Research Article

Spray-Dried Microparticles Containing Polymeric Micelles Encapsulating Hematoporphyrin

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Abstract. The purpose of this study was to examine the properties of a new pulmonary delivery platform of microparticles containing micelles in which a therapeutic photosensitizing drug, hematoporphyrin (Hp), was encapsulated. Different poloxamers were used to form micellar Hp, and one of these, Pluronic L122-Hp, was subsequently incorporated into lactose microparticles by spray-drying. Spectral and morphological analyses were performed on both micellar Hp, and lactose microparticles containing micellar Hp (lactose-micellar Hp) before and after dissolution of the microparticles in water. Photodynamic activity of the various Hp samples were evaluated in human lung epithelial carcinoma A549 cells using a light-emitting diode (LED) device at a wavelength of 630 ± 5 nm. No significant difference was observed between micellar Hp and lactose-micellar Hp regarding the generation of singlet oxygen. The mean particle size of the microparticles was $2.3\pm 0.7\mu\text{m}$ which is within the size range for potential lung delivery. The cellular uptake of micellar Hp and lactose-micellar Hp measured on A549 cells was at least twofold higher than those obtained with the Hp at equivalent concentrations. Micellar Hp exhibited higher cytotoxicity than Hp due to reduced formation of Hp aggregates and increased cellular uptake. The spectral properties as well as the photodynamic activity of the micellar Hp was retained when formulated into microparticles by spray-drying. Microparticles containing micelles have the potential for delivering micelle-encapsulated hydrophobic drugs in targeted therapy of pulmonary diseases.

KEY WORDS: micelle; microparticle; photosensitizer; pulmonary; spray-drying.

INTRODUCTION

Pulmonary inhalation systems are used for local delivery of active agents, especially in diseases of the lung including cancer, asthma, and chronic pulmonary infections (1). The advantages of local delivery include rapid onset of action, reduced dose requirements, reduced exposure of drug to the systemic circulation, and decreased side effects (2,3). However, there are additional obstacles that must be overcome with inhalation aerosol delivery before administered drugs can reach the site of drug action (4,5). To begin with, mucociliary clearance can exclude drugs and foreign particles introduced to the airways. In addition, large molecules or particles may not be able to cross the epithelium or the cytoplasmic membrane, thus the therapeutic agents may not be able to reach their site of action (6).

Studies suggest that mucociliary clearance can be overcome by using nano-size suspensions in aqueous droplets (7). *In vitro* studies using nano-size carriers have demonstrated avoidance of macrophages phagocytosis and enhanced uptake by epithelial and cancer cells (8–12). Therefore, the use of nano-size carriers may aid in preventing undesirable mucociliary clearance and may further enhance efficient drug transport to the epithelium. However, nano-size carriers for pulmonary delivery of drugs suffer from the disadvantage of being easily exhaled from the respiratory tract. Sham (13) *et al.* demonstrated that nanoparticles can incorporate into microparticle carriers and that the incorporation of nanoparticles did not affect the fine particle fraction or mass median aerodynamic diameter of the microparticles. Their results demonstrated that nanoparticles can be delivered to the lungs in microparticles that subsequently dissolve following contact with the aqueous environment of the lung epithelium.

In the present study, we developed carrier microparticles composed of lactose, an FDA-approved material for several inhalation products. Polymeric micelle nanocarriers were contained in these microparticles. Figure 1 shows a schematic representation as to how the nanoparticle would be delivered to the epithelium in a microparticle carrier. The size ($1\sim 5\mu\text{m}$) (14) of type carrier particles can allow sufficient deposition of the bronchial region or the alveolar region. When these microcarriers deposit in the lungs, the lactose

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Fig. 1. Schematic of polymeric micelle loaded into microparticles for lung delivery

matrix is expected to rapidly dissolve and release the polymeric micelle.

