SYNTHESIS AND CHARACTERISATION OF A MACROPHAGE-DERIVED HYBRID NANOSYSTEM FOR DOXORUBICIN DELIVERY TO GLIOBLASTOMA

Girstautė Dabkevičiūtė[1], Christian Celia[2], Vilma Petrikaitė[1,3]

[1]Life Sciences Center, Institute of Biotechnology, Vilnius University, Lithuania
[2]Department of Pharmacy, University of Gabriele dAnnunzio Chieti Pescara, Italy
[3]Laboratory of Drug Targets Histopathology, Lithuanian University of Health Sciences, Lithuania
girstaute.dabkeviciute@bti.stud.vu.lt

Glioblastoma (GBM), a highly aggressive brain cancer, has a median survival of 12-14 months with limited therapy options [1]. The challenging GBM microenvironment hinders conventional drug delivery, motivating nanoparticles (NPs) exploration. Liposomes (LPs), notable for their biocompatibility and targeted drug delivery capabilities, and naturally released extracellular vesicles (EVs), are promising drug carriers. Combining LPs and EVs creates a hybrid system with enhanced therapeutic properties [2]. Our study focuses on synthesising and characterising a LPs-EVs nanosystem for DOX delivery to GBM cells.

Two LP formulations, F1 and F2, were created via the thin layer evaporation (TLE) method, each with specific lipid molar ratios: 6:3:1 for DPPC:CHOL:DSPEmPEG2000 in F1 and 7:4:6:1 for DPPC:DPPS:CHOL:DSPEmPEG2000 in F2. Lipid films were hydrated with either a PBS solution (10 mM, pH 7.4), ammonium sulphate solution (250 mM, pH 5.5), or M0 macrophage-derived EVs in PBS, following extrusion through membrane filters (pore sizes: 800 to 100 nm). For therapeutic LPs, DOX was entrapped using a pH gradient and remote loading procedure, with entrapment efficiency determined by fluorescence. Size, polydispersity index (PdI), and zeta potential (ZP) were assessed using dynamic light scattering. Long-term stability of empty, DOX-loaded, and hybrid NPs at 4°C was evaluated weekly for a month. Short-term stability of empty and DOX-loaded LPs in PBS and DMEM with 10% FBS at 37°C under stirring was assessed at various time points for 72 hours.

The average size of F1, F2, M0-F1 and M0-F2 NPs was below 136 nm, while DOX-loaded liposomes increased in diameter by up to 30 nm. The PdI of empty NPs was lower than 0.14 and DOX loading increased it up to 0.18. ZP of empty NPs was –25.7 ± 1.5 mV (F1), –19.4 ± 1.9 mV (F2), -24.6 ± 2.4 mV (M0-F1), and -36.4 ± 3.9 mV (M0-F2), while DOX loading altered ZP from 3 to 6 mV. F1, F2, DOX-F2, M0-F1 and M0-F2 remained stable at 4°C for 4 weeks, showing no significant changes in size, PdI, and ZP, However, DOX-F1 exhibited an increase in PdI up to 0.3 after 17 days. Results showed that both empty and DOX-loaded NPs remained stable in PBS and DMEM with 10% FBS at 37°C under stirring for 72 hours.

To conclude, the F2 lipid formulation exhibited desirable properties as a base for the synthesis of hybrid macrophage-derived nanosystem, and M0-F2 NPs are worthy of further studies for DOX delivery to GBM cells.

[1] S. Bahadur et al. Current promising treatment strategy for glioblastoma multiform: A review. (Oncol. Rev. 2019)
[2] Z. Liu et al. Nanoscale drug delivery systems in glioblastoma (Nanoscale. Res. Lett. 2022)