行政院國家科學委員會專題研究計畫成果報告

利用超微聚合粒子進行基因與蛋白質傳送評估

Evaluation of gene and protein delivery to cell via polymeric micelles

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1. ABSTRACT

Purpose. Determine aortic endothelial cells permeation ability and mechanisms of the aqueous block copolymeric micelles, poly(ethylene oxide)-poly(benzyl aspartate) (PEO-PBLA) chemically conjugated with FITC by transport study and confocal laser scanning microscopy. **Methods.** The block copolymers' PEO-PBLA-FITC was first synthesized and characterized by GPC and CMC, confocal microscopy. Permeation ability and mechanisms of polymeric micelles in aortic endothelial cells were evaluated by incubating with NaF, NaN_3, wortmannin, cytochalasin B inhibitors, at 20°C, and under reverse condition. The extent of localization of uptake polymeric micelles was established by confocal microscopy. *Results*. The size of the aqueous PEO-PBLA-FITC polymeric micelles was detected around 56 nm with unimodal distribution by AFM. The CMC test revealed the fluorescence intensity increased to around 0.01~0.05 mg/ml. NaF, NaN_3, wortmannin, cytochalasin B inhibitors, at 20°C, and under reverse condition inhibited the absorption of polymeric micelles through aortic endothelial cells with apparent permeability coefficients of 18.07±1.03, 12.98±0.93, 11.31±0.77, 12.44±1.23, 6.40±0.23, 11.11±0.46, 10.22±1.09x10^{-7} cm/sec, respectively.. Confocal laser microscopy showed that fluorescent compounds were distributed in the intracellular cytopolasm and nucleus. *Conclusion*. PEO-PBLA-FITC copolymeric micelles in an aqueous system were transported by energy dependent endocytosis and were localized on transcellular and nucleus endothelial cells.

Key words: Polymeric micelles, endocytosis, endothelial, FITC..

中文摘要

因藥物傳送系統中，具有(PEO)的水溶性超微聚合粒子，漸漸開始發展。其主要因素為此類特殊水溶性超微聚合粒子，除本身粒子在 nm 範圍中，在藥劑學上，並具有延長藥效劑型、容易製備與良好安定性的特性外，此粒子可以載程非水溶性藥物於其內，並可避免一般聚合物引起生物體內之免疫反應，或者延長、避免肝臟代謝之現象。然而這類超微聚合粒子如何傳送吸收至組織中，或免除組織排除之機制，至目前為止，仍須探討與研究。因此本計畫主要目的是利用已知 PEO-PBLA 所形成之超微聚合粒子來探討其本身特性與組織、細胞相互關係。第二年計畫主要方向是，第一利用具有螢光物質 FITC 與超微聚合粒子化學結合後，以利增加觀察與組織相互互動之關係能力。其結果經合成及純化後，在利用 GPC 偵測時，發現聚合物 PEO-PBLA-FITC 之濃度大於 0.01 ~ 0.05 mg/ml 時，可形成超微聚合粒子，並以 DLS 測量，其粒子在 56 nm 範圍並且粒子分布非常均一(分散性=1.17)。同時在螢光動脈細胞組織進行穿透中，超微聚合粒子對溫度、NaF、NaN_3、wortmannin、

cytochalasin B、反向穿透中均會受其抑制作用。進而以對焦螢光顯微鏡中，發現超微聚合粒子會以能量胞飲作用，在 15 分鐘內有效分散於細胞核與細胞質中。

關鍵字：超微聚合粒子，對焦螢光顯微鏡，動脈細胞

2. INTRODUCTION

The selective delivery of anticancer drugs to non-operable tumors via drug carriers is one approach to rational drug therapy(1-2). These drug delivery systems, including many different drug carriers are often directed attach epitopes present on tumor cells and carry drug which interfere with tumor cells. Usually, these maromolecular carriers have to cross the tumor blood vessel wall consisting of endothelial cells and a basement membrane which are a major barriers for delivery (3). Polymeric micelles, one of several macromolecule delivery carriers, are made from adriamycin conjugated block copolymer PEO-PBLA for selective drug delivery and have a reported high in vivo anticancer activity against leukemia and solid tumors as well as form stable micelles in the present of serum and circulate in the blood stream for a long amount of time. In order to effectively utilize this character of the long circulation of polymeric micelles in the blood stream, understanding the transport ability of PEO-PBLA polymeric micelles in the aortic endothelial membrane may be essential. Thus, the purpose of the present study was to elucidate the nano range of polymeric micelles penetration mechanisms and their ability in bovine aortic endothelial cells by chemically using conjugated FITC with PEO-PBLA polymeric micelles. In addition, the study aimed at determining the localization of PEO-PBLA-FITC polymeric micelles in the aortic endothelial cells by using confocal laser scanning microscopy.

3. RESULTS AND DISCUSSION

The conjugation, purification and micelle formation of PEO-PBLA-FITC in Hank's buffer solution were similar to these in previous study (2). The total fluorescence intensity increases of a fluorescent probe upon micellization have been utilized to determine CMC for a host of surfactants. At low concentrations of PEO-PBLA-FITC (below 0.01 mg/ml), negligible changes in the total fluorescence intensity were observed. All the polymeric micelles solution permeation, and AFM and confocal microscopic measurements were carried out to at least above the CMC of the polymers.