Polymeric micelles have been used as drug carriers for intravenous administration and exhibit many advantages including their ease of production, long circulation times and having surfaces that are readily modified with targeting ligands (15). It was previously shown that various hydrophobic agents could be successfully incorporated into polymeric micelles (16–22). Commercially available Pluronic polymers are amphiphilic surfactants that can self-assemble into polymeric micelles in aqueous solutions above their critical micellar concentrations (CMC) (23). Pluronic triblock copolymers have PEO_(a)–PPO_(b)–PEO_(a) as their basic structure formed by hydrophobic propylene oxide (PO) and hydrophilic ethylene oxide (EO) blocks where the a and b chain lengths vary from 2–130 and 16–70 units, respectively (24). In this study, micelles containing a hydrophobic photosensitizer drug (hematoporphyrin, Hp) were prepared using three different Pluronic polymers: F127, P105, and L122 (Fig. 2). The highly drug-loaded micelles encapsulating Hp were subsequently entrapped in spray-dried lactose microparticles. The physicochemical properties of these microparticles were determined and their cellular uptake and cytotoxic effects following photoactivation were compared to free Hp and micellar Hp that was not entrapped in microparticles.

Fig. 2. Characteristics of Pluronic block copolymers

MATERIALS AND METHODS

Materials

Hematoporphyrin dihydrochloride (Hp) was purchased from ChromaDex (Irvine, CA), Pluronic block copolymers (P105 and L122), α -lactose monohydrate, dimethylformamide (DMF), chloroform, dichloromethane, methanol, histidine, *n*, *n*-dimethyl-4-nitroso-aniline (RNO) were purchased from Sigma (St Louis, MO, USA). Pluronic F127 (PF127) was purchased from Wei Ming Pharmaceutical (Taipei, Taiwan). Absolute ethanol was used immediately after opening and experiments were performed on freshly prepared samples. A Milli-Q system (Academic, ZMQS60001) was used to purify H₂O, and all other chemicals were analytical-grade reagents obtained from Sigma.

Preparation and Characterization of Hp Entrapped in Micelle

Three types of Pluronic block copolymers, L122, P105, and F127 were used in this study. Hp was entrapped into micelles by the film formation method (25). Briefly, Hp in methanol was added to a solution of L122 or F127 in chloroform, or P105 in dichloromethane to obtain 100:1, 100:2, 100:4, and 100:10 polymer/drug (*w/w*) ratios. The solvent was removed by rotary evaporation to form a thin film. The film was hydrated using 1 mL of distilled water at room temperature to give a final 10% *w/v* micelle solution. The hydrated preparation was kept overnight at room temperature and then passed through a 0.22 μ m PVDF filter (Millipore®, Volketswill, Switzerland) to remove the untrapped Hp. Size distribution was measured with dynamic light scattering using a particle size analyzer (Coulter N4 Plus Submicron, Beckman Coulter).

Determination of Drug Loading and Entrapment Efficiency in Micelle

An accurately weighed amount of freeze-dried micellar Hp was dissolved in absolute ethanol. The amount of Hp entrapped was determined by measuring its absorption at 397 nm. The drug loading and the entrapment efficiency were calculated as follows (26):

$$\text{Drug loading (\%)} = \frac{\text{Mass of Hp in micelles}}{\text{Mass of micellar Hp}} \times 100(\%) \quad (1)$$

$$\begin{aligned} \text{Theoretical drug loading (\%)} \\ = \frac{\text{Mass of initial Hp}}{\text{Mass of initial pluronic and Hp}} \times 100(\%) \quad (2) \end{aligned}$$

$$\text{Entrapment efficiency(\%)} = \frac{\text{Drug loading}}{\text{Theoretical drug loading}} \times 100(\%) \quad (3)$$

