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The fate of poly(2-dimethyl amino ethyl)methacrylate-based polyplexes after intravenous administration☆

F.J. Verbaan [a,*], C. Oussoren [a], I.M. van Dam [a], Y. Takakura [b], M. Hashida [b], D.J.A. Crommelin [a], W.E. Hennink [a], G. Storm [a]

[a] *Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, PO Box 80082, 3508 TB, Utrecht, The Netherlands*

[b] *Graduate School of Pharmaceutical Sciences, Department of Drug Delivery Research, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan*

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Abstract

Poly(2-dimethyl amino ethyl) methacrylate (pDMAEMA) cationic polymers have been shown to be efficient vectors for gene delivery in vitro. This contribution deals with the in vivo properties of polyplexes based on this polymer. In mice, pDMAEMA/[^{32}P]-pLuc complexes distributed primarily to the lungs. The gene expression profile matched the biodistribution profile. In vitro turbidity experiments in serum showed severe aggregation upon addition of cationic polyplexes, pointing out the involvement of aggregates in the dominant lung uptake of the positively charged polyplexes. Incubations of polyplexes with albumin yielded a decline of the zeta potential of the complexes to negative values, making an electrostatic mechanism in the dominant lung uptake less likely. Hemagglutination experiments showed that the polyplexes induce the formation of extremely large structures when incubated with washed erythrocytes. Altogether, the present data indicate that aggregate formation and trapping of the formed aggregates in the lung capillary bed is probably responsible for the dominant lung uptake and transfection. Poly(ethylene)glycol (PEG) of the polymeric structures prevented the increase in the observed turbidity in serum seen with polyplexes and was also able to reduce interactions with erythrocytes. Currently, the in vivo fate of the PEGylated polyplexes is under investigation.

Keywords: Poly(2-dimethyl amino ethyl)methacrylate; Transfection; Distribution; Zeta potentials; Albumin; Hemagglutination; Erythrocytes; Hemagglutination

Viral vectors have been investigated extensively as carriers for genetic material, and have been shown to provide for significant transfection efficiencies. However, there are also a number of serious disadvantages associated with the use of viral vectors, such as concerns about immunogenicity, safety and large-scale production of viral

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* Corresponding author. Tel.: +31-30-2536899; fax: +31-30-2517839.

E-mail address: f.j.verbaan@pharm.uu.nl (F.J. Verbaan).

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vectors (Lehn et al., 1998). Therefore, much attention is focused on the development of non-viral vectors for the formulation of genes for in vivo gene transfer. Non-viral vectors include for example cationic lipids (lipoplexes) and cationic polymers (polyplexes). The in vivo behavior of these carrier systems needs to be well understood in order to be able to develop safe and efficient gene delivery systems. Recently, we started to investigate the utility of the cationic polymer poly(2-dimethylamino ethyl)methacrylate (pDMAEMA) (Fig. 1) as a non-viral vector. Binding of this cationic polymer to DNA led to condensation of the DNA. The size and zeta potential of the condensates, called polyplexes, were shown to depend on the polymer/DNA ratio and to be important factors in determining the transfection efficiency (Cherng et al., 1996). Highly charged (30 mV) polyplexes, around 200 nm in size, possessed the highest transfection activity in vitro, when evaluated in COS-7, OVCAR-3 and B16 cell cultures (van de Wetering et al., 1997). Since the first step in the transfection process is binding of the polyplexes to the cell surface, possibly followed by internalization of the polyplexes by the cell, fluorescent activated cell scanning experiments were performed on OVCAR-3 cells using DNA labeled with ethidium homodimer-1. Incubation of the cells with the free probe and naked

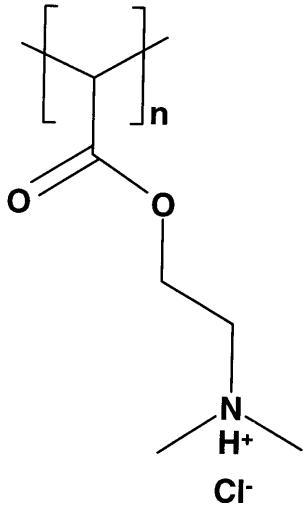

Fig. 1. Structure of poly(2-dimethyl amino ethyl) methacrylate.

plasmid did not result in an increase in fluorescence of the cells. Polyplexes prepared at a low ratio polymer/DNA ratio (0.19 w/w), bearing a net negative zeta potential, did not cause an increase in fluorescence of the cells as well. OVCAR-3 cells incubated with polyplexes prepared at higher ratios (3 and 12 w/w), exhibiting positive zeta potentials, resulted in an increase in the cell-associated fluorescence. This indicated that cellular association of fluorescent plasmid occurred at these ratios. Confocal scanning laser microscopy experiments showed that internalization followed the binding of polyplexes to the cells (Zuidam et al., 2000). The results suggested that a positive zeta potential is required for achieving cellular association and transfection in vitro.

The aim of the present study was to assess the behavior of pDMAEMA-based polyplexes in mice after intravenous administration. To this end, plasmid DNA was labeled with [α-^{32}P]-dCTP by nick translation. Approximately 6-week-old, female Balb/c received positively charged polyplexes (polymer/DNA ratio 3:1 w/w) labeled with trace amounts of radioactivity, in an injection volume of 200 μl by tail vein injection. At various time points, blood was collected from the vena cava under ether anesthesia and subsequently the mice were killed. Radioactivity levels in each organ were determined. It was observed that the positively charged pDMAEMA/[^{32}P]-DNA polyplexes distributed primarily to the lungs. Within minutes, 80% of the injected dose was recovered from the lungs. In a second set of experiments, transfection studies using plasmid DNA encoding for the firefly luciferase enzyme were performed. Twenty-four hours after intravenous administration of pDMAEMA-based polyplexes (polymer/DNA ratio, 3:1 w/w), luciferase levels were determined in lungs, liver, spleen, kidneys and heart. The results showed that the gene expression profile matched the biodistribution profile of the administered positively charged polyplexes. Most of the expression was seen primarily in the lungs. A third set of experiments was designed to shed more light on the mechanism involved in the dominant lung uptake of polyplexes. In vitro turbidity experiments in serum were performed providing