Tumor-Selective Anticancer Drug Delivery System Mediated by Dendritic Poly(L-Lysine)

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ABSTRACT SUMMARY

Previously, we found that PEG-modified dendritic poly(L-lysine)s of 6th generation (KG6) accumulated to tumor in mouse after intravenous injection. In this study, we prepared hydrophobic cavity between PEG and KG6 by inserting peptide, then, examined encapsulation of doxorubicin (DOX) and its delivery to tumor. PEG-penta-phenylalanine-KG6 (PEG-5Phe-KG6) could encapsulate larger number of DOX than other control molecules, and could deliver DOX to tumor after intravenous injection.

INTRODUCTION

Selective delivery of anti-cancer drugs to tumor is an important key for chemotherapy without side effect caused by non-specific distribution of the drugs. Previously, we reported that mono-dispersed dendritic poly(L-lysine)s of the 6th generation (KG6) formed complex with DNA and RNA, and showed high transfection efficiency into several cultivated cells with low cytotoxicity (Fig. 1A).¹⁻⁴ The transfection efficiency was not seriously reduced, even when 50% serum was added to the transfection medium. The zeta potential of the DNA complex with KG6 was kept at neutral when the charge ratio (+/-) was increased to 8, supporting the stability in the presence of serum at high concentration. In addition, the DNA complex could circulate in blood flow for long time at least 3 h after intravenous injection into mice, then the DNA was finally accumulated into liver.⁵

Previously, we found that KG6 modified with polyethyleneglycol (PEG) showed efficient accumulation into tumor tissue by enhanced permeation and retention (EPR) effect.⁶,⁷ In this study, we prepared hydrophobic cavity between PEG chains and KG6 by introducing oligo-phenylalanines or oligo-alanines (Fig. 1B), then, examined encapsulation of doxorubicin (DOX) into the hydrophobic layer and its delivery to tumor.

EXPERIMENTAL METHODS

KG6 was synthesized by coupling Boc-Lys(Boc) step by step as previously reported.¹ Amino groups of phenylalanine, alanine, penta-phenylalanines and penta-alanines were reacted with PEG-NHS. Resultant PEG-modified amino acids and penta-peptides were coupled with KG6. Modification rates of the PEG-amino acids and the PEG-penta-peptides on KG6 were evaluated by measuring their molecular weights using gel permeation chromatography.

DOX was encapsulated into the dendrimers by mixing them in DMSO in presence of triethylamine followed by dialysis in water. Numbers of encapsulated DOX in the dendrimers were evaluated by measuring fluorescence intensity of the samples. Release of DOX from the dendrimers was evaluated by a dialysis method.

Biodistribution of DOX delivered by the dendrimer was examined by fluorescence bioimaging system (Maestro, Caliper). Amount of DOX delivered by the dendrimer was quantified by HPLC after extraction of DOX from the tumors.

RESULTS AND DISCUSSION

PEG-modified mono-phenylalanine, mono-alanine, penta-phenylalanines, and penta-alanines were coupled to terminal amino groups of KG6. Their modification rates were 63%, 50%, 29%, and 28%, respectively. Coupling efficiencies of the PEG-penta-peptides were lower than the PEG-mono-amino. It would be due to steric hindrance of the peptide moiety.

Next, we encapsulated DOX, an anticancer drug for lung and breast cancers, in the dendrimers. DOX and the dendrimers were dissolved in DMSO, then dialyzed in water. After lyophilization, we got a powder of dendrimers encapsulating doxorubicin. The PEG-penta-
peptide-modified KG6 (PEG-5Phe-KG6 and PEG-5Ala-KG6) encapsulated larger numbers of DOX than PEG-mono-amino acid-modified KG6 (PEG-1Phe-KG6 and PEG-1Ala-KG6) (Fig. 2). In the case of PEG-1Phe-KG6 and PEG-1Ala-KG6, encapsulation abilities were the same to PEG-KG6. It is suggested that single amino acid was not enough to make cavity for the encapsulation, and most of bound DOX located in the PEG layer. In the case of PEG-5Phe-KG6 and PEG-5Ala-KG6, DOX could enter into hydrophobic cavity provided by the penta-peptides.

Next, we examined sustained release of doxorubicin from PEG-5Phe-KG6 using a dialysis system. Slower release was observed in the case of PEG-5Phe-KG6 than doxorubicin alone.

PEG-5Phe-KG6 was modified with Cy5 dye, then, its biodistribution after intravenous injection into tumor-bearing mouse was observed by a fluorescence bioimaging system (Fig. 3). Strong fluorescence was observed from tumors, indicating that PEG-5Phe-KG6 is promising drug carrier to tumor. Finally, we evaluated accumulation of doxorubicin delivered by PEG-5Phe-KG6. As a result, significant larger amount of doxorubicin was observed compared with case of DOX alone, indicating that PEG-5Phe-KG6 could act as DOX carrier to tumor.

CONCLUSION

The penta-phenylalanins in the dendritic molecule acted as a hydrophobic cavity for delivery of DOX to tumor.

REFERENCES


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CONCLUSION

Fig. 2. Encapsulation of DOX in PEG-X-KG6. Number of DOX was standardized by number of PEG chain on KG6.

Fig. 3. Biodistribution of Cy5-modified PEG-5Phe-KG6 after intravenous injection to tumor-bearing mouse.