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Localized delivery to CT-26 tumors in mice using thermosensitive liposomes

Jeffrey Wells, Arindam Sen, Sek Wen Hui*

Molecular and Cellular Biophysics Department, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA

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Abstract

A heat-sensitive liposomal drug delivery system was tested using Colon-26 (CT-26) cultured cells and tumors in mice. Lucifer yellow iodoacetamide (LY) was used as a fluorescence marker. The heat-sensitive liposomes exploit the temperature-dependence of critical micellar concentrations of the poloxamer, F127. LY release from unilamellar liposomes at different temperatures was measured. Onset of LY release occurred near 33 °C, and reached plateau above 42 °C when 90% of the LY was released. Temperature-treated liposomes were mixed with CT-26 cells to measure the binding of the released LY to cell surface. Temperature-dependency of cell-bound LY corresponds to the release curve. CT-26 tumors were grown subcutaneously in both hind legs of Balb/c mice. Mice received heat-sensitive or plain liposomes via tail vein injections, or no liposomes. For each mouse, one tumor was kept at 31.5 °C, while the counterlateral tumor was heated to 42 °C during injection and for 30 min after. LY released in tumors was determined from fluorescence intensity. Tumors receiving heat-sensitive liposomes plus heat treatment showed 2.5-fold greater fluorescence than all other tumors, which were at the background level. This study demonstrates the possible use of poloxamer-containing liposomes as a heat-sensitive drug delivery system in vivo.

Keywords: Liposome; Poloxamer; Hyperthermia; Controlled release; Fluorescence; Colon tumor

1. Introduction

Targeting of drugs to specific areas of the body is a major goal in drug delivery (Yatvin et al., 1978; Weinstin et al., 1979). The toxicity of many drugs, especially chemotherapeutic drugs, is an important reason to try to limit the release of drugs to a localized site. Often, local injection of drugs is not a viable option, since drugs reach only a very short distance from the injection site into the immediately surrounding tissue. For systemic delivery, encapsulation of drugs within a biocompatible delivery vehicle is likely to reduce the overall toxicity. Methods for releasing encapsulated drugs at treatment sites are greatly desired.

Liposomes provide a stable, biocompatible carrier of encapsulated drugs. Unmodified liposomes are rapidly cleared from circulation to accumulate significantly in specific sites. Incorporating one or more mole percent of phospholipids with conjugated polyethylene glycol chains (PEG-lipid) prolongs circulation times by slowing clearance by the mononuclear phagocytic system (MPS) (Woodle and Lasic, 1992). Liposomes have been shown to preferentially localize within tumors when injected systemically (Ogihara et al., 1986; Papahadjopoulos and Gabizon, 1990; Blume and Cevc, 1993; Yuan et al., 1994), especially with hyperthermia (Huang et al., 1994; Chelvi and Ralhan, 1997). A major goal of drug

* Corresponding author. Tel.: +1-716-845-8595; fax: +1-716-845-8899.
E-mail address: sekwen.hui@roswellpark.org (S.W. Hui).

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delivery for many years has been to create liposomes that can be activated to release their contents only at the site of targeted therapy.

Several physical methods have been applied to change the integrity of the liposomal membrane, to achieve triggered local release on demand. These methods include: photolysis of lipid components (Grossweiner et al., 1982; Pidgeon and Hunt, 1983; Anderson and Thompson, 1992; Thompson et al., 1996), photo-induced polymerization (Bisby et al., 1999), enzymatic cleavage of membrane lipids (Kibat et al., 1990, Wymer et al., 1998), pH lability (Yatvin et al., 1980; Connor and Huang, 1985; Straubinger et al., 1985; Meyer et al., 1998; Mills et al., 1999; Zignani et al., 2000), temperature-sensitive phase transitions of the liposomal bilayer conformation (Yatvin et al., 1978; Bassett et al., 1986; Tomita et al., 1989), and thermosensitive magnetoliposomes (Viroonchatapan et al., 1998). Many commonly used temperature-sensitive release techniques involve content leakage during phase transitions of the liposomal membrane. A main problem of previous methods involving gel to liquid phase transitions for liposomal content release have been significant content release at physiological temperatures or low content release efficiencies within the optimal therapeutic temperature range of 39–42 °C (Gaber et al., 1995).

