Bifunctional Lipid-like 4-(N-Alkylpyridinium)-1.4-Dihydropyridines: Contribution of Molecular Structure to Physico-chemical Properties and Cytotoxicity

Pavels Dimitrijevs [1, 2], ***Martins Rucins*** [1], ***Oksana Pertichenko*** [1, 3], ***Ludmila Jackevica*** [1], ***Anita Gulbe*** [1], ***Mara Plotniece*** [1, 2], ***Karlis Pajuste*** [1], ***Arkadij Sobolev*** [1], ***Ilona Domracheva*** [1], ***Dace Bandere*** [2], ***Aiva Plotniece*** [1]

[1] *Latvian Institute of Organic Synthesis*
[2] *Rīga Stradiņš University, Department of Pharmaceutical Chemistry, Latvia*
[3] *University of Latvia, Department of Physics, Laboratory of Magnetic Soft Matter*

Introduction. Discovery and development of various delivery systems remains important. Cationic 1.4-dihydropyridine (1.4-DHP) amphiphiles, capable of transfecting pDNA into different cell lines in vitro were developed by Latvian Institute of Organic Synthesis. It was demonstrated that the 1.4-DHPs with double pyridinium moieties showed high transfection efficiencies in vitro (Hyvönen et al., 2000). This research was the study of novel non-viral delivery systems such as putative delivery systems – original synthetic lipid-like compounds containing pharmacophore group.

Aim, Materials and Methods. The aim of the study is to characterise the physico-chemical, self-assembling and cytotoxic properties for 1.4-DHPs and to clarify the relationships with biological activity.

Cationic 1.4-DHPs were synthesized according to Rucins (Rucins et al., 2014, 2015). Thermogravimetric (TG) and differential thermal analysis (DTA) of 1.4-DHPs were evaluated (SHIMADZU DTG-60). Cytotoxicity of 1.4-DHPs in vitro was assessed using the MTT assay on two monolayer tumor cell lines – HT-1080 and MH-22A in comparison with their action on normal mouse fibroblasts. The Neutral Red Uptake Assay was performed on 3T3 cells according to Strokes (Strokes et al., 2008). Data from the *in vitro* tests was used for estimation of the starting dose for acute oral systemic toxicity tests *in rodent*. Samples for characterisation of self-assembling properties, dynamic light scattering (DLS) studies (Zetasizer Nano ZSP), were prepared by injection method.

Results. Nine cationic synthetic lipids on the 1.4-DHP core containing 4-(N-alkylpyridinium) substituent and/or propargyl moiety/ies as pharmacophore groups were synthesized. Eight out of them had the same trend of TGA – decomposed in one step and showed weight loss in the range 179 and 280 °C. Cytotoxicity tests showed that 4-(N-ethylpyridinium)-1.4-DHPs did not demonstrate any cytotoxic effect on tumor HT-1080 and MH-22A cell lines and their estimated acute oral toxicity LD50 was defined as practically non-toxic. 4-(N-hexylpyridinium)-1.4-DHP derivatives and 4-(N-dodecylpyridinium)-1.4-DHP derivatives possessed high cytotoxicity on tumor cell lines (IC50 1–80 mM) and their estimated oral toxicity LD50 was defined as slightly toxic or non-toxic. The average size of the nanoparticles formed by 1.4-DHP derivatives varied from 30 to over 1000 nm for freshly prepared samples, depending on compound structure.

Conclusions. Variation of propargyl moiety number and position in the 1.4-DHP cycle and alkyl moiety length at quaternised nitrogen atom at position 4 of the 4-(N-alkylpyridinium)-1.4-DHP molecule strongly affects the self-assembling properties and cytotoxicity of tested lipid-like 1.4-DHP derivatives. Presence of a N- dodecylpyridinium moiety at the 1.4-DHP cycle is essential for formation of stable nanoparticles with average diameter values in the rage of 106–147 nm while introducing propargyl moieties at positions 3 and 5 of the 1.4- DHP ring decreases stability of formed nanoparticles. All tested compounds possess thermal stability at the temperature range below their melting points.

Funded by the Latvian National Research Programme BIOMEDICINE (2014–2017).