33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

PEG-b-PCL and PEG-b-PLA Polymeric Micelles as Nanocarrieres for Lamellarin N delivery

Hathaichanok Pungkham, Nattharin Swatdipakdi, Man Theerasilp, Supattra Karnkla, Montakarn Chittchang, Poonsakdi Ploypradith, and Norased Nasongkla

***Abstract*—Lamellarin N (Lam N) is a member of an interesting marine natural product class isolated from mollusks and subsequently found in ascidians and sponges. The limited aqueous solubility of Lam N hinders further studies on its cytotoxic activity against cancer cells. In this study, micelles comprising poly(ethylene glycol)-block-poly(ε-caprolactone) (PEG-b-PCL) and poly(ethylene glycol)-block-poly(D,L-lactide) (PEG-b-PLA) were developed to circumvent Lam N limited aqueous solubility. The results showed that an increase in the molecular weight of both core materials resulted in higher Lam N loading content, whereas the release of Lam N decreased as a function of molecular weight of PCL and PLA. With less hydrophobic property, PLA micelles provided faster Lam N release. Thus, polymeric micelles could be utilized as controlled-release delivery systems for poorly water soluble Lam N.**

I. Introduction

Tremendous efforts have been made to develop methods for cancer treatment, such as chemotherapy, surgery, radiation therapy, immunotherapy, and monoclonal antibody therapy. These therapies mostly affect healthy cells, leading to adverse effects, such as hair loss, weakness, and nausea. Drug targeting technology is a new choice for cancer treatment with reduced side effects and increased plasma half-life of drugs. Drug delivery systems in the form of nanoparticles can not only increase drug solubility and half-life, but also avoid clearance by the reticuloendothelial system (RES). Also considered as one type of nanoparticles, micelles, especially those with very low critical micelle concentrations (CMC), are very stable in aqueous environments, and thus, can be given by intravenous injection into bloodstream [1], [2]. Moreover, their nanoscale size (20-200 nm) is small enough for preferential accumulation in tumors by enhanced permeability and retention (EPR) effect [3].

Anticancer drugs can either be extracted from natural products or synthesized, such as doxorubicin (closely related to daunomycin, a natural product) and 7-ethyl-10-hydroxy-camptothecin (SN-38, an active metabolite of irinotecan in high-grade glioma (HGG) treatment). A new group of anticancer drug candidates, so-called "lamellarins", has been discovered, initially from mollusks and subsequently frolm ascidians and sponges. Lamellarins are cytotoxic against various cancer cells by inhibiting topoisomerase I, which is important for cell division [4]. As a result, they have been investigated for potential therapeutic use in the treatment of some human cancers. For an environmental reason, extraction of lamellarins from marine organisms can affect a number of natural equilibria. Therefore, lamellarin synthesis is the preferred choice, in which additional modifications around the lamellarin core can also be made to improve their selectivity toward cancer cells over the normal ones. Herein, we focused on lamellarin N (Lam N) with limited aqueous solubility, as indicated by its logP value of 3.37, compared to –3.4 for highly water-soluble glucose. As shown in Equation 1, this parameter can be calculated from the ratio of the concentration in aqueous immiscible solvent to the concentration in aqueous phase. The logP values clearly indicate that the aqueous solubility of Lam N is much lower than that of glucose.

$$\log P_{oct/water} = \log\left(\frac{[solute]_{octanol}}{[solute]^{unionized}_{water}}\right) \quad (1)$$

Such a low aqueous solubility has limited Lam N from further studies, especially on its antitumor efficacy, despite a number of benefits [5]. The objective of this study was to increase aqueous solubility of Lam N through encapsulation of the drug inside polymeric micelles in order to improve its pharmacokinetic behavior. The amphiphilic block copolymers between poly(ethylene glycol) (PEG) and poly(D,L-lactide) (PLA), or poly(ε-caprolactone) (PCL) were used in this study because of their excellent properties, including biocompatibility, low toxicity, as well as absence of antigenicity and immuno-genicity. Lam N-micelles were first prepared from diblock copolymers using 2 different methods, namely the solvent evaporation and film sonication techniques. Then, Lam N loading content and *in vitro* release were determined using a UV-Vis spectrophotometer and a fluorometer, respectively. The aqueous solubility of free Lam N was compared with the amount of Lam N brought into an aqueous solution by micelles to determine the enhancement in solubilization.

Manuscript received April 11, 2011. This work was supported by Mahidol University, Thailand. This research was supported in part by the Office of the National Research Council of Thailand (NRCT).

