Angiopep-2-conjugated Liposomes Encapsulating Dibenzazepine for Glioblastoma Multiforme

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ABSTRACT SUMMARY

Dibenzazepine (DBZ) is one of the most potent γ-secretase inhibitors (GSIs), which block the Notch signaling pathway and have been proven to have anti-tumor effects.

However, because of its non-selective biodistribution, severe diarrhea and suppression of lymphopoiesis were occurred. To overcome these problems, an efficient delivery system, such as liposome, is urgently required. In this study, we developed DBZ–loaded PEGylated liposomes modified with angiopep-2 (PEG-Lipo-DBZ-Angio) as targeting drug delivery system to transport across the blood-brain barrier (BBB) to treat brain glioma.

INTRODUCTION

The cancer stem cells (CSCs) hypothesis is currently the most widely accepted therapy regarding tumor initiation and self-renewal ability. Low efficient chemotherapy of glioblastoma multiforme (GBM) is probably due to the CSC which is known to cause the GBM resistance to various agents. CD133 has recently been considered as a general CSC marker for brain tumor types, such as GBM U87 cell line, and U87-CD133+ cells display glioma stem cells (GSCs) properties and appear to be highly tumorigenic. GBM, the most devastating malignant brain tumors in adults, has remained poor prognosis for decades. Despite recent advances in surgery, chemotherapy and radiotherapy, a median survival is just about 1 year with a 5-year mortality rates as high as 95%.

Notch signaling pathway is known to play a critical role in the regulation of self-renewal and differentiation in neural stem cells, however, aberrant Notch signaling has been known to cleave the Aβ precursor protein (APP) to yield of Aβ peptides associated with Alzheimer’s Disease. Recently GSIs (such as DBZ) have gained more attention as novel anti-cancer drugs due to their ability to block the Notch signaling pathway, but clinical use of Notch inhibitors is restricted by severe side effects and there is a demand for alternative cancer-targeted therapy.

Angiopep-2, a new and effective ligand of low density lipoprotein receptor-related protein (LRP), possesses a high brain penetration capability in both in vitro model of BBB and in situ brain perfusion in mice. And based on LRP overexpression on the BBB and GBM U87 cells, angiopep-2 could be used not only for enhancing delivery across the BBB but also for targeting to GBM tumors.

As the liposomes are well known drug delivery systems to reduce the drug toxicity and increase the therapeutic efficacy, we used liposomes as targeted delivery of DBZ to block Notch signaling. And angiopep-2 was conjugated to the liposome. To overcome the rapid clearance by reticular endothelial system (RES), the surface of liposomes were also modified with flexible hydrophilic polymers such as polyethylene glycol (PEG).

In this study, PEG-Lipo-DBZ-Angio was evaluated for their physical characterization and in vitro study using sorted U87-CD133+ cells.

EXPERIMENTAL METHODS

U87-CD133+ cells were separated from U-87 GBM cells by using the magnetic bead separation method. The sorted U87-CD133 cells formed spheroid bodies in serum-free media supplemented with b27, bFGF and EGF.

Angiopep-2 conjugated liposomes were prepared by lipid film hydration method. The lipid mixtures of EPC, cholesterol, DSPE-mPEG2000-maleide with DBZ were dissolved in chloroform and evaporated to dryness. The dried lipid films were hydrated in PBS (pH 7.4). Then, for the conjugation of angiopep-2 to liposomes, angiopep-2 was added into the suspensions and incubated at room temperature for overnight, followed separation by dialysis.

PEG-Lipo-DBZ-Angio formulation was evaluated in terms of particle size, zeta potential, encapsulation efficiency and serum protein adsorption assay. To measure the inhibitory effects of DBZ in various formulations to the sorting U87-CD133+ cells, cytotoxicity assay and sphere formation assay were performed. And to evaluate the angiopep-2-mediated internalization was further supported by competition experiments with free angiopep-2.

RESULTS AND DISCUSSION

U87-CD133+ cells were separated using the CD133 cell isolation kit. The efficiency of sorting was verified by FACS. Fig. 1A shows the shapes of unsorted U87 cells and sorted U87-CD133+ cells. U87-CD133+ cells were spheroid-shape suspended cells and CD133 was more than 90% expression, but unsorted was only 5.4% (Fig. 1B).

The molar ratio of phospholipids was EPC : cholesterol : DSPE-mPEG2000-maleide = 60 : 40 : 1, and which was reacted with angiopep-2 at the ratio 1:1 in PBS (pH 7.0) for 24 h at room temperature. This composition was used for the rest of the experiments.

The mean diameter of prepared PEG-Lipo-DBZ-Angio was 110 ± 5 nm and the average zeta potential was −5.3 ± 5.5 mV. The encapsulation efficiency of DBZ was about 56.6 ± 8.2%. During the storage at 4℃ up to 14
days, drug precipitation of liposomal aggregation did not occur.

Figure 1. The shape of unsorted and sorted U87-CD133+ cells (A). The expression of CD133 analyzed by FACS (B)

The protein adsorption of PEGylated liposomes and Angiopep-2-conjugated PEGylated liposomes was 4-fold lower, compared to conventional liposomes (Non-PEG-Lipo) at 48 h in serum protein adsorption assay (Fig. 2).

Figure 2. In vitro protein adsorption assay of non-PEGylated liposome, PEGylated liposome encapsulating DPBS, PEGylated liposome encapsulating DBZ and Angiopep-2 conjugated PEGylated liposome encapsulating DBZ after incubation with 1% BSA at 37°C. Data were reported as absorbance by protein adsorption. (** P < 0.01)

The cytotoxicity effect of different DBZ formulations on U87-CD133+ cells was evaluated by MTT assay. The results showed that PEG-Lipo-DBZ-Angio exhibited the highest inhibitory effect to the proliferation of U87-CD133+ cells among various formulations (Fig. 3).

Also, spheroid-colony formation, an in vitro cell culture model to identify CSCs, is considered an indication of self-renewal ability and would be consistent with a CSC like phenotype or property. Untreated U87-CD133+ cells successfully produced spheroid colonies, but PEG-Lipo-DBZ-Angio could greatly impact the ability of U87-CD133+ cells to form CSC-like spheroids in vitro.

Figure 3. Cytotoxicity of liposomal DBZ formulations treated for 48 h on U87-CD133+ Cells. Cell viability was evaluated by MTT assay. The data is reported as percentage of control (DPBS). The data represent the mean ± SD. (** P < 0.01)

CONCLUSION
DBZ–loaded liposomes modified with angiopep-2 showed less protein adsorption that DBZ could be circulated much longer in vivo than free drug and has more opportunity to accumulate into the solid tumor by EPR effect. Also, PEG-Lipo-DBZ-Angio had the capacity to deliver the liposomal contents effectively to transport across the BBB and target the GBM cells. DBZ–loaded liposomes modified with angiopep-2 showed promising cytotoxicity effect and anti-spheroid-colony formation against the sorted U87-CD133+ cells.

It is concluded that the developed immunoliposome delivery system can provide stability of DBZ. It is expected that GSI loaded angiopep-2 - liposome could be used as a potential therapeutic agent for GBM therapy.

REFERENCES

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