A Method for Drug Delivery Using Magnetoliposomes

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This paper describes the initial stage of the formation of a toxin delivery system. The system will allow for the selective killing of tumor cells by using magnetoliposomes to deliver a toxin. Magnetoliposomes are liposomes with magnetic particles embedded into the lipid bilayer; they provide for the controlled release of toxin upon exposure to an oscillating magnetic field. Our objectives were: 1) to develop a solenoid that is capable of producing a magnetic field and heating a magnetic fluid; 2) to create calcein-filled liposomes; and 3) to create stable magnetoliposomes. These magnetoliposomes will then be used as the foundation of further work, striving to formulate a drug delivery system more efficient and specific than any other treatment currently available.

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Introduction

It is well known that current methods of systemic drug delivery lack the specificity needed to target tumors. It is almost impossible to deliver a therapeutic amount of toxin to a tumor, because current systems leave the drug circulating throughout the body. Chemotherapies damage both healthy cells and cancer cells, resulting in the destruction of both, which is why many patients experience symptoms such as fatigue, hair loss, and infection.

The purpose of our research was to develop a tissue-specific drug delivery system using magnetoliposomes that are targeted to cancer cells. We intended to use these magnetoliposomes to create a toxin delivery system that will target individual tumor cells, leaving healthy cells unharmed. Magnetoliposomes are liposomes with magnetic particles embedded into the lipid bilayer.[1,2] Liposomes are microscopic spherical vesicles that form when phospholipids are hydrated. When mixed in water and under the proper conditions, the phospholipids arrange themselves into sheets, where the phospholipid molecules are side by side. These sheets then join to form a bilayer membrane, which encloses some of the water in a phospholipids sphere. The microscopic spherical vesicles may be used to deliver toxins, vaccines, enzymes, or any other substance to the body. When the magnetic particles lodged in the lipid bilayer are exposed to an oscillating magnetic field, the particles will align with it, causing the particles to flip. This will cause the surface of the magnetoliposome to heat, melting away the envelope and releasing any substance the vesicle may be holding. With this delivery system, toxin will be released.

Our research focused on the making of a solenoid that would produce a uniform magnetic field, forming calcein-filled liposomes, and forming calcein-filled magnetoliposomes. Calcein was used in both liposome experiments in order to gauge whether we had formed liposomes and the manner in which we could cause them to release their content.

Methods

Experiment 1

Our aim was to construct a solenoid that would generate a uniform magnetic field when electric current was passed through it. If we were successful in our construction, any magnetic solution placed in the solenoid would heat due to its magnetic particles aligning and flipping with the magnetic field. To do this, we wrapped iron wire around a section of PVC tubing and connected the ends of the wire to an AC-generator. To prevent the magnetite solution from heating simply because the iron wire was hot, we hooked the solenoid up to a circulating bath. The water from the bath would run through the solenoid, inhibiting any heat transfer from the iron wire to the magnetite solution. To test for heating, we put 1 mL of magnetite solution in the solenoid and the same amount of H_2O as the control. Placing each in at separate times, we measured their temperatures periodically. If successful, the magnetite's temperature would rise while the H_2O's temperature would remain at nearly room temperature.

Experiment 2

Our aim was to form calcein-filled liposomes using an already tested protocol.[3] At high concentrations, calcein is self-quenching and therefore gives a low

fluorescence signal. If the liposomes are broken, the calcein is diluted and the fluorescent signal increases. Following a reverse-phase evaporation protocol, we proceeded in trying to make liposomes. First, an appropriate amount of dry phospholipids was mixed with chloroform. To remove any solvent, the solution was placed in a rotary evaporator. The resultant substance was then redissoved in 6mL of diethyl ether, 2mL of TES buffer, and .1g of calcein. The mixture was then blended together using a sonicator for five minutes. The solution was then placed in the rotary evaporator in order to remove the organic solvent (diethyl ether). It was then passed through a Sepharose 4B column to separate any potential liposomes from the non-encapsulated material. To test our experiment, we took 14 samples of the solution in simultaneous order from the column. Using Luminescence Spectrometer the amount of free calcein was measured in each sample. Triton was then added to each sample to rupture the liposomes, releasing any encapsulated calcein.

