The preparation of Multivesicular Liposome formulation of deca-peptide (TKII-10) with sustained release characteristics

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ABSTRACT SUMMARY

The deca-peptide (TKII-10) was found to have significant anti-angiogenic effect after direct injection into the eye. We attempted to prepare a sustained release formulation of TKII-10 based on the multivesicular liposome structure. Their in vitro and in vivo release profiles and activities were evaluated.

INTRODUCTION

Therapeutic proteins and peptides administered are often cleared rapidly in vivo and therefore need to be injected frequently in order to maintain therapeutic levels. Various types of liposome formulations had been examined for sustained release of proteins and peptides including multivesicular liposomes [1, 2]. A unique feature of the multivesicular system is that the vesicles contain discontinuous internal aqueous chambers bounded by a continuous non-concentric network of lipid membranes, rendering a higher aqueous volume-to-lipid ratio for higher loading capacity.

The deca-peptide (named TKII-10) was developed based on the Arg54-Trp63 sequence of human t-PA kringle 2. It had been shown to have significant anti-angiogenic effect after direct injection into the eye [3]. But it required frequent injection in order to achieve therapeutic efficacy [4]. Therefore we would like to develop a sustained delivery formulation of the peptide and examine their in vitro and on vivo behaviors.

EXPERIMENTAL METHODS

The multivesicular liposome particles containing TKII-10 were prepared based on the typical two-step double emulsification process developed by Kim et al [5, 6], with a slight modification. Briefly, 1 mL chloroform containing the lipids (molar ratio HSPC: cholesterol: DPPG: triolein, 5:8:1:1) was emulsified for 3 min (10,000 rpm) with 1 mL aqueous solution (the first aqueous solution) containing 82mg TKII-10 and 4% sucrose (w/v), to obtain a water-in-oil emulsion (the first emulsion). A subsequent emulsification for 5 s (7,500 rpm) with a second aqueous solution, 8 mL of 4% glucose (w/v) /20 mM lysine resulted in a ‘water-in-oil-in-water’ double emulsion (the second emulsion). The second emulsion was transferred and added to another aliquot of the aqueous solution in a 100 mL Egg type flask. Chloroform was removed by flushing the surface of the mixture at 37 °C with nitrogen for approximately 15 min. The resultant multivesicular liposomes were harvested by centrifugation for 10 min at 1000 × g and washed twice with PBS (phosphate-buffered saline) to remove unencapsulated TKII-10. After washing, the particle pellet fraction was resuspended by adding an equal volume of PBS. The whole progress should be kept in sterile environment.

All multivesicular liposomes were examined using an Olympus BX61 optical microscopy, and the Malvern Mastersizer 2000 particle sizer. The TKII-10 concentration in MVLs was determined by Waters UPLC & AB SCIEX SelexION Triple Quad™ 5500 system. The encapsulation efficiency was calculated with the following equation:

\[ \text{En (\%)} = \left( 1 - \frac{D_{\text{free}}}{D_{\text{tot}}} \right) \times 100\% \]

RESULTS AND DISCUSSION

Multivesicular liposomes containing TKII-10 were prepared according to the standard double-emulsion method. The preparations were highly reproducible, usually yielding MVL with similar size distributions and
encapsulation efficiencies. A typical particle size distribution profile was shown in Figure 1. The MVL had a size distributions ranging from 7 μm to 30 μm in diameter. The median size was approximately 14 μm and the encapsulation efficiencies was about 86%.

A significant advantage of the MVL formulations is its high drug loading capacity. Compared with conventional liposomes, which often have limited encapsulation for hydrophilic proteins, the MVL offered a much larger internal space and had higher encapsulation efficiencies. Triolein was used as a hydrophobic space filler to stabilize the junctions [6].

The in vitro release profile of these MVL particles were assayed at 37°C in 0.5 ml PBS to simulate the environment inside the eye (Figure 2). The duration of release from MVL (7 μm–30 μm) could last for more than 20 days. There was an initial burst release during the first three days and the release rate slowed down and was quite consistent afterwards. Further optimization would be helpful to tune the release profile for therapeutic applications.

Figure 1. Particle size distribution of TKII-10 MVL.

Figure 2. TKII-10 in vitro release profiles from MVL (4°C and 37°C) in PBS at various incubation time points. n=3. Mean±SD.

CONCLUSION
In summary, we demonstrated that TKII-10 MVL formulation can achieve high encapsulation efficiency, good stability and sustained release profile. Further optimization is needed in order to develop a clinically valuable sustained release formulation of TKII-10.

REFERENCES

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