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Antitumor efficacy following the intracellular and interstitial release of liposomal doxorubicin

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ABSTRACT

pH-triggered lipid-membranes designed from biophysical principles are evaluated in the form of targeted liposomal doxorubicin with the aim to ultimately better control the growth of vascularized tumors. We compare the antitumor efficacy of anti-HER2/neu pH-triggered lipid vesicles encapsulating doxorubicin to the anti-HER2/neu form of an FDA approved liposomal doxorubicin of DSPC/cholesterol-based vesicles. The HER2/neu receptor is chosen due to its abundance in human breast cancers and its connection to low prognosis. On a subcutaneous murine BT474 xenograft model, superior control of tumor growth is demonstrated by targeted pH-triggered vesicles relative to targeted DSPC/cholesterol-based vesicles (35% vs. 19% *decrease* in tumor volume after 32 days upon initiation of treatment). Superior tumor control is also confirmed on SKBR3 subcutaneous xenografts of lower HER2/neu expression. The non-targeted form of pH-triggered vesicles encapsulating doxorubicin results also in better tumor control relative to the non-targeted DSPC/cholesterol-based vesicles (34% vs. 41% *increase* in tumor volume). Studies in BT474 multicellular spheroids suggest that the observed efficacy could be attributed to release of doxorubicin directly into the acidic tumor interstitium from pH-triggered vesicles extravasated into the tumor but not internalized by cancer cells. pH-triggered liposome carriers engineered from gel-phase bilayers that reversibly phase-separate with lowering pH, form transiently defective interfacial boundaries resulting in fast release of encapsulated doxorubicin. Our studies show that pH-triggered liposomes release encapsulated doxorubicin intracellularly and intratumorally, and may improve tumor control at the same or even lower administered doses relative to FDA approved liposomal chemotherapy.

1. Introduction

PEGylated liposomal doxorubicin (Doxil®) is an FDA approved therapeutic modality for several types of cancer including metastatic breast cancer, ovarian cancer and AIDS-related Kaposi's sarcoma. Enhancement of drug accumulation and retention within vascularized tumors – a process described by the EPR effect [1]- is among the benefits of this approach. The targeted form of liposomal doxorubicin is expected to become the next generation of this therapeutic modality [2,3]. The targeted approach has been demonstrated [4] to exhibit *in vivo* superior efficacy against vascularized tumors that is attributed to increased internalization of the delivery carriers by cancer cells comprising the tumors [5]. Interestingly, comparison with identical non-targeted liposomal doxorubicin that exhibits relatively inferior control of tumor growth, demonstrates same accumulation levels for both forms of liposomal doxorubicin within tumors, but significantly lower internalization by cancer cells of the non-targeted modality. Active internalization, therefore, seems to be associated with accelerated intracellular trafficking of doxorubicin *in vivo*.

At the cellular scale, the above findings point to the same direction with previous *in vitro* studies demonstrating the importance of intracellular trafficking of doxorubicin in affecting its cytotoxicity. In particular, following selective targeting of and internalization by cancer cells, a triggered mechanism for fast release of doxorubicin from internalized carriers has been correlated with greater accumulation of doxorubicin at the cell nucleus and with greater cytotoxicity [6]. Towards acceleration of intracellular trafficking, triggered release of doxorubicin from internalized liposomes stimulated by the acidification of the endosomal pH has been extensively explored [6–8] and several lipid membrane designs have been developed [9,10]. Intracellularly, not only the rate and extent of release but also the particular pH values at which release starts to occur may significantly affect the intracellular trafficking of the released agent [11]. Especially for the case of

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