Experiment 3

Our aim was to form magnetoliposomes with the same protocol used to form blank calcein liposomes. To make magnetoliposomes, 50 µl of magnetite was added during the phospholipid/chloroform phase. The same protocol in Experiment 2 was used to test Experiment 3. We also added an iron chelator after the Triton to remove any iron oxide that may be dampening the florescence of the calcein and inhibiting the reading. After producing magnetoliposomes, we placed the sample in the solenoid for 5 minutes to see if the magnetic field would cause calcein release.

Experiment 4

This is a separate attempt at forming magnetoliposomes. A similar protocol was used as in the previous liposome-forming experiments. The solution was centrifuged at 2000 g to separate any encapsulated material from the rest of the free calcein-containing solution. This was done instead of separating these materials using the column. The magnetoliposomes settled to the bottom leaving the excess solution on top to be pipetted off. Critic acid buffer was then added to the magnetoliposomes solution, mixed by hand, spun down, and pipetted off. This was repeated three times, resulting in a condense solution of magnetoliposomes. The solution was then placed in a magnetic separator, where the magnetolipomes were attracted to the magnetic side. This step showed that we had formed magnetoliposomes. The solution was placed in a Luminescence Spectrometer and measured as in the previous experiments.

RESULTS

Experiment 1

The magnet successfully heated a magnetic fluid; its temperature rose swiftly and leveled off at 99°C. The temperature of the H_2O control rose only 2°C in four minutes. Thus, we proved that the solenoid did produce a magnetic field strong enough to bring a magnetic substance to its boiling point.

Experiment 2

Some amount of free calcein was detected in every sample collected from the column. When we applied Triton, the fluorescent signal increased, showing that liposomes were formed and that they encapsulated calcein. By the last of the samples, more calcein was detected before Triton was applied. This was due to the high amount of free calcein flowing through the column by the time these samples were taken. The results from this experiment indicate that calcein-filled liposomes were formed.

Experiment 3

Although the amount of free calcein rose after each application of Triton and iron chelator, the experiment did not produce the type of results we expected. The amount of calcein released was much less than in experiment 2 and we are not sure why we do not get an increasing amount of free calcein in the latter samples. There are several possibilities for why these results differ from Experiment 2. The magnetic particles may have interfered with the formation of stable liposomes. It is also likely that the procedure was not done perfectly since this was our first attempt at forming magnetoliposomes. One observation we made is that there was a lot of magnetite trapped at the top of the column. We think that maybe the magnetoliposomes do not travel smoothly through the column, so we decided to try a different separation method.

Experiment 4

When the sample from this experiment was placed in the Luminescence Spectrometer, the sample gave a reading of 293. After Triton was added, the reading jumped to 820. This gives a fluorescence increase similar to the samples from experiment 2, indicating that we have produced stable magnetoliposomes. It seems that the centrifugation method was more effective than the column in separating the magnetoliposomes from the free calcein. Although we think that the experiment gave stable liposomes, we do not see any release of calcein when the sample is exposed to the oscillating magnetic field for five minutes.

CONCLUSION

Current methods of toxin delivery are insufficient, because they lack the specificity needed to deliver therapeutic amounts of a drug to a specific area of the body. The purpose of our research was to begin the development of an effective toxin delivery system that will have the ability to specifically target tumor cells.

We worked to develop magnetoliposomes, which are an important part of the system. We accomplished the first three of our goals. We produced a solenoid that heated a magnetic fluid to its boiling point: created calcein filled liposomes; and created calcein filled magnetoliposomes. However, when the magnetoliposomes were placed in the solenoid we did not observe calcein release.

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