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Category: Biomedical Health and Sciences

Title: Electrostatic Targeting of Feraheme Using Doxorubicin Conjugates for Prostate Cancer

1. Rationale

Prostate cancer is a prevalent disease where one in seven men will be diagnosed (Kaittanis, 2017). Death from prostate cancer is mostly caused by delayed diagnosis because the cancer has spread to other parts of the body. The tumor will metastasize into other organs or become resistant to chemotherapeutics which leads to death. The current methods for treating prostate cancer cells include removal of the prostate gland followed by continuous treatment by prostate cancer treatment drugs in order to prevent recurrence of the cancer from marginal tissues after surgical removal (Ruggiero, 2011). Many of these drugs are non-specific, which means they can kill off healthy cells rather than just cancerous cells. Feraheme is a type of iron oxide nanoparticle, which are sized between 10-300nm (stock concentration of 30mg/ml). Past research has shown that treatment drugs have been successfully loaded onto these particles, and they also show contrast under magnetic resonance imaging for tracking inside the body (Daldrup-Link, 2017). Studies have shown that Feraheme successfully delivered therapy to lung and prostate carcinoma cells with different combinations of treatment (Santra, 2009; Kaittanis, 2014). In order to increase the amount of drug that is delivered by these particles, a prostate-specific membrane antigen (PSMA) targeting agent can be conjugated onto the drug through chemical modifications before loading onto the Feraheme particles (Kalia, 2010).

Doxorubicin is a naturally fluorescent drug and can be modified for treatment purposes and for the conjugation of a targeting agent. Modifications can be done through amine reaction conjugations, EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

hydrochloride) conjugations, and maleimide thiol reactions (Spicer, 2014). Doxorubicin has been shown to load well onto Feraheme in past research (Kaittanis, 2014). Therefore, it is expected to load well onto the particles after the modifications. The attached prostate-specific membrane antigen (PSMA) targeting agent will increase uptake by the prostate cancer cells, and the Feraheme acts as a carrier to deliver the drug into the cells through a different mechanism. It will also offer a method of clearance from the body because iron oxide nanoparticles tend to distribute to the liver and spleen. This means that the particles will not remain in the body for an extended period of time. Past research has shown how different types of chemotherapeutics can be loaded onto iron oxide nanoparticles and how multiple drugs could be delivered at the same time (Kaittanis, 2014). However, these studies have not tested whether a drug with a targeting component can be delivered and taken up by the cells effectively. Research on increasing the uptake by cancer cells can increase the efficacy of different drugs and help combat the non-specificity of different treatment drugs.

2. Research Question(s), Hypothesis(es), Expected Outcomes

a. Research Questions

- Can the conjugation of Doxorubicin and the prostate-specific membrane antigen (PSMA) targeting agent be loaded onto Feraheme?
- Will the targeting agent loaded onto Feraheme increase uptake into the cells by prostate cancer cell lines?

b. Hypotheses

- The modified Doxorubicin will successfully load onto Feraheme because past research has shown successful loading of free Doxorubicin onto Feraheme (Kaittanis, 2014)
- There will be a difference in the method of uptake by prostate cancer cells for the Feraheme with the targeting agent loaded onto the nanoparticles compared to the drug alone

c. Expected Outcomes

- It is expected that the Doxorubicin conjugated with a targeting agent will load successfully onto Feraheme because Doxorubicin alone has been shown to load well onto Feraheme in experiments (Kaittanis, 2014). This combination is also expected to increase uptake by the prostate cancer cells because of the presence of a prostate-specific membrane antigen (PSMA) targeting agent on the Feraheme nanoparticles. The uptake method will be different because the cells take in drugs and iron through different mechanisms.

d. Procedure

Role of Mentor:

The mentor provided all of the basic lab materials and equipment such as lab coats, well plates, goggles, gloves, safety hoods, etc. He also provided the prostate cancer cell lines Du145, 22rv1, LNCaP, and PC3, which were all obtained from the American Type Culture Collection (ATCC) company. There were no tissues used in this study, only cell lines. The mentor will provide guidance for new procedures such as performing a bioconjugation reaction and fluorescence assays.