Anyarambhatla and Needham (1999) published a formulation of heat-sensitive liposomes containing monophosphatidylcholine (MPPC) incorporated into DPPC with distearylphosphatidylethanolamine (DSPE)-PEG 2000. MPPC acts as a mild detergent producing disruption of the membrane when the bilayer is in a fluid state. Incorporating MPPC into the liposome membrane lowers the phase transition temperature of DPPC from a pure component temperature of 41.5 °C, to a mixed component temperature range of 39–42 °C. They achieved over 80% release of carboxyfluorescein (CF) after heating to 41 °C within 30 min, even in the presence of 50% bovine serum. The majority of the dye release occurred within the first 5 min of heating. This type of liposomes has been applied to trigger drug release in tumors (Kong et al., 2000).

Kono et al. (1994, 1999) and Kono (2001) tested liposomes bearing multiple copolymers of *N*-isopropylacrylamide (NIPAM), alone and with acryloylpyrrolidine having characteristic lower critical solution temperature (LCST) around 38 °C. NIPAM molecules were mixed with non-bilayer forming phosphatidylethanolamine (PE) lipids. When the temperature was raised above the copolymer's transition temperature, un-mixing of polymers and lipid triggered liposome leakage. Addition of 550 Da PEG-PE lipids suppressed content release below the LCST, but not above. Nearly 100% calcein leakage occurred at 42 °C. Serum significantly decreased the efficiency of content release.

Kim et al. (1997) reported synthesizing poly-NIPAM copolymers with varying degrees of acrylic acid additions. Copolymer transition temperatures occurred at 30, 33, 37, and 43 °C. Greater amounts of acrylic acid increased the transition temperature of the copolymers. Copolymers were found to induce leakage of dyes from the liposomes at the LCST. Dye leakage also occurred below the LCST, which may have been due to the phase transition of the dimyristoylphosphatidylcholine (DMPC)/DPPC lipids used.

Poloxamers are polyethylene oxide–polypropylene oxide–polyethylene oxide (EO–PO–EO) block copolymer surfactants. They have detergent-like qualities that enable them to be used in place of mild detergents in products for human use, such as toothpastes and contact lens cleaners. Poloxamers such as F127 are listed in the US Pharmacopeia as inactive excipients and are widely used in a variety of clinical applications. The temperature-dependent nature of poloxamers' critical micelle concentrations (CMCs) has been extensively studied (Alexandridis et al., 1994, 1995). The poloxamer, F127, has a four-fold decrease in its CMC with an increase in temperature from 31 to 35.5 °C. This is believed to be caused by a dehydration of the PO block of the copolymers at higher temperatures. The rationale for encapsulating poloxamers into liposomes is to hold a reservoir of molecules inactive at physiological temperature, and is capable of disrupting the membrane bilayer once heated. When the temperature is raised, the CMC of the poloxamer lowers dramatically, causing the poloxamers in solution to interact with the bilayer from the inside of the liposome (Kostarelos et al., 1995, 1997; Castile et al., 1999, 2001). As the poloxamers incorporate into the bilayer, the membrane becomes disrupted, allowing entrapped molecules of considerable size to escape.

Poloxamers have been used in clinical formulations as a slow content releasing gel coating (Moghimi et al., 1991; Woodle et al., 1992; Khattab et al., 1995). Chandaroy et al. (2001) utilized the heat-sensitive properties of the poloxamer F127 to heat-trigger liposome content release. In separate experiments, the poloxamer F127 was encapsulated along with CF or FITC labeled bovine serum albumin (BSA-FITC, 66 kDa). The liposomal formulation used was 1:1 DOPC:cholesterol ratio. At 0.04% (w/v) concentration of F127, CF release began above 30 °C, and reached a plateau around 90% at 38 °C. At 0.08% F127 concentration, 20% BSA-FITC was released from liposomes at 38 °C, and reached 90% release at 42 °C. Liposomes incorporating 3% PE-PEG 2000 lipids showed a similar temperature release profile to liposomes without PEG lipids. The onset temperatures of CF leakage matched published poloxamer CMT values closely.

This study is aimed to demonstrate the feasibility of using a heat-sensitive, poloxamer-containing liposome system for drug delivery in vivo. The lipid formulation used for this study was slightly modified from that used in Chandaroy et al. (2001) to improve content release and ease of production. The same liposome and poloxamer system was used throughout the project including temperature-dependent content release experiments in "test tubes," cell binding and tumor uptake.

