Diblock Polymeric Micelles

Yu et al. (1998) used amphotericin B (AmB), an antifungal drug, as a model to study the release profile of poly(ethylene oxide)-block-poly(benzyl-aspartate) (PEO-*b*-PBLA) block copolymer micelles. AmB is a poorly water-soluble drug that is currently formulated with the bile salt deoxycholate (Fungizone®). This formulation induces hemolysis and slowed release of AmB is shown to prevent this toxic side effect. In addition, Yu et al. investigated the effect of freeze-drying and reconstitution on the micelles.

The drug-loaded PEO-*b*-PBLA micelles were prepared as follows. The PEO-*b*-PBLA was first dissolved in dimethylfomamide and the AmB added to this solution. The polymer/drug solution was then dialyzed against distilled water to remove the DMF and unencapsulated AmB. The pH of the dialysis medium was maintained at pH 11.3 to ensure ionization of the unencapsulated AmB that facilitates its removal. The dialysis medium was neutralized to pH 5.6 upon completion. The isotonicity of the micellar solution in the dialysis bag was adjusted with dextrose. Adjustment of the isotonicity was not made for the micelles that were freeze-dried. The loading of AmB was measured by UV-vis spectroscopy after dilution with DMF that breaks down the PEO-PBLA micelles to release the AmB. The loading efficiency was approximately 30%, and the molar ratio of AmB to polymer ranged from 0.40 to 1.0.

TEM can resolve nanoscopic colloids such as polymeric micelles. TEM pictures revealed spherical PEO-*b*-PBLA micelles, confirming earlier dynamic light-scattering measurements on the polymeric micelles. The mean diameters of the unloaded PEO-*b*-PBLA micelles and AmB-loaded PEO-*b*-PBLA micelles were 20.0 ± 3.9 and 25.8 ± 4.2 nm, respectively, indicating a slight increase in size upon drug loading. The size distributions of the PEO-*b*-PBLA micelles are very narrow, characteristic of polymeric micelles.

Intravenous solutions of AmB were administered at levels of 50–100 mg/mL. The loading of AmB into PEO-*b*-PBLA micelles drastically lowers the hemolytic activity of AmB, even at an AmB level of 10 μg/mL. When deoxycholate solubilizes AmB (Fungizone), AmB is very hemolytic, reaching a 100% hemolysis at a level of ca. 3.0 μg/mL. Without deoxycholate, which exhibits its own hemolytic activity, micelles of AmB itself formed by dialysis are slightly less hemolytic. In contrast, AmB-loaded PEO-*b*-PBLA micelles caused no hemolysis at 3.0 μg/mL for a period of up to 5.5 h versus 30 min. This indicates that the release of AmB from PEO-*b*-PBLA micelles is slow. The lack of hemolytic activity may also reflect the release of monomeric AmB from PEO-*b*-PBLA micelles as opposed to Fungizone, which releases both aggregated and monomeric forms of the drug.

The nonhemolytic effect of AmB-loaded PEO-*b*-PBLA micelles was supported by another study on the *in vitro* dissociation of antifungal efficacy and toxicity for these diblock copolymeric micelles (Yu et al., 1998). Since the aggregation state of AmB is a determinant factor for toxicity, AmB has been loaded into PEO-block-PBLA micelles in a monomeric state, resulting in a formulation that has uncoupled efficacy from toxicity for concentrations up to 15 μg/mL as measured by hemolytic activity and minimal inhibitory concentration (MIC). The antifungal efficacy of AmB-loaded PEO-*b*-PBLA micelles in terms of MIC is greater than Fungizone, perhaps owing to a stabilizing role of the polymeric micelles and/or enhanced interaction of AmB at a membrane level. The results support findings on the selective activity of monomeric AmB against fungal cells over mammalian cells.

It was found that unloaded PEO-*b*-PBLA micelles cause no hemolysis, even at a level of 0.70 mg/mL. PEO-*b*-PBLA has an extremely low critical micelle concentration (Kwon et al., 1993a,b), and thus, there is little monomeric PEO-*b*-PBLA for the lysis of lipid bilayer membranes. In addition, PEO-*b*-PBLA micelles may break apart slowly to monomers. The lack of hemolytic activity of PEO-*b*-PBLA contrasts strongly with other amphiphiles used for drug solubilization and intravenous drug administration. Sodium deoxycholate causes l00% hemolysis at a level of 0.32 mg/mL. This is due to disruption of lipid bilayer membranes of red blood cells.