Preparation of Lactose Microparticle Containing Hp-Entrapped Micelle

The spray-drying process to form lactose microparticles containing micellar Hp (lactose-micellar Hp) was performed

using an EYELA SD-1000 spray dryer (Japan). The adjustable parameters include inlet and outlet temperature, solution pump flow rate, and the aspirator partial vacuum. In the present experiments, the inlet air temperature was 150°C, outlet temperature was 80~85°C, the pump flow rate was 4 mL/min, the aspirator was set to 0.45 m³/min, and the atomizing air flow rate was 250 kPa. Two grams of lactose were dissolved in 99 mL of distilled water and mixed with 1 mL of a suspension comprised of 0.1 g Pluronic micelles containing 2 mg Hp and subsequently pumped into the feeding system of the spray dryer. The glass chambers of the spray dryer were shielded from light. After spray-drying for 25 min, the powders were removed from the collector vessel and stored at 4°C under light protection.

Spectroscopic Analysis

Absorption spectra (300~650 nm) of free Hp, micellar Hp and lactose-micellar Hp being were measured with a Beckman COULTER DU800 spectrophotometer.

Singlet Oxygen Generation

Aggregation of the hydrophobic photosensitizers could possibly result in self-quenching of the excited state in aqueous medium (27). Aggregated photosensitizers generally produce very little singlet oxygen and have much lower photodynamic activity. If the micellar Hp was successfully loaded in lactose microparticles and did not degrade or aggregate following addition of the microparticles to aqueous media, then the singlet oxygen production would be expected to be similar to Hp entrapped in micelles but greater than free Hp. The detection of singlet oxygen was performed by an RNO (*p*-nitrosodimethylaniline) bleaching assay. The free Hp (2 μ g/mL), micellar Hp and lactose-micellar Hp were exposed to green light using a LED (540 $\pm$ 10 nm, 22 mW) device in the presence of histidine (0.01 M) and RNO (50 μ M) in PBS (pH 7.4). The bleaching rate of RNO was followed at 440 nm. The slope (*S*) of the plot of bleached absorption ($-A$) at 440 nm vs. irradiation time is proportional to the rate of production of singlet oxygen. This method is based upon secondary bleaching of RNO as induced by the reaction of ¹O₂ with the histidine ring which results in the formation of a transannular peroxide intermediate (transannular peroxide) capable of inducing the bleaching of RNO (28). The solution, taken in a 3-mL glass cuvette placed at a distance of 2.5 cm from the light source, was continuously stirred during irradiation. The irradiation was carried out in an open cuvette in equilibrium with the atmosphere (29). The irradiation experimental setup and spectroscopy instruments were set nearby, and absorbance measurements followed by irradiation were carried out every 1 min using a UV-Vis spectrometer.

Scanning Electron Microscopy

Surface morphology of the lactose-micellar Hp particles was examined by Scanning Electron Microscopy (SEM; Hitachi, S-2400, Japan). SEM was performed on the dried microparticles coated with gold.

Cell Culture

The cytotoxic and cellular uptake effects of free Hp, micellar Hp, and lactose-micellar Hp on A549 lung cancer cell lines were assessed. Human lung adenocarcinoma A549 cells were maintained in a humidified incubator containing 5% CO₂ at 37°C. A549 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin (GIBCO BRL, USA). Cells were routinely grown in a tissue culture 75 T flask and harvested with a solution of 1% trypsin while in the logarithmic phase of growth. Cells were maintained in these culture conditions for all experiments.

Intracellular Distribution of Hp Fluorescence in Cells

The subcellular distribution of Hp formulated in free, micelle, and micelle-loaded lactose microparticle was examined in A549 cells. Cells were grown on coverslips in a six-well plate at (3×10^5 cells per well) for 18 h. The cells were subsequently treated with 5 µg/mL of free Hp, micellar Hp, or lactose-micellar Hp at 37°C for 3 h. For the last 30 min of incubation, cells were stained with 75 nM of LysoTracker® green. Cellular Hp and LysoTracker® green fluorescence were visualized with a confocal spectral microscope (ZEISS, LSM 510 META). The excitation source was a 405 nm for Hp, 488 nm for LysoTracker® green. To detect the fluorescence images of Hp and LysoTracker® green, the 575 nm long pass for Hp and 505±550 nm band pass for LysoTracker® green were used.