2. Materials and methods

2.1. Materials

Lucifer yellow iodoacetamide potassium salt (LY) was purchased from Molecular Probes (Eugene, OR). All poloxamers (Pluronics) were kindly donated by BASF Corp. (Mount Olive, NJ). RPMI 1640 cell culture medium was purchased from Gibco BRL (Grand Island, NY). Triton X-100, bicinchoninic acid protein assay kit, and sodium dodecyl sulfate were purchased from Sigma-Aldrich (St. Louis, MO). Lipids were purchased from Avanti Polar Lipids (Alabaster, AL).

Centricon YM-100 cellulose centrifugal filters were purchased from Amicon (Bedford, MA). Two hundred, 400, and 800 nm Nucleopore polycarbonate membrane filters were purchased from Whatman (Clifton, NJ). Polyprep chromatography spin columns were purchased from BioRad (Hercules, CA). The hypodermic needle thermocouple probe, model HH23, was purchased from Omega (Stanford, CT). An Amicon, model MMC, diafiltraion device was used to concentrate large unilamellar vesicles (LUVs) prior to animal injections.

2.2. Thermosensitive liposome preparation

All liposomes were composed of 67 mol% dioleoylphosphatidylcholine (DOPC), 32 mol% cholesterol, and 1 mol% PEG 5000-conjugated [1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE)]. Lipids in chloroform were mixed at the given molar ratio, dried initially under a stream of nitrogen gas, then under a vacuum for 1 h. Five micromoles lipid was used for test tube experiments, and 50 μmol for in vitro cell binding and in vivo tumor uptake experiments. Four milligrams per millilitre LY was dissolved in PBS buffer (0.25 Osm). LY solutions were used within 2 weeks of preparation and stored at 4 °C to avoid degradation. All poloxamer-containing solutions were 0.04% (w/v) F127 in PBS. The dried lipids were hydrated at 4 °C with 2 ml LY/F127 solution for the test tube experiments, and 3 ml for the in vitro and in vivo experiments. The samples were vortexed gently to create multilamellar vesicles. LUVs prepared for test tube and in vitro experiments were extruded 10 times through a 400 nm pore size polycarbonate filter at 4 °C. LUVs prepared for in vivo experiments were extruded 3 times through an 800 nm filter, followed by 3 times through a 400 nm filter, and 4 times through a 200 nm filter. The free LY and F127 were separated from the LUV encapsulating the dye and poloxamer by using Sephadex G-25 column chromatography. Test tube experiments used a gravity driven column. In vitro and in vivo experiments used spin columns centrifuged at 1000 × g for 2 min. Unencapsulated (free) dye and poloxamers are less than 10% of encapsulated ones in eluted LUV samples. For in vivo experiments, the eluted volume was then concentrated four-fold in a diafiltration device using 300 kDa pore size cellulose filters. CF was encapsulated in the same manner as described in Chandaroy et al. (2001), but used the same lipid mixtures as described above for LY.

2.3. Content release (test tube) assay

One millilitre LUV samples were aliquotted into plastic tubes and placed in water baths maintained at 21, 30, 33, 36, 39, 42, and 45 °C for 30 min. As a standard, complete dye release samples were prepared by adding 30 μl of 2% Triton X-100 solution and heated at 45 °C. After heating, samples were put into 30 kDa centrifugal filter tubes and spun at 1000 × *g* for 1 h at 4 °C to remove intact LUVs. The filtrate was then assayed by fluorimetry for LY content. Fluorimetry assays were performed using a spectrofluorimeter, model 8000 C, from SLM Instruments (Urbana, IL). Fluorescence emission scans from 490 to 580 nm were taken. The LY fluorescence peaks, with an excitation at $\lambda =$ 427 nm, were at emission $\lambda =$ 528–533 nm. CF fluorescence was measured with an excitation $\lambda =$ 475 nm and emission $\lambda =$ 515–519 nm.

