Tunable Release of Doxorubicin in Cancer Tumors Using Polymeric Micelles

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Statement of Purpose: This study explores a novel drug delivery system using smart pH-sensitive polymer micelles which can achieve tunable delivery of doxorubicin (DOX) while minimizing toxicity and maximizing efficacy. Micelles have been shown to be a promising delivery system because of their high water solubility and high drug loading capacity, as well as their ability to achieve targeted drug delivery via the enhanced permeation and retention effect. The amphiphilic block copolymers in this system are pH sensitive and contain linkers with different hydrolysis rates which allow drug release to be controlled.

Methods: Ring-opening polymerization of β-benzyl L-aspartate N-carboxy anhydride (BLA-NCA) was conducted by using α-methoxy-ω-amino-poly(ethylene glycol)(PEG: MW 12,000 kDa) as a macroinitiator in anhydrous DMSO at 45ºC for two days. Increasing the amount BLA-NCA yielded three compositions of block copolymers containing 5, 15, and 35 pBLA repeating units. This provided PEG-poly(β-benzyl L-aspartate) block copolymers whose side chains were deprotected using 0.1 N NaOH further resulting with poly(ethylene glycol)-poly(aspartic acid) block copolymers [PEG-p(Asp)]. PEG-p(Asp) was chemically modified with spacers (e.g. glycine methyl esters (Gly) and methyl 4-aminobenzoate (Abz)) using HBTU in DMF at 40ºC overnight. Hydrazide (Hyd) was used to replace the methyl esters of the spacers making PEG-p(Asp-Gly-Hyd) and PEG-p(Asp-Abz-Hyd) block copolymers. In DMSO, at 30ºC these block copolymers were conjugated with DOX. Through the hydrazone drug linkage, pH-sensitive release of DOX with differential hydrolysis rates at the acidic intratumoral or intracellular environment (pH 4.0-6.8) was achieved. DMSO and unreacted drugs were removed through ether precipitation. A Sephadex LH20 column was used to further remove DOX which may have been physically bound instead of conjugated to the polymer backbone. ^{1}H-NMR was used to characterize the block-copolymers. Block copolymer–drug conjugates were used to prepare polymer micelles using the dialysis method. Size distributions of the micelles were analyzed by dynamic light scattering (DLS) measurements. Drug release studies were performed under sink conditions at physiological pH (7.4), as well as intracellular pH (5.0).

Results: Polymers are denoted as X-Y, where X stands for PEG MW$\times 10^{-3}$ and number of pBLA repeating units, respectively. ^{1}H-NMR analysis confirmed each step of the synthesis process. Six different block copolymers were synthesized consisting of 12kDa PEG and number of pASP repeating units (5, 15, and 35). Hydrazide-modified drug-binding spacers (Gly or Abz) were introduced to the block copolymers (Figure 1).

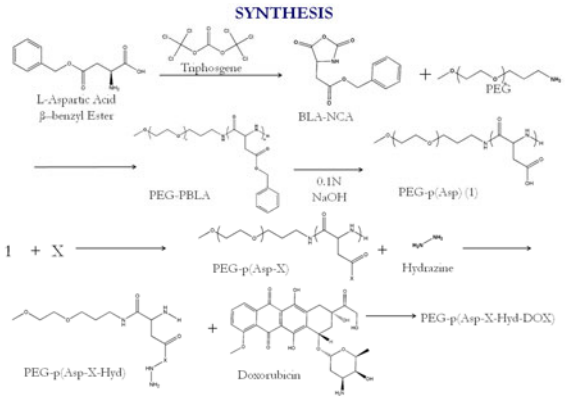

Figure 1. Synthesis of amphiphilic block-copolymers with different spacers (X: glycine or aminobenzoate).

UV-VIS spectroscopic analysis at 480 nm confirmed successful DOX conjugation to the block copolymers. Dialysis was used to form six different micelles, which DLS analysis showed that they ranged between 50-100 nm in diameter. UV-VIS was also used determine drug loading content which ranged from 2.8 to 31.7 weight percent depending on the micelle formulation. The effects of different drug binding linkers, and number of different repeating units were observed in the drug release study. Results indicated that tunable release of DOX was achieved by simply changing drug binding spacers that can alter electron density of hydrazone-drug linkers. Intriguingly, side-chain modifications to micelle-forming block copolymers made almost no effects on both micelle integrity and chemical stability of drug-binding linkers. As block copolymer chain lengths increased, however, drug release patterns decreased presumably due to micelle stabilization.

Conclusions: A collection of six micelle-forming block copolymers, possessing drug binding linkers with different hydrolysis rates became available. Doxorubicin was successfully conjugated to each of the block copolymers. This polymeric micelle delivery system shows great potential for further in vivo development of effective combination cancer chemotherapy.

References:

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