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Making transport possible: Flurbiprofen-loaded nanoparticles for the treatment of Alzheimer's disease

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PROBLEM

Alzheimer's disease (AD) is an irreversible, progressive brain disease, resulting in horrendous social-economic burdens. Causal interventions are still lacking. A promising anti-AD drug is R-flurbiprofen which modulates γ-secretase activity and can selectively lower pathogenic Aß42 levels [1]. Unfortunately, R-flurbiprofen exhibits a poor ability to cross the blood-brain barrier (BBB) in patients [2]. Here we show that flurbiprofen-loaded nanoparticles are capable of binding to *in vitro* BBB model cells and are taken up into the cells.

EXPERIMENTAL PROCEDURES

For cell culture experiments, we used the brain capillary endothelial cell lines HBMEC (human) and bEnd3 (murine). Our primary culture system is based on porcine brain capillary endothelial cells (pBCEC). The Lumogen® Orange fluorescence label of the fluribiprofen-laoded poly lactic acid nanoparticles (PLA-FBP-NP) allowed detection at 524/539 nm We investigated the cellular binding characteristics by flow cytometry experiments. Cellular uptake was examined by confocal laser scanning microscopy (CLSM).

SUMMARY

In order to revisit flurbiprofen as an anti-Alzheimer's drug, we designed a nanoparticle formulation for the transport of flurbiprofen over the BBB. We tested the binding capability of PLA-FBP-NP in various *in vitro* BBB model systems in order to assess transport capacity. By flow cytometry **(Figure 1 B, C)** and CLSM **(Figure 1D)**, we verified cellular binding and uptake of the flurbiprofen-loaded PLA nanoparticles. Furthermore, the nanoparticle formulation showed no obvious toxic effects on barrier integrity as indicated by unaffected transendothelial electrical resistance (TER) measurement after exposure **(Figure 1A)**. Data for drug release on the brain-representing compartment of the BBB model by high-pressure liquid chromatography is in progress right now. In future we plan an apolipoprotein E modification of the nanoparticles in order to allow a targeted transport over the BBB as shown earlier for human serum albumin-based nanoparticles [3, 4, 5].

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RESULTS

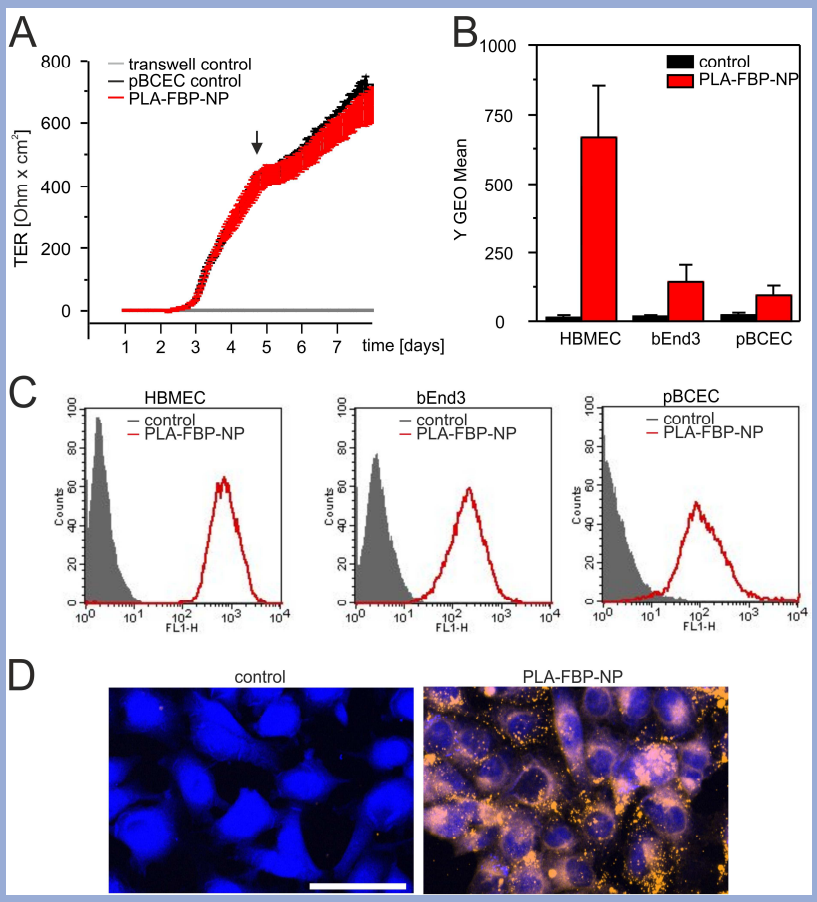

Figure 1. Flurbiprofen-loaded poly lactic acid nanoparticles (PLA-FBP-NP) do not interfere with barrier integrity and are taken up into *in vitro* BBB model cells. A Our primary BBB model is composed of pBCEC and displays excellent TER in cellZscope® experiments. The TER values were not obviously impaired after application of PLA-FBP-NP. Hence, we assume no toxic effects of our nanoparticles on barrier integrity. **B** Flow cytometry experiments displayed binding of the PLA-FBP-NP (red) compared to untreated control cells (black). Data is shown as Y Geo Mean values. **C** Exemplary histograms for the different cell types corresponding to B. Cell bound PLA-FBP-NP were detected in the FL1-H-channel. **D** CLSM images of HBMEC incubated with or without PLA-FBP-NP. The cytosol was stained with CellTracker™ Blue (blue); signals for nanoparticle detection are displayed in orange. Nanoparticle signals correlate with the cell bodies, and seem to be particularly located in the cytosol. For CLSM and flow cytometry experiments, PLA-FBP-NP were incubated for 4 hours at 37 °C in a concentration of 100 µg/cm² which represents a FBP concentration of 33 µM. A Lumogen® Orange fluorescent label was used for detection at 524/539 nm. The scale bar is 50 µm.