H. Pungkham, N. Swatdipakdi, and M. Theerasilp are with the Biomedical Engineering Department, Mahidol University, Nakorn Pathom 73170, Thailand.

S. Karnkla, M. Chittchang, and P. Ploypradith are with Chulabhorn Research Institute and Chulabhorn Graduate Institute, Bangkok 10210, Thailand.

N. Nasongkla is with the Biomedical Engineering Department, Mahidol University, Nakorn Pathom CO 73170, Thailand (corresponding author to provide phone: 662-889-2138 ext 6357; fax: 662-899-2138 ext 6367; e-mail: egnns@mahidol.ac.th).

II. MATERIALS AND METHODS

A. Materials

Lam N was synthesized according to the method developed by Ploypradith et al [6]. D,L-Lactide (LA), ε-caprolactone (CL), and poly(ethylene glycol) (PEG, M_W = 5 kDa), were purchased from Acros Organics (NJ, USA). LA was purified by recrystallization from ethyl acetate, whereas CL was purified by distillation over calcium hydride (CaH_2). Dimethyl sulfoxide (DMSO), chloroform, and tetrahydrofuran (THF) were purchased from RCI Lab-scan Ltd. (Milwaukee, WI, USA) and used as received.

B. Synthesis of Diblock Copolymers

PEG-b-PLA was synthesized by ring opening polymerization of D,L-lactide under dry argon at 110°C in the presence of methoxy-terminated poly(ethylene glycol). Stannous(II) octoate ($Sn(Oct)_2$) was used as a catalyst, and the polymerization was carried out for 4 h. PEG-b-PLA was purified by redissolving in acetone and precipitating in hexane three times.

Similarly, PEG-b-PCL was synthesized by ring opening polymerization of ε-caprolacton under dry argon at 140 °C in the presence of methoxy-terminated poly(ethylene glycol), and $Sn(Oct)_2$ was used as a catalyst. The polymerization was carried out for 24 h, and the product was purified by redissolving in acetone and precipitating in hexane three times. Copolymer composition was analyzed by ^1H NMR.

TABLE I

COMPOSITION OF PEG-b-PCL AND PEG-b-PLA MICELLES

Hydrophilic block	Hydrophobic block
poly(ethylene glycol) (M_w = 5 kDa)	poly(ε-caprolactone) (M_w = 3.1, 5.8, and 12.6 kDa) poly(D,L-lactide) (M_w = 2.9, 6.5, and 9.7 kDa)

C. Preparation of Lam N-Micelles

Two different methods were used to prepare Lam N-loaded micelles.

1) Film sonication

Lam N-containing polymeric micelles were fabricated by film sonication technique in an aqueous phase. This procedure was carried out by dissolving 1 mg of Lam N and 10 mg of the diblock copolymer (with different molecular weights of the hydrophobic components as shown in Table I) in a mixture of methanol and dichloromethane (1:1 v/v). The solvent was then evaporated to yield a solid film, to which 10 mL of water was subsequently added and then vigorously sonicated (Vibra-Cell™, Model CV.18, Sonics & Materials, Inc., Newtown, CT, USA) at a power level of 60% for 1 min. Micelles were then filtered through centrifugal filter devices with a molecular weight (MW) cut-off of 50 kDa (Millipore, Billerica, MA, USA) to remove free Lam N by sequential centrifugation until there was no observable changes in the retained volume.

2) Solvent evaporation

This technique was carried out by dissolving 1 mg of Lam N and 10 mg of the diblock copolymer (with different molecular weights of the hydrophobic components as shown in Table I) in THF. The solvent was evaporated to yield a solid film, which was subsequently redissolved in 1 mL of THF and transferred to a beaker containing 10 mL of water. The mixture was sonicated at a power level of 60% for 1 min and then gently stirred at room temperature until the organic phase was completely evaporated. Free Lam N was later removed from the micelle solution using the same method as described above.

D. Lam N Loading Content Determination

Lam N loading content is defined as the weight percentage of Lam N in the micelles, which was measured by a UV-Vis spectrophotometer, over the total weight of the micelles. First, micelle solutions were freeze-dried to yield solid micelle samples. Then, the dried micelles were weighed and redissolved in a mixture of DMSO and chloroform (1:1 v/v) before subjected to the UV-Vis analysis. The absorbance of the micelle solution at 390 nm was measured to determine the Lam N content using the previously established calibration curve. The percentage of the loaded Lam N was calculated by Equation (2).

$$\%\ \text{Lam N loading} = \frac{\text{Weight of Lam N in micelles}}{\text{Weight of micelles}} \times 100\ \% \qquad (2)$$

E. Determination of Lam N-Micelle Size by Dynamic Light Scattering (DLS)

DLS was performed using a Zetasizer (Malvern Instruments Ltd., Worcestershire, UK). Scattered light was detected at 90° at room temperature. The data for each sample were obtained in triplicates, and the average values were reported with standard deviations.

