Development of novel nanovesicles consisting of an antitumor agent tocopheryl succinate for cancer combination therapy

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ABSTRACT SUMMARY

We have developed a novel drug delivery system (DDS) using an antitumor agent, α-tocopheryl succinate (TS). Although TS itself readily forms nanovesicles (TS-NVs), TS-NVs are unstable for encapsulating drugs and in vivo DDS. In the present study, to improve the stability of vesicles, we developed novel nanovesicles consisting of TS and egg phosphatidylcholine (TS-EPC-NVs). The stability of TS-EPC-NVs was significantly higher than that of TS-NVs. As the results, the antitumor activity of TS-EPC-NVs was more potent than that of TS-NVs. The enhanced antitumor activity of TS-EPC-NVs was found to be due to its effective intratumoral distribution, homogenous cellular uptake, enhanced cytosolic delivery and mitochondrial injury. In summary, TS-EPC-NVs represent a novel and attractive drug delivery system for combination therapy in cancer. The system shows antitumor activity of the encapsulated drug and the carrier itself.

INTRODUCTION

In past clinical research, combination therapy using several drugs, each of which has a different mechanism in cancer, has been shown to enhance the therapeutic effect compared to monotherapy. To enhance the availability of combination therapy, it is necessary to develop the drug carriers for multidrug therapy. Previously, we have developed a novel DDS carrier consisting of an antitumor agent, tocopheryl succinate (TS). TS has attracted attention as a unique anti-cancer drug for its ability to induce apoptosis in various cancer cells². Furthermore, TS itself readily forms nanovesicles (TS-NVs) and is a prospective tool for use as an antitumor DDS². Because TS-NVs have low vesicle stability in the presence of divalent cations and serum, TS-NVs are not suitable to encapsulate other drugs and passive targeting delivery to tumor tissue via enhanced permeation and retention effect. Therefore, to put TS-NVs into practice for actual tumor therapy, increasing the stability of TS-NVs is necessary. In this study, to improve the stability of TS-NVs, we developed novel nanovesicles consisting of TS and egg phosphatidylcholine (EPC) which can form a stable lamellar structure (TS-EPC-NVs)³ (Figure 1). We compared the anti-tumor effects of TS-EPC-NVs with TS-NVs. Furthermore, we studied the mechanism of the antitumor effect of TS-EPC-NVs.

EXPERIMENTAL METHODS

TS-NVs and TS-EPC-NVs were prepared by simple hydration method as reported previously⁴. To control the particle size, TS-NVs and TS-EPC-NVs were passed through a mini-extruder using a polycarbonate membrane (0.1 μm pore size). The particle size and surface potential of the nanovesicles were measured by dynamic light scattering and laser doppler electrophoresis, respectively. The cellular uptake and intracellular trafficking of fluorescent labeled nanovesicles were examined by flow cytometer and confocal laser scanning microscopy. The antitumor activity was examined by WST-1 assay in B16-F1 cells, mouse melanoma cell line, and by monitoring tumor volume in B16-F1 cell-bearing mice.

RESULTS AND DISCUSSION

TS-EPC-NVs were successfully constructed by mixing TS and EPC (1:7:1 mol/mol). In response to divalent cations or serum, the diameters of TS-NVs were significantly increased by aggregation of the vesicles, but TS-EPC-NVs remained almost constant. The results suggest that TS-EPC-NVs showed higher stability in the
presence of divalent cations and serum compared with TS-NVs.

To determine the influence of increased vesicle stability on the tumor inhibitory effect, we compared the in vivo antitumor effects of TS-EPC-NVs and TS-NVs (administered intravenously) on the growth of mouse melanoma. TS-EPC-NVs were more effective at preventing tumor growth than TS-NVs. To clarify the reason why TS-EPC-NVs was more efficacious than TS-NVs, we next compared the intratumoral distribution of both nanovesicles. Fluorescent-labeled TS-EPC-NVs showed a broader and more homogenous distribution in the tumor tissue compared to TS-NVs. It is suggested that the effective intratumoral distribution of TS-EPC-NVs may contribute to the potent antitumor effect by TS-EPC-NVs.

We next examined the effects of TS-EPC-NVs on in vitro cancer cell growth. When the cells were treated with 25 or 50 μM TS, TS-EPC-NVs showed an almost two-fold greater inhibitory effect than did TS-NVs. Furthermore, we next characterized the cytotoxicity of nanovesicles containing TS. The results from Annexin V-staining and caspase 3/7 activities indicated that TS-EPC-NVs were more potent at inducing apoptosis than were TS-NVs. Then, to clarify why TS-EPC-NVs induces apoptosis effectively, we measured the amount of nanovesicles taken up per cell by flow cytometric analysis. Although total cellular uptake of nanovesicles was almost the same following treatments with TS-NVs and TS-EPC-NVs, the variation in the amount of TS-EPC-NVs taken up per cell was smaller than that observed with TS-NVs. We next investigated the intracellular trafficking of TS-EPC-NV. Cellular uptake of TS-EPC-NVs was inhibited by the addition of sucrose, suggesting that TS-EPC-NVs were taken up through the classical endocytotic pathway. Furthermore, TS-EPC-NVs-induced cell death was accelerated by co-treatment with chloroquine, suggesting that TS-EPC-NVs were delivered to cytoplasmic target proteins for inducing apoptosis following endosomal escape. To obtain further evidence for cytosolic delivery of TS-EPC-NVs, siRNA was encapsulated into TS-EPC-NVs. In B16-F1 cells expressing luciferase stably, TS-EPC-NVs encapsulating anti-luciferase siRNA showed approximately 45% knockdown efficiency, suggesting that TS-EPC-NVs were delivered to the cytosol. Previously, it was suggested that mitochondria are reaction sites of TS-induced apoptosis4. Thus, to obtain further information regarding apoptosis induced by TS-EPC-NVs, effect of the TS-EPC-NVs on mitochondrial intactness was examined. As shown in Figure 2, TS-EPC-NVs decreased more potently the mitochondrial membrane potential than TS-NVs, indicating that TS delivered to cytoplasm would injure mitochondria. As a result, mitochondrial injury-induced apoptosis would be occurred.

CONCLUSION

In the present study, we developed novel nanovesicles consisting of TS and EPC as a co-delivery system for anticancer drugs. TS-EPC-NVs, as stable vesicles, showed more potent antitumor effects than TS-NVs. The enhanced antitumor effects of TS-EPC-NVs were attributed to the effective intratumoral distribution, cellular uptake, delivery to the cytosol, and mitochondrial injury, all of which were due to increased vesicle stability. Collectively, TS-EPC-NVs are attractive drug carriers that show both anti-tumor activity of the encapsulated drug and the carrier itself.

**Figure 2.** Effect of TS-NVs and TS-EPC-NVs on mitochondrial membrane. B16-F1 cells were treated with TS dissolved in ethanol (denoted as TS-EtOH), TS-NVs, or TS-EPC-NVs. After staining with Rhodamine 123, mitochondrial membrane potential was measured by flow cytometer.

**REFERENCES**


**ACKNOWLEDGMENTS**

This work was supported in part by the Japan Society for the Promotion of Science and by the Kyoto Pharmaceutical University Fund for the Promotion of Scientific Research.