PEG-Farnesylthiosalicylate Conjugate with a Drug Interactive Motif as an Improved Micellar System for Delivery of Anticancer Agents

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ABSTRACT SUMMARY
We have recently developed a dual-functional drug carrier that is based on polyethylene glycol (PEG)-derivatized farnesylthiosalicylate (FTS, a nontoxic Ras antagonist). In this study, we report that incorporation of a drug-interactive motif (Fmoc) into PEG5k-FTS2 micellar system led to further improvement in both drug loading capacity and formulation stability. Interestingly, delivery of anticancer drug paclitaxel (PTX) via PEG5k-Fmoc-FTS2 micelles led to superior antitumor activity over other treatments including Taxol and PTX formulated in PEG5k-FTS2 micelles in prostate cancer model. Our study suggests that incorporation of a drug-interactive group represents a simple and effective approach to improve the performance of micellar system.

INTRODUCTION
The current drug discovery process is costly and inefficient. Many promising drug candidates failed to reach the market mainly due to low bioavailability and toxicity issues. In recent decades, micelles have gained considerable attention due to their simplicity, small sizes, and ability to improve the pharmacokinetic and efficacy of anticancer drugs.

Recently we have developed a novel self-assembling nanomicellar system based on pegylated FTS (1). We found that PEG5k-FTS2 conjugate formed small-sized micelles (20-30 nm) and were highly efficient in solubilizing PTX. Additionally, PTX formulated in PEG5k-FTS2 micelles demonstrated enhanced antitumor activity in vivo over Taxol. Furthermore, our group demonstrated that incorporation into a surfactant a drug-interactive motif (9-fluorenylmethoxycarbonyl, Fmoc) at interfacial region could enhance drug-loading capacity and formulation stability (2). Thus, we hypothesized that the incorporation of Fmoc into our previous developed PEG5k-FTS2 delivery system will further facilitate drug-loading capacity and formulation stability.

We designed and synthesized a newly micellar carrier composed of an FTS-based hydrophobic domain, a PEG hydrophilic segment and a drug-interactive Fmoc motif (PEG5k-Fmoc-FTS2). We further characterized the biophysical properties of drug-loaded micelles including particle size, loading capacity. Finally, delivery of anticancer agents (PTX) via PEG5k-Fmoc-FTS2 was assessed in a prostate cancer model.

EXPERIMENTAL METHODS
The drug-free and PTX loaded micelles were prepared and PTX loading efficiency was quantified by high performance liquid chromatography (HPLC) as described before (1). The size was assessed by dynamic light scattering (DLS) and stability means that there was no noticeable size change during the follow-up period as described before (1).

A human prostate cancer (PC-3) xenograft model was used to assess the therapeutic activities of PTX loaded PEG5k-Fmoc-FTS2 micelles as described before (3). All results were reported as the mean ± standard deviation unless otherwise indicated. Statistical analysis was performed by Student’s t-test for two groups and one-way ANOVA for multiple groups.

RESULTS AND DISCUSSION
PEG5k-Fmoc-FTS2 conjugate readily formed micelles in aqueous solution and had small sizes around 30 nm (Table 1), which will allow effective passive targeting to various types of cancers.
PEG<sub>5K</sub>-Fmoc-FTS<sub>2</sub> micelles effectively solubilized PTX in aqueous solution in a molar ratio as low as 0.5:1 with size around 30 nm and were able to stable for about 12 h (Table 1). The loading capacity for PTX is extremely high (21.8%), which is a 3.9-fold increase compared with the PEG<sub>5K</sub>-FTS<sub>2</sub> micelles. This is likely due to the unusual properties of Fmoc motif involved in interaction with the incorporated drug. Fmoc group can provide strong hydrophobic interaction and forming π-π stacking with compounds carrying aromatic moieties. Most polymer micelles have been used for delivery of poorly water-soluble drugs are primarily based on hydrophobic interactions and deficient in loading hydrophilic drug. Compared with these polymeric micelles, both hydrophobic interaction and the π-π stacking contribute to the overall carrier/drug interaction by the addition of Fmoc motif (Figure 1).

As shown in Figure 2, Taxol formulation showed a modest effect in inhibiting the tumor growth at a dose of 10 mg PTX/kg. In contrast, PTX formulated in PEG<sub>5K</sub>-Fmoc-FTS<sub>2</sub> micelles showed a much more pronounced antitumor activity at the same dosage. More importantly, PEG<sub>5K</sub>-Fmoc-FTS<sub>2</sub> micelles exhibited even better tumor growth inhibition than PEG<sub>5K</sub>-FTS<sub>2</sub> micelles. The superior tumor inhibition of PEG<sub>5K</sub>-Fmoc-FTS<sub>2</sub> micelles could be explained by the enhanced stability compared to analog without Fmoc. Increasing the PTX dosage to 20 mg/kg resulted in a further improvement in the therapeutic effect.

Figure 2. Enhanced antitumor activity of PTX formulated in PEG<sub>5K</sub>-Fmoc-FTS<sub>2</sub> micelles in a human prostate cancer xenograft model (PC-3).

CONCLUSION

We designed and synthesized a new delivery system that was composed of an FTS-based hydrophobic domain, a PEG hydrophilic segment and a drug-interactive Fmoc motif. The drug-loading capacity and formulation stability were much enhanced by inclusion of a drug-interactive Fmoc motif. More importantly, PTX loaded PEG<sub>5K</sub>-Fmoc-FTS<sub>2</sub> led to superior antitumor activity in prostate cancer model. PEG<sub>5K</sub>-Fmoc-FTS<sub>2</sub> may represent a promising micellar system that could effectively deliver anticancer agents to tumors.

REFERENCES


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