F. In Vitro Release of Lam N from Polymeric Micelles

Polymeric micelles were transferred into a dialysis tubing with a MW cut-off of 50 kDa (Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) and immerged into 20 mL of phosphate buffered saline (pH 7.4). Release studies were performed at 37°C in a shaker-incubator (BIOSAN Environmental ES-20/60, Biosan Ltd., Riga, Latvia). At selected time intervals, the buffer solution outside the dialysis tubing was removed and superseded with a fresh buffer solution. Lam N concentrations were determined based on the fluorescence intensity at the excitation and emission wavelengths of 280 nm and 310 nm, respectively. Standard deviations were obtained from triplicate samples.

G. Solubility of Lam N in Water

Solubility of free Lam N in water was determined by first dissolving 1 mg of Lam N in 0.5 mL of THF. The solution was then added to 500 mL of water and stirred at 60°C for 24 h. Additional 100 mL portions of water were sequentially added with continuous stirring at 60°C for a period of 24 h until no insoluble Lam N could be detected under a fluorescence microscope. The total volume of water added was recorded and used to calculate the solubility of Lam N.

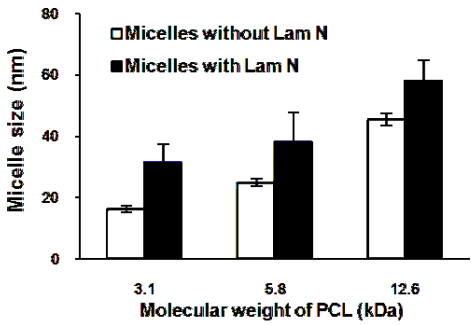
The amount of Lam N brought into aqueous solution by micelles was inferred from the Lam N loading content and the minimum retained volume of micelle solution, in which micelles did not aggregate, in the centrifugal filter devices.

III. Results and Discussion

A. Determination of Lam N-Micelle Size by Dynamic Light Scattering (DLS)

Micelle size plays an important role in tumor accumulation. Cancer cells can grow to reach 1-2 mm^3 in size. Nutrients and oxygen required by these cells can be transported by a fast-growing neovasculature. These situations lead to an abnormality of the endothelial cell structure. For example, the vascular pore size becomes larger than 400 nm, constituting a leaky vasculature from which micelles can leave for tumor tissues. In this study, the micelle size was analyzed using DLS. In general, the micelle size depends on the molecular weight of the hydrophobic block, as shown in Figure 1. For PCL, the sizes of the Lam N-containing polymeric micelles were considerably different from those without Lam N (Figure 2A). In contrast, encapsulation of Lam N in the PLA micelles did not significantly increase the micelle size (Figure 2B).

(A)



(B)

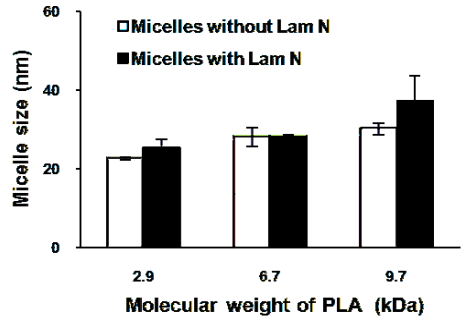


Fig 1. Size comparison of different lengths of hydrophobic core of A) PEG-b-PCL (3.1, 5.8, and 12.6 kDa) micelles and B) PEG-b-PLA (2.9, 6.5, and 9.7 kDa) micelles with and without Lam N by dynamic light scattering.

B. Lam N Loading Content Determination

Figure 2 shows that the Lam N loading content in both PEG-b-PCL and PEG-b-PLA micelles linearly increased as a function of PCL and PLA molecular weights. Lam N loading content increased from 6.4 to 12.2 and 3.7 to 8.7 when the molecular weights of PCL and PLA were increased from 3.1 to 12.6 kDa and 2.9 to 9.7 kDa, respectively. These results were consistent with the previous study of paclitaxel loading in MPEG-b-PCL micelles [7]. With the longer segments of PCL and PLA in the micelles, the Lam N loading content increased due to their higher hydrophobic interactions with Lam N in a larger hydrophobic core. Comparing the two polymers, using PCL as a core material provided a higher loading content of Lam N, possibly because PCL is more hydrophobic than PLA. Since Lam N is highly hydrophobic, it can be entrapped more efficiently in the hydrophobic core of the PEG-b-PCL micelles than in that of the PEG-b-PLA micelles. This observation is in accordance with the fact that drug hydrophobicity is one of the main factors governing the loading process.

