



Cover Story

An integrated assessment of PEGylated liposomal doxorubicin products

Doxil® is a multi-component liposomal formulation of doxorubicin, of which therapeutic performance is controlled by a complex array of interrelated physicochemical and biological properties [1]. Doxil is indicated for HIV-related Kaposi's sarcoma in patients having low CD4 count and extensive mucocutaneous or visceral diseases, advanced ovarian cancer, and multiple myeloma. Doxil's patent expired recently and its production now ceased. In February 2013, the US Food and Drug Administration (FDA) approved a follow-on version of Doxil made by Sun Pharma Global FZE. There are other Doxil generic versions, but manufactured and marketed only in certain countries.

The generic products are bioequivalent to the reference listed drug, Doxil in this case. FDA has generated a draft documentation regarding 'non-binding recommendations' for evaluation of follow-on formulations of injectable poly(ethylene glycol)-conjugated (PEGylated) liposomes with entrapped doxorubicin [2]. Although, such draft recommendations address many pharmaceutical attributes for comparison, the complexity of liposomal formulation makes it difficult for exact reproduction and characterization. This is not only important in terms of bioequivalence, but also in relation to the reported adverse effects of Doxil comprising idiosyncratic infusion-related reactions as well as palmar-plantar erythrodysesthesia ('hand-foot' syndrome) [1]. Accordingly, more concerted actions are necessary to better understand inter-linked physicochemical and biological mechanisms that regulate Doxil's performance and its benefit-to-risk ratio. In addition, there is a need to validate integrated multi-platform analytical approaches for improved characterization and assessment of similarities between the original and generic versions.

A paper by Professor Moghimi and his colleagues in this issue [3] introduces an integrated bio-analytical assessment of liposomal size, number and morphology. These parameters highlight differences not only among tested batches of PEGylated liposomal products that have been considered similar to Doxil, but also among two tested batches of the same product (Doxil and Caelyx® in this case). Difference in vesicular morphology was assessed by cryogenic transmission electron microscopy (cryo-TEM) followed by a complex image analysis determining the apparent aspect ratio of vesicles. Nanoparticle Tracking Analysis was used for estimation of particle concentration. The results show that liposomal batches with high proportion of near-spherical vesicles with encapsulated short-length needle-shaped doxorubicin crystals had more vesicles to account for the stated doxorubicin content (2 mg/mL) as compared with batches showing relatively more prolate ellipsoidal vesicles entrapping longer doxorubicin needles. These observations were further reflected in differences in average zeta potential values, which may be a reflection of the curvature effect on conformation of the surface projected PEG chains, and hence their shielding efficacy [3].

Differences in liposomal morphology (and population aspect ratios) and number are expected to play an important role not only in vesicular pharmacokinetics and extravasation into solid tumors, but also in relation to adverse reactions. Accordingly, such variation could account for differences seen in formulation performance in clinical settings [4]. For instance,

inadvertent activation of the complement system is considered to be a causal factor for liposomal-mediated infusion-related reactions in human subjects [4]. Indeed, this study by the Moghimi group [3] shows a link between vesicular morphology (and aspect ratio) and the extent of complement activation. For instance, formulations containing more spherical vesicles were less effective in inciting complement compared with batches or formulations bearing more populations of vesicles with a prolate ellipsoid morphology. Complement profiling further revealed that there could be other vesicular-related physicochemical parameters, which are not easily distinguishable with current analytical tools, which can modulate complement activation [3]. For this reason, complement activation profiling was introduced as a part of the analytical characterization portfolio of liposomal products together with cryo-TEM analysis. The study by the Moghimi group may open a simple way in minimizing liposomal-mediated adverse reactions. For instance, one could test complement reactivity of patients' plasma (or whole blood) in vitro with different lots of the liposomal products, and identify the least reactive (or ideally a non-reactive) batch for infusion. Similarly, differences in vesicular morphology and deformability may account for differences in the extent of 'hand-foot' syndrome. Perhaps, the frequency of such reactions is greatest with batches containing more populations of prolate ellipsoidal vesicles entrapping long doxorubicin needles. Further studies with larger batches are still necessary, but these finding opens a door for designing and engineering of improved formulations for better therapeutic outcomes.

In summary, the work of Moghimi and colleagues [3] has introduced an integrated biophysical and immunological toolbox for better analysis and identification of physical differences among vesicular populations. This is the beginning of an exciting paradigm shift in vesicular characterization strategy, which could not only allow for better and wider liposomal doxorubicin product design, but also for better implementation and shaping of regulatory 'non-binding' recommendations for Doxil follow-on products. The approach taken by the Moghimi group can be easily applied to other injectable formulations.

References

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