Life prolongation in rats with malignant glioma by intranasal siRNA/drug co-delivery to the brain with cell-penetrating peptide-modified polymer micelles

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ABSTRACT SUMMARY
To develop a novel therapeutic strategy for managing brain disorders, we investigated the therapeutic effects on a rat model of malignant glioma treated by siRNA/anti-cancer drug co-delivery system to the brain using the MPEG-PCL-Tat micelles with a nose-to-brain delivery system.

INTRODUCTION
Although the siRNA-based therapies against CNS disorders are expected, the function of BBB poses a major challenge to the drug development efforts aimed at treating CNS disorders. Therefore, the development of effective strategies that would enhance siRNA delivery to the brain is of great interest for both the clinical and pharmaceutical fields.

In a previous study, we showed that Tat peptide, which is representative cell-penetrating peptide (CPP), modified poly (ethylene glycol) and poly (ε-caprolactone) block copolymers (MPEG-PCL-Tat) are capable of forming stable complexes with anti-VEGF siRNA and, after systemic delivery, exerts significantly higher suppression of the tumor growth than does naked siRNA in tumor-bearing mice. In addition, we reported that application of MPEG-PCL-Tat micelles could facilitate the anti-cancer drugs delivery to the brain by intranasal delivery, thus prolonging the survival of rats with intracranial C6 glioma.

In order to develop the an efficient siRNA/anti-cancer drug co-delivery system to the brain, we investigated the therapeutic effects on a rat model of malignant glioma treated by anti-Raf-1 siRNA (siRaf-1)/anti-cancer drug (camptothecin, CPT) co-delivery system to the brain using the combination of nose-to-brain delivery system and CPP-modified polymer micelles.

EXPERIMENTAL METHODS
CPT was loaded into the micelles using a film method. Briefly, a 100 mg of MPEG-PCL-Tat and 5 mg of CPT were dissolved into 50 mL of chloroform, and sonicated for 3 min. After sonication, the chloroform was evaporated. The siRaf-1/MPEG-PCL-Tat or siRaf-1/CPT-loaded MPEG-PCL-Tat complexes were prepared by mixing the components at room temperature at N/P ratio 30 for 30 min before usage.

Rat C6 glioma cells, seeded in a 96-well plate at a density of 20,000 cells per well, were allowed to adhere to the plate for 24 h. The cells were washed and treated with various formulations in FBS-free F-12K for 12 h at 37°C under 5% CO2. Then cells were incubated with CCK-8 solution for 3 h. The absorbance of viable cells was measured at 450 nm using a microplate reader. The absorbance of control cells indicated 100% cell viability.

To establish the intracranial tumors, each rat anesthetized by intra-peritoneal injection of pentobarbital (50 mg/kg) was given C6 glioma cells (1 × 10^6 cells) in 10 µL F-12K medium using a syringe fitted with a 30-gauge needle inserted into the left cerebral hemisphere to a depth of 7 mm at 3 mm anterior to the lambda and 3 mm lateral to the midline. The animals were monitored for 1 h after surgery and then daily. The tumor-bearing rats were randomly divided into each treating groups. Treatment was administered via intranasal injection daily for one week. The survival time of each rat was recorded and evaluated using the Kaplan-Meier method.
RESULTS AND DISCUSSION

The MPEG-PCL-Tat/siRNA complex and CPT-loaded MPEG-PCL-Tat/siRNA complex exhibited 60-80 nm and the approximately 15 mV. We subsequently assessed the cytotoxicity induced by Raf-1 gene silencing or CPT in C6 rat glioma cells transfected with siRaf-1, control siRNA (siControl), or CPT with MPEG-PCL-Tat using the WST-8 assay. Fig. 1 shows that the cell viability of MPEG-PCL-Tat/siRaf-1 complex, and CPT-loaded MPEG-PCL-Tat, and CPT-loaded MPEG-PCL-Tat/siRaf-1 complex significantly decreased compared with that of siRaf-1 alone, indicating that these formulations induce the cell death in rat glioma cells. Then, the CPT-loaded MPEG-PCL-Tat/siRaf-1 complex showed the lowest cell viability.

Fig. 1 In vitro cytotoxicity in C6 glioma cells. Cytotoxicity was determined by the WST-8 assay. The C6 cells were, a: untreated, or transfected with b: MPEG-PCL-Tat/siControl, c: MPEG-PCL-Tat/siRaf-1, d: CPT-loaded MPEG-PCL-Tat/siControl, and e: CPT-loaded MPEG-PCL-Tat/siRaf-1 at an N/P ratio of 30. The mean ± S.D. (n = 3). Significance analysis was used an ANOVA followed by Dunnett’s test. *P < 0.05, **P < 0.01.

We finally evaluated the in vivo therapeutic effects in glioma model rats of intranasally administered siRaf-1 alone without any micelles, MPEG-PCL-Tat/control siRNA, MPEG-PCL-Tat/siRaf-1 complex, CPT-loaded MPEG-PCL-Tat micelles/control siRNA complex, and CPT-loaded MPEG-PCL-Tat micelles/siRaf-1 complexes. As shown in Fig. 2, MPEG-PCL-Tat/siRaf-1 complex, CPT-loaded-MPEG-PCL-Tat micelles/control siRNA complex, and CPT-loaded MPEG-PCL-Tat micelles/siRaf-1 complexes prolongs the survival period of rats after seven days of continuous delivery. These results indicate that MPEG-PCL-Tat improved the delivery of siRNAs and CPT to the brain, and showed a marked prolongation of the survival period.

CONCLUSION

Our results indicate that cell-penetrating peptide-modified block copolymer accelerates the nose-to-brain delivery of siRNA/drug, and we propose that this agent may be useful for the clinical therapy of brain tumors and CNS disorders.

REFERENCES


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