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TAT peptide-based micelle system for potential active targeting of anti-cancer agents to acidic solid tumors

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Abstract

A novel drug targeting system for acidic solid tumors has been developed based on ultra pH-sensitive polymer and cell penetrating TAT. The delivery system consisted of two components: 1) A polymeric micelle that has a hydrophobic core made of poly(L-lactic acid) (PLLA) and a hydrophilic shell consisting of polyethylene glycol (PEG) conjugated to TAT (TAT micelle), 2) an ultra pH-sensitive diblock copolymer of poly (methacryloyl sulfadimethoxine) (PSD) and PEG (PSD-*b*-PEG). The anionic PSD is complexed with cationic TAT of the micelles to achieve the final carrier, which could systemically shield the micelles and expose them at slightly acidic tumor pH. TAT micelles had particle sizes between 20 and 45 nm and their critical micelle concentrations were 3.5 mg/l to 5.5 mg/l. The TAT micelles, upon mixing with pH-sensitive PSD-*b*-PEG, showed a slight increase in particle size between pH 8.0 and 6.8 (60–90 nm), indicating complexation. As the pH was decreased (pH 6.6 to 6.0) two populations were observed, one that of normal TAT micelles (45 nm) and the other of aggregated hydrophobic PSD-*b*-PEG. Zeta potential measurements showed similar trend substantiating the shielding/deshielding process. Flow cytometry and confocal microscopy showed significantly higher uptake of TAT micelles at pH 6.6 compared to pH 7.4 indicating shielding at normal pH and deshielding at tumor pH. The confocal microscopy indicated that the TAT not only translocates into the cells but is also seen on the surface of the nucleus. These results strongly indicate that the above micelles would be able to target any hydrophobic drug near the nucleus.
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Keywords: TAT specificity; Tumor pH; pH-sensitive polymer; Micellar carrier

1. Introduction

Tumor targeting of a cytotoxic agent refers to the passive accumulation of nano-scaled drug carriers to solid tumors, followed by active internalization into tumor cells [1]. The internalization of drug, either alone or along with a carrier, is required for cell death because most cytotoxic drugs act intracellularly [2,3]. Important considerations of nano-sized carriers for improved passive tumor targeting include the surface property, shape and size for a given tumor. Surface PEGylation of the carriers is regarded as the gold standard for longer residence time in the blood and improved biocompatibility [4]. Spherical nanocarriers with diameters from 40 to 300 nm are typically used for passive targeting [5].

Active targeting carriers have either monoclonal antibodies (mAb) [6], binding fragments [7] specific to a tumor associated surface antigen or a ligand binding to its corresponding receptor on the tumor cell surface. It has been clearly established that active targeting results in higher accumulation of carriers in tumors [8] albeit mAb or ligand conjugated to carriers may not guarantee long-range interactions with tumor cells. The term 'active targeting' is a misnomer as the carriers do not actively seek their target, in this case the tumor areas, but exert specific interactions with tumor cells only upon contact.

Most therapeutic systems rely on the receptor-mediated endocytotic pathway for internalization into cells. This pathway leads to the entrapment and, to a large extent, degradation of transported biomolecules in lysosomes. The use of cell penetrating peptides, like the HIV peptide TAT, has the advantage of avoiding this pathway and taking the cargo directly into the cell. TAT-mediated cytoplasmic uptake of drug conjugated polymers [9,10], plasmid DNA [10], bacteriophages [11], magnetic nanoparticles of about 10–20 nm in diameter [12] and

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even liposomes having a diameter of 200 nm [13] has been documented in the literature [14–17]. Until now the mechanism of internalization of TAT peptide is unclear. However a number of evidence show that the internalization does not necessarily involve the presence of a specific cellular receptor or transporter as uptake seems to be temperature and cell type independent [18–21]. There appears to be two kinds of mechanisms involved depending upon the size of the cargo. Smaller molecules attached to TAT seem to transduce directly into cells by the energy independent electrostatic interactions and hydrogen bonding [22] but larger cargos get into the cells by the energy dependent macropinocytosis pathway [23]. For over a decade various attempts have been made to make use of this versatile tool but only recently has some progress been made [24,25]. The main hurdle has been to bestow specificity and target the TAT peptide to the place of interest.

The delivery system described here is a ‘smart micellar nanoplatform’ that can possibly hide the non-specific TAT peptide in normal body. It involves assimilation of two components: 1) chemotherapeutic polymeric micelles — consisting of polyethylene glycol (PEG) outer shell, with TAT attached to the PEG and a hydrophobic core made of poly (L-lactic acid) into which any chemotherapeutic can be incorporated; 2) the TAT shield — an ultra pH-sensitive smart block copolymer PSD-*b*-PEG (Poly sulfonamide). Physical mixing of the two components forms the final carrier. When the PSD-*b*-PEG polymer is mixed with the TAT micelle, the TAT peptides which are positively charged get shielded by the negatively charged PSD of the block copolymer.

