Biodegradable particles for co-delivery of doxorubicin and CpG ODN oligonucleotides as an improved therapy for tumors
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ABSTRACT SUMMARY
Doxorubicin (dox) is an anthracycline based drug that induces apoptosis in tumors but also has adverse effects on healthy cells. Dox-treated apoptotic tumor cells can release a variety of tumor-specific and tumor-associated antigens. Bacterial DNA and synthetic oligodeoxynucleotides containing the CpG motif (CpG ODN) have shown significant potential in acting as a strong adjuvant to tumor-associated antigens for generating strong immune responses against tumors. We have developed different poly(lactide-co-glycolide) (PLGA) particle-based formulations for co-delivery of dox and CpG ODN to tumors. The efficacies of various formulations were evaluated on the basis of survival enhancement in tumor bearing mice. We successfully developed PLGA particles co-encapsulating dox and CpG ODN that promoted significant enhancement of survival of tumor-bearing mice compared to other standalone formulation strategies.

INTRODUCTION
Recent data compiled by the American Cancer Society and National Cancer Institute suggests that one in three woman and one in two men in the United States will develop cancer in their lifetime [1]. While survival of cancer patients has improved over recent decades, there are increasing incidences of recurrences highlighting the inability of current therapies to achieve complete eradication of the cancer. These potentially lethal recurrences may be avoided by combining immunotherapy with conventional treatments. Immunotherapy involves stimulation of antigen specific immunity against tumor-associated antigens that can specifically eliminate cancer cells which survive first-line treatment. Many clinical and preclinical studies have established that administration of a bacterial adjuvant with a chemotherapeutic drug has significantly reduced tumor progression and metastasis [2]. It is now recognized that tumor cells treated with dox undergo an immunogenic apoptosis, releasing tumor antigens which can stimulate tumor-specific T cell immunity and this antitumor immunity may be further enhanced by the presence of CpG ODN. We hypothesized that the maximum potential of combination therapy could be achieved by co-delivery of dox and CpG ODN oligonucleotides to the local tumor environment using biodegradable particle depots. These particles should provide sustained release of dox and CpG ODN in the tumor microenvironment. Dox is a weakly basic drug (pKa 8.2) that carries positive charge and CpG ODN due to its phosphate backbone carries a negative charge. Co-encapsulation of such oppositely charged molecules can be designed in a number of unique ways. In this study, we have proposed different formulation prototypes of PLGA particles for the co-delivery of dox and CpG ODN into the tumor. These formulations were developed and characterized to achieve PLGA particles that can provide sustained release of dox and CpG ODN in tumors leading to enhanced survival.

EXPERIMENTAL METHODS
Three different formulations of poly(lactide-co-glycolide) (PLGA) particles were prepared: A) Dox-CpG-t; B) Dox-CpG-d; C) admixture of Dox and CpG ODN particles

Figure 1: Three prototypes of PLGA particles for co-delivery of dox and CpG ODN.
These particles were fabricated using the modified double emulsion solvent evaporation process. Briefly, PLGA was dissolved in dichloromethane (DCM). For the preparation of Dox-CpG-t, complexes of Dox and CpG ODN in 1% poly(vinyl alcohol) (PVA) were encapsulated in PLGA particles. For Dox-CpG-d, Dox and CpG ODN solutions were independently emulsified in PLGA solution. These emulsions were combined and then emulsified in 1% PVA to prepare particles. Precipitated particles were collected via centrifugation, washed twice with purified water and lyophilized. All particles were characterized for size, shape, surface charge and surface morphology using the Zetasizer Nano ZS, scanning electron microscopy and powder X-ray diffraction.

A therapeutic C57BL/6 murine tumor model was used to evaluate antitumor activity of the microparticle formulations. Mice (n = 5) were challenged subcutaneously with 10^6 EL4 tumor cells. An intratumoral (i.t.) injection of different treatment groups was given on day 3 post tumor challenges and the tumor volume were measured over time with equation:

\[ \text{Tumor volume} = \frac{\pi}{6} \times \text{length} \times \text{breath} \times \text{height} \]

Mice were sacrificed if the growing tumor shows inflammation or grew more than 20 mm in one dimension.

RESULTS AND DISCUSSION

Complexes of dox and CpG ODN were prepared at different ratios to fabricate the Dox-CpG-t formulation. It was found that Dox-CpG complexes were unstable assemblies with large polydispersity in size. Also, complexation of dox with CpG ODN led to a decrease in the anti-cancer activity of dox when tested on \textit{in vitro} EL4 lymphoma cell line. Thus, the Dox-CpG-t formulation was not developed for further studies. Dox-CpG-d and admixtures of particles were successfully prepared with the modified double emulsion solvent evaporation method. These were spherical particles with 3 μm mean diameter. These particles showed sustain release of dox and CpG ODN.

All formulations when tested in EL4 tumor bearing mice showed improved survival compared to untreated tumors (Figure 2). Additionally, Dox-CpG-d showed significantly high enhancement in the survival compared to all other treatment groups.

![Figure 2: Survival curve of mice bearing EL4 tumors. Mice were injected i.t. with particulated formulations encapsulating various combinations of dox and CpG ODN. Survival curves of each treatment were compared with naive group. (n = 5).](image)

CONCLUSION

A successful PLGA particle based formulation was developed for co-delivery of dox and CpG ODN to tumors. This formulation significantly enhanced the survival in tumor bearing mice confirming that co-delivery of dox and CpG ODN in the same particle is necessary to achieve the maximum potential of combination chemo-immunotherapy.

REFERENCES


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