

Referee1#

The paper by Isanta et al. describes an original family of prospective transfection agents for cellular delivery of a specific polypurine hairpin type of triplex-forming oligonucleotide, which has been previously studied as a potential gene silencing tool. The transfection agents based on a symmetrical tripod and 4-alkoxy pyridinium structure with long pendant alkyl chains were employed to successfully deliver the oligonucleotidic hairpins into a row of cancer cell lines, and decrease the level of anti-apoptotic target (survivin) mRNA.

I think this work may be an important contribution to the field of therapeutic oligonucleotides, and I will be glad to see it published in Biomolecules after a few corrections, which are specified below.

1. The main problem is a very peculiar structure of the survivin oligonucleotide to be delivered. Its polypurine (dA and dG only) sequence is very specific and somewhat distant from either a typical antisense oligonucleotide or siRNA. It would have been great if the authors could additionally provide at least a few examples of how their liposomal system work with regular mixed sequence oligonucleotides around 20 some nt in length characteristic of many antisense agents, and short RNA duplexes such as siRNAs. Inclusion of any examples of successful delivery of the above, especially siRNAs, will tremendously expand the scope of the present work and make it much more compelling.

We performed viability assays and gene silencing experiments by transfecting an antisense oligonucleotide against survivin (previously validated with DOTAP in Coma et al., 2004) but now using TRIFAPY C12. We observed a reduction in cell viability upon the transfection of aODN at two different concentrations (500 nM and 800 nM) in combination with T12C at 1.5 or 2 µg/mL. Moreover, we observed a reduction in mRNA levels of survivin quantified by RT-qPCR after the incubation of 800 nM aODN with TRIFAPY C12 at 2 µg/mL. Thus, we demonstrated the efficacy of this novel tricationic transfectant in the delivery using different types of therapeutic oligonucleotides.

2. Table 1: The structures of the hairpin oligonucleotides in the 2nd and 3rd rows appear to be scrambled, which makes poor impression; may be better to present these in a pictorial format rather than simple textual.

We corrected the table in the manuscript.

3. Unfortunately, the authors did not compare the efficiency of their system with any other common transfection agents such as PEI, which was used in ref 27.

We incorporated into the manuscript the results of cytotoxicity, internalization, and gene silencing experiments conducted using the commercially available liposome DOTAP in the prostate cancer cells (PC-3). Both TRIFAPYs and DOTAP demonstrated efficient results in PPRH transfection. However, it is worth noting that the working concentrations used in experiments with the newly synthesized TRIFAPY compounds were approximately 1 μ M, which is ten times lower than the effective concentrations used for DOTAP (10 μ M).

4. There are no 'oligonucleotide only' controls without liposomes but with DMSO; there is also no separate 'DMSO only' control.

We conducted experiments with PPRHs alone without TRIFAPYs and with DMSO only, which did not produce any effect, and incorporated the viability results of the proposed conditions into the manuscript.

5. Figure 1: replace 'alcoxy' with 'alkoxy'.

Corrected into the manuscript.

Thus, it is formally a major revision as there are some essential controls missing.