Surface Chemistry Modification of Paclitaxel Nanocrystals and In Vitro Evaluation

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ABSTRACT SUMMARY
The goal of our study is to modify surface chemistry of paclitaxel nanocrystals (PTX-NCs) in order to enhance tumor accumulation and minimize systemic toxicity. For this, polymeric materials with and without tumor-targeting ligands have been attempted in the production of PTX-NCs. The results showed that surface chemistry modification effected little change to the particle morphology and size of PTX-NCs and enhanced the physicochemical stability.

INTRODUCTION
Poorly-soluble chemotherapeutic agents can be formulated into nanocrystals to improve their clinical efficacy. Formulated directly as nanosized crystalline particles (nanocrystals) without any encapsulating or solubilizing carriers, a nanocrystal system may be administered directly. Our previous studies have shown that PTX-NCs were effective against tumor in murine models, and their effectiveness was stronger than or comparable with that of Taxol (drug solution). More importantly, the side effects of the NCs were significantly reduced, possibly due to the absence of solubilizing or encapsulating chemicals. Nonetheless, the biodistribution results indicated that for the nanocrystals (as well as Taxol), the EPR (enhanced permeability and retention) effect was minimal in terms of targeting the tumor. Both delivery systems showed less than 1% of the total injected dose “leaked” out to the tumor. The nanocrystals were rapidly cleared from the blood circulation by the MPS (macrophage phagocytic system) and more than 40% of the injected dose found in the liver. When the animals were treated by the nanocrystals, the drug concentration in tumor was maintained quite steadily from 8 to 72 hours, suggesting the prolonged retention and possible release of the drug from engulfed nanocrystals in the macrophages. Given the significant liver uptake and limited EPR effect demonstrated by the “bare” nanocrystals, therefore, we aim to modify the surface chemistry of PTX-NCs to improve biodistribution with a particular focus on sparing core organs and maintaining a constant drug concentration in the tumor.

EXPERIMENTAL METHODS
Paclitaxel nanocrystals were prepared by an anti-solvent crystallization method as reported in our previous publications. In this study, we used sulforodamine B (SRB), a fluorescent dye, to physically integrate into PTX-NCs as guest molecules during the crystallization process. These hybrid nanocrystals were expected to achieve simultaneous cancer treatment and bioimaging, as demonstrated in our earlier studies.

To treat the nanocrystal surface, PTX-NCs were added to a dopamine solution and by adjusting pH, self-polymerization of dopamine was initiated via its hydroxyl and amino groups. Further, the dopamine-coated nanocrystals (DP-PTX-NCs) were dispersed in Tris–HCl solution (10 mmol/L, pH = 8.5) under sonication, which subsequently incubated with 2 mg/mL folic acid (FA)-conjugated PEG2000-NH2 for 2 h at room temperature. The FA-PEG2000-NH2 was synthesized by coupling folic acid to the amino groups of tBOC-PEG2000-NH2. The polymer-coated nanocrystals (FA-PEG-DP-PTX-NCs) were further tested together with PTX-NCs and DP-PTX-NCs.

Particle size and zeta potential of the NCs were measured by dynamic light scattering (DLS) analysis. Scanning electronic microscopy (SEM) was utilized to characterize particle morphology and size of the NCs. Cellular uptake of the NCs was studied in KB cells. The cancer cells were seeded in glass-bottom petri dishes and cultured in FA-deficient medium overnight. Then the cells were treated with the SRB hybrid nanocrystals with or without the coating polymers (100
ug/mL) for different durations (5, 15, 30 min, 1, and 2 h). Afterwards, the cells were fixed with 4% PFA for 10 min, and the nuclei were dyed with Hoechst for 15 min. Finally, KB cells were re-suspended in 1mL PBS at 4 °C prior to confocal analysis.

RESULTS AND DISCUSSION

Dynamic light scattering (DLS) analyses showed that NCs had narrow particle size distribution (Fig.1). The mean particle sizes of PTX-NCs DP-PTX-NCs and FA-PEG-DP-PTX-NCs were 239, 243, and 232 nm, respectively. The zeta potentials of three types of nanocrystals were -14.9, -42.8, and -18.5 mV, respectively. All NCs were stored at 4 °C. After 4 months, the mean particle size of PTX-NCs became 709 nm, but other two surface-coated nanocrystals showed no significant change. The surface chemistry treatment of the nanocrystals seemed to help maintain the stability of the particles.

Figure 1. DLS result of bare PTX-NCs.

Figure 2. SEM micrograph of PTX-NCs(A), DP-PTX-NCs (B), FA-PEG-DP-PTX-NCs (C).

SEM results showed that PTX-NCs were uniform rods. The surface of polymer-coated NCs became rough, possibly due to the presence of surface coating. DP-PTX-NCs and FA-PEG-DP-PTX-NCs were similar to pure PTX-NCs regarding their morphology and size, suggesting that the surface modification of the nanocrystals had little influence on the particle size and shape.

Confocal results showed that SRB hybrid PTX-NCs (red) were taken by KB-cells within 5 min and started to concentrate around the nuclei. In addition, the presence of intracellular vesicles in the cytoplasm of KB cells suggested that the actin and lysosome might be involved into the internalization of the hybrid nanocrystals. Preliminary data (not shown here) also indicated that the folic acid-coated NCs could enhance tumor accumulation.

Figure 3. Confocal images of SRB-PTX-NCs (red).

CONCLUSION

Surface treatment of PTX-NCs with polymers appeared to effect little change to the particle morphology and size but enhance the physical stability. It is anticipated that the polymer-coated NCs could have a positive impact on reducing systemic toxicities of using drug nanocrystals for cancer therapy.

REFERENCES

1. Hollis CP, Weiss HL, Evers BM, Gemeinhart RA, Li TL, Pharm. Res. 2013, ASAP

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