Nanoparticle Delivery of a Highly Toxic Metal Chelator to Cancer Cells

You Jung Kang[1], Chung-Fan Kuo[1], and Sheereen Majd[1],[2]*
Departments of [1]Biomedical Engineering and [2]Engineering Science and Mechanics, The Pennsylvania State University

Statement of Purpose: Malignant cells are known to have higher iron requirements compared to healthy cells presumably due to their higher proliferation rate. Therefore, the use of metal chelators has become a promising approach to sensitize and eliminate various cancer cells by depriving these cells from iron. Among the recently developed metal chelators, Di-2-pyridylketone-4,4-dimethyl-3-thiosemicarbazone (Dp44mT), has shown great antiproliferative properties in a number of cancers including lung, melanoma, and neuroepithelioma. However, low solubility and high cytotoxicity ($IC_{50} \approx 30 \pm 10$ nM) of this metal chelator are major concerns for its application in cancer treatment. Here, we propose to employ poly(lactic-co-glycolic acid) (PLGA) nanoparticles to efficiently encapsulate Dp44mT for targeted delivery of this agent to tumor cells while sparing normal cells. In this study, we investigate the potential of PLGA nanoparticles for delivery of Dp44mT and explore the effectiveness of this strategy in defeating cancer cells in vitro.

Methods: To prepare Dp44mT-loaded nanoparticles (NP-Dp44mT), we applied a modified nanoprecipitation technique. Briefly, a mixture of PLGA and Dp44mT in acetone was injected into deionized water containing polyvinyl alcohol (PVA), which was used for improved colloidal stability. During the injection, the aqueous phase was magnetically stirred for better mixing. After evaporation of organic solvent from the final mixture by gentle stirring, the PLGA polymeric nanoparticles containing Dp44mT were formed. The resultant nanoparticles were then collected and stored. We evaluated the quality of the obtained nanoparticles in terms of their size distribution, shape, colloidal stability, encapsulation efficiency, and drug release profile in physiologically relevant media. Moreover, we tested the therapeutic effectiveness of the proposed nanoparticles encapsulating Dp44mT in glioma cells and normal endothelial cells.

Results: We evaluated the size and shape of NP-Dp44mT using dynamic light scattering (DLS) and scanning electron microscopy (SEM) and demonstrated that these particles were relatively homogenous in size (62.7 ± 9.9 nm) and shape (Figure 1A and B). The obtained nanoparticles also exhibited a good stability without aggregations in a physiological solution over a week (Figure 1D). Moreover, we compared the encapsulation efficiency of Dp44mT in the proposed nanocarrier system to that of nanoliposomes and showed a significantly higher encapsulation (Figure 1C). Finally, the obtained NP-Dp44mT showed a good and controllable release profile (Figure 1E). Next, we tested the effect of NP-Dp44mT on the glioma cell line, U251. These experiments revealed that the uptake rate of NP-Dp44mT in U251 cells was significantly higher than that of free form (Figure 2A). Moreover, the cytotoxicity of NP-Dp44mT was higher than that of the free from in U251 cells presumably due to the higher uptake rate (Figure 2B). Importantly, these NP-Dp44mT did not induce significant cell death in healthy endothelial cell line, BBMVEC, indicating that these NP-Dp44mT might offer a safe and effective strategy for selectively killing malignant cells.

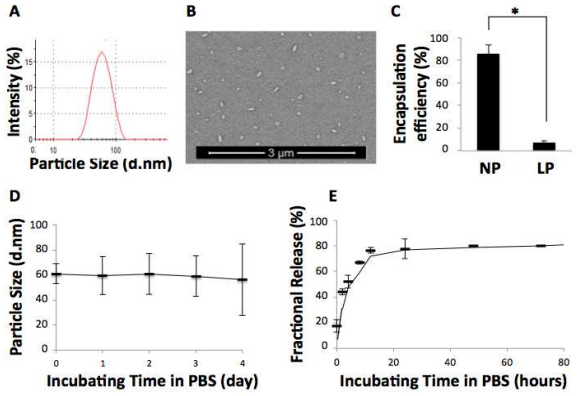

Figure 1. Characterization of NP-Dp44mT prepared by the modified nanoprecipitation method. **A.** Size distribution of NP-Dp44mT. **B.** SEM image of NP-Dp44mT. **C.** Comparison of encapsulation efficiency in PLGA nanoparticles and nanoliposomes. **D.** Stability of nanoparticles in a solution of PBS supplemented with FBS. **E.** Release profile of Dp44mT from PLGA NPs under agitation in a solution of PBS.

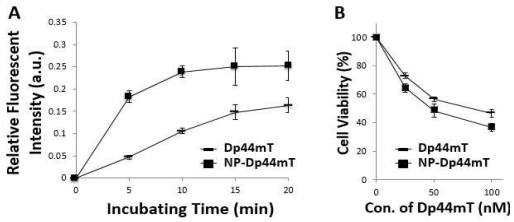

Figure 2. Effectiveness of Dp44mT encapsulated in the nanoparticles to defeat cancerous cells. **A.** Comparisons of cellular uptake rate between free form and NP-encapsulated Dp44mT in the U251 cell line. **B.** Cellular viability induced by Dp44mT in the U251 cells.

Conclusions: In summary, we present a promising approach to selectively deliver a highly toxic metal chelator, Dp44mT, to tumor cells using PLGA NPs. Our results show that the PLGA NPs can encapsulate Dp44mT with high efficiency and exhibit an improved solubility and a good stability in physiological solutions compared to the free drug. These NPs also showed a controlled release profile. Moreover, the proposed nanocarrier was able to maintain drug activity, enhance cellular uptake rate, and kill the cancer cells efficiently while sparing normal endothelial cells. Overall, the proposed formula may offer an attractive strategy for effective treatment of a number of solid tumors.

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