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Cover story

Rational drug loading of liposomes revisited

Liposomes are a leading drug delivery system for parenteral use. Doxil®, the first nano-liposomal formulation approved by the U.S. Food and Drug Administration (FDA) [1], paved the way for an additional 13 approved liposomal drugs currently in clinical use. The success of liposomal drugs stems from a combination of various factors including their extensive physico-chemical characterization, biocompatibility, and familiar pharmacokinetic profiles. Not all drugs, or active pharmaceutical ingredients (API), however, are suitable for delivery by liposomes. A viable liposomal formulation must carry a sufficient API concentration to achieve therapeutic efficacy in humans. Also, APIs have to be associated/loaded into liposomes during circulation in the blood with a stable 2 year shelf-life.

APIs can be loaded into liposomes either by association with the liposomal membrane (exemplified by AmBisome®) or by loading the API into the intra-liposomal aqueous phase which is the case for most liposomal formulations. A stable association with the liposomal membrane requires that the APIs have very high ($> 10^8$) partition coefficient of liposome membrane/aqueous medium without disrupting the liposome bilayer. Two different approaches have been used for passive loading of APIs into the liposomal aqueous phase. One is the conventional ("old fashioned") hydration of the liposomes by aqueous solution of the desired API. The other is by passive remote loading, as described by Wehbe [2] (in this issue), in which the API and a liposome-membrane permeability enhancer such as ethanol are added to preformed desired liposomes. Solubility of the API can be increased by elevating the temperature during lipid hydration or during passive remote loading. The major drawback of passive loading is the aqueous solubility limit of the API.

In contrast to the passive loading, active loading is defined by the ability to reach intra-liposome concentration that is many folds higher than the API solubility in the loading aqueous medium. In active remote loading the API accumulates in the intra-liposome against a concentration gradient. Liposomes exhibiting a transmembrane ion gradient (usually ammonium sulfate for weak bases and calcium acetate for weak acids) are prepared. The drug is added to the medium and its unionized form influxes across the liposome membrane; it is then ionized inside the liposome and trapped by forming an insoluble salt with the impermeable counter ion (usually sulfate or calcium). Concomitantly, the permeable protonated/deprotonated gradient form ion (ammonium/acetate) effluxes through the liposome membrane. For each molecule that is effluxed, one molecule of drug is influxed. This method requires APIs which are either amphipathic weak bases (e.g., doxorubicin, vincristine, topotecan, etc.) or amphipathic weak acids (e.g., mupirocin). Amphipathic weak acids or weak bases are defined by having logD at pH 7 in the range of -2.5 to 2, where logD is the octanol-water partition coefficient as a function of pH. Amphipathic weak bases should have a $pK_a \leq 11$ and weak acids should have $pK_a > 3$. This is especially relevant for nano-liposomes that are the preferred form for parenteral administration. Nano-liposomes have a very low intra-liposome aqueous phase ($(133 \pm 18)\cdot 10^3$ nm^3) for which passive loading may not allow reaching the desired API concentration in the liposome. The active loading method enabled the successful development of Doxil®, DaunoXome® and Onivyde®.

To identify APIs that are suitable for active remote loading, Cern et al. [3] in this issue developed computational models to predict both loading into and leakage from nano-liposomes. A good candidate in terms of loading should have a drug (API) to lipid mole ratio of ≥ 0.2 or 0.3 with a loading efficiency $\geq$ 90% or 70%, respectively, to achieve a human dose of 150 mg per single administration (assuming a molecular weight of 500 Da). A dataset was collected for 67 molecules loaded to rigid membrane liposomes and 194 molecular descriptors were calculated to create models. The models created using the Iterative Stochastic Elimination (ISE) algorithm [4] are capable of identifying molecules suitable for remote loading into rigid nano-liposomes based on their molecular structures. The same computational method was used to model the stability of the liposomal API in terms of leakage upon storage. Both loading and leakage models were used for the screening of 13,700 molecules. Among them, 318 molecules were highly scored by both models, of these, 67 molecules are APIs in clinical use.

The computational screening by Cern et al. [3] can be used as the first line of drug screening, which can be followed by other physicochemical and pharmacological considerations. The utility of the computational approach was exemplified by the development of a nano-liposomal formulation of mupirocin (Nano-mupirocin) which was identified using a previous modeling attempt [5] as a good candidate for remote loading. The main advantage of the computational screening is bypassing extensive experiments to identify the suitable APIs for active remote loading. It would be even more advantageous, if the computational screening can predict the *in vivo* efficacy and safety, which will ultimately determine the usefulness of liposomal formulations.

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