Rigid Model Membranes with pH-Sensitive 'Raft-switches': Applications in Liposome-mediated Drug Delivery for Cancer Therapy

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Abstract: - pH-sensitive liposomes have been extensively studied to increase drug bioavailability within targeted cancer-cells, but they do not behave ideally *in vivo*. We use 'raft-switches' to develop pH-sensitive liposomes that retain their long circulation times, and preserve their pH-sensitivity in the presence of serum proteins.

Key-Words: - pH-sensitive liposomes, pH-sensitive lipid rafts, drug delivery, cancer therapy

1 Introduction

In cancer therapies, advances in liposome research show strong potential *in vitro*, but, in the clinic, disseminated metastatic cancer is still incurable. This is largely due to low tumor absorbed doses, low drug bioavailability within cancer cells, and high toxicities at normal organs. In drug delivery to metastatic tumors with developed vasculature, the preferential tumor accumulation and retention of liposomes is adequate and primarily dependent on their size. One major issue of concern, however, is the inadequate release of drug inside cancer cells that constitute the tumor. pH-sensitive liposomes that release their contents after their endocytosis by cancer cells have been extensively studied for this purpose, but have limited *in vivo* application.

2 Problem Formulation

In our view, three major structural parameters in liposome design for drug delivery are: membrane rigidity, surface modification (architecture), and the pH-sensitive character. Increased phospholipid-membrane rigidity enhances drug retention by liposomes during blood circulation, and also prevents liposome clearance, thus, increasing liposome accumulation in tumors. But, after endocytosis, conventional rigid-membrane liposomes have limited capacity to release their contents during the acidification of the endosomal lumen, resulting in low drug bioavailability in cancer cells. On the other hand, conventional pH-sensitive liposomes have significantly shorter blood-circulation times which translate into decreased tumor uptake, and higher toxicity. In conventional pH-sensitive liposomes, addition of PEGylated lipids that increase the circulation times, challenges the pH-sensitive character [1]. We have developed pH-sensitive liposomes with rigid membranes that combine long circulation times with complete release of contents in the endosome. Other types of pH-sensitive liposomes that have been studied include surface-grafted ionizable peptides that can cause phase separation and domain formation of lipids on charged membranes. But, such structures are unlikely to be 'stealth' *in vivo*. We propose a much simpler strategy for release of contents that will not alter what has been proved as 'stealth' surface *in vivo*.

We developed liposomes comprised of ionizable 'domain-forming' ('raft'-forming) rigid lipids that are triggered to form domains as a response to the endosomal acidic pH. Domain formation (lateral lipid-separation) at the endosomal pH causes the encapsulated contents to be released due to imperfections in 'lipid packing' around the domain 'rim'. At physiological pH (during circulation) the lipids are charged, the liposome membrane is 'mixed' and the contents cannot leak. The liposomal membrane is composed of rigid lipids (all lamellar-forming), and is covered with PEG-chains so as to increase the blood circulation times. PEGylation does not interfere with the pH-sensitive properties of the developed liposomes.

We have experimentally shown that domain formation can potentially occur when both lipid

constituents (both lamellar-forming) have long saturated rigid hydrocarbon-chains, but of different lengths. We use pH-tuned 'raft'-forming membrane-'switches' as a mechanism to create rigid-liposomes that will efficiently release the encapsulated drugs after liposome endocytosis by cancer cells.

3 Problem Solution

Content retention in pH-activated rigid-liposomes is controlled by pH. Endosomal uptake of the drug carrier has to be accompanied by fast leakage of drug from the carrier and release into the cytoplasm while the carrier is still in the endosomal or early lysosomal stage (30 minutes upon endocytosis within a pH range: 6.5-5.0), to prevent enzymatic degradation of drugs at the lysosome, or excretion of the carrier from the cell [2]. We show that content release from liposomes containing pH-activated domains increases strongly with decreasing pH.

