

Targeted Stimuli-Responsive Dextran Conjugates for Doxorubicin Delivery to Hepatocytes

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Abstract – A targeted, stimuli-responsive, polymeric drug delivery vehicle is being developed in our lab to help alleviate severe side-effects caused by narrow therapeutic window drugs. Targeting specific cell types or organs via proteins, specifically, lectin-mediated targeting holds potential due to the high specificity and affinity of receptor-ligand interactions, rapid internalization, and relative ease of processing.

Dextran, a commercially available, biodegradable polymer has been conjugated to doxorubicin and galactosamine to target hepatocytes in a three-step, one-pot synthesis. The loading of doxorubicin and galactose on the conjugates was determined by absorbance at 485 nm and elemental analysis, respectively. Conjugation efficiency based on the amount loaded of each reactant varies from 20% to 50% for doxorubicin and from 2% to 20% for galactosamine. Doxorubicin has also been attached to dextran through an acid-labile hydrazone bond.

Doxorubicin acts by intercalating with DNA in the nuclei of cells. The fluorescence of doxorubicin is quenched when it binds to DNA. This allows a fluorescence-based cell-free assay to evaluate the efficacy of the polymer conjugates where we measure the fluorescence of doxorubicin and the conjugates in increasing concentrations of calf thymus DNA. Fluorescence quenching indicates that our conjugates can bind to DNA. The degree of binding increases with polymer molecular weight and substitution of doxorubicin.

In cell culture experiments with hepatocytes, the relative uptake of polymer conjugates was evaluated using flow cytometry, and the killing efficiency was determined using the MTT cell proliferation assay. We have found that conjugate uptake is much lower than that of free doxorubicin. Lower uptake of conjugates may increase the maximum dose of drug tolerated by the body. Also, non-galactosylated

conjugate uptake is lower than that of the galactosylated conjugate. Microscopy indicates that doxorubicin localizes almost exclusively at the nucleus, whereas the conjugates are present throughout the cell. Doxorubicin linked to dextran through a hydrazone bond was used to achieve improved killing efficiency. Following uptake, the doxorubicin dissociates from the polymer in an endosomal compartment and diffuses to the nucleus. The LC₅₀ of covalently linked doxorubicin is 7.4 µg/mL, whereas that of hydrazone linked doxorubicin is 4.4 µg/mL.