Dual Modality Nano-Particles for Tumor Uptake

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Abstract

Nanoparticles can be used as effective carriers of drugs and imaging agents for tumors. To measure the uptake in tumors, fluorophores and MR contrast agents can both be incorporated into the same nanoparticles. The goal of our project was to prepare new super paramagnetic iron oxide (SPIO) nanoparticles that are also labeled with a near infrared fluorescence dye (DACT815-Dioctadecyl-3,3,3,3-tetramethylindodicarbocyanine-5,5-dithiolic acid) which would then be measurable using MR and fluorescence microscopy. The experimental design consisted of injecting the nanoparticles into mice via tail vein followed by uptake evaluation in vivo by the aforementioned methods. The super-paramagnetic iron oxide particles are used as a T2 contrast agent. The nanoparticles had an average size of 22 nanometers as measured by dynamic light scattering and transmission electron microscopy. The concentration of iron oxide in the nanoparticles was 1 mg/mL. The nanoparticles in the tumors were visualized 24 hours post injection by MR and then by fluorescence microscopy using a Cy5 filter. By using dual-labeled nanoparticles, we expect to obtain more detailed information on tumor uptake and distribution of nanoparticle sized drug formulations. Also, these dual labeled nanoparticles will allow for a more accurate analysis of tumor vasculature and changes post heating.

Materials and Methods

The nanoparticles were made consisted of a monolayer of lipid with super paramagnetic iron oxide particles on the inside and a fluorescent dye. It took multiple trials to figure out what ratio of these ingredients would work best. The final Iron Oxide nano-particles consisted of:

200μL of SPIO at the concentration of 5mg of iron per mL.
50μL of PEPIE (the lipid suspended in chloroforn)
50μL of fluorescence dye

Procedure for making SPIO nanoparticles:
- First, the ingredients above are all measured out and mixed in a round bottom flask (vortexing is not necessary)
- Next, the solvent is evaporated out of the solution. This was done using a rotary evaporator machine. This takes approximately 40 minutes
- After all the solvent is evaporated, the thin films of lipids left on the side of the flask is re-suspended with 1 mL of saline. This was done using a combination of heating, and sonication.
- From here, the nanoparticles may need to be extruded and sized to ensure their effectiveness. This can be done using an extruder. The size distribution can be checked using a laser.

Background

Iron oxide nanoparticles are composed of an iron core surrounded by lipids. The lipid can then have other molecules attached to it. Dye can be attached which allow for the nanoparticles to target certain cells. A fluorescent dye can be attached to the outside allowing for the particle to be looked at under a Flourescence microscope.

The reason nanoparticles are used to carry drugs and agents is because they collect in tumors. This is known as enhanced permeability and retention (EPR), and occurs because of the leaky vasculature inside tumors. The nanoparticles are able to escape, but become trapped once inside the tumor. They are to large to escape elsewhere in the body, which effectively targets them to tumors.

Objective

The objective was to see if subjecting mice to heat treatment could help increase tumor vasculature: Because that nanoparticles can be made with a dye and MR contrast agent, they could be used to help look at tumor vasculature. Their uptake and diffusion into the tumor is a good marker of blood flow. The MR agent being used is SPIO-Paramagnetic Iron Oxide because of its relatively high contrast and because of the body’s ability to expel iron through natural processes.

Imaging the Nanoparticles:
- The 200 μL of nanoparticles were injected into each of three tumor bearing mice to measure perfusion. Two of the mice were heated to see if heat would effect the penetration of the nanoparticles.
- The liposome’s diffusion into the tumor, and other parts of the body, was first measured through magnetic resonance imaging (MRI). Three sets of scans were taken for each of the three mice. The scans were taken pre-injection, immediately post injection, and 24 hours post injection.
- These scans were taken, the mice were optically imaged. After this the tumors were excised, imaged, sectioned, and fluorescence microscopy performed on the sections to measure fluorescence.

Optical Analysis of Tumor:
- Through reflective fluorescence imaging, the tumors were looked at In Vivo; Control
- Heated

Conclusions

Through our experiments, we were able to show that dually labeled nanoparticles could be produced at a consistent size through our procedure. These nanoparticles collected in tumors, but it also seems they collected in other organs of the mouse. Heat also seems to have had an effect, but to what extent is still unknown. Future experiments will test larger size nanoparticles to what effect size has for increasing contrast in the MR scan. Hopefully future experiments will lead to clearer results with regard to iron oxide uptake in tumors and the effect of heat treatment in uptake of these nanoparticles.

References
- "Mol Cancer Ther Aug 2006 vol. 5 no. 8 1909-1917"

Acknowledgements

This work was done with immense help from my mentors, and the Repasky lab.
Mentors: Additional Help from: Arindam Sen Ph.D. Soumya Ullas Elizabeth Repasky Ph.D. Timothy Winslow Joseph Spernyak Ph.D. Steven Turoske*