Chemotherapeutic Delivery Based on Tumor pH Using an in Situ Affinity Change

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Statement of Purpose: Due to high toxicity of many chemotherapy drugs, a system which can deliver preferentially in the presence of tumors is preferred. We have explored use of affinity interactions to control the rate of drug release in many therapeutic settings [1]. To further alter the drug delivery profile, herein we have incorporated a high affinity moiety onto doxorubicin, preventing its premature release. In the low pH of a tumor, the high affinity group is cleaved off resulting in drug with a lower affinity. The free drug, Doxorubicin (DOX) has previously been shown to have slow, sustained delivery from our polymers, and capacity to clear human xenograft glioblastomas. Adamantane-modified Doxorubicin (AD-DOX) with a pH-sensitive hydrazone linker was loaded into beta-cyclodextrin (β-CD) polymers and dextran polymers. In high affinity settings drug was not released until cleaved at low pH.

Methods: PH sensitive AD-DOX was synthesized according to a previously published protocol [2], evaluated by NMR, FTIR, and surface plasmon resonance spectroscopy (SPR) to confirm correct synthesis and evaluate its interaction with β-CD. An MTT assay to demonstrate the killing capacity of modified drug was performed with unmodified Doxorubicin (DOX) as the positive control; treating U-87 MG human glioblastoma cells. To evaluate the effect of the high affinity moiety, an in situ drug release study was performed with AD-DOX over 87 days in triplicate with four separate conditions including β-CD and dextran polymers in acetate pH 5.0 buffer and in phosphate buffered saline (PBS) pH 7.4. Buffers were selected to model the slightly acidic tumor microenvironment and the neutral environment of healthy tissue.

Results: Chemical analyses demonstrated that we synthesized the AD-DOX drug as previously described. Through the MTT assay, we observed that the conjugate typically had equivalent cytotoxicity to DOX; however, Figure 1 indicates that when AD-DOX had a concentration between 3.6-36 μM it had more significant killing capacity than DOX. Specifically, at a concentration of 10 μM AD-DOX killed 69.74% of the cells compared to DOX killing 27.89%.
The drug release study results in Figure 2 confirm slow, sustained drug release from the affinity-based β-CD compared to the dextran polymers. Further, the figure demonstrates that AD-DOX had a more rapid release from the polymers in the acidic buffer.

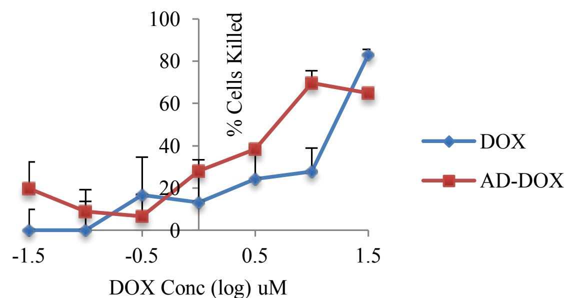

Figure 1: Cytotoxicity comparison between modified and unmodified drug. While for the most part toxicity was comparable, at some concentrations the modified drug was even more toxic.

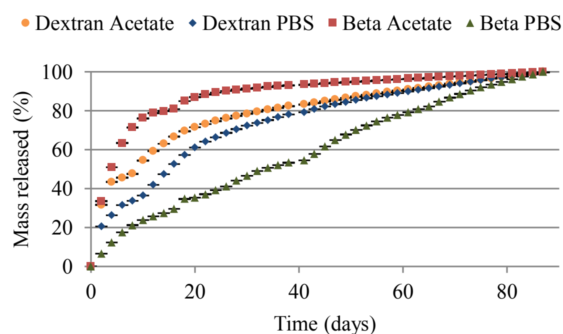

Figure 2: Drug released at each time point. Non-affinity dextran based polymers showed typical biphasic diffusional release of drug regardless of pH. However the affinity-based cyclodextrin polymer showed slow, sustained release of AD-Dox at pH 7.4, and more rapid release once the AD is cleaved off at pH 5.0.

Conclusions: The increased cytotoxicity of AD-DOX at reduced concentrations indicates the potential of utilizing a decreased dosage of AD-DOX to achieve a more significant cell killing capacity than DOX. The sustained released from the affinity-based polymers is indicative of complex formation with β-CD and its resulting delay in release. The prolonged release rate from the β-CD polymers disappears when in acidic buffer, representative of the cleaving of the high affinity moiety from DOX. AD-DOX loaded onto affinity-based polymers is capable of selectively delivering DOX more rapidly in slightly acidic (tumor-like) environments. Future study includes analysis of the collected FTIR and SPR data.

References: [1]: (Wang NX. Macromol. Biosci. 2011;11:321-332), [2]: (Luo GF. ACS Appl. Mater. Interfaces 2012;4:5317-5324)

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