New insights in the development of solid lipid nanoparticles for active brain-targeted drug delivery

Joana Fontes Queiroz, Ana Rute Neves, Sofia A.Costa Lima and Salette Reis

REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313 Porto, Portugal
fontes_joana@hotmail.com

Abstract

The challenging of cross the blood-brain barrier (BBB) and reach the brain in an appropriated therapeutic concentration is the Holy Grail for effectively treat and cure brain diseases. The BBB is not only a physical barrier, it constitutes a dynamic, semi permeable and highly selective barrier that protects and supplies the brain. However the BBB represents a considerable obstacle to brain entry of the majority of drugs and thus severely restricts the therapy of many brain diseases including brain tumors, brain HIV, Alzheimer and other neurodegenerative diseases. The traditional drug delivery systems with no brain targets release the drugs in systemic circulation failing the delivery into the brain. Therefore, there is a huge need to develop and design approaches with specific target to brain in a better and more effective way for the treatment of brain diseases. Here, the nanotechnology can be an important tool to improve the specificity and permeability of drugs in the BBB [1].

In this work we developed a new delivery system to direct drugs to the brain, by functionalizing solid lipid nanoparticles (SLNs) with apolipoprotein E (Apo E), aiming to enhance their binding to low-density lipoprotein (LDL) receptors overexpressed on the BBB endothelial cells.

SLNs were successfully functionalized with Apo E, using two distinct strategies which took advantage of the strong interaction between biotin and avidin. The functionalization of SLNs with ApoE was demonstrated by infrared spectra and fluorimetric assays. Transmission electron microscopy (TEM) images revealed spherical nanoparticles, dynamic light scattering (DLS) gave a Z-average under 200 nm, polydispersity index below 0.2 and zeta potential between -10 mV and -15 mV. A stability study revealed that these characteristics remained unchanged for at least 6 months. In vitro cytotoxic effects were evaluated by MTT and LDH assays in the hCMEC/D3 cell line, a human BBB model, and revealed no toxicity up to 1.5 mg/ml for 4 hour of incubation. The BBB permeability was also evaluated in transwell devices with hCMEC/D3 monolayers and it was found a 1.5-fold increase in the permeability of functionalized SLNs when compared with non-functionalized ones.

In order to clarify the transport pathways of the nanoparticles through the BBB, the different molecular mechanisms of endocytosis and transcytosis processes were carefully studied using flow cytometry system (FCS), confocal laser scanning microscopy (CLSM) and fluorimetric assays with tracers and different pathway inhibitors. The transport of SLNs across the hCMEC/D3 monolayer was found through a transcellular but not a paracellular route. Functionalized SLNs exhibited higher

intracellular uptake compared with non-functionalized ones and were found to enter the cells through a specific clathrin-mediated mechanism, related to the expression of LDL receptors on BBB.

The results suggested that these novels ApoE-functionalized SLNs resulted in dynamic stable systems capable of being used for an improved and specific brain delivery of drugs through the BBB.

Acknowledgments

ARN acknowledges the FCT for financial support through the Ph.D. grant (SFRH/BD/73379/2010). The authors are greatly indebted to financing sources of EU through the Project ON2, NORTE-07-0124-FEDER-000067 and from FCT through the project Pest-C/EQB/LA0006/2013.

References

[1] Kaur I. P.; et al., Journal of Controlled Release**. Potential of solid lipid nanoparticles in brain targeting** (2008) 127, 97-109.