Exploring the Potential of the Tat Cell Penetrating Peptide in Drug Delivery Using Both Drug Conjugation and Self-Assembly Strategies

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Abstract Summary:
Cellular entry is critical for delivery of drugs with intracellular target. Two different types of Tat peptide conjugates were produced in the experiments to improve the drug intracellular accumulation: qC8-Tat, with Tat peptide bound to four octanoic acids, and Tat-drug conjugates with paclitaxel (PTX) bound at either the N-terminus (NTP) or C-terminus (CTP) of the peptide. The qC8-Tat conjugate was observed to self-assemble into nanofibers, which were capable of carrying PTX for cancer therapy. The NTP and CTP conjugates overcame multidrug resistance (MDR) in NCI/ADR-RES ovarian cells, with their cytotoxicity being conjugation site dependent.

EXPERIMENTAL METHODS
All the peptides were synthesized by standard solid phase synthesis techniques. PTX-buss was synthesized and purified according to our previous report, and reacted with Tat peptide (AcCGRK2R2QR3P2Q or AcGRK2R2QR3P2QC) using the same procedure we reported before to obtain N-terminal Tat-PTX conjugate (NTP) and C-terminal Tat-PTX conjugate (CTP). qC8-Tat was synthesized by reacting Octanoic acid (C8) with (K2)KGRK2R2QR3P2Q. The complete peptides were cleaved from the solid support using a mixture of TFA/TIS/H2Owater in a ratio of 95:2.5:2.5 for 3 h. All the crude products were purified by preparative RP-HPLC, and characterized with mass spectroscopy.

PTX-loaded nanofibers (PTX-N) were prepared by dissolving PTX and qC8-Tat in hexafluoro-2-propanol, removing the solvent, and reconstitution Dulbecco's Phosphate-Buffered Saline (DPBS, pH 7.4) at 2 mM (in qC8-Tat). The non-encapsulated PTX was removed using centrifuge. Blank nanofibers (Blank-N) were prepared with the some method except no PTX was added during the process. The morphology of nanofibers was characterized by transmission electron microscope, and the drug loading of PTX-N was determined by analytical HPLC.

RESULTS AND DISCUSSION
To explore the effect of conjugation and conjugation site on the efficacy of linked drug, a pair of Tat-PTX conjugates were synthesized (Figure 1a), by binding PTX to either N-terminal (NTP) or C-terminal (CTP) of the Tat peptide. Both conjugates overcame the MDR of NCI/ADR-RES when compared with free PTX, while CTP showed advantage over NTP (Figure 1b). The results demonstrate that Tat conjugation could improve the intracellular accumulation of PTX in MDR cells, and N-terminal conjugation could probably hinder the Tat-membrane interaction more than C-terminal conjugation.
When flexible alkyl tails was introduced to Tat peptide, the resulting qC₅-Tat (Figure 2a) was able to self-assemble into nanofibers (Blank-N) in DPBS (Figure 2b). PTX could be encapsulated into the nanofiber, producing drug loaded nanofibers (PTX-N) with altered stiffness (Figure 2c). When KB-3-1 cells were treated with PTX, PTX-N or Blank-N, comparable anticancer activity between PTX and PTX-N was observed, while the blank-N didn’t show significant toxicity (Figure 2d). These results demonstrated that hydrophobic modification to Tat peptide can produce conjugates with the ability to self-assemble into nanostructures, which can function as nanocarriers for hydrophobic drug delivery.

CONCLUSION

Tat peptide can be engineered for efficient intracellular drug delivery. The therapeutic cargo will significantly affect the function of Tat peptide in its cell penetrating efficacy as well as self-assembly. These effects require thorough consideration during the development of further Tat peptide conjugates.

REFERENCES


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