Cytotoxicity Studies to Characterize Self-Assembling Amphiphiles for Targeted Cancer Drug Delivery
Anne E. Kim, Se Hye Kim, Samantha King, Aileen Shieh, and Jon R. Parquette*
OSU College of Arts and Sciences, Department of Chemistry

Background

Advantages of nanodrugs in anticancer research
- Improves water solubility and drug stability
- Prolongs circulation time
- Increases target specificity
- Facilitates passive accumulation in tumors via enhanced permeation and retention effect

Camptothecin (CPT)
- Possesses potent antitumor properties that derive from its inhibition of topoisomerase I
- Showed remarkable anticancer activity in preliminary clinical trials
- Drawbacks: poor solubility under physiological conditions and unstable E-lactone ring

Self-Assembly of CPT-peptide conjugates
- Peptide conjugation to CPT increases stability of drug
  - Preliminary data testing stability shows hydrolysis occurs at ester bond, not at lactone
  - Hydrophobic portion (peptide) and hydrophobic portion (CPT) spontaneously assemble into nanotubes
  - Once inside tumor cells, esterases will cleave ester bond → CPT will open to toxic form
  - We have developed mono-, di-, and tetra-peptide conjugates

Analysis of Drug

Compound
- Compound of interest is NH₂-K(CPT)-NH₂
- Monopeptide derivative is appealing due to simplicity, as opposed to di- and tetra-peptide
- Amide derivative is most favorable due to increased solubility, as opposed to methyl ester and free acid derivatives

Analysis of Drug

Cytotoxicity Tests
- Cytotoxicity tests were conducted to determine half maximal inhibitory concentration (IC₅₀) levels to test efficacy of drug
- 3 non-small cell lung cancer cell lines (A549, H460, H23) with varying BCRP expression were used
- Drug was administered at varying concentrations and incubated with cells for 96 h
- Concentrations were diluted with PBS from 10 mM stock solution (5.75 µg of drug in 1.0 mL of PBS) that was aged at 23 °C for 3 days, stored at 4 °C in between trials
- Preliminary data

Future Steps

- Run E-lactone stability tests at pH 7.4 using high performance liquid chromatography
- Run more cytotoxicity trials to confirm preliminary data
- Quantitate accumulation of drug in vitro via flow cytometry
- Examine drug flow in cells with confocal images
- Determine biodistribution in vivo

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Conclusions
- NH₂-K(CPT)-NH₂ shows promise based on collected data (TEM and cytotoxicity) so far
- There is formation of nanotubes due to amphiphilicity of molecule
- Cytotoxicity studies
  - Seemed to have similar effectiveness as Ac-KK, but is less effective than NH₂-KK
  - Still more effective than CPT-11 (clinically approved)

Stability

NH₂-K vs. Other CPT Derivatives

IC₅₀ Levels of NH₂-K were compared with CPT, CPT-11 (clinically approved), NH₂-KK, and Ac-KK
- NH₂-K appeared to be comparable to Ac-KK and less effective than NH₂-KK

Ester

Formation – 250 µL in Phosphate Buffered Saline (PBS), was diluted from 10 mM stock solution (5.75 µg of drug in 1.0 mL of PBS) that was stored at 4 °C for 1 month, aged for 3 days 23 °C

Nanotube formation – 250 µL in PBS, was diluted from 10 mM stock solution (5.75 µg of drug in 1.0 mL of PBS) that was stored at 4 °C for 1-month, aged for 3 days 23 °C

Stability

Data

Time Dependent HPLC Studies for Stability of Ester in PBS

Ac-K(K(CPT)-NH₂)

NH₄,K(K(CPT)-NH₂)

Ac-K(K(CPT)-NH₂)

NH₂-K(CPT)-NH₂

NH₂-K(CPT)-NH₂

Ac-K(K(CPT)-NH₂)

Ac-K(K(CPT)-NH₂)

Ac-K(K(CPT)-NH₂)

Ac-K(K(CPT)-NH₂)

Ac-K(K(CPT)-NH₂)

Ac-K(K(CPT)-NH₂)

Ac-K(K(CPT)-NH₂)