Modulation of Tumor Cytotoxicity and Targeting Selectivity of Nanocarriers via a Dual Ligand Targeting Approach

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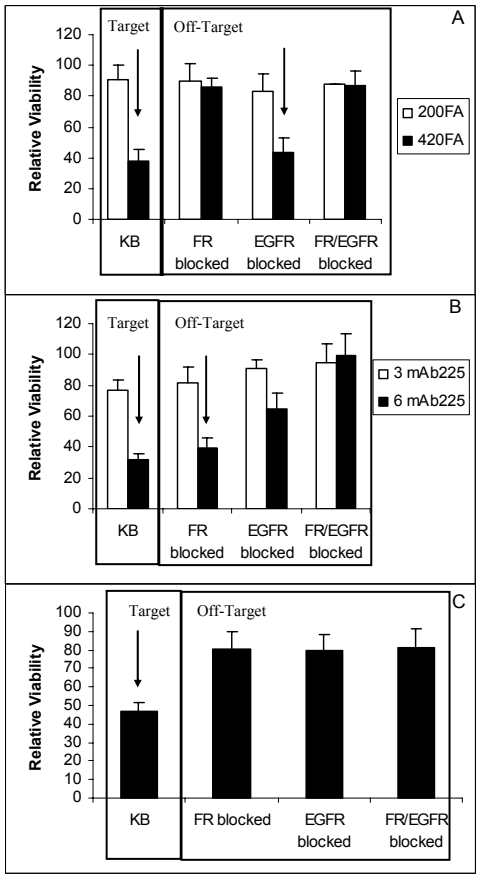
Statement of Purpose: Ligand-targeted nanocarriers directed at over-expressed receptors on tumor cells (target) are useful for the delivery of various therapeutic agents including chemotherapy drugs. However, the expression of the targeted receptors on healthy (off-target) cells can lead to accumulation of drugs in these cells, leading to unintended side effects. In this series of studies, we developed a liposomal nanocarrier system in which two types of targeting ligands were simultaneously presented on the carrier surface in quantities such that only target cells expressing each of the targeted receptors would take up sufficient quantities of drug to elicit cytotoxicity. By minimizing toxicity to off-target cells not bearing the full array of targeted receptors, selectivity enhancement was demonstrated *in vitro*.

Methods: Liposomal nanocarriers were constructed from distearoyl-sn-glycero phosphocholine (DSPC), cholesterol, and distearoyl-sn-glycero-phospho-ethanolamine-poly(ethylene glycol) of molecular weight 2000Da with a terminal maleimide group (DSPE-PEG_{2000}-maleimide). After extrusion of liposomes to 100nm diameter, ammonium sulfate buffer was exchanged for a saline solution to obtain an ammonium sulfate gradient. A DSPE-PEG_{3350}-folic acid conjugate[1,2] was inserted into the pre-formed liposomes.[2] A monoclonal antibody (mAb225) against the epidermal growth factor receptor (EGFR) was then coupled to the terminal maleimide groups by thiol linkage. Liposomes were loaded with doxorubicin via the ammonium sulfate gradient. KB cells (target cells) bearing both targeted receptors (folate receptor and the EGFR) and off-target cells (modeled through receptor-blockade) were subjected to a two hour treatment with the doxorubicin-loaded liposomes. Off-target cells had only one (either folate receptor or the EGFR) or no receptors available. Cells were washed to remove non-associated doxorubicin and were re-incubated for 72 hours. Cytotoxicity was determined by formazan. Liposomes bearing only folic acid or mAb225 (single ligand liposomes) were utilized as controls. Selectivity enhancement (defined here as off-target cell : target cell toxicity) was determined at a 10μM doxorubicin concentration and by an LC50 comparison.

Results / Discussion: The number of folic acid and mAb225 per liposome (or ligand:lipid ratio) was determined by an optimization process with single ligand liposomes utilizing cytotoxicity from doxorubicin as an outcome measure. Cytotoxicity was measured as viability relative to untreated cells. The optimal number of folic acid ligands was found to be ~ 400 ligands per liposome while the optimal number of mAb225 was found to be ~ 6 ligands per liposome. Based on these results, sub-optimal numbers of one-half the optimal number (200 folate and 3 mAb225 per liposome) were utilized for dual ligand liposomes to ensure (1) equal contribution from each ligand and (2) minimize toxicity from either ligand alone.

Figure 1 shows the ability of all optimal single ligand (folic acid, A, and mAb225-targeted, B) and dual ligand formulations (C) to achieve toxicity in the target cell line (KB), see arrows. Sub-optimal single ligand formulations (200FA, A, and 3mAb225, B) did not achieve toxic effects in either target or off-target cells. Only the dual ligand formulation (C) was able to achieve a toxic effect selectively in the target cells (KB) (no reduced viability in off-target cells). Selectivity was found to be ~1.6 with dual ligand liposomes at 10μM DXR. Selectivity enhancement was found to be ~3-5 fold with dual ligand liposomes as measured by LC50 values (not shown).

Figure 1. Demonstration of selectivity enhancement with dual ligand, but not single ligand, liposomes



Conclusions: Use of ligands to direct nanocarriers at over-expressed receptors on tumor cells is nearing clinical reality. This study demonstrates the ability to tailor nanocarriers to match the cell receptor profile, thereby improving tumor-cell selectivity.

References:

1. Gabizon et al. Bioconjug Chem, *10:* 289-298, 1999.
2. Saul, J. M., et al. J Control Release, *92:* 49-67, 2003.