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Meeting Abstract

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Cytotoxicity, self-assembling and physico-chemical properties of bifunctional lipid-like 4-(*N*-alkylpyridinium)-1,4-dihydropyridines as putative delivery systems

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Background: Design of biologically active delivery materials is an ideal strategy for biomedical applications. Previously, cationic 1,4-dihydropyridine (1,4-DHP) amphiphiles capable of transfecting pDNA into cell lines *in vitro* were developed by our group [1].
Objectives: The aim of the study is characterization of physico-chemical, self-assembling and cytotoxic properties of 4-(*N*-alkyl-pyridinium)-1,4-dihydropyridines.
Methods: Cationic 1,4-DHPs were synthesized according to Rucins *et al.* [2,3]. 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 NRU assay was performed on 3T3 cells according to Stokes [4]. Data from the *in vitro* tests were used for estimation of the starting dose for acute oral toxicity (LD_{50}) tests in rodents. Thermogravimetric (TGA) and differential thermal analysis were evaluated by Shimadzu DTG-60 analyzer. Samples for characterization of self-assembling properties by dynamic light scattering (DLS) measurements (Zetasizer Nano ZSP) were prepared by injection method.
Results: Nine cationic amphiphilic 1,4-DHPs containing 4-(*N*-alkyl-pyridinium) substituent and/or propargyl moiety/ies as pharmacophore groups were synthesized. Cytotoxicity tests showed that 4-(*N*-ethylpyridinium)-1,4-DHPs did not demonstrate any cytotoxic effect on tumor cell lines, their LD_{50} was defined as practically non-toxic. 4-(*N*-hexylpyridinium) and 4-(*N*-dodecylpyridinium)-1,4-DHPs possessed high cytotoxicity on tumor cell lines (IC_{50} 1–80 mM) and their LD_{50} was defined as slightly toxic or non-toxic. The average size of the nanoparticles varied from 52 to over 1000 nm for fresh samples, depending on the compound structure. TGA data demonstrated decomposition in one step and showed weight loss in the range of 179–280 °C.
Conclusions: Increasing of length of the alkyl chain at quaternized nitrogen in 4-(*N*-alkylpyridinium)-1,4-DHPs or the introduction of propargyl moieties in the 1,4-DHP molecule significantly influences the cytotoxicity against cancer cells. The 4-(*N*-alkylpyridinium)-1,4-DHPs form nanoparticles, but from all tested nanoparticles only the ones formed by 4-(*N*-dodecylpyridinium)-1,4-DHPs were stable after two weeks of storage. The presence of a cationic charge and *N*-dodecylpyridinium moiety at the 1,4-DHP cycle is essential for the formation of stable and homogenous nanoparticles. All tested compounds possess thermal stability at the temperature range below their melting points. These lipid-like compounds would be a promising tool for cancer therapy developments.

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Keywords: cytotoxicity – synthetic lipids – nanoparticles – dynamic light scattering

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