Efficient, non-toxic gene delivery by negatively charged polyprenyl-based lipoplexes: Application in RNA delivery and the effects on cell physiology

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The development in the field of DNA and RNA delivery into cells and progress in understanding pathogenesis of many diseases resulted in nucleic acids becoming actually drugs and their delivery one of the top molecular biology techniques applicable in clinics. Still, one of the major challenges facing the development of gene therapy is lack of efficient and safe gene vectors.

We have examined a new class of polyprenyl-based cationic lipids for gene transfer. Studies have shown that semisynthetic polyprenyltrimethylammonium iodides (PTAI**)** in formulations with co-lipids (DOPE, DC-cholesterol, DOPC) have the ability to effectively transfect plasmid DNA in a wide range of cell types *in vitro* both in the presence and absence of serum. Although generally it is considered that bigger lipoplexes bearing positive zeta potential are more efficient, our data clearly demonstrate that small (90 – 150 nm), negatively charged (about -30 mV) polyprenyl-based lipoplexes are efficient and have parameters making them promising candidates for *in vivo* gene delivery.

As it was demonstrated that lipofection procedure may have several side effects on cell physiology, we tested the effects of PTAI formulation on cell motility, proliferation, viability and gap junctional intercellular coupling (GJIC). We have tested four derivatives: amino-Pren-7, amino-Pren-8, amino-Pren-11 and amino-Pren-15. Cell motility of a model DU-145 (human prostate cancer) cells was estimated by time-laps monitoring of movement of individual cells and GJIC intensity measured using donor cells labelled with calcein plated onto monolayers of acceptor cells transfected with PTAI-based lipoplexes. The dynamics of calcein transfer from donor to acceptor cells was analyzed. Antimicrobial activity was evaluated by colony reduction assay and the hemolytic activity against human red blood cells (RBCs) was tested using PBS suspension prepared from fresh blood.

The results show that lipoplexes based on PTAI have no effects on cell physiology that is cell viability, proliferation and morphology. Moreover, they also occurred to have no effect on GJIC and cell motility (24 hours after transfection all the cells cover the distance of about 210-240 µm showing a displacement of 70-80 µm). Some PTAI-based vectors exhibit potent bactericidal activity against *Streptococcus aureus* and *Escherichia coli*, while showing no toxic effect on eukaryotic cells, which can be beneficial during prolonged storage of formulations. Furthermore, (as we suggest *in vivo* application of PTAI vectors) we have also proved their safety towards human RBSs, which membranes are not disrupted in the presence of all the examined concentrations of PTAI-based lipoplexes. Moreover, the formulations tested in plasmid DNA transfer into cells are also effective in gene silencing techniques utilizing RNA delivery. We have successfully introduced shRNA inducing GFP gene silencing into DU145, XC (rat sarcoma) and B16F10 (mouse melanoma) cells expressing pEGFP-C1 plasmid achieving GFP gene silencing. Additionally, PTAI-based formulations can be safely stored for extended periods (up to 18 months) at 4°C.

In conclusion, lipoplexes based on PTAI provide ability to introduce DNA or RNA into cells with satisfying efficiency, easily and safety, as they exhibit no toxic activity and no side effects on cell proliferation, motility and GJIC. What is more, PTAI-based formulations show advantages important for convenient use (both – DNA and RNA delivery, antimicrobial activity, prolonged storage) and *in vivo* applications (no RBCs rupture in the presence of PTAI-based lipoplexes, effectiveness in the presence of serum).

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