USE OF THE POST-INSERTION METHOD FOR THE FORMATION OF LIGAND-COUPLED LIPOSOMES

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The use of targeted liposomes is recognized as a promising strategy for improving the selective targeting of drugs to diseased tissues *in vivo*, leading to reductions in drug toxicity and improvements in therapeutic outcomes. A large variety of targeting molecules have been attached to the surface of liposomes to date, using a variety of coupling methods [1-5]. If targeted liposomes are to be useful in clinical applications, simple and flexible preparation methods are required so they can be tailored to the patient's disease profile without the need for separate manufacturing procedures for each ligand and drug combination. In addition, the coupling chemistries for attaching antibodies to liposomes are sometimes incompatible with the optimal conditions for loading drugs into liposomes, e.g. requirement for a particular pH for loading and a different pH for coupling. In our laboratory we have developed an approach that allows us to insert desired ligands, covalently attached to polyethylene glycol (mPEG)-lipid micelles, into preformed drug-loaded liposomes

Ligands coupled to the terminus of polyethylene glycol (PEG) could be inserted into the outer monolayer of preformed, drug-loaded liposomes in a time and temperature-dependant manner [6]. Efficient transfer of sheep IgG-PEG-DSPE from micelles was achieved above the phase transition temperature of the lipids (around 60°C). The amount of IgG and mPEG-DSPE transferred into the liposomes was dependant upon the concentration of the IgG-PEG-DSPE micelles added. In our studies we achieved up to 0.67 nmol Ab/µmol PL and 3 mol % mPEG-DSPE could be transferred from micelles into liposomes. As the amount of mPEG-DSPE pre-existing in the recipient liposomes increased, the amount of IgG-PEG-DSPE that could be transferred into the liposomes decreased. We observed that, except for Caelyx/Doxil (9 mol % mPEG-DSPE in the outer monolayer of the liposome), we could achieve adequate transfer of IgG-PEG-DSPE micelles onto the preformed liposomes, so as to achieve good target binding. This suggests there is an upper limit to the amount of mPEG-DSPE in the recipient liposomes that will allow transfer from the micelles. The resulting formulation was found to have good stability in human plasma; after 48 h at 37°C no transferred IgG or mPEG-DSPE appeared to have dissociated from the liposomes. Incubation of antibody-micelles with the preformed liposomes did not seem to accelerate the release of doxorubicin from the liposomes [6].

HSPC:CHOL:PEG-DSPE, 2:1:0.1 liposomes coupled to anti-CD19 (PCPIL[anti-CD19] and immunoliposomes formed by the conventional Mal-PEG-DSPE

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coupling procedure (PCSIL[anti-CD19]) had similar levels of binding to CD19+ B-lymphoma cell line (Namalwa) at similar levels of coupled antibody [7]. The cytotoxicity of DXR-loaded PCPIL[anti-CD19] against Namalwa cells, was similar to that of PCSIL[anti-CD19], and both were higher than non-targeted formulations. PCPIL[anti-CD19] and PCSIL[anti-CD19] had similar pharmacokinetic profiles and therapeutic efficacy in a murine model of human B-cell lymphoma [7]. Both the targeted formulations were cleared significantly more rapidly than the non-targeted liposomes in BALB/c mice, likely though binding to normal B cells (via CD19 epitopes) and to mononuclear phagocyte cells (via Fc receptors) in these immune-competent mice. Further, *in vivo* survival studies demonstrated comparable mean survival times for SCID mice injected with Namalwa cells and treated with doxorubicin-loaded PCPIL[anti-CD19] or PCSIL[anti-CD19] [7].

We have also used the post-insertion method for inserting anti-CD19 as either whole Ab or Fab' fragments into SM:CHOL (55:45 mol/mol) liposomes loaded with vincristine (Vinc-SMPIL[anti-CD19]) [Sapra, P., unpublished results]. The rate of leakage of vincristine from Vinc-SMPIL[anti-CD19] was found to be similar to that from Vinc-SMSIL[anti-CD19] (Mal-PEG-DSPE coupling procedure). In addition, *in vitro* cytotoxicity of these immunoliposomes against Namalwa cells was found to be comparable to Vinc-SMSIL[anti-CD19]. Vinc-SMPIL[anti-CD19] had significantly improved therapeutic efficacies compared to vincristine-loaded non-targeted liposomes or free vincristine in the Namalwa model of human B-cell lymphoma.

The post–insertion method is also suitable for the insertion of peptide ligands into preformed Stealth immunoliposomes. Antagonist G-targeted DXR-loaded liposomes were prepared by the post-insertion method and tested in a human small cell lung cancer (H69) cell line. A maximum of 0.36 nmoles antagonist G/μmol PL could be inserted into HSPC:CHOL liposomes (PLG) and these PLG demonstrated similar binding to liposomes prepared by the conventional Mal-PEG-DSPE method. The cytotoxicities of the DXR-loaded PLG (DXR-PLG) were higher than those of conventional liposomes prepared by the Mal-PEG-DSPE method (DXR-SLG), likely because this hydrophobic peptide could non-specifically associate with the conventional liposomes through hydrophobic interactions [8]. This points out another advantage of the post-insertion method; it can decrease non-specific interactions of ligands with liposomes.

In conclusion, the post-insertion approach to preparing targeted liposomal formulations seems to be simple, rapid and flexible, all desirable properties from both a research and a manufacturing viewpoint. The method allows a combinatorial approach to the design of targeted liposomes, where a variety of ligands can be inserted into a variety of pre-formed liposomes containing a variety of drugs, allowing the therapy to be tailored to the needs of individual patients.

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