Rigid liposomes consisting of DPPC, DSPA, cholesterol and 2% mole PEG (2000MW) with encapsulated self-quenching concentrations of calcein, were incubated in media (RPMI with 10% serum) at 37^{o}C at different pH values (Figure A). Content release was instantaneous and complete at pH=4 (corresponding to the lysosomal environment), and very rapid and almost complete at pH=5.5 (mean endosomal value). After one hour of incubation at pH=7.4 liposomes were stable retaining 80% of contents. This value dropped to 68% after 1 day, and to 52% on the third day in serum-supplemented media. In PBS (pH=7.4) retention remained stable at 95% after 3 days. Content retention at physiological pH is essential, since this pH corresponds to the blood environment during circulation when content leakage is not desirable. Leakage was compared to DSPC rigid-membrane liposomes because of their pH-stability. DSPC liposomes showed low degree of leakage only at pH=4 (Figure B). We also tested in these conditions, standard pH-sensitive DOPE-CHEMS liposomes (without PEG) that showed less significant pH-response in serum-supplemented media (Figure C). Loss of pH-sensitivity by DOPE-CHEMS liposomes has been reported to occur in media and also upon addition of surface-grafted PEG [3].

To elucidate the release mechanism, FRET of liposome suspensions was studied in PBS, RPMI (cell growth medium), and 10% serum supplemented RPMI at pH=7.4, 5.5, and 4.0. For FRET measurements, NBD-PE and RhD-PE lipids with appropriate hydrocarbon tails were chosen, so that the energy donor and the energy acceptor to partition at a different extent in the stearoyl- and palmitoyl-rich phase. The 'lipid-separation' fractions obtained by FRET were similar in all solutions. FRET performed on liposomes consisting of DPPC and DSPA in serum supplemented media at room temperature showed 86% decrease in 'lipid mixing' at pH=5.5 and 96% decrease at pH=4.0 compared to 100% 'mixed' eggPC membranes, consistent with DSPA protonation and DSPA-domain formation.

In parallel FRET studies, the liposomal structures remained intact even at the more acidic pH. No fusion among liposomes occurred as evaluated by FRET measurements in suspensions comprised of two liposome populations: one population labeled with the fluorescence energy donor, and the other population labeled with the energy acceptor.

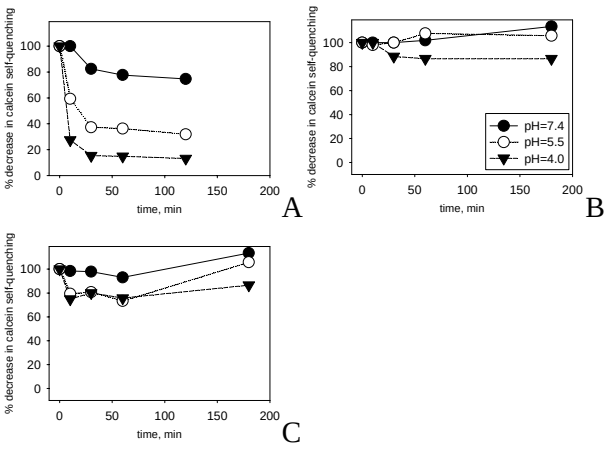

Figure. Relief of calcein self-quenching as a result of leakage from liposomes at different pH values in media (10 % serum, 37^{o}C); A: pH-active rigid-liposomes; B: DSPC rigid liposomes, C: DOPE-CHEMS pH-sensitive liposomes: that reportedly lose their pH-sensitive character in media with serum [3].

In vitro and *in vivo* studies are in progress with antiHER2/neu antibody-conjugated liposomes to enhance endocytosis by SKOV3 ovarian cancer cells.

4 Conclusion

Our results show that pH-sensitive rigid PEGylated liposomes containing 'raft-switches' hold great promise as drug carriers in the sense that they should: a) enhance drug bioavailability within cancer cells, and b) retain the long circulation times of rigid

PEGylated liposomes, and thus exhibit significant tumor uptake.

References:

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