Development of Temperature Sensitive Liposomes for targeted Drug Delivery

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ABSTRACT

Hyperthermia and the liposomal drug delivery have been extensively evaluated in the past decades for drug delivery. More recently, these technologies have been used together in clinical trials. As an initial evaluation, luciferin was encapsulated in the liposome to provided release characteristics after heating. We have developed, through an easy and accurate process, temperature sensitive stable liposomes that release their drug content upon local heating. Different formulations have given very encouraging results for the stability and release profiles after heating in vitro in presence different buffers, serum and cells. In addition, these formulations have been shown to be non-toxic *in vitro*. The long-term goal of this project is to use focused ultrasound to heat and release chemotherapy in tumor lesions using targeted, heat-sensitive liposomes.

Keywords: temperature-sensitive liposomes, targeted drug delivery, ultrasound, imaging, chemotherapy and tumor.

1 INTRODUCTION

The liposomes are self-assembled colloidal particle that occur naturally and can be prepared artificially, as show by Bangham team in the mid-1960s [1]. They have served as drugs carriers for therapeutic intervention. In addition, to their use as anticancerous agents, they have also been used in anesthetic, anti-inflammatory, anti-parasitic, enzymatic [2], gene therapy, vaccine agents, and finally imaging agents. Many of them are approved in numerous countries and are commonly used for more than 15 years. Different strategies have been used these past decades to improve their stability and increase their circulation time after intravenous injection by introducing hydrogenated synthetic and/or pegylated lipids. However, these approaches reduced the release of the active agents from the liposome into the site of action, even when co-localization was clearly demonstrate or the targeting ligand was used. This resulted in an increase in the administrated dose and subsequently, an increase in unwanted side effects in parasitic and cancer therapy or increase in background signal for imaging.

Since the studies of Papahadjopoulos [3] and Yatvin [4] in which they showed the controlled release of agents using liposomes under various temperature settings, new thermotherapy technologies are being developed for use in the clinic [5]. Many teams have attempted to exploit synergetic interactions of hyperthermia and liposomal drug delivery for cancer therapy, monitoring and/or imaging [6]. Needham et al. [7-9] have develop different thermosensitive liposome formulations of which, one, ThermoDox®, has been approved by the FDA foruse in clinical trials. Since 2003, ongoing trilas with ThermoDox in prostate cancer patients in association with radiofrequency ablation therapy has been under evaluation. Similar formulations have been used in imaging for thermo-mapping or monitoring.

In this present paper, as an initial evaluation, we have developed an easy process to formulate thermosensitive liposomes for cancer therapy in conjunction with focused ultrasound. The luciferin was used as a model and imaging agents for ease of visualization. After optimization of the process, the size and the stability was studied in the buffer and serum. Red blood cells were used to determine possible cell and membrane toxicity and to evaluate cell aggregation.

2 MATERIALS AND METHODS

2.1 Liposome Preparation

DPPC, 1,2- dipalmitoyl-sn-Glycero-3-Phosphocholine; MPPC, 1-palmitoyl-2-hydroxy-*sn*glycero- 3-phosphocholine; DSPE-PEG-2000, 1,2-distearoyl-*sn*glycero-3-phosphoethanolamine-*N*-polyethylene glycol 2000 were purchased from Avanti Polar Lipids (Alabaster, AL, USA). D-luciferin thermosensitive liposome (TSL) preparation was adapted from the method described by Anyarambhatla and Needham (1999). Briefly, the lipids, in ratio DPPC: MPPC: DSPE-PEG-2000: 86:10:4, were dissolved in chloroform, and deposited as a film on a flat-bottomed flask by slow evaporation under nitrogen followed overnight vacuum drying. The dried lipids were vortexed with in phosphate buffered saline (PBS, Gibco, Carlsbad, CA, USA) containing 10 mg/ml D-luciferin (Caliper Life Sciences, Alameda, CA, USA) and homogenized size reduction was carried out by sonication for

10 min at 50°C. Unencapsulated luciferin was removed by dialysis of the liposomes in PBS overnight.

2.2 Liposome Analysis

Particle size was determined by dynamic laser light scattering using unimodal and differential size distribution processor analysis (Zeta Pals, Brookhaven Instruments Corporation, Holtsville, NY, USA). The results were represented by the mean ± esm of three measurements.

2.3 Evaluation of Heating on the liposome

After dilution in PBS or serum, each size formulation was measured before and after heating at 45ºC in a water bath for 10 minutes.