Cellular Uptake

A549 cells were seeded in a six-well plate at 2×10^5 cells per well (2 mL cell suspension) and the plates were incubated 24 h at 37°C under a 5% CO₂ atmosphere. The medium was removed and 2 mL of DMEM medium containing free Hp, micellar Hp, or lactose-micellar Hp were incubated for 3 h at 37°C under a 5% CO₂ atmosphere. The medium was removed and the cells were washed twice with 2 mL PBS. To lyse the cells, 1 mL of lysis buffer (0.1 N NaOH) was added followed by incubation on ice for 10 min. The suspension was

homogenized and centrifuged at 14,000 rpm for 20 min. The fluorescence of the supernatant was measured using a spectrophotometer (Ex, 397 nm; Em, 633 nm). A 25-µL sample of the cell lysates was analyzed using the MicroBCA™ protein assay. The uptake of Hp was calculated as fluorescence per microgram of cellular protein.

Photocytotoxicity

A549 cells were grown in 96-well plates at a density of 8×10^3 cells/well for 24 h. The culture medium was removed and replaced with free Hp, micellar Hp or lactose-micellar Hp in DMEM medium (100 µL/well). The cells were incubated for 3 h (avoiding light) and washed once with 100 µL PBS/well. The medium without phenol red (100 µL/well) was added to the cells and subsequently irradiated with 4, 6, 8 J/cm² using a LED (635±5 nm, 60 mW/cm²) light source. After light irradiation, the medium was replaced with DMEM containing 10% FBS. Twenty-four hours later, cell survival was measured using an MTT [3(4,5-dimethyl-thiazoyl-2-yl) 2,5 diphenyl-tetrazolium bromide] assay. The MTT assay is based on the activity of mitochondria dehydrogenases, which can reduce a water-soluble tetrazolium salt to a purple insoluble formazan product. The amount of MTT formazan product was analyzed spectrophotometrically at 570 nm.

RESULTS

Loading and Entrapment Efficiency of Drugs in Micelles

Hp was entrapped into micelles using the thin film method. Figure 3 shows a linear increase in the amount of Hp loaded into the polymeric micelles as the amount of Hp added to the Pluronic solutions is increased. Maximum drug loadings of 8.4%, 6.5%, and 4.4% were obtained with the L122, P105, and F127 micelles, respectively. No matter what initial amount of Hp was used in the preparation, the loading efficiency of L122 is statistically greater than those of P105 and F127 ($P < 0.05$). The optimal polymer:Hp ratio for maximum entrapment efficiency was at 100:2 for all Pluronic micelles. Using this ratio, the entrapment efficiency was

Fig. 3. Effect of initial amount of Hp used to prepare Hp-entrapped micelles on drug loading (a) and entrapment efficiency (b)

98.3% for the L122 micelles which has the longest PO chain and shortest EO chain, and was indicative of optimal micellar stabilization. In view of the high drug loading and entrapment efficiency, Pluronic L122 micelles encapsulating Hp were used in the subsequent studies to form the micelle-containing microparticles.

Characterization of Micellar Hp Before and After Spray-Drying

The absorption spectra of Hp in water and ethanol as well as micellar Hp and lactose-micellar Hp resuspended in water are shown in Fig. 4.

The particle diameters of Pluronic L122 micellar Hp and lactose-micellar Hp dispersed in water were 105 ± 30 and 112 ± 46 nm, respectively ($P > 0.05$). The relative $\lambda_{397}/\lambda_{372}$ intensities of micellar Hp (1.351 ± 0.001) and lactose-micellar Hp (1.359 ± 0.007) were slightly lower than Hp in ethanol (1.428 ± 0.014), but higher than Hp in PBS (0.831 ± 0.037) indicating that a higher level of monomerization of Hp occurred after spray-drying and re-dissolving in water.

