POLYPLEXES BASED ON POLYCARBONATE POLYMERS AND siRNA AGAINST HDAC7 FOR A TARGETED ANTI-ANGIOGENIC THERAPY

Antoine Frère[1], Michal Kawalec[3], Paul Peixoto[2], Brigitte Evrard[1], Philippe Dubois[3], Laetitia Mespouille[3], Denis Mottet[2], Géraldine Piel[1]

[1]Laboratory of Pharmaceutical Technology and Biopharmacy - CIRM, University of Liege, Liege, Belgium
[2]Metastasis Research Laboratory – GIGA Cancer, University of Liege, Liege, Belgium
[3]Laboratory of Polymeric and Composite Materials, University of Mons, Mons, Belgium
E-mail: antoine.frere@ulg.ac.be

The growth and spread of cancerous tumors is possible through angiogenesis: the formation of new blood vessels from the pre-existing vasculature. These new capillaries allow a supply of oxygen and nutrients necessary for the tumor development. They also allow the spread of the tumor in the body and the formation of metastases.

A family of enzymes, named histones deacetylases (HDAC), play key role in angiogenesis. Among the different HDAC members the specific inhibition of HDAC7 disturbs the angiogenic process, making it an attractive target for an anti-angiogenic therapy (1). To specifically knockdown HDAC7 expression, siRNA technology is used. This specific siRNA recognizes and leads to the degradation the mRNA encoding for HDAC7 protein. The delivery of siRNA into the cytoplasm of target cells to exert their effects remains a significant challenge. Novel nanoparticle-based approaches that enable more efficient delivery of siRNA sequences are constantly progressing towards the goal of meeting the challenge of delivery.

Vectors used in this work are polyplexes formed by the self-assembly of biodegradable polycarbonate polymers and siRNA specifically targeted against HDAC7. To be effective *in vivo*, polyplexes must meet several physico-chemical characteristics. To get the best physico-chemical characteristics, many parameters can be modified like the polymer structure, the polyplexes formulation, the preparation method, ... (2). The main characteristics are the incorporation of the siRNA into the polyplexes (determined by agarose gel electrophoresis or by the Quant-iT™ RiboGreen® kit), the size (measured by dynamic light scattering), the charge (zeta potential measured by laser Doppler velocimetry) and the buffering capacity (measured by titration). The transfection capacity of polyplexes with good physicochemical characteristics has been examined in HeLa cells (determined by flow cytometry and microscopy). A western blot has been performed to assess the expression level of HDAC7 protein in treated cells compared to a control.

Different architectures of biodegradable polycarbonate polymers at different ratios (N/P, polymer/siRNA) have been tested. Most of them show a complete incorporation of the siRNA at N/P above 10. At these N/P ratios, size measurements show an average diameter between 200 and 500 nm. Ideally, the diameter should be around 200 nm; not smaller to avoid renal excretion and not too big to escape the monomolecular phagocytic system. This scale also allows accumulation of nanoparticles in the tumor due to the enhanced permeability and retention (EPR) effect (3). Zeta potential is slightly positive, around +5mV. To interact with cell membranes, nanoparticles must have a slight positive charge, lower than 10 mV to avoid a too high toxicity. *In vitro*, flow cytometry shows a high transfection level for most selected polyplexes, up to 90% of transfected cells. Unfortunately, no decrease of the expression of HDAC7 has been observed by western blot.

The two main hypotheses to explain the lack of efficacy of our new polyplexes are either a too high affinity between the polymer and siRNA that prevents the release of the siRNA in the cytoplasm or either the use of an endocytosis pathway without vesicle acidification. This acidification allows the endosome bursting using the buffering capacity of the polymer through the « proton sponge effect » (4). These two hypotheses will be further studied.

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