The time transport profile of 0.5 mg/ml PEO-PBLA-FITC polymeric micelles across aortic endothelial monolayers in Hank's buffer solution was found about 4% after 120 minutes. Table 1 shows that 0.05-0.5 mg/ml polymeric micelles transport through the aortic endothelial monolayer and apparent permeability coefficients were 18.89±1.60, 15.05±1.90, and 18.07±1.03 $x10^{-7}$ cm/sec, respectively It was shown that the transport properties were statistically independent of the initial concentration. Without endothelial monolayers on cell culture insert, polymeric micelles penetration rate increased around 10 times. Adding 5 mM NaF with polymeric micelles to endothelial cells caused the permeability to be inhibited to 12.98±0.93 $x10^{-7}$ cm/sec. In addition, NaN_3, induced a similar reduction in the transport of polymeric micelles in the case of NaF (table 1). Third, 100 nm wortmanin inhibited 32% permeation as well as reduced 65%

penetration by 0.1 mM chtochalasin B. The uptake and influx of polymeric micelles in low temperature significantly decreased(P=11.31±0.77 x10^{-7} cm/sec) as compared to the control group. On the other hand, in the reverse permeation studies, there was a marked asymmetry in the two unidirectional fluxes of polymeric micelles across the endothelial cells as shown in Table 1. However, the transport of polymeric micelles in the presence of both chambers of 10% FBS serum did not influence the apparent permeability coefficient (P). Finally, FITC only move through the PET membrane of cell culture insert and it was found to be similar to polymeric micelles through only the cell culture insert (table 1).

Table 1. Polymeric micelles PEO-PBLA-FITC transport on aortic endothelial monolayers.

	App. Perm. Coeff. P ± SEM (10^{7} cm/sec)
Control	173.90 ±7.07
0.05mg/mlPolymeric Micelles	18.89±1.60
0.1mg/ml Polymeric Micelles	15.05±1.90
0.5mg/ml Polymeric Micelles	18.07±1.03
0.5 mg/ml+5 mM NaF	12.98±0.93
0.5 mg/ml+5 mM NaN_3	11.31±0.77
0.5mg/ml+100nM wortmannin	12.44±1.23
0.5mg/ml+0.1mM cytochalasin B	6.40±0.23
0.5mg/ml+20° C temperature	11.11±0.46
0.5 mg/ml+reverse effect	10.22±1.09
0.5mg/ml +10% serum	18.75±2.14
FITC only	202.50±13.70

4. LOCALIZATION OF PEO-PBLA-FITC POLYMERIC MICELLES ON AORTIC ENDOTHELIAL CELLS BY CONFOCAL LASER SCANNING MICROSCOPY

Fifteen minutes after incubation of 0.5 mg/ml PEO-PBLA-FITC polymeric micelles in confocal laser scanning microscopy, FITC (red color) was found to be distributed over the intracellular space area, primarily at parts of endothelial monolayer cells with cytoplasm (Fig. 1a). The distributions were numerous and due to this, their close proximity, and their small particle size (around 50 nm), they were difficult to resolve individually in fluorescent microscopy. Simultaneous imaging of FITC and nuclear live stain Hoeschst 33342 (green color) showed the majority of FITC was located around the cytoplasm and nucleus (Fig. 1b), but not between adjacent intercellular spaces. In a vertical cross section FITC fluorescence could only be found distributed inside cells, but not on top of cells.

5.REFERENCES

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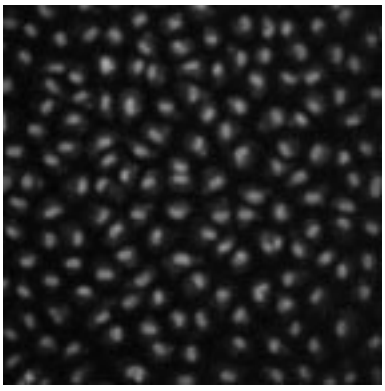
a)



b)

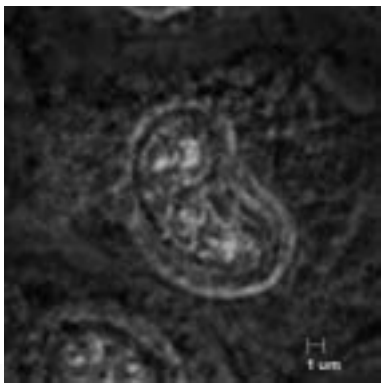


Figure 1. Transport of 0.5 mg/ml PEO-PBLA-FITC polymeric micelles across the aortic endothelial cell monolayer for 15 minutes by confocal fluorescence microscopy. a) FITC transport (red) were stained in the cytoplasm and nucleus. Live nucleus-staining by Hoeschst 33342 (green) was observed in the nucleus. b) a closer look inside the cytoplasm, and some FITC can also be observed in the nucleus area.