Relative Singlet Oxygen Generation of Free Hp, Micellar Hp, Lactose-Micellar Hp

The generation of singlet oxygen from free Hp in PBS, micellar Hp dispersed in PBS, re-dissolving of lactose-micellar Hp in PBS was detected by spectrophotometric measurement of *p*-nitrosodimethylaniline (RNO) bleaching induced by imidazole as a singlet oxygen specific substrate. As shown in Fig. 5, there are no significant differences ($P > 0.88$) between the rates of RNO photobleaching ($-A_{440}$) in the presence of histidine (caused by singlet oxygen production) by micellar Hp and of the resuspended lactose-micellar Hp. However, the values of both of these samples are significantly different than that of free Hp. ($P < 0.002$).

Optical and Fluorescence Microscope Images of Lactose-Micellar Hp

The morphology of the spray-dried powder made of micellar Hp loaded into lactose microparticles was examined

Fig. 4. Absorption spectra of Hp

Fig. 5. Oxidation of RNO by singlet oxygen in the presence of histidine produced by illumination of Hp in PBS, micellar Hp and resuspended lactose-micellar Hp measured by loss of absorbance at 440 nm

using optical and fluorescence microscope (Fig. 6). The spray-dried powder produced spherical microparticles. Figure 6b shows fluorescence microscopic images of the lactose-micellar Hp. The images demonstrate the distribution of micellar Hp

Fig. 6. Optical (a) and fluorescence (b) microscopic images ($\times 1,000$ magnification) of microparticle containing spray-dried micelle-Hp

Fig. 7. Spray-dried powder morphology visualized by scanning electron microscopy

throughout the lactose microparticle visualized using green light as an excitation source to produce fluorescent red light.

Size and Morphology of Lactose-Micellar Hp

The particle dimensions and surface morphology of the spray-dried powders were visualized using scanning electron microscopy (Fig. 7). Spray-dried lactose particles containing micellar Hp were perfectly spherical with a smooth surface. The mean geometric particle size of spray-dried powders was $2.3 \pm 0.7 \mu\text{m}$, within the respirable particle size range ideal for maximizing pulmonary deposition of dry powders.

Intracellular Distribution of Hp Fluorescence in A549 Tumor Cells

The distribution of Hp formulations in A549 tumor cells was examined and visualized by confocal microscopy. After incubation of free Hp, micellar Hp, and lactose-micellar Hp with the cells for 3 h, intracellular red fluorescence was clearly observed in the cytoplasm (Fig. 8). The LysoTracker®

Free Hp

Micellar Hp

Lactose-micellar Hp

Fig. 8. Visualization of intracellular fluorescence of lung adenocarcinoma cells (A549) after treated with free Hp, micellar Hp and lactose-micellar Hp for 3 h and then processed for confocal microscopic examination: *a* Hp fluorescence (red), *b* LysoTracker® green staining (green), *c* co-localization of *a* and *b*

green probes permeated freely through cell membranes and typically concentrated in the lysosomes. After incubation for 30 min, the intracellular green fluorescence spots were observed in the lysosomes. It is also known that nanocarriers can undergo endocytosis and localize in lysosomes. After incubation, all formulations were taken up by A549 cells and were delivered to the cytoplasmic region but not to lysosomes. Cellular uptake was quantified demonstrating that Hp in all forms entered the cells in a concentration-dependent manner (Fig. 9). The fluorescence intensities measured on A549 cells treated with micellar Hp and lactose-micellar Hp were at least twofold greater than those obtained with free Hp at equivalent concentrations.

