**p-Hydroxybenzoic Acid (p-HA) Modified Polymeric Micelles for Brain-targeting**

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**ABSTRACT**

Chemotherapy for brain diseases has been hampered due to the inability of transport of drug across the blood-brain barrier (BBB). In order to overcome the barrier, a small molecule, benzamide analogue p-hydroxybenzoic acid (p-HA), was used as a ligand for brain-targeting.

**INTRODUCTION**

Blood-brain barrier (BBB) is a physical dynamic barrier between brain and blood, which serves as a protection barrier to effectively prevent certain foreign substances from entering the brain blood circulation, it hampers treatment and diagnosis of CNS diseases such as reduce Alzheimer's diseases, brain tumors, and Parkinson's diseases. Benzamide analogue is a kind of small molecules used for the treatment of central nervous diseases which have high affinity with dopamine receptors that are prominent in most parts of CNS. In this study, p-HA-modified micelles were first established, then in vitro and in vivo targeting effect of the micelles was investigated. The anti-glioblastoma efficacy of DTX-loaded micelles was studied on intracranial glioblastoma bearing nude mice.

**EXPERIMENTAL METHODS**

1. **Preparation and characterization of p-HA-PEG-DSPE micelles**

   p-Hydroxybenzoic acid (p-HA) was conjugated to NH2-PEG-DSPE, which was then characterized by 1H-NMR. Micelles loaded with coumarin 6, DiR or DTX (Docetaxel) were prepared using the thin-film hydration and extrusion method. The particle size and size distribution of micelles were determined by dynamic light scattering and AFM techniques.

2. **Targeting ability study of p-HA-PEG-DSPE micelles**

   Cellular internalization of coumarin 6-loaded micelles were studied using BCECs cells. Potential brain-targeting effect of p-HA-PEG-DSPE micelles was evaluated in normal nude mice. time-dependent biodistribution of the DiR-labeled micelles was observed at different time points.

3. **Anti-Glioblastoma study of p-HA-PEG-DSPE/DTX micelles**

   Therapeutic efficacy of DTX-loaded micelles was investigated in a xenograft mouse model bearing human U87 multiforme glioblastoma[27]. The intracranial U87MG bearing nude mice were randomly divided into four groups ($n=9$) and treated with the p-HA-PEG-DSPE/DTX micelles, unmodified mPEG-PEG-DSPE/DTX micelles, Taxotere and saline (dose: 8 mg per kg of body weight at 6, 9, 12, and 15 days post-tumor implantation. The survival times were recorded.

**RESULTS AND DISCUSSION**

1. **Characterization of p-HA-PEG-DSPE**

   The NMR spectrum showed a characteristic peak of p-HA at 6.8-7.8 ppm. The chemical shifts at 3.2-4.0 ppm and 0.8-1.5 represent the PEG (-O-CH2-CH2-O-) and the DSPE, respectively. The 1H-NMR spectrum results indicated the successful synthesis of p-HA-PEG-DSPE.

2. **Characterization of p-HA-PEG-DSPE micelles**

   All micelles exhibited narrowly-distributed vesicle sizes around 20 nm as determined by dynamic light scattering method, satisfying the requirement of brain-targeted delivery system. The AFM images showed the spherical morphology of DTX-loaded micelles was studied on intracranial glioblastoma bearing nude mice.

3. **Targeting ability of p-HA-PEG-DSPE micelles**

   In vitro cellular uptake was qualitatively determined by fluorescent images and quantitatively as a percentage of coumarin 6 positive cells. As shown in Figure 2, p-HA-PEG-DSPE/C6 micelles were internalized by BCECs cells more efficiently than mPEG-PEG-DSPE/C6 micelles. Flow cytometry experiments showed the percentage of coumarin 6-positive cells increased from 72.4% to 92.9%. All these results indicated that p-HA could enhance micelles uptake by BCECs cells in vitro.
p-HA-PEG-DSPE/DiR micelles and mPEG-DSPE/DiR micelles were injected via caudal vein in normal nude mice. Visible fluorescence accumulation appeared much stronger in the brains of p-HA-PEG-DSPE/DiR micelle group, compared with the normal micelle group through the entire studies. Ex vivo fluorescent image and semi quantitative of excised organs further confirmed that fluorescence accumulation in the brains treated with the p-HA-PEG-DSPE/DiR micelles were 1.3-1.8 times higher compared with the normal micelle group at any time post-injection ranged from 2 h to 12 h (Fig. 3).

Fig.3. Ex vivo imaging and semi-quantitative analysis of dissected organs at different time points. (A) mPEG-DSPE/DiR micelles; (B) p-HA-PEG-DSPE/DiR micelles.

4. Anti-Glioblastoma study of p-HA-PEG-DSPE/DTX micelles

Fig. 4 represented Kaplan–Meier survival curves of the DTX-loaded formulations. The median survival time of the p-HA-PEG-DSPE/DTX micelle group, the mPEG-DSPE/DTX micelle group, Taxotere® group and saline group were 45.8, 32, 27 and 22 days, respectively. These results demonstrated that the survival of the p-HA-PEG-DSPE/DTX micelle group was significantly longer than those of other groups ($P<0.05$). These survival data suggested that p-HA could mediate BBB transport of polymeric micelles, thus enhancing therapeutic efficacy of chemotherapy drugs against brain tumors.

Fig.4. Kaplan-Meier survival curves of mice bearing intracranial U87 glioblastoma.

CONCLUSION

The p-HA-modified micelles exhibited strong enhancement on the uptake against the BBB both in vitro and in vivo. All data indicated that this novel small molecular (p-HA) can be used in the anti-tumor drug delivery system which targeting to the brain, and, p-HA-conjuncted micelles could have a potential value of clinical application.

REFERENCES


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