Transport of L-DOPA Loaded PLGA Nanoparticles across Blood-Brain Barrier

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ABSTRACT SUMMARY
The aims of this study were to evaluate the mechanisms of uptake and transport of poly(D, L-lactide-co-glycolide) (PLGA) nanoparticles (NPs) carrier systems using in vitro blood-brain barrier (BBB) model.

INTRODUCTION
PLGA is a widely used polymer for NPs preparation due to its biocompatibility, biodegradability, low toxicity and ease of fabrication. Compounds loaded in polymeric devices are released either by diffusion through the polymer matrix, erosion of the polymer, or by both.

L-DOPA (LD) is the gold standard for Parkinson disease treatment. The main limitations of LD in clinical use are its low solubility, high sensitivity to chemical and enzymatic oxidation and peripheral decarboxylation. Only 1% of the given dose is transported unchanged into the brain. In order to improve its bioavailability, a number of particulates systems have been employed as delivery systems.

Polymeric NPs are nano-size carriers for drugs to be encapsulated into, adsorbed or chemically linked to their surface. These carriers possess high stability in biological fluids and are resistant to the enzymatic metabolism. NPs have been reported to transport across BBB via various mechanisms. In this study, we examined the possibility of delivery LD across BBB using PLGA NPs as a carrier.

EXPERIMENTAL METHODS
Preparation of FITC-conjugated PLGA NPs. The FITC-labeled PLGA was prepared by covalent conjugation of FITC to EDC-activated carboxylic terminal groups of PLGA polymers using a modified carbodiimide method. The PLGA NPs were prepared using a double emulsion solvent evaporation method. Briefly, the primary emulsion of LD and FITC-conjugated PLGA was dispersed in an aqueous phase to form secondary emulsion. Subsequently, the NPs precipitated by removing the organic solvent and recovered by ultracentrifugation. The particles were then washed and freeze-dried.

Development and characterization of in vitro BBB model. The BBB model was developed by co-culturing rat brain microvascular endothelial cells (RBMVECs) and astrocytes on a Transwell filter as illustrated in Figure 1.

RESULTS AND DISCUSSION

Uptake of PLGA NPs by RBMVECs. The uptake of FITC-PLGA NPs containing LD was investigated in confluent RBMVECs monolayer cultures on Petri dish. Cells were treated with NPs suspension at concentrations ranging from 10 to 1000 µg/ml over 4 h. At the indicated times, the NPs suspension was aspirated off, and the cells were rinsed with ice-cold HBSS. The cells were then transferred to FACS tube. The extent of NPs being taken up by the cells was determined with fluorescence activated flow cytometry (FACS). The intracellular localization of FITC labeled NPs was also examined with confocal laser scanning microscopy (CLSM).

Transport of PLGA NPs by BBB model. The transport studies were performed using BBB cell model with transendothelial electrical resistance (TEER) values higher than 400 Ω cm². The efflux of sucrose was measured to evaluate the paracellular permeability. The cells were washed with HBSS before studies. Thereafter, 1.5 ml of transport buffer was added to the receiver chamber in 12-well plates. 0.4 ml of NPs suspension at various concentrations (10-1000 µg/ml) was added to the donor chamber and incubated at 37°C. At the designated times (15 to 240 min), the inserts containing NPs were removed to the new wells containing 1.5 ml fresh buffer. The samples were collected from the receiver chambers at each time point and the donor chamber at the last time point. Sample solutions were treated with equal volume of 1% Triton 100/1 M NaOH at 37°C. The intensity of fluorescence of the extracted NPs was determined using fluorescence spectrophotometer (λex 495, λem 525). The amount of NPs that has transported across the BBB model was calculated from the calibration curve. At the end of the experiments, TEER and the transendothelial transport of sucrose were determined to confirm the integrity of cell monolayers.
Characterization of PLGA NPs. Under scanning electron microscope (SEM), the NPs were nearly spherical with irregular and porous outer surface with a particle size ranging from 185 to 415 nm. All particles possessed negative surface charge with zeta potential values of -10 to -25 mV. The encapsulation efficiency was 60%.

Characterization of BBB model. The TEER of BBB model was ~ 450 Ω cm² and the permeability (P_app) of sucrose across the model was 5.28x10⁻⁶ cm/s indicating an integrated barrier. Figure 3 shows the uptake of low density lipoprotein (Dil-Ac-LDL). The fluorescence microscopic image shows the tight junctions between adjacent cells.

Uptake of PLGA NPs. FACS results showed that a greater fraction of Tween 80 coated NPs was taken up by the cells than uncoated particles. The mean fluorescence intensity increased with increasing concentration of NPs. This suggested that the uptake of NPs via receptor-mediated process was concentration-dependent. On the other hand, the uptake efficiency of NPs decreased at higher concentration, suggesting that the endocytosis pathway was saturable. The uptake of NPs was temperature-dependent; confirming the uptake of particle is an energy consuming metabolic process. At 37°C, the uptake of NPs occurred as early as 30 min and linearly increased with time during the first 4 h before a saturation uptake was achieved.

Transport of NPs across BBB model. After incubation of NPs at concentration of 200 µg/ml at apical side, PLGA NPs appeared in the receptor chamber by 15 min. The cumulative mass of NPs linearly increased up to 60 min of incubation time. The P_app calculated from the first hour transport was 3.13x10⁻⁵ cm/s. The flux of NPs (µg/min/cm²) was directly proportional to the particle concentration. Model fitting indicating that the active flux followed one binding-site kinetics, with a K_m of 417±66.8 µg/ml, and V_max of 0.995±0.007 µg/min/cm².

CONCLUSION
In this paper, we established a co-culture BBB model with high paracellular resistance. This in vitro model was close enough to resemble the in vivo situation for examining the permeability of PLGA NPs. PLGA NPs at a defined concentration can be successfully delivered across the BBB model without changing the BBB. LD encapsulated within the particles can be protected from chemical and enzymatic degradation. PLGA NPs have great potential to deliver therapeutic agents to CNS.

REFERENCE