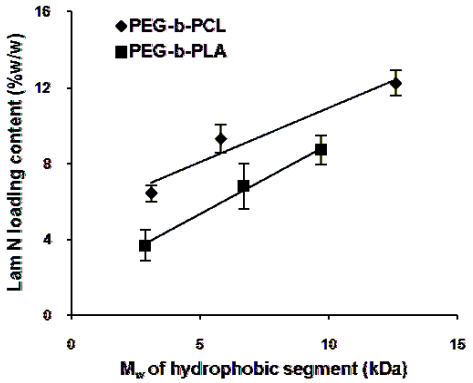


Fig 2. Comparison of Lam N loading contents in PEG-b-PCL and PEG-b-PLA micelles prepared by solvent evaporation method as a function of PCL and PLA length.

Micelle preparation techniques have also been found to affect the drug loading content [8]. The solvent evaporation method drastically increased the Lam N loading content in PEG-b-PCL micelles up to 3 folds throughout the whole range of PCL molecular weights used, compared to the film sonication method as shown in Figure 3.

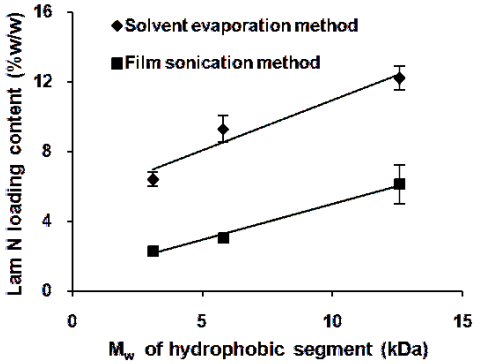


Fig 3. Comparison of Lam N loading contents in PEG-b-PCL micelles using film sonication method and solvent evaporation method.

C. In Vitro Release of Lam N from Polymeric Micelles

Lam N release from micelles with different molecular weights of PCL and PLA was studied (Figure 4). The release

rate was lower when the molecular weights of PCL and PLA were increased due to more limited diffusion of drug molecules through the polymer matrix and more entanglement of the higher molecular weight hydrophobic chains.

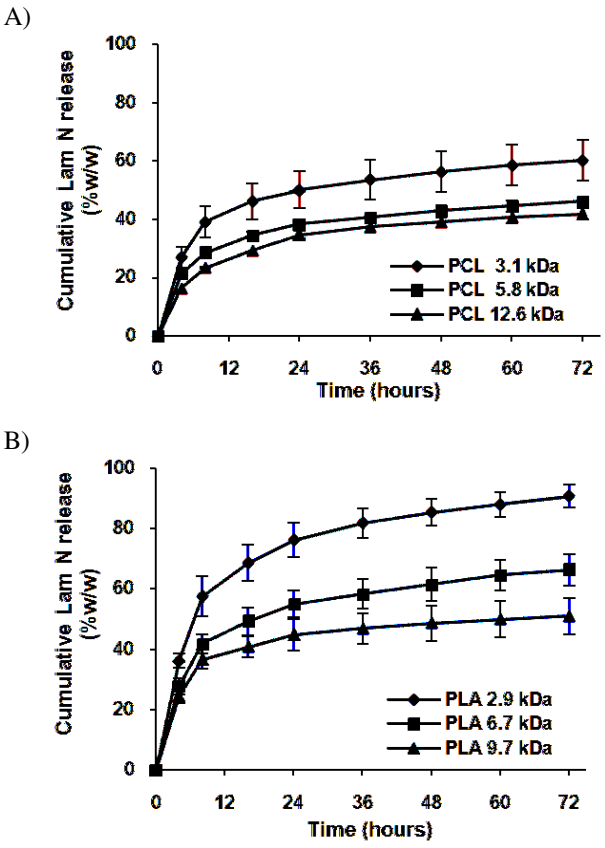


Fig 4. Cumulative release of Lam N from A) PEG-b-PCL and B) PEG-b-PLA micelles by solvent evaporation method.

