Antibody-Directed and Ultrasonically-Activated Drug Carrier

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Statement of Purpose: Our lab has been investigating the site-directed delivery of drugs using polymeric nanocarriers that release their contents upon irradiation by ultrasound (US).[1-4] In a typical application, drug sequestered in a carrier is infused into the circulatory system, and the US is focused on a tumor site, such that as the carriers flow through the insonated volume, they release their contents to the tumor tissue. However, US only releases about 10% of the drug content per insonation event. We believe that it would be much more efficient to have the carriers remain in the insonated volume so that all of their drug load can be released through multiple insonation events. To this end we are developing techniques to attach targeting moieties, such as antibodies, to the poly(ethylene glycol) (PEG) chains on the surface of these nanosized drug carriers. This report presents our progress in this direction.

Methods: PEG Activation. Dry monomethoxy-PEG (5,000 MW) was dissolved in dry dichloromethane along with excess acryloyl chloride and triethylamine and stirred under N_2 overnight at 25°C. The resulting PEG-acrylate was dried with anhydrous $MgSO_4$, precipitated in ether, characterized by NMR, and stored at -20°C.[5]

Fab' Preparation and Attachment to PEG. Sheep IgG was purchased from Sigma. The $F(ab')_2$ fragments were prepared using an ImmunoPure $F(ab')_2$ Preparation Kit (Pierce, Rockford, IL). The fragments were dialyzed and then reduced with 2-mercaptoethylamine as described elsewhere.[6] The reduced Fab' was immediately added to the PEG-acrylate and let stand for 12 h at 25°C. Un-reacted PEG was separated from the PEG-Fab' using a Sephadex-G25 column. Product was proven by NMR.

Results and Discussion: This synthetic route of attaching Fab' to PEG chains takes advantage of the reaction of reduced sulfhydryl (-SH) with a vinyl group. The preparation of the PEG-acrylate is straightforward, and the NMR spectra showed a pure product. The preparation of the Fab' fragment was slightly more complicated, but greatly aided by the use of a commercial kit. NMR analysis showed that the desired product was obtained. The yield was about 50%.

This same chemistry scheme can be used to attach specific antibodies to our ultrasonically activated drug carriers, which are composed of triblock or diblock copolymers of a PEG hydrophilic block and a hydrophobic block. In most of our studies we use Pluronic P105 and PEO-pNIPAAm block copolymers that can be similarly derivatized. Antibodies specific to the tumor tissues or another targeted tissue will attach the carrier directly to the target. Application of US in short bursts will release drug in large concentrations only at that site.

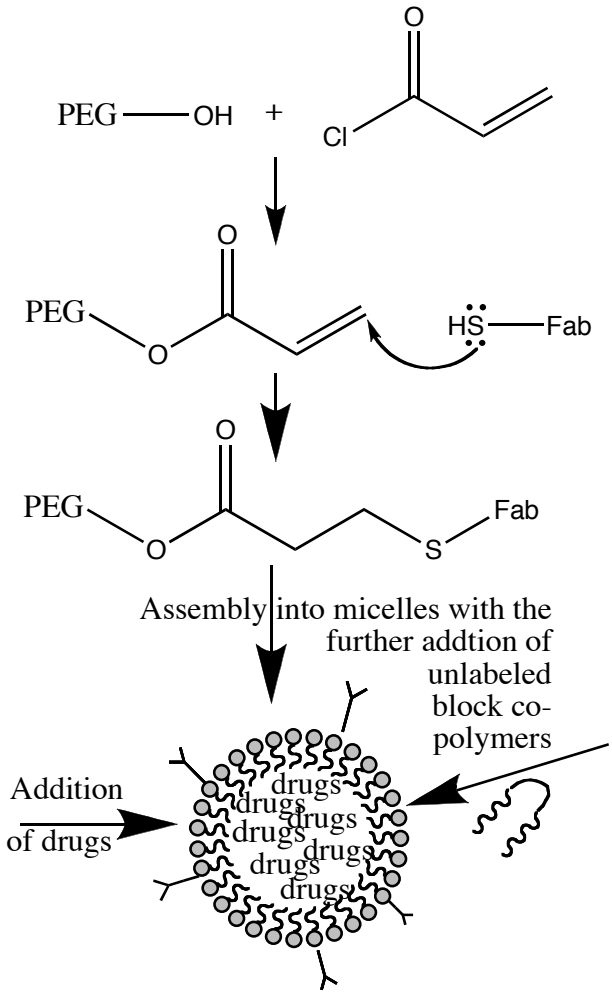

Figure 1. Synthesis of Drug-Carrying-Targeting Micelles

An additional advantage of site attachment is that as the carrier eventually degrades into harmless molecules, any drug remaining in the carrier following ultrasonic activation will be released at the targeted site. Other tissues and systems in the body will be spared the unwanted side effects of chemotherapy.

Conclusions: This synthetic pathway is useful in constructing drug-releasing micelles with specific targeting abilities to carry the sequestered drug directly to the site. Then the drug can be released on demand by focusing ultrasound at this site.

References:

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