2.4. In vitro cell binding assay

Heating of LUV samples was performed the same manner as done for the test tube experiments. After heating, the liposome samples were allowed to cool down to 20 °C. Colon-26 (CT-26) tumor cells were grown in RPMI 1640 medium. Cells were washed 3 times with PBS, scraped from petri dishes, vortexed to suspend cells, and aliquotted into plastic tubes with 1.5×10^6 cells in 500 μl PBS in each tube. One ml LUV samples were added to each tube of cells to allow for binding of the LY to cell surfaces. The cells were shaken gently on a rotating platform for 30 min at 20 °C. After incubation, the tubes were centrifuged at 1000 × *g* for 5 min. The supernatant was removed and the cells were washed with PBS to remove LUVs and unbound LY. Cells were washed twice more. Five hundred microlitres of a mild cell lysis buffer (4% Triton X-100, 50 mM SDS, 10% ethanol in PBS) was added to each tube. Lysis buffers containing sodium hydroxide were found to permanently destroy most of the LY fluorescence and therefore not used. Samples were vortexed and incubated at 40 °C for 24 h to completely dissolve the cells in the lysis buffer. Samples were then assayed by fluorimetry.

2.5. In vivo tumor uptake assay

CT-26 cells excised from tumor bearing mice were dispersed and frozen immediately. The frozen cells are capable of forming tumors. Hair on the hind legs of BALB/c mice was removed with Nair® hair removal lotion 1 day prior to tumor cell innoculation. Frozen CT-26 cells were thawed and washed with PBS buffer. 1×10^7 cells were subcutaneously injected onto each hind leg of the mice. Tumors grew to about 1 cm after 3 weeks. The day preceding heat treatments, hair on the hind legs of the mice was again removed. Immediately prior to treatment, mice were anaesthetized intraperitoneally with 50 mg ketamine/kg and 5 mg xylazine/kg mouse body weight. One mouse at a time was immobilized. A needle probe thermocouple was inserted into the center of the tumor to be heated. Temperatures of unheated tumors were also taken. Heating was accomplished by shining a heat lamp directly upon a piece of black felt placed over the tumor. The area around the tumor was surrounded by three layers of aluminum foil. This ensured that radiative heating only occurred through the tumor surface. The heating was constantly monitored to maintain an intratumor temperature of 42.0 ± 0.6 °C. Unheated part of the anaesthetized mouse body is normally at or below 35.0 °C. Once the intratumoral temperature reached 42 °C, the mouse was injected with 150 μl LUV sample via a tail vein. Heating continued for 30 min after injection of the liposomes. After heating, the mouse was immediately sacrificed and the tumors excised. For each treatment group, six or more tumors were used. Tumors were frozen and thawed twice before homogenization. PBS was added to each tumor at a 3:1, PBS:tumor volume ratio. The tumors were homogenized in a Polytron tissue homogenizer. After homogenization, cell lysis buffer was added to the tumor extract at a 1:1 ratio. The extracts were incubated 24 h at 40 °C and vortexed. The extracts were centrifuged at 10,000 × *g* for 2 h to remove undissolved cell matter. Tumor lysates were assayed by fluorimetry.

3. Results

3.1. Content release ("test tube") experiments

LUVs encapsulating LY were treated at various temperatures, and the amounts released were assayed using a filtration method. LY remaining entrapped within LUVs was separated from that released by heating, using centrifugal filter tubes. The accuracy of this assay

