Brain Specific Gene Delivery by Bubble liposomes and Ultrasound

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ABSTRACT SUMMARY

Bubble liposomes (BLs) are ultrasound (US) sensitive liposomes entrapping perfluoropropane gas. It has been reported that BLs and US can enhance gene delivery efficiency. In this study, we assessed the property of BLs focused on their perfluoropropane entrapping efficiency and stability. BLs which were made from DSPC as main lipid component were more stable and had much more perfluoropropane entrapping efficiency than those made from DMPC and DPPC. We also attempted to establish brain specific gene delivery method. Systemic administration of BLs and plasmid DNA and transcranial US exposure could deliver plasmid DNA into brain. Therefore, the combination of BLs and US might be useful system to establish minimally invasive and brain specific gene delivery.

INTRODUCTION

Recently, it was reported that microbubbles, which were US contrast agents, improved the gene transfection efficiency by cavitation with US exposure. In addition, some nanobubbles which were submicron sized bubbles have been developed. To apply for gene delivery tool, they had some problems regarding stability and targeting ability. To solve these problems, we paid attention to liposomes that were good drug and gene delivery carriers. In this situation, we succeeded to prepare BLs which were liposome technology based nanobubbles. BLs could be utilized for contrast enhancement in ultrasonography, easily modified with targeting moiety on their surface to accumulate for targeting site and changeable lipid components to optimize their stability and the ability of gene association efficiency. Although BLs have much potency for gene delivery by the combination of US, there are many parameters such as lipid composition, surface charge, size distribution, US exposure condition etc. for optimization. We cannot simultaneously optimize these parameters. Lipid composition is very important for liposome stability. Therefore, in this study, we assessed the effect of lipid component on stability of BLs. Then, we also examined the feasibility of more stable BLs as gene delivery tools utilized cavitation by US exposure. In addition, we attempted to establish minimally invasive and brain specific gene delivery system.

EXPERIMENTAL METHODS

Assessment of BLs Stability

Liposomes were composed with dimyristyl phosphatidylcholine (DMPC), diparmityl phos phatidylcholine (DPPC) or distearyl phosphatidy lcholine (DSPC): DSPE-PEG2k-OMe = 94: 6 (mol ratio). Then, these liposomes were contained perfluoropropane to form BLs. The BLs were put in 20 degree for 0 to 4 hr. After that, perfluoropropane in the BLs were quantitated by gas chromatography.

Gene delivery for brain using BLs and US

Luciferase cored plasmid DNA (100 µg/mouse) and BLs (as amount of perfluoropropane 1.85 µL/mouse) were intravenously injected. Then, the mice were transcranially exposed US (Frequency: 1 MHz, Intensity: 0.7 W/cm², Duty: 10 %, Time: 1 min.) to brain. After 24 hours, luciferase expressing site were assessed by in vivo imaging system (IVIS) and luciferase activities in various organs were measured.

RESULTS AND DISCUSSION
BLs which were composed with DSPC were higher amount of perfluoropropane than other compositions. And the amounts of perfluoropropane in the BLs which were composed with DMPC and DPPC rapidly decreased after preparation (Fig. 1). On the other hand, the BLs which were composed with DSPC were stable for at least 4 hours after preparation at 20 degree. This result suggested that DSPC was relatively suitable phosphocholine for preparation of BLs. Therefore, we used DSPC as main lipid component of BLs for gene delivery.

We assessed gene delivery for brain using BLs and US. Gene expression in major organs such as liver and kidney was low level. On the other hand, the brain, which was US exposure site, was higher gene expression than other organs. This result suggested that the BLs and US could selectively deliver plasmid DNA into brain.

**CONCLUSION**

The combination of BLs and US might be useful tools to establish minimally invasive and tissue specific gene delivery.

**REFERENCES**


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