MULTILAYER NANOCARRIERS – A PROMISING TOOL FOR DELIVERY OF NEUROPROTECTIVE DRUGS THROUGH BLOOD-BRAIN BARRIER

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Despite an enormous progress in understanding molecular basis of age-related neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, no efficient neuroprotective strategy has been invented so far. One of the major limitations is an inefficient delivery of neuroprotective drugs through the blood-brain barrier (BBB). The very poor water solubility of most promising neuroprotectants limits their delivery to the affected part of the brain. Nowadays, nanoparticles (NPs) have attracted much attention as promising drug carriers that could deliver therapeutics to their specific molecular targets. Herein, we present a novel methodology for delivering hydrophobic neuroprotective substances through BBB using multifunctional polymeric-based nanoparticles (NPs).

The drug-loaded NPs were prepared from nanoemulsion template methods, i.e., the spontaneous emulsification solvent evaporation method [1]. Subsequently, NPs were modified using the layer-by-layer approach by creating multifunctional polyelectrolytes shells. Developed nanocarriers were characterized by determination of their size (below 250 nm), zeta potential and encapsulation efficiency (~ 100 %). For initial tests we have chosen two types of empty nanocarriers abbreviated as AOT/$(PLL/PGA)_2$-g-PEG and PCL/$(PLL/PGA)_2$-g-PEG without or with rhodamine B as fluorescent marker. As neuroprotectants we selected cyclosporine A (CsA) and tacrolimus (FK506) due to their anti-apoptotic, immunosuppressive and anti-inflammatory properties. Human neuroblastoma SH-SY5Y cell line was used to estimate the biocompatibility of nanocarriers loaded with appointed drug in the cellular viability quantification and cell death assessment using WST-1 and LDH tests, respectively. In parallel, we examined the neuroprotective potential of encapsulated drugs against oxidative stress-induced cytotoxicity. We also evaluated the capability of designed nanocarriers labeled with rhodamine B to pass through hCMEC/D3 cell monolayer to test if they can be considered as promising platforms for drug delivery. The immortalized human brain microvascular endothelial cell line (hCMEC/D3) was selected as well-characterized *in vitro* model of BBB and suitable for studying barrier permeability for neuroprotectants and drug carriers.

Results showed that the both kinds of new designed polymeric nanocarriers affected viability of SH-SY5Y cells only when used in the highest concentrations - in higher dilutions are devoid of cytotoxicity. We demonstrated that encapsulated CsA and FK506 moderately reduced H_2O_2-evoked cell damage in SH-SY5Y cells. In addition, both types of nanoparticles crossed BBB in its *in vitro* model in time-dependent manner. The maximum fluorescence intensity, which corresponds to the maximum quantity of NPs that passed BBB in chosen model, was obtained after 48 hour of treatment. Overall, these data point to biocompatibility and potential utility of proposed polymeric-based nanoparticles for transporting neuroprotective substances to central nervous system.

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References

[1] M. Szczęch, K. Szczepanowicz, Nanomaterials, 13 (2020) p. 496.