83-14 monoclonal antibody-grafted solid lipid nanoparticles for targeting delivery of saquinavir across the blood–brain barrier

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ABSTRACT SUMMARY

83-14 monoclonal antibody (MAb) was modified on solid lipid nanoparticles (SLNs) to improve the brain-targeting delivery of saquinavir (SQV). An increase in the weight fraction of poloxamer 407 (P407) in surfactant layer slightly reduced the permeability across the blood–brain barrier (BBB). However, an increase in the concentration of surface 83-14 MAb significantly enhanced the permeability across the BBB.

INTRODUCTION

An application of peptide or peptidomimetic MAb can promote the drug delivery into the brain by targeting specific endogenous receptors on brain-microvascular endothelial cells (BMECs). 83-14 MAb, an insulin-like peptidomimetic MAb with molecular weight of 150 kDa, has a strong affinity to brain capillary and can highly bind with \( \alpha \)-subunit of human insulin receptor (IR).\textsuperscript{1} In addition, the size of 83-14 MAb is similar to that of neuroactive molecule.\textsuperscript{2} Thus, 83-14 MAb may raise the transport efficiency of drug carriers across the BBB.

EXPERIMENTAL METHODS

Dynasan 114, palmitic acid, SQV, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] were dissolved in methanol at 400 rpm and 75°C. 0.4% (w/v) cholesteryl hemisuccinate and 1% P407, Tween 80, and sodium dodecyl sulphate (SDS) were mixed in ultrapure water. 250 \( \mu \)L of organic phase were then added into 750 \( \mu \)L of aqueous phase at 400 rpm and 75°C for 5 min. The emulsified fluid was added into ultrapure water at 1000 rpm and 3°C for 15 min. After filtration and centrifugation, the pellet was resuspended, frozen, and lyophilized for 24 h, suspended in 4 mL of Dulbecco’s phosphate buffered saline into 0.25 mg/mL, mixed with 50 \( \mu \)L of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride of 1 mM and 50 \( \mu \)L of N-hydroxysuccinimide of 1.5 mM at 100 rpm and 4°C for 1 h. After centrifugation, the pellet was resuspended in a solution of 83-14 MAb at 100 rpm and 3°C for 6 h, dialyzed in an ultra centrifugal filter device at 7000 \( \times \)g for 10 min, frozen, and lyophilized. Grafted 83-14 MAb was analyzed with a bicinchoninic acid kit and quantified by an enzyme-linked immunosorbent assay spectrophotometer at 562 nm.

The protocol for culturing human BMECs (HBMECs) and human astrocytes (HAs) in a transwell system comprising a polyethylene terephthalate insert was proposed previously.\textsuperscript{3} 83-14 MAb/SQV-SLNs and SQV-SLNs of 250 ppm were added into the donor, incubated in a humidified CO\textsubscript{2} incubator at 37°C for 4 h. 20 \( \mu \)L of fluid in the receiver was sampled every 2 h and analyzed by a high performance liquid chromatograph with an ultraviolet detector at 239 nm. The total volume in the receiver was compensated with 20 \( \mu \)L of fresh medium immediately. The permeability of SQV across cocultured HBMECs and HAs, \( P_{HBMECs/HAs} \) was calculated by \( 1/P_{HBMECs/HAs} = 1/P_e - 1/P_m \) and \( P_i = J/\Delta C = V_d(C_i/dt)/(A \cdot \Delta C) \), where subscript i are e or m.\textsuperscript{4} Transendothelia electrical resistance (TEER) of cocultured HBMECs and HAs in fresh medium with the influence of SLNs was evaluated by the Millicell electrical resistance system.

RESULTS AND DISCUSSION

Fig. 1 shows the permeability of SQV across the BBB using SQV-SLNs and 83-14 MAb/SQV-SLNs. The value of TEER can be an...
index of the compactness in a BBB model. The TEER value of the present BBB model was about 220 $\Omega \times \text{cm}^2$. When SQV-SLNs and 83-14 MAb/SQV-SLNs were applied, the TEER value was about 180-200 $\Omega \times \text{cm}^2$. A BBB model with a TEER value higher than 120 $\Omega \times \text{cm}^2$ could be proper for assessing drug delivery into the brain.\textsuperscript{5} The permeability of SDS-stabilized SQV-SLNs was the lowest among all preparations (Fig. 1). This was because SDS did not contain ligands for HBMEC recognition. In addition, the electrostatic repulsion between anionic SDS and HBMECs hampered the uptake of SDS-stabilized SQV-SLNs. P407 and Tween 80 favored the permeability across the BBB. The BBB permeability enhanced slightly with a decreasing weight fraction of P407 (Fig. 1). Colloids covered with surface Tween 80 could mimic the low-density lipoprotein (LDL) particles and could be recognized by LDL receptors, rendering endocytosis via the pathway of receptor-mediated transcytosis into brain endothelia. In addition, Tween 80 could inhibit the efflux function of P-glycoprotein on BMECs. Moreover, P407, P118, or Tween 80 on polymethyl methacrylate nanoparticles could advantage the transport into brain endothelia. The graft of 83-14 MAb on SQV-SLNs significantly benefited the BBB permeability (Fig. 1). The BBB permeability elevated with an increasing concentration of 83-14 MAb (Fig. 1). The main reason for this improved BBB delivery was that 83-14 MAb recognized IRs on HBMECs and accelerated the transport of 83-14 MAb/SQV-SLNs. 83-14 MAb/SQV-SLNs with mixed P407 and Tween 80 were more efficacious in enhancing the BBB permeability than those with pure P407 (Fig. 1). In human brain capillaries, the uptake quantity of 83-14 MAb was about 4% injected dose after administration for 3 h. The permeability-surface area product in the primate BBB using 83-14 MAb was about 10 times that using antitransferrin to target transferrin receptors.\textsuperscript{6} In addition, several circulated peptides, such as insulin, insulin-like growth factors, leptin, and transferrin could also target the BBB and deliver drugs into the brain via receptor-mediated transcytosis.\textsuperscript{7}

CONCLUSION

83-14 MAb/SQV-SLNs were prepared for delivering SQV across the BBB. 83-14 MAb/SQV-SLNs could diminish the dosage of SQV and could be very useful in brain-targeting efficacy. The combination of surface 83-14 MAb, P407, and Tween 80 with lipid core containing mixed Dynasan 114 and palmitic acid can be a promising nanocarrier for carrying hydrophobic antiviral reagents into the brain.

REFERENCES


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