A Method for Drug Delivery Using Magnetoliposomes

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Studies show that current methods of systemic drug delivery lack the specificity needed to effectively target tumors. Our current efforts are a continuation of our work presented through the NIDDK program last year. The aim of our research is to develop a toxin delivery system that will safely and effectively target individual tumor cells without harming the healthy cells along side it. Ultimately our goal is to formulate a method of toxin delivery using Magnetoliposomes. Such a system will allow for the selective killing of tumor cells by using magnetoliposomes to deliver toxin. Magnetoliposomes are liposomes with magnetic particles embedded into the lipid bilayer.

In this study, we expand on findings from our previous study by employing a method of fusion to rupture magnetoliposomes. Polyethylene glycol (PEG) was used to promote aggregation of magnetoliposomes.

While magnetoliposomes were produced and ruptured using an oscillating magnetic field in our first study, we are exposing magnetoliposomes to a constant magnetic field. Exposure to a permanent magnetic field will closely mimic the results of adding PEG to magnetoliposomes. By employing both systems, maximum fusion will be achieved.

Our current objectives include: 1) determining the lipid composition that will best promote fusion upon introduction to PEG; 2) using the composition to add magnetic particles to the lipid composition and testing the amount of fusion after exposure to a permanent magnetic field; and 3) combining the two methods, finding the exact concentration of PEG to facilitate the fusion between the magnetoliposomes after exposure to the constant magnetic field. 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.

Introduction

The goal of our research is to develop a toxin delivery system that will target tumor cells with a much higher specificity then any other treatment available. To achieve this goal, we will modify an already effective treatment and use it as the basis of a novel delivery system that can be activated by externally applied magnetic fields.

The system will be targeted to tumor cells that overexpress a cell surface receptor called human epidermal growth factor receptor 2 (HER-2). Overexpression of HER-2 is one of the most efficient irregularities found in most tumors and is therefore an ideal target for toxin delivery. Our delivery system improves on treatments that use drug-filled liposomes. The external surface of our liposomes will be coated with antibodies that bind selectively to HER-2, targeting liposomes to cells that overexpress HER-2. After binding to a cell, the liposomes become engulfed into the cell. Although more liposomes will become engulfed by tumor cells, healthy cells throughout the body also express some amount of HER-2 and will receive unacceptable amounts of toxin. To restrict the release of toxin to only cancerous tissue, we will incorporate the use of magnetoliposomes into the system.

Magnetoliposomes are liposomes with magnetic particles embedded into the lipid bilayer.[1–3] Liposomes are microscopic spherical vesicles that form when phospholipids are hydrated. When mixed in water and under the proper conditions, phospholipids arrange themselves into sheets, in which the phospholipids molecules are side by side. These sheets then join to form a bilayer membrane that encloses some of the water into a phospholipid sphere, or liposome. Liposomes have been used as an efficient means of delivery of toxins, vaccines, enzymes, or any other substance to the body.

When exposed to a constant magnetic field, the polar ends of each magnetic particle in the bilayer of the magnetoliposome will align. The magnetic field will cause the magnetic liposomes to aggregate, coming near enough to each other to fuse. The fusion will cause the magnetoliposomes to release their toxin. By focusing a strong constant magnetic field over the tumor, contents of the magnetoliposomes will only be released in that tissue.

Methods

Vesicle Preparation

Mixtures of cholesterol and lipids at appropriate ratios were dried by using a rotary evaporator for 20 mins to remove the organic solvent. A calcium buffer was heated in a warm bath at 42°C before being added to the dry lipid material. The calcein liposome solution was then mixed with a bath sonicator. The mixed solution was then poured into a tube for further use. 4-mL of solution were removed from the tube to be sonicated for 20 mins. After 20 mins, the 4-mL solution was run through a Sepharose 4B column to separate any liposomes from the nonencapsulated material.[4]

Fluorescence Testing

Three samples of the liposome material were taken from the column. Typically, the second sample was used for florescence testing. Five measurements of 100 μL was taken from the second sample and placed in cuvets. Twenty microliters of water were added to one measurement as the control.

Varying percentages of polyethylene glycol (PEG) were then added to the others. The first sample was mixed with 20 μL of 10% PEG, the second mixed with 15 μL 10% PEG, the third with 10 μL 10% PEG, and the fourth with 5 μL 10% PEG. Water was added to the second, third, and fourth samples to equal the volume of the first sample. After two minutes of settling, each sample was added to 3 mL chloroform and placed in the luminescence spectrometer for a base reading. Once the reading was taken, 5 μL triton was added to the solution to rupture the liposomes, causing them to release more calcein. A second reading was then taken, giving us a final fluorescence reading. On the basis of the comparison between the first and second readings, we were able to determine each liposome composition using the same method for formation and fluorescence reading.Through this method, the best possible liposome composition was found for optimal fusion of the liposomes. This was the 2:1 ratio of dioleoylphosphatidylethanolamine (DOPE) to dioleoylphosphatidylcholine (DOPC).

Magnetoliposome Vesicle Preparation

200 mL of magnetite were added to a test tube, and it was rinsed 3x with 3 mL chloroform. 3 mL of chloroform were then added to the magnetite before the mixture was dried with a rotary evaporator. A liposome composition was then added to the dry magnetite. The rest of the formation and fluorescence reading follows the previous protocol.

Magnetic Testing

A 2:1 DOPE to DOPC composition was used as in previous magnetolipsome formation. Two 100-μL samples were placed in a warm water bath at 37°C. One was placed next to a constant magnetic field and one was not. A fluorescence reading was then taken from each sample according to the usual protocol. A stronger fluorescence reading was taken from the sample placed next to a permanent magnet. A second manner of testing was then used in which two samples were each administered a 2.5% PEG dose. The samples were then placed in a warm bath at a temperature of 37°C. One sample was again placed next to a permanent magnet and the other was not. A fluorescence reading was then taken from both samples. A stronger florescence reading was again shown by the sample placed within a constant magnetic field.

Results

The 2:1 ratio of DOPE to DOPC was found to be the beat liposome composition to promote the optimal function of liposomes. This composition was therefore used in the formation of magnetoliposomes.

The first set of magnetoliposome testing followed the same method of PEG-mediated fusion. The second sets of magnetolipsomes tested were fused by using a permanent magnetic field while in a warm water bath. The third set used both forms of fusion, in which a 2.5% PEG supplement was added to the samples before they were placed in the warm bath and one was exposed to a permanent magnetic field.

CONCLUSION

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

To achieve this goal we worked to form magnetoliposomes that would fuse and release their toxin when exposed to a permanent magnetic field. Thus far, our experiments have focused on the following: 1) finding the lipid ratio that would promote optimal fusion of liposomes; 2) forming magnetoliposomes that would fuse to release their encapsulated calcein; and 3) causing magnetoliposomes to fuse when exposed to permanent magnetic field. We accomplished all three of these goals to some extent. A stronger magnetic field will be used in the future to cause greater fusion of magnetoliposomes. We produced liposomes that would fuse greatly when PEG was added, mimicking the protein-mediated fusion found in the biomembrane process. We produced magnetoliposome that showed the same kind of fusion results as the plain liposomes. Mangnetoliposomes were fused with a permanent magnetic field. However, we believe that a more powerful magnet would produce a strong enough magnetic field to fuse the magnetoliposomes for ideal toxin release.

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