2.4 In vitro Toxicity on Red Blood Cells

Hemoglobin (Hb) release from erythrocytes: Freshly isolated human erythrocytes (RBC) were incubated with the liposomal formulations at 37°C for 30 minutes. After washing, the RBC pellet was lysed with sterile water and the remaining hemoglobin was estimated from its absorption at 560 nm for comparison with control RBC's. The results were expressed as Hb_{50}, calculated from the mean ± esm of triplicates.

Influence of Liposomes on red cell aggregation: A 1% suspension of fresh human red blood cells was mixed with the liposomal formulations on a microplate, and the mixture was incubated at 37°C for 1 hour and at 4°C for 1 and 3 hours. The pattern of red cell aggregation was then evaluated by microscopy.

3 RESULTS

3.1 Liposome Analysis

After the evaluation of the stability of the TSL and preservation of release profile upon heating *in vitro* in serum, the shelf-life stability was evaluated. The liposomes are stable at least one month at 4ºC (Figure 1). Similar results have been observed in the HEPES buffer.

Temperature sensitive liposomes are stable in both serum and buffer (Figure 3) even after storage of 3 months at 4°C.

3.2 *In Vitro* Toxicity Evaluation

The temperature sensitive liposomes with or without Luciferin did not show any membrane toxicity (Figure 2), or cause red blood cell aggregation at the concentrations tested (from zero to 1mg/ml of lipids and/or 5 μg/ml of Luciferin) at 37°C and 4°C after 1 and 3 hours (data not shown).

Live Time	Day 0	Day 7	Day 30
A	71 ± 4	81 ± 3	92 ± 5
B	88 ± 3	75 ± 1	97 ± 2
C	76 ± 1	77 ± 2	107 ± 8

Figure 1: Shelf-life: Liposome size stability at 4ºC during one month. A: TSL without Luciferin, B: TSL with Luciferin before dialysis, C: TSL with Luciferin after dialysis. The results are represented as the mean ± stdiv of three measurements.

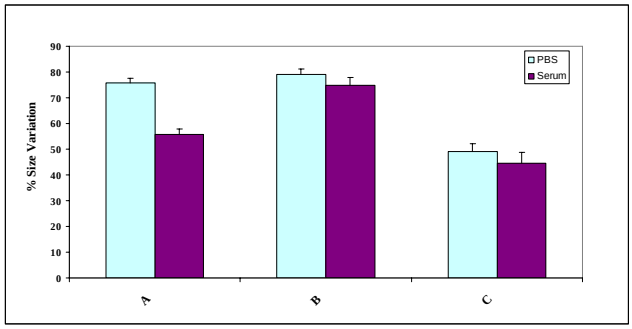

Figure 3: Influence of serum on liposome size. A: TSL without Luciferin, B: TSL with Luciferin before dialysis, C: TSL with Luciferin after dialysis

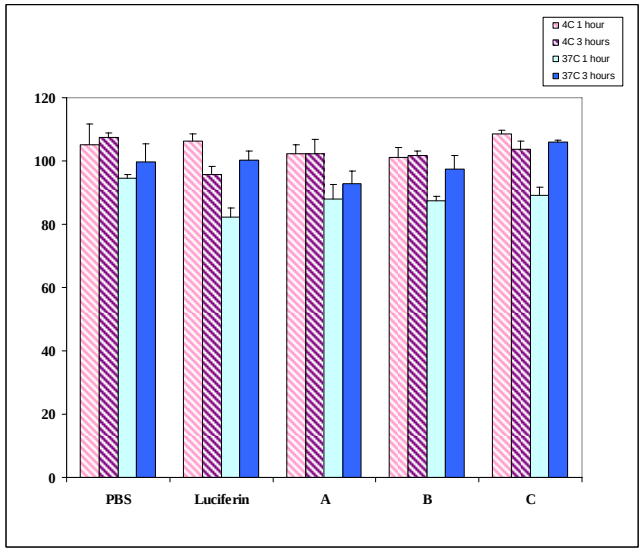

Figure 2: Hemolysis effect of the liposomes on of human red blood cells. A: TSL without Luciferin, B: TSL with

Luciferin before dialysis, C: TSL with Luciferin after dialysis. The results are presented as the mean ± stdiv of triplicate measurements.

4 DISCUSSION AND SUMMARY

The tested formulations have shown encouraging results with regards to stability and release profiles upon heating *in vitro*, in serum and in presence of cells. We have developed an easy one step process to make stable and safe temperature sensitive liposomes that release their drug content upon local heating. We employed luciferin as a method for *in vitro* and *in vivo* imaging, tracking and analysis (in preliminary study with the mouse foot, data not show). The long-term goal of this project is to use focused ultrasound to heat and release chemotherapy at the local tumor site using targeted, heat-sensitive liposomes.

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