Role of Student Researcher:

Bioconjugation Reaction

- Doxorubicin (CAS No.: 25316-40-9), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (CAS No. 25952-53-8), N,N-diisopropylethylamine (DIEA) (CAS No. 88996-23-0), 2-PMPA (CAS No. 173039-10-6), and hydroxybenzotriazole (HOBt) (CAS No. 123333-53-9) will be obtained from the Sigma-Aldrich company.
- Doxorubicin will be diluted in dimethyl sulfoxide (DMSO) to make a 10mM solution.
- 2-Phosphonomethyl pentanedioic acid (PMPA) will be diluted in MES buffer (pH 6.28) to make a solution of 10mg/ml
- 1 equivalent of Doxorubicin, 1 equivalent of 2-Phosphonomethyl pentanedioic acid (PMPA), 2 equivalent of EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride), 2 equivalent of hydroxybenzotriazole (HOBt), and 3 equivalent of N,N-diisopropylethylamine (DIEA) will be mixed in a reaction vial for the conjugation
- The mixture will be left to stir overnight to make the product of PMPA conjugated onto Doxorubicin
- Liquid chromatography mass spectrometry will be performed to determine if the reaction succeeded
- The Amount of reagents used in the reaction will be altered in order to increase the amount of products produced

Cell Culture

- Du145, LNCaP, 22Rv1, and PC3 human prostate cancer cells will be obtained from ATCC
- LNCaP and 22Rv1 cells will be cultured in T75 flasks with Roswell Park Memorial Institute (RPMI) media with 10% fetal bovine serum (FBS) and 1% penicillin streptomycin (PS)
- Du145 cells will be cultured in T75 flasks with Minimum Essential Medium (MEM) media with 10% fetal bovine serum (FBS) and 1% penicillin streptomycin (PS)
- PC3 cells will be cultured in T75 flasks with F-12K media with 10% fetal bovine serum (FBS) and 1% penicillin streptomycin (PS).
- The cells will be plated in 96-well plates for treatment with Doxorubicin combined with the targeting agent alone and the Dox-targeting agent loaded onto Feraheme
- The treatment will be applied for 24 hours before the fluorescence assays are performed

Data Collection

- The cells will be examined under the microscope, and various photos will be taken of the different treatments such as free drug treatment and drug loaded onto Feraheme treatment
- Intensity will be quantified to determine percent uptake by the prostate cancer cells after treatment

- Flow cytometry will be used because of the natural fluorescence of Doxorubicin (excitation wavelength: 470nm and emission wavelength: 585nm) to determine percent uptake

e. Risk and Safety

- Doxorubicin, EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride), and hydroxybenzotriazole will be used for my project. These chemicals can be handled because gloves, lab coats, and safety goggles are provided to prevent spills and contamination. Doxorubicin will be delivered to the prostate cancer cells to detect uptake. EDC and hydroxybenzotriazole were used for the conjugation of Doxorubicin with 2-PMPA.

f. Data Analysis

- Flow cytometry and microscopy will be used to analyze the fluorescence intensity of the prostate cancer cells after treatment. The fluorescence is associated with the Doxorubicin drug and indicates percent uptake by the cells. The signal intensity data will be normalized to the untreated control cells. The fluorescence data will be plotted using the Prism software (Version 8).

g. Bibliography

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3. **Vertebrate Animal Research**

    a. No vertebrate animals were used for my project. The mentor nor I came into contact with live animals, and none were harmed for my experiments. Live cells were used (LNCaP, 22Rv1, PC3, and Du145) and were ordered from the American Type Culture Company (ATCC)

4. **Potentially Hazardous Biological Agents Research**

    a. Cell lines are obtained from American tissue type culture company (ATCC). The cell lines are 22Rv1 (CRL-2505m BSL 2), Du145 (HTB-81 BSL 1), PC3 (CRL-1435 BSL 1), LNCaP (CRL-1740 BSL 1). The prostate cancer cell lines are accepted human cell lines and are used in cell cultures. BSL levels are given by ATCC. BSL 2 is the highest level I will be working with.

5. **Hazardous Chemicals, Activities, and Devices**

    a. Everyone working in the lab will wear lab coats, closed toed shoes, long pants, and gloves. All materials will be disposed of in hazardous waste bins and properly disposed according to MSKCC policy. All biological materials are autoclaved and destroyed off site by a professional waste cleanup company. All materials used during cell culture are sent to this waste stream.