Photocytotoxicity of Free Hp, Micellar Hp, Lactose-Micellar Hp

The photocytotoxicity of free Hp, micellar Hp, and lactose-micellar Hp in A549 cells measured by the MTT assay following irradiation with LED is depicted in Fig. 10. Under dark conditions, none of the Hp formulations had a cytotoxic effect, but all elicited a light-dose-dependent decrease in cell survival. After a 3-h incubation of A549 cells with 0.5 $\mu\text{g/mL}$ Hp in all formulations and subsequent irradiation at 4 J/cm^2 , cell survival was 89% of the control for free Hp, 47% for micellar Hp, and 44% for lactose-micellar Hp. When irradiation power was increased to 8 J/cm^2 , cell survival was decreased to 75% of control for free Hp, 12% for micellar Hp, and 11% for lactose-micellar Hp.

DISCUSSION

In this study, the photosensitizer Hp entrapped in polymeric micelles was incorporated into lactose microparticles as a pulmonary drug delivery system. Polymeric micelle cores may act to improve the apparent solubility of lipophilic drugs. Poorly soluble drugs can be loaded into the micelle core by hydrophobic interactions or covalent/non-covalent

Fig. 9. Influence of the drug concentration on cellular uptake of free Hp, micellar Hp, and lactose-micellar Hp. The A549 mammary tumor cells were incubated at different equivalent drug concentrations in DMEM medium for 3 h. The values shown are means of three independent experiments and bars are the standard error of the mean. *** $P < 0.001$

Fig. 10. Effect of light dose on photocytotoxicity of Hp, micellar Hp, and lactose-micellar Hp. The A549 tumor cells were incubated at equivalent drug concentration of 0.5 $\mu\text{g/mL}$ for 3 h and irradiated at light doses of 4, 6, 8 J/cm^2 . Cell viability was assessed by MTT assay 24 h after light irradiation. The values shown are means of three independent experiments and bars are the standard error of the mean. *** $P < 0.001$

bond formation. The total copolymer molecular weight, chain length of hydrophilic (corona), and hydrophobic (core) portions, and drug concentration are factors that influence micelle size, loading capacity, and loading efficiency (21,24,30). Polymeric micelles were selected for the entrapment of the photosensitizer Hp due to ease of production, small particle size, and high drug loading capacity. The maximum Hp entrapment efficiency was obtained with Pluronic L122 micelles, which has a longer PO chain and shorter EO chain than the Pluronic F127 and P105 micelles, thus enabling higher drug loading capacity.

No significant change in the particle size was observed after spray-drying. The photophysical properties of Hp strongly depend on Hp aggregation state (31). In this study, the λ_{max} of Hp aggregates in water was observed at 372 nm. Hp in ethanol is known to reduce aggregation of Hp; in this solvent, a λ_{max} red shift to 397 nm was observed typical of monomer Hp (32). Similar patterns were observed for Hp entrapped in micelles and lactose microparticles containing micellar Hp after dispersed in water. The red shifts to 398 nm can be interpreted as arising from the monomerization of Hp aggregates when bonded to the non-polar regions of the micelle. The relative absorption intensities of the Hp monomer and aggregate ($\lambda_{397}/\lambda_{372}$) can be used as a measure of the aggregation of Hp in solution (33).

The well-established absorption spectrum of Hp and singlet oxygen generation was utilized to characterize Hp entrapped in micelle or lactose microparticle containing micellar Hp. This result indicates that micellar Hp after spray-drying with lactose and re-dissolving the carrier particles in water did not change the photochemical properties of Hp-entrapped micelle.

