.

For immunohistochemical analysis, mice treated with free doxorubicin resulted in higher percentages of proliferating cell nuclear antigen (PCNA)-positive cells compared to the control group in the tumor sections (Fig. 2). In contrast, tumors from mice that received the PDT or combined therapy (doxorubicin-PPN-PDT) had significantly decreased cell proliferation. Therefore, PPN-based PDT or PCI reduced the number of proliferating cells, while free doxorubicin alone was ineffective within the drug-resistant subcutaneous tumors.

4 CONCLUSION

In summary, a powerful nanocarrier with photodynamic activity has been developed, the resulting nanoparticles loaded with doxorubicin, and this combination used to deliver two types of cancer therapy simultaneously. This functionalized nanomedicine is a potential carrier for the synergetic combination of photodynamic therapy, chemotherapy, and P-gp inhibitor for the treatment of cancer.

REFERENCES


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