Polymeric Micelles for Controlled Delivery of Dexamethasone to Tumors

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Statement of Purpose: Despite the development of an arsenal of highly potent chemotherapeutic agents, clinical success in treating cancer remains a challenge. This can frequently be attributed to the obstacles a drug must encounter en route to its site of action that cannot be accounted for in laboratory studies. In the case of tumors, an irregular vasculature accompanied by high interstitial fluid pressure (IFP) prevents significant accumulation of the drug. Studies show that pretreatment with dexamethasone (DEX) may act to reduce the IFP [Kristjansen, 1993] and increase the amount of drug taken up by tumors [Wang, 2007], resulting in enhanced efficacy and/or decreased toxicity [Wang, 2004; Leggas, 2009]. However, due to the immunosuppression seen when DEX is administered systemically, a localized drug delivery system is preferable. In this study, we have sought to develop and characterize a series of DEX-loaded polymeric micelles formed from poly(ethylene glycol)-poly(amino acid) block copolymers that may localize in tumors through the EPR effect and deliver DEX in physiologically relevant amounts. Various linkers were employed in an effort to optimize the location and time profile of drug release.

Methods: Polymers were synthesized according to the methods previously optimized with slight modifications [Bae, 2009]. Briefly, poly(ethylene glycol)-poly(β-benzyl-L-aspartate) (PEG-PBLA) with two different molecular weights (12-20 and 12-35, where 12 represents a PEG MW of 12,000 and 20/35 represents the number of BLA repeating units, respectively) was deprotected with 0.1 N NaOH to give PEG-poly(aspartic acid). This was subsequently coupled with methyl glycinate at the side chain using O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) as a coupling reagent. The resultant product was divided in half. The first half was deprotected with 0.1 N NaOH and then coupled to DEX through a DIC/DMAP (1,3-diisopropylcarbodiimide/4-dimethylaminopyridine) esterification reaction to give PEG-poly(aspartate-glycine-DEX) (PAGD). The second half was reacted with hydrazine to generate a hydrazide group suitable for reaction with levulinic acid. This introduced a pH-sensitive hydrazone bond to the polymer. The final step involved coupling this product to DEX, again through a DIC/DMAP esterification reaction to give PEG-poly(aspartate-glycine-hydrazone-levulinic ester-DEX) (PAGHLD). The final four polymer compositions are denoted as 12-20 PAGD, 12-35 PAGD, 12-20 PAGHLD, and 12-35 PAGHLD. The products were evaluated by GPC and ^{1}H-NMR (DMSO-*d6* and D_20). Micelles were prepared through aqueous reconstitution of the block copolymers that had been freeze-dried from a 10% acetonitrile solution. Size exclusion chromatography (SEC) and dynamic light scattering (DLS) measurements were conducted for micelle characterization.

Results: GPC measurements showed that PEG-PBLA block copolymers 12-20/12-35 were successfully synthesized. ^{1}H-NMR spectra of the two PAGHLD compositions in DMSO-*d6* showed DEX peaks at 6-8 ppm. Precipitation problems with the PAGD compositions in DMSO-*d6* prevented accurate spectra from being obtained. The NMR spectra obtained in D_20 showed only PEG and solvent peaks for all four compositions, indicating micelle formation in aqueous solutions. SEC chromatograms showed the presence of both aggregate and unimer peaks. DLS confirmed that micelles were formed through our simple freeze-drying method, showing small micelle sizes (Figure 1). Average micelle sizes, however, varied depending on polymer composition as well as linkers introduced.

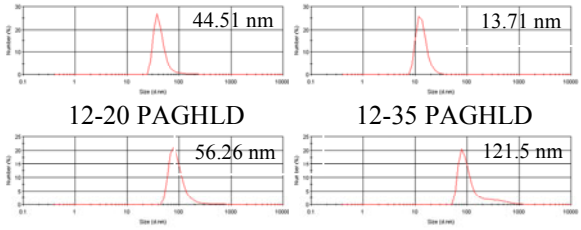

Figure 1. DLS number distributions of micelles prepared through a freeze-drying method.

Conclusions: DEX-conjugated polymer compositions possessing different chain lengths (12-20 and 12-35) and linkers (ester and hydrazone) were successfully prepared and characterized. SEC and DLS measurements indicated that micellization occurred in aqueous solutions. Micelles are of a small size suitable for taking advantage of the EPR effect, irrespective of the linkers introduced. It is expected that these micelles will form a protective core for DEX to be delivered to tumors. The use of different linkers and molecular weights may allow for the optimization of drug release.

References:

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