It has been shown that the micelle core materials also play a key role in the control of drug release rate. For Lam N, changing the core polymer from PCL to PLA clearly resulted in a faster release, possibly because PLA is more hydrophilic, and thus, has less interaction with Lam N than PCL. Changing the y-axis to cumulative Lam N release (mg) (data not shown) revealed that PLA (6.7 kDa) provided the fastest release among three types of PLA materials where there was no different between PCL micelles. Also, Lam N released from PLA (6.7 kDa) was faster than that of PCL with MW of 5.8 kDa.

D. Solubility of Lam N

It was found that the solubility of Lam N in water was very poor (0.56 mg/L). The amount of Lam N brought into an aqueous solution by the polymeric micelles was significantly increased up to 200-folds as shown in Figure 5. This result confirms that the encapsulation of Lam N in PCL and PLA micelles offers a major advancement in improvement of solubility of this drug.

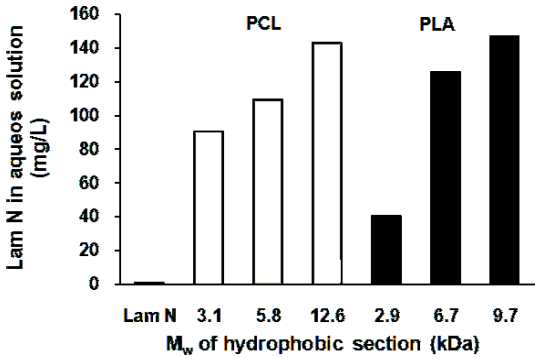


Fig 5. Amount of Lam N in aqueous solution.

IV. Conclusion

The results of this study showed that Lam N was successfully encapsulated in micelles. Factors, such as molecular weight and types of core materials, directly affected the Lam N loading content and release rate. An increase in the molecular weight of the core materials resulted in the higher Lam N loading contents. In contrast, the release of Lam N decreased as a function of the molecular weights of PCL and PLA.

References

[1] N. Nasongkla, E. Bey, J. Ren, H. Ai, C. Khemtong, J. S. Guthi, S. F. Chin, A. D. Sherry, D. A. Boothman, and J. Gao, "Multifunctional polymeric micelles as cancer-targeted, MRI-ultrasensitive drug delivery systems," *Nano Lett.*, vol. 6, pp. 2427-2430, Nov. 2006.

[2] X. Shuai, H. Ai, N. Nasongkla, S. Kim, and J. Gao, "Micellar carriers based on block copolymers of poly(epsilon-caprolactone) and poly(ethylene glycol) for doxorubicin delivery," *J. Control. Release*, vol. 98, pp. 415-426, Aug. 2004.

[3] D. Sutton, N. Nasongkla, E. Blanco, and J. Gao, "Functionalized micellar systems for cancer targeted drug delivery," *Pharm. Res.*, vol. 24, pp. 1029-1046, Jun. 2007.

[4] C. Tardy, M. Facompre, W. Laine, B. Baldeyrou, D. Garcia-Gravalos, A. Francesch, C. Mateo, A. Pastor, J. Jimenez, I. Manzanares, C. Cuevas, and C. Bailly, "Topoisomerase I-mediated DNA cleavage as a guide to the development of antitumor agents derived from the marine alkaloid lamellarin D: trimethylester derivatives incorporating amino acid residues," *Bioorg. Med. Chem.*, vol. 12, pp. 1697-1712, Apr. 2004.

[5] M. Chittchang, P. Batsomboon, S. Ruchirawat, and P. Ploypradith, "Cytotoxicities and structure-activity relationships of natural and unnatural lamellarins towards cancer cell lines," *ChemMedChem*, vol. 4, pp. 457- 465, Mar. 2009.

[6] P. Ploypradith, T. Petchmanee, P. Sahakitpichan, N. Litvinas, and S. Ruchirawat, "Total synthesis natural and unnatural lamellarins with saturated and unsaturated D-ring," *J. Org. Chem.*, vol. 71, pp. 9440- 9448, Dec. 2006.

[7] X. T. Shuai, T. Merdan, A. K. Schaper, F. Xi, and T. Kissel, "Core-cross-linked polymeric micelles as paclitaxel carriers," *Bioconjug. Chem.*, vol. 15, pp. 441-448, May-Jun 2004.

[8] E. Blanco, E. A. Bey, Y. Dong, B. D. Weinberg, D. M. Sutton, D. A. Boothman, and J. Gao, "Beta-lapachone-containing PEG-PLA polymer micelles as novel nanotherapeutics against NQO1-overexpressing tumor cells," *J. Control. Release*, vol. 122, pp. 365-374, Oct. 2007.