A model of the proposed drug delivery system is shown in Fig. 1. The model is proposed to have a final size of about 50–200 nm after drug loading, and having a PEG shell it has a strong possibility of preferentially accumulating in the tumor tissue by Enhanced Permeation and Retention Mechanism (EPR) [26]. The PSD that is negatively charged at pH 7.4 has been shown to become neutral below pH 7.0 [27] (extracellular tumor pH). Hence deshielding of TAT micelle is triggered by the lower pH of the tumor milieu [28,29]. As a consequence, at the tumor site the TAT micelles will be exposed to the surrounding. The TAT would then help target the drug loaded micelle into the cells and nucleus where the cytotoxic effect takes place.

Another possible advantage of this carrier system could be attributed to its potential to kill the so called ‘cancer stem cells’. These cells, which have been implicated for tumor relapse and metastasis, are very difficult to kill with conventional methods and active targeting. They are usually buried deep inside the tumors and are extremely difficult to reach with conventional therapies [30]. More importantly, these tumor cells have a different phenotype compared to other cancer cells, so active targeting, which is usually designed for a particular antigen (present in a predominant variety of tumor cells), will be ineffective against them [31]. On the other hand this micelle-based delivery system uses TAT for internalization, so in the acidic environment as TAT is exposed there is a potential for penetrating into these cells and kill them.

2. Materials and methods

2.1. Materials

Sulfadimethoxine [4-amino-*N*-(2,6-dimethoxy-4pyrimidynyl)benzenesulfonamide] (SD), *N*-hydroxysuccinimide (HOSu) and dicyclohexyl carbodiimide (DCC) were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and used without further purification. α-Hydroxy-ω-carboxymethyl poly(ethylene oxide) (PEG monoacid) was synthesized and purified by Zalipsky's method [32]. Methacryloyl chloride (Aldrich) was distilled under reduced pressure (10 mm Hg) at 30 °C; dimethyl sulfoxide (DMSO, Aldrich) was purified by vacuum distillation at 75 °C at 12 mm Hg. 2, 2′-Azobisisobutyronitrile (AIBN) (Aldrich) was recrystallized in methanol twice. TAT labeled with FITC was synthesized at protein synthesis core facility at the University of Utah. All other chemicals were of reagent grades and were used without further purification.

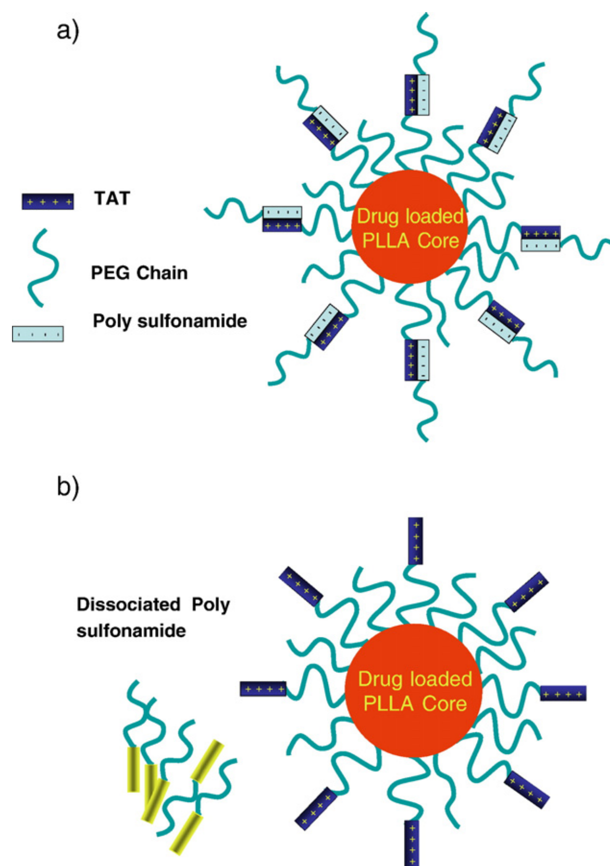

Fig. 1. Schematic model for the proposed drug delivery system: the carrier system consists of two components, a PLLA-*b*-PEG micelle conjugated to TAT and a pH-sensitive diblock polymer PSD-b-PEG. a) At normal blood pH, the sulfonamide is negatively charged, and when mixed the TAT micelle, shields the TAT by electrostatic interaction. Only PEG is exposed to the outside which could make the carrier long circulating; b) when the system experiences a decrease in pH (near tumor) sulfonamide loses charge and detaches, thus exposing TAT for interaction with tumor cells.

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