CELLULAR INTERNALIZATION ANALYSIS OF FOLATE-TARGETED PLA-PEG FILOMICELLES LOADED WITH NEW BETULIN DERIVATIVE

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Introduction

The use of natural plant-derived compounds has been considered to be an interesting aspect for the treatment of human neoplastic diseases because they are relatively easily available due to their commonly occurrence in the nature. Betulin has been shown to elicit anticancer properties by inhibiting cancer cells growth [1]. Moreover, series of new betulin derivatives have been synthesized, which characterize better cytotoxicity compared to betulin [2]. However, these agents are poorly soluble in water and it is important to develop dosage form that can effectively solubilize drug but also provide biocompatibility. Further progress is needed to develop bioresorbable carriers for targeted delivery of betulin derivatives which could be used for treatment of different kinds of tumors. PLA-PEG filomicelles were developed for targeted delivery of anticancer compounds. Folic acid (FA) was used as a targeting moiety. Thus, folate-drug delivery systems can enter cells by receptor-mediated endocytosis [3,4]. Investigation of uptake stage of micellar drug delivery system is crucial step to achieve effective anti-cancer therapy.

Materials and Methods

Filomicelles from the combination of poly(L-lactide)-Jeffamine-folic acid and poly(L-lactide)-poly(ethylene glycol) for delivery of betulin derivative 30-diethoxyphosphoryloxy-28-O-propynoylbetulin as an anticancer agent were prepared. HeLa human cervixadenocarcinoma cell line was used for uptake analysis. Flow cytometry and confocal laser scanning microscopy (CLSM) were used to study the cellular uptake of PLA-Jeff-FA/PLA3000PEG2000 micelles.

Results and Discussion

Fluorescence intensity of the HeLa cells incubated with PLA-Jeff-FA/PLA3000PEG2000 micelles with fluorescein was much higher than that of cells cultured in the presence of free fluorescein and the control with untreated cells.
CLSM analysis revealed subcellular distribution of PLA-JeffFA/PLA3000PEG2000 micelles with fluorescein in HeLa cells. Treated cells with folate-targeted filomicelles exhibited intense intracellular fluorescence. In contrast, almost no fluorescence was observed in the case of normal human connective tissue cells.

Conclusions

The successful internalization of PLA-Jeff-FA/PLAPEG micelles by FAR-positive HeLa cell line was confirmed by flow cytometry and confocal laser scanning microscopy. Effective cell uptake is a prerequisite to obtain carrier for efficient, targeted delivery of new anticancer agent and will allow to investigate internalization cell pathways.

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