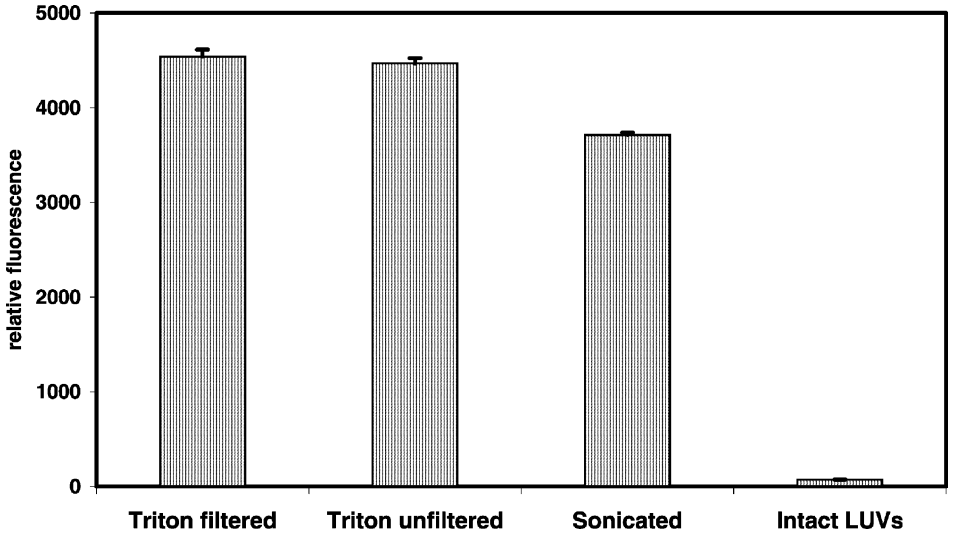

Fig. 1. A comparison of fluorescence intensities of unfiltered LUV encapsulating CF and lysed by Triton X-100, and of filtrates of LUV before or after lysis by Triton X-100 or by sonication. Filtrate samples were collected from 30 kDa centrifugal filter tubes after spun at $1000 \times g$ for 1 h at 4 °C. The result verifies the separation of LUVs from free dye for the "test tube" content release assay.

method was verified with LUVs containing a 4 mg/ml CF solution without poloxamer. The LUVs for all experiments had the same lipid composition. Fig. 1 shows a comparison of fluorescence of LUV samples filtered before and after lysis with Triton X-100. (MLV samples lysed by this method show no sign of remaining vesicles when viewed by microscopy.) There was no difference in fluorescence between filtered or unfiltered Triton-lysed samples. The fluorescence values of filtrates of intact LUV samples were less than 1.6% of that of the Triton-lysed samples. This verifies the validity of this method for separating LUVs from free dye. Sonication of the liposomes for 10 min is also shown to compare its efficiency versus Triton treatment for releasing entrapped dye from liposomes. Sonicated samples had 83% as much fluorescence as Triton lysed samples.

Temperature-dependent dye release after 30 min heating in test tubes is shown in Fig. 2. Results were normalized to percent release, compared to samples lysed with Triton. LY release from heated vesicles is depicted as closed circles. LY release reached 82 and 90% at 42 and 45 °C, respectively. Values plotted represent an average of three experiments. Error bars of standard deviations are given. LY release occurred over a 9 °C temperature range. Test tube results were used as a reference for in vitro cell binding and in vivo tumor uptake fluorimetry experiments.

3.2. In vitro cell binding experiments

The fluorescence intensity of LY bound to CT-26 tumor cells was measured as a function of liposome treatment temperature. LY-encapsulating LUV were treated at different temperatures before adding to cells at 20 °C. The minimum fluorescence level of LY detectable above cell autofluorescence was predetermined to be at a concentration around 25 μg/ml, for the number of cells used. Average cell autofluorescence of negative control samples was subtracted from the raw data values. A positive control reference for 100% release was made by sonication, before adding to cell aliquots. Triton was not used to lyse the LUVs, to avoid the problem of Triton lysing cell membranes when added to cells.

For comparison, LY bindings to cells after heat-induced release from liposomes are plotted as filled triangles shown in Fig. 2. All data are normalized to the value measured with positive control sample. Cell-bound LY released from liposomes at 22 °C was 17%, and reached 51 and 98% with