If the micelle had disintegrated after spray-drying with lactose and dissolving the microparticle in water, aggregation of the Hp might result with subsequent self-quenching effect of the excited state in aqueous medium (27). As it has been known from previous reports, aggregated photosensitizers

generally produce very little $^1\text{O}_2$ and have much lower photodynamic activity. In our study, after resuspending the micellar Hp loaded into lactose microparticles, the λ_{max} and $\lambda_{397}/\lambda_{372}$ ratio of Hp was similar to that observed for Hp in ethanol. We employed RNO as a probe to monitor the singlet oxygen formation and found that there were no significant differences in the rates of decay of RNO (as monitored by the decrease in absorbance at 440 nm) between micellar Hp and lactose-micellar Hp. Analysis of absorption spectra and singlet generation studies of micellar Hp and lactose-micellar Hp indicate that by dissolving the lactose carrier particles, the resultant nanocarriers were not disrupted and thus maintained high levels of Hp monomer in the micelle.

Figure 6b shows fluorescence microscope images of the micellar Hp loaded into lactose carrier particles. The appearance of red fluorescence in the microparticles indicates that micellar Hp had been successfully entrapped into carrier particles. These lactose microparticles were spherical in shape and were $2.3 \pm 0.7 \mu\text{m}$ in diameter as demonstrated by SEM micrographs, making them ideal for maximizing pulmonary deposition as dry powders. When the particle size is greater than about $5 \mu\text{m}$, dry powders generally do not reach the lungs due to impact in the airways. Particles having diameters smaller than about $1 \mu\text{m}$ can be easily exhaled. Thus, particles to be delivered to the lungs are preferentially within the size range of $1\text{--}5 \mu\text{m}$ for distributing in the alveoli.

In vitro cell culture was used to determine whether the micellar Hp loaded into lactose microparticles still maintained their cellular uptake and photocytotoxic effect. We also employed confocal spectral microscopy to study the subcellular localization of the micellar Hp. It has been reported that micelles can be internalized through an endocytosis pathway and subsequently localized in acidic endocytic compartments (i.e., endosomes and later lysosomes) (34–36). LysoTracker® green, which is a specific fluorescence dye, was used for the staining of lysosomes. It was observed that the red fluorescence caused by the Hp diffused throughout the cytoplasm and cannot be perfectly superimposed with the green fluorescence caused by the LysoTracker® green. This observation indicated that micellar Hp and the lactose-micellar Hp after dissolution of the lactose microcarrier were not exclusively localized in the lysosomes.

The Hp, micellar Hp, lactose-micellar Hp were tested for cellular uptake and photocytotoxicity. The survival ratio of A549 non-small cell lung carcinoma cell lines after PDT revealed that micellar Hp and lactose-micellar Hp were more effective at cell killing than free Hp. In order to clarify the enhanced effect of the photocytotoxicity, we investigated the uptake of Hp by tumor cells. The intracellular concentration of Hp was found to be greater for micellar Hp and lactose-micellar Hp than with free Hp. These results suggested that the phototoxicity of the photosensitizer was enhanced by Hp entrapped in micelles.

In this investigation, micelles were successfully loaded into lactose microparticles, thus offering a number of potential advantages over conventional drug delivery techniques. These include enhanced uptake by cancer cells or epithelium cells, reduced macrophage clearance, uptake by RES and accumulation into solid tumors and inflamed tissues through the enhanced permeation retention effect (EPR effect). It has been reported that polymer micelles composed of Pluronic®

block copolymers can overcome multi-drug resistance (23). Using micelles in lactose microparticles of a selected particle size, we expect to be able to deliver the microparticles to the deeper part of the lung. After the dissolution of the lactose microparticles, the nano-sized micelles will be in intimate contact with lung epithelium. Thus, this delivery platform has potential for use in the treatment of various lung diseases including cancer, cystic fibrosis, and asthma.

CONCLUSION

In this study, micelles containing the photosensitizer Hp were successfully loaded into lactose microparticles. The size, absorption spectral properties, and singlet oxygen generation of Hp entrapped in micelles showed that the micelles remained stable after dissolution of the lactose microparticles. Thus, the incorporation of drug-containing micelles into lactose microparticles using spray-drying is a potentially effective drug delivery platform, especially for pulmonary applications.

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