Polyplex based on polycarbonate polymer for an efficient delivery of anti-angiogenic siRNA

Antoine Frère[a], Sarah Tempelaar[d], Paul Peixoto[b], Olivier Peulen[b], Brigitte Evrard[a], Philippe Dubois[d], Laetitia Mespouille[d], Denis Mottet[c] ,Géraldine Piel[a]

[a] *Laboratory of Pharmaceutical Technology and Biopharmacy, University of Liege, Liege, Belgium; E-mail: antoine.frere@ulg.ac.be*

[b] *Metastasis Research Laboratory – GIGA, University of Liege, Liege, Belgium*

[c] *Protein Signalisation and Interaction – GIGA, University of Liege, Liege, Belgium*

[d] *Laboratory of Polymeric and Composite Materials, University of Mons, Mons, Belgium*

Introduction

To meet the challenge of gene therapy and deliver small-interfering RNA (siRNA) into the cytoplasm of target cells, several barriers must be overcome. The most widely used polymeric vector is polyethylenimine (PEI) but its cytotoxicity limits its use *in vivo*. As a safe alternative, we designed and synthesized guanidium and morpholino functionalized biocompatible polycarbonate polymer that are able to complex the siRNA with a significantly lower cytotoxicity than PEI [1]. The siRNA used in the study is specifically targeted against histone deacetylase 7 (HDAC7) mRNA. The specific shutdown of HDAC7 protein disturbs the angiogenic process of endothelial cells, making it an attractive target for an anti-angiogenic therapy, interfering with the cancerous tumor growth and metastasis development [2].

Materials & Methods

To be effective *in vivo*, polyplexes must meet several physicochemical characteristics. Polyplexes are characterized at different ratios (N/P, polymer/siRNA). The incorporation of the siRNA into the polyplexes is determined by agarose gel electrophoresis and by the Quant-iT™ RiboGreen® kit. Size and zeta potential are measured using a Zetasizer Nano ZS®, and the buffering capacity of polymers by acid-base titration. The transfection capacity of polyplexes has been examined in HeLa cells (determined by flow cytometry and microscopy). RT-qPCR and western blot have been performed to assess the expression level of HDAC7 mRNA and protein in treated cells compared to a control. To assess the toxicity of polycarbonate polymers, a WST-1 assay was performed.

Results & Discussion

Among the different tested polycarbonate polymers, only the PTMC-b-PCG-b-PCM polymer shows a significant efficacy in the shutdown of the targeted mRNA, coding for the HDAC7 protein. At the optimal N/P ratio, polyplexes formed by the self-assembly of PTMC-b-PCG-b-PCM polymer and HDAC7 siRNA completely incorporate the siRNA, have a diameter around 200 nm and a slightly positive ζ potential value, around 10 mV. Experiments have shown that the buffer capacity of co-polymers, an important characteristic to escape from the endosome through the "proton-sponge effect" after the cellular uptake, is directly related to the morpholine/guanidine ratio. The buffer capacity of polymers with an equal ratio of these functions is close to that of PEI. Flow cytometry and fluorescent microscopy show a high cellular uptake of polyplexes, with more than 90% of transfected cells. Moreover, this co-polymer has a significantly lower cytotoxicity than PEI.

Conclusion

Synthetic guanidium and morpholino functionalized biocompatible polycarbonate polymers proved high ability to form polyplexes with low cytotoxicity compared to well-known PEI used as reference in this work. PTMC-b-PCG-b-PCM polyplexes possess promising physicochemical characteristics with a possible future use in gene therapy.

References

1. W. Y. Seow, Y.Y. Yang, *Journal of Controlled Release*, 2009, **139**, 40-47.
2. D. Mottet, A. Bellahcene, S. Pirotte, D. Waltregny, et al., *Circulation Research*, 2007, **101**, 1237-1246.