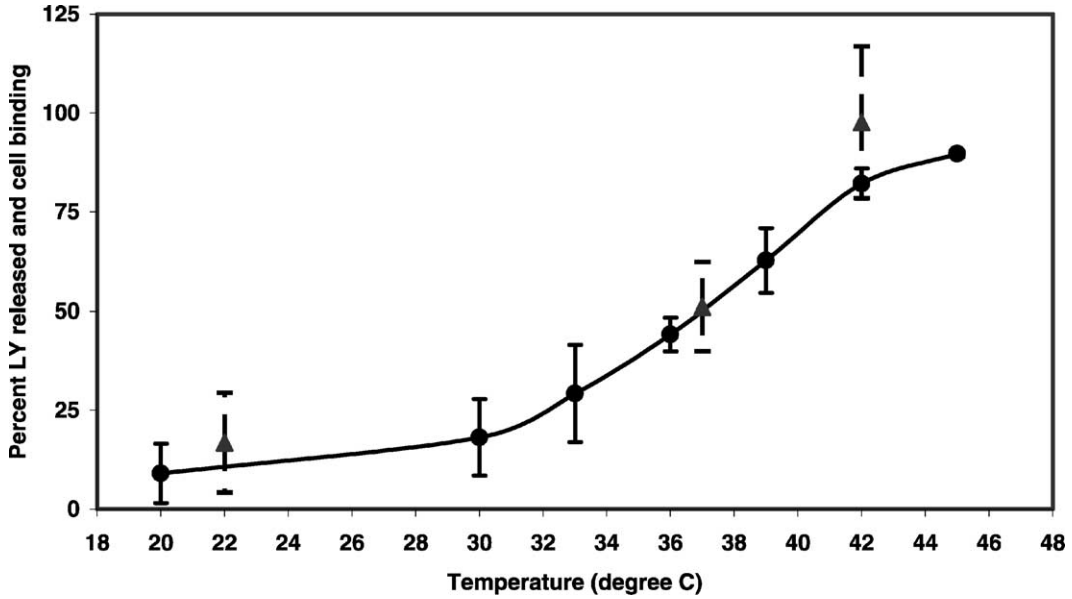

Fig. 2. Temperature-dependent LY release from F127-containing LUV, as determined by the "test tube" method (filled circles). Results are normalized to percent release, compared to samples lysed with Triton. Values plotted represent an average of three repeated experiments. Error bars represent standard deviations. For comparison, LY binding data to CT-26 cells after heat-induced release from liposomes are plotted as filled triangles.

liposomes treated at 37 and 42 °C, respectively. The cell binding data corresponds remarkably well with the test tube results. Autofluorescence of tumor cells was not a large factor in these results. Differences in fluorescence values of test tube and cell binding data were within standard deviations.

3.3. *In vivo tumor uptake experiments*

The intensity of LY fluorescence detected in dissolved tumor tissue is much lower in comparison to those from in vitro experiments. Overlapping spectra of LY and the autofluorescence of tumor tissue is a concern. Fig. 3 shows the emission spectra of pure LY and average unheated tumor autofluorescence excited at 427 nm, normalized to the same scale. Considerable overlap of fluorescence peaks obscures much of the LY signal. LY fluorescence is detectable only if it exceeds the autofluorescence spectrum of the tumor tissues. LY fluorescence values of tumors were averaged from readings in the wavelength range of 528–533 nm. This corresponds to the LY fluorescence peak found in test tube experiments.

Tumored mice were injected with liposomes during local heating of one tumor to 42 °C. Tumors were divided into six treatment groups:

1. heat-sensitive liposome injection plus local heat treatment (FH),
2. heat-sensitive liposome injection without local heating (FC),
3. non-heat-sensitive liposome injection plus local heat treatment (NH),
4. non-heat-sensitive liposome injection without local heating (NC),
5. no injection of liposomes but with local heat treatment (CH), and
6. no injection of liposomes but without local heating (CC).

Fluorescence intensities of tumor treatment groups are given in Fig. 4. Each individual data point represents the fluorescence intensities of an individual tumor normalized for protein content. Protein content was measured separately with a bicinchoninic acid assay. Comparing heated and unheated tumors from the same mice also allowed determination of whether

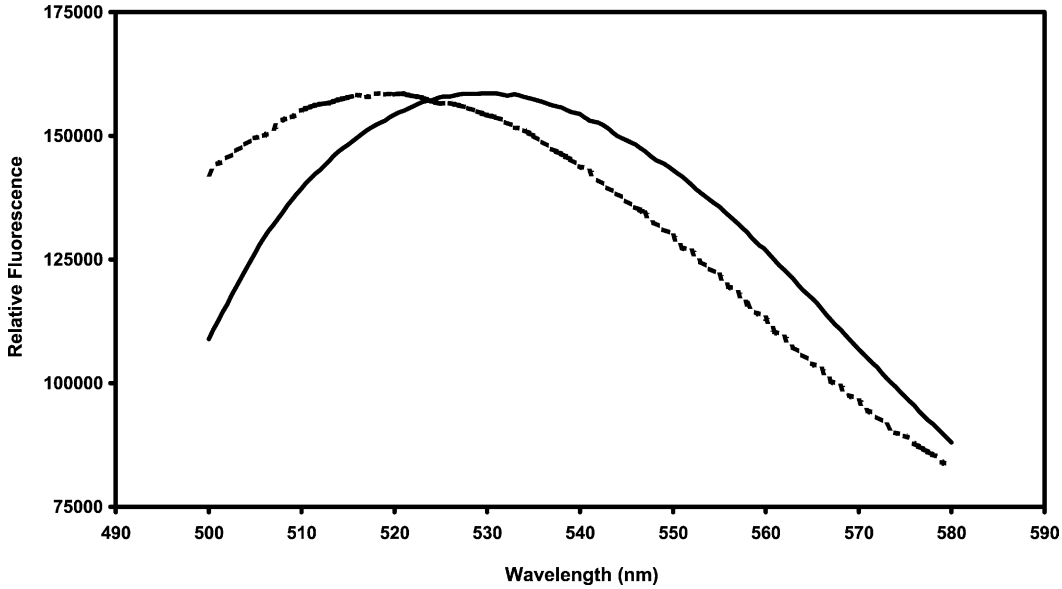

Fig. 3. Fluorescence emission spectra of LY (solid curve) and of unheated CT-26 tumor autofluorescence (dotted curve), both excited at 427 nm, are shown normalized to the same scale. Considerable overlapping is apparent.

the dye traveled away from the site of heat treatment through the circulation to the contralateral tumor. Comparing heated or unheated control tumors receiving no liposomes demonstrated variability of tumor autofluorescence values.

Unheated tumors receiving heat-sensitive liposome injection showed similar fluorescence to control uninjected groups. This indicates that dye release was insignificant at 31.5 °C, the temperature at which unheated tumors were maintained. In vitro, a small

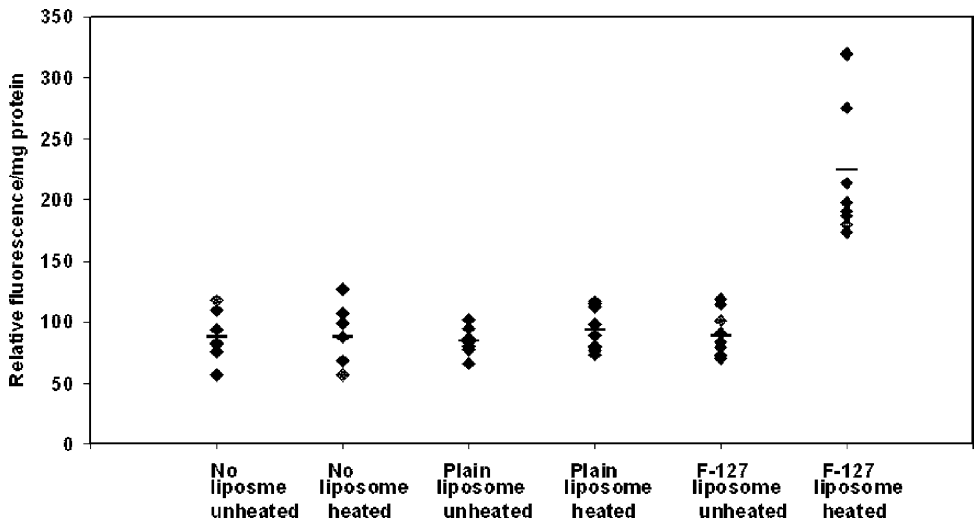

Fig. 4. Fluorescence values of individual CT-26 tumor, after normalized for protein content, are plotted for each treatment group. The bar in each data group represents the mean value.

amount of dye release was detected at 30 °C. The low LY quantum yield and the overlap of the LY and tumor autofluorescence spectra obscured the differences between CC, CH, NC, NH, and FC group values. However, values of the FH data group were significantly higher than those of the other groups ($P < 0.00002$). The lowest value of normalized LY fluorescence for any tumor in the FH group was higher than the highest value for any tumor outside of this group. It also shows that LY released at the heated tumor site bound rapidly and efficiently close to the site of release.

4. Discussion

We have demonstrated the in vitro and in vivo applications of the temperature-sensitive liposome system developed by Chandaroy et al. (2001), using the poloxamer F127. Minor modifications were made from the protocol used by Chandaroy to facilitate use of this system in vivo. We screened a large number of poloxamers for better heat-induced dye release at varying concentrations and temperatures, but F127 remained the best choice in the 30–42 °C temperature range. Alexandridis and Holzwarth (1997) and Pandit et al. (2000) showed salt species dependent decreases in CMC with the addition of phosphate and chloride ions. Replacing the buffering ions from phosphates to 3-[*N*-Morpholino] propanesulfonic acid (MOPS) did not show a noticeable improvement in the onset temperature or range of heat-induced release. Attempts to produce the poloxamer-containing liposomes at 20 °C resulted in low content release efficiency. Lowering the ambient temperature during hydration and extrusion of the lipids was found to be necessary to avoid incorporating poloxamers into the bilayer. No effort was made to optimize the ambient temperature at which the liposomes could be produced while maintaining release efficiency.

LY, instead of the general fluorescence indicators CF and FITC-BSA used by Chandaroy et al. (2001), was chosen as the dye for heat-induced release from liposomes and in vitro cell binding experiments for two reasons. The iodoacetamide moiety stably binds to cell surface proteins at room temperature and physiological pH. It was also the most water-soluble of several commercially available, protein-binding dyes tested. Water solubility of LY is approximately 10 mg/ml at room temperature. Release of liposomal contents occurred within a 30-min period of heat treatment. A significant drawback in using LY for this study was its low fluorescence quantum yield. This fact complicated analysis of in vivo data especially.

CT-26 tumor cells were used because these cells can be cultured in vitro and grown as subcutaneous solid tumors in mice. CT-26 cells were in suspension during the in vitro LY-binding experiment to maximize cell surface exposure to the dye. Even so, the majority of the LY released by the liposomes was not bound to cells, but remained in the supernatant. This could explain the relative proportion of LY bound to cells reflecting the amount released in solution, as shown in Fig. 2.

In comparison to cell-bound LY signal in vitro, the LY fluorescence of in vivo samples was much lower in relation to cell autofluorescence. This was partly due to limited exposures of tumor cells to the released dye. The significant tumor cell autofluorescence in the same range as LY fluorescence obscured the measurement of weak LY spectra. Much of the variability of fluorescence between tumors in the control and treatment groups could also be related to differences in the amount of blood contamination in tumor lysates. Tumor to tumor differences in autofluorescence further added to the dispersion of results.

In addition to LY release at the site of heated tumors, other factors would also influence the fluorescence intensity at heated sites. These factors include: heat-induced vasodilation within the tumors leading to increased accumulation of liposomes (Huang et al., 1994), extravasation of liposomes caused by the insertion of thermocouple needles, the short time period that the circulating liposomes passing through the heated region, and the continued circulation of released LY not immediately bound at the site of heating. In the temperature-insensitive vesicle treatment groups (NC, NH), heating had a small and statistically insignificant ($P = 0.229$) effect upon the amount of fluorescence detected. The time necessary for efficient release of LY from LUVs is not known precisely. The unheated tumor may receive heat-sensitive liposomes as well as released dye from the heated tumor. In addition, the unheated tumor temperatures remained around 31.5 °C. Twenty-three percent of LY is

released from liposomes around 32 °C (Fig. 2). Thus, unheated tumors are expected to have higher fluorescence levels than control tumors (without injection). Yet none of the variation was observed when comparing unheated tumors exposed to injected liposomes (NC, FC) to tumors without injection (CH, CC). The differences, if any, are below the high background autofluorescence of the tumor tissue. The insertion of thermocouple needles in unheated tumors did not cause measurable liposome extravasation, as compared to control tumors without needle insertion. Had the autofluorescence been weaker, a more direct comparison of in vivo and in vitro data could have been made. The fact that the heated, heat-sensitive liposome treatment group (FH) had much higher fluorescence than the unheated (FC) group ($P = 0.00002$) demonstrates that LY was released from liposomes upon heating, and that a sufficient amount of released LY remained at the heated site.

5. Conclusion

This study proves the feasibility of using a heat-sensitive poloxamer system to release liposomal contents in vivo. Dye delivery enhancement with thermosensitive liposomes and mild hyperthermia was much greater than the effect of hyperthermia alone. Further formulation optimization may be made of this system. Release of small polar molecules such as LY with the heat-sensitive poloxamer-liposomes was very efficient in test tubes and in cell binding experiments in vitro. Despite the 9 °C temperature range from onset to plateau of release, enhancement of in vivo dye delivery to locally heated CT-26 tumors was statistically significant.

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