Dual-targeting paclitaxel-loaded nanoparticles for the treatment of glioma in mice

Zhiqing Pang¹ ², Bo Zhang¹ ² ³, and Yu Hu³

¹Department of Pharmaceutics, School of Pharmacy, Fudan University, 826 Zhangheng Road, Shanghai 201203, China; ²Key Laboratory of Smart Drug Delivery, Ministry of Education & PLA, 826 Zhangheng Road, Shanghai 201203, China; ³Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan, Hubei 430022, China

zqpang@fudan.edu.cn

ABSTRACT SUMMARY

The dual-targeting paclitaxel-loaded nanoparticles (PNP-PTX) were developed by decoration with peptide-22, a novel peptide with special affinity to the low density lipoprotein receptor (LDLR), for transporting drug across the BBB and then targeting brain tumor cells. Dual targeting effects in vitro demonstrated that peptide-22 decoration significantly increased the transport ratio of PTX across the BBB model and induced the apoptosis of C6 glioma cells below the BBB. Fluorescence microscopy indicated that PNP labeled with a near-infrared dye could permeate the BBB and accumulate more in the glioma site than unmodified NP. Pharmacodynamics results revealed that the median survival time of glioma bearing mice administered with dual-targeting PNP-PTX was significantly prolonged as compared with that of any other group. TUNEL assay and H&E staining showed that PNP-PTX treatment induced significantly more cell apoptosis and tumor necrosis compared with other treatments.

INTRODUCTION

Chemotherapy for brain glioma has been of limited benefit due to the inability of drug to penetrate the blood-brain barrier (BBB) and non-selective accumulation of drug in the entire brain. To conquer the BBB in brain tumor therapy, dual-targeting strategies were presented. LDLR is highly expressed on both the BBB and glioma cells, which makes the peptide-22, a novel peptide targeting to LDLR¹, a good candidate for dual targeting drug delivery system to treat glioma. In this paper, peptide-22-conjugated nanoparticles were constructed as a novel effective dual targeting drug delivery system (PNP) for glioma therapy.

EXPERIMENTAL METHODS

Nanoparticles (NP) were prepared through an emulsion/solvent evaporation technique using MePEG–PLA and COOH–PEG–PLA block copolymers. Peptide-22 was conjugated to the surface of NP using an EDC/NHS technique. Particle size and zeta potential were determined by dynamic light scattering using a zeta potential/particle sizer (NICOMP 380 ZLS, USA). The morphology of nanoparticles was observed by a transmission electron microscope (H-600, Hitachi, Japan) following negative stain with 2% sodium phosphotungstate solution. LDLR expression on C6 cells, BCECs and H92c(2-1) cells was assayed by ELISA. In vitro cellular uptake of coumarin 6-labeled nanoparticles by BCECs, C6 cells and H92c(2-1) cells was investigated by fluorescence microscope (Leica, Germany) and FACS Aria Cell Sorter (BD, USA). BCECs were seeded onto polycarbonate 24-well Transwell membrane (FALCON Cell Culture Insert) to construct an in vitro BBB model. Dual targeting effects were evaluated by the transport ratio of PTX across the BBB model and the apoptosis induction of C6 glioma cells below the BBB. The distribution of PNP in glioma were observed under fluorescence microscope at 8 h after glioma bearing mice were i.v. injected with DiR-labeled NP or PNP. In vivo anti-glioma effect was investigated by monitoring the survival curve of glioma bearing mice after the treatment of PNP-PTX, NP-PTX, Taxol (PTX dose of 5 mg/kg) or saline. H&E staining and TUNEL were used to detect the apoptosis in...
RESULTS AND DISCUSSION

The mean diameters of both NP-PTX and PNP-PTX were around 120 nm with spherical shape and a uniform distribution. ELISA analysis revealed that low density lipoprotein receptor (LDLR) was over-expressed on C6 cells and BCECs but little on H92c(2-1) cells. The nanoparticle uptake demonstrated that peptide-22 decoration on nanoparticles significantly increased the cellular uptake of nanoparticles by C6 cells and BCECs but not by H92c(2-1) cells, and excessive free peptide-22 significantly inhibited the cellular uptake of PNP by C6 cells and BCECs but not by H92c(2-1) cells. In addition, peptide-22 modified NP exhibited enhanced BBB transport capacity (Figure 1) and apoptosis induction of C6 cells after crossing the in vitro BBB model as compared with other PTX formulations.

PNP labeled with Dir could permeate the BBB and accumulated more in the glioma site than unmodified NP (Figure 2). When the glioma bearing mice were subjected to 4 cycles of 5 mg/kg of PTX in different formulations, a significant extension of median survival time was observed in the group of PNP-PTX as compared with that in any other group (Figure 3). Furthermore, PNP-PTX could extensively induce the glioma cell apoptosis and tumor necrosis visualized by TUNEL and H&E staining (Figure 4).

Figure 1 The transport ratio (%) of PTX across the in vitro BBB model during 24 h. Statistically significant differences by Student’s t-test when compared with the corresponding value of PNP-PTX: aP < 0.05; bP < 0.01; cP < 0.001.

Conclusions
Peptide-22 decoration not only improved the cellular uptake of nanoparticles by C6 and BCECs, but also enhanced BBB permeability of PTX and C6 apoptosis blow the BBB in vitro. In vivo tests revealed dual targeting PNP-PTX showed significantly stronger brain permeation, glioma targeting, and enhanced chemotherapeutic effects of PTX than other treatments for glioma-bearing mice models.

REFERENCES