

Delivery of complexes based on dendronized thiacalix[4]arenes and small RNAs into HeLa tumor cells

A.I. Stanavaya^{1*}, V.M. Abashkin¹, I.E. Shiabiev², P.L. Padnya², I.I. Stoikov², D.G. Shcharbin¹

¹ *Institute of Biophysics and Cell Engineering of NASB, Minsk, Belarus*

² *A.M. Butlerov Chemistry Institute, Kazan Federal University, Kazan, Russia*

* *alesiastanovaya@gmail.com*

Key words: thiacalix[4]arenes; cellular uptake; HeLa tumor cells; siRNA

One of the promising areas of cancer therapy is targeted delivery of genetic material with the help of nanoparticles. In our studies, we used three conformations of thiacalix[4]arenes (*1,3-alternate*, *cone*, and *partial cone*) that have been modified with cationic dendrons of three generations (G1, G2, and G3). These structures were studied as nanocarriers of small RNAs.

The aim of this study was to analyze cellular uptake of dendronized thiacalix[4]arenes and siRNA complexes into HeLa tumor cells using flow cytometry and fluorescence microscopy.

Flow cytometry analysis showed that complexes based on the studied nanoparticles and small RNAs internalized into HeLa cells. In the case of TCA G1 1,3-alt, a high level of cellular uptake was observed – about 80% after 24 hours of incubation at the N:P ratios of 10:1 and 15:1. TCA G2 1,3-alt showed the lowest level of cellular uptake, whereas TCA G3 1,3-alt showed the highest cellular uptake at the N:P ratio of 15:1. Fluorescence microscopy data showed that TCA G1 1,3-alt accumulated in cells after 24 hours of incubation, while in the case of TCA G2 1,3-alt and TCA G3 1,3-alt, no uptake of complexes was detected. TCA G1 Cone also demonstrated a high level of cellular uptake of the complexes after 24 h of incubation at the N:P ratios of 5:1, 10:1 and 15:1. In case TCA G2 Cone, the level of cellular uptake of the complexes was about 50% at the N:P ratio of 15:1 after 24 hours of incubation, while for the third generation compounds it was the lowest. Fluorescence microscopy study showed that only TCA G1 in cone conformation accumulated in HeLa cells. TCA G1 showed a high level of cellular uptake of the complexes (about 90%) was observed at the N:P ratios of 5:1, 10:1, and 15:1 after 24 hours of incubation. TCA G2 PaCo demonstrated a fairly high level of cellular uptake at the N:P ratio of 15:1, while the third generation compounds showed the lowest level of cellular uptake. Fluorescence microscopy images demonstrated that TCA G1 PaCo accumulate in HeLa cells.

Modified thiacalix[4]arenes of *1,3-alternate*, *cone*, and *partial cone* conformations of all three generations accumulate to varying degrees in HeLa cells, but the highest level of cellular uptake was observed for the first generation compounds.

Funding: The study is supported by Belarusian Republican Foundation for Fundamental Research (BRFFR, Grant No. B23RNFM-041) and by the Russian Science Foundation (Grant No. 24-43-10005, <https://rscf.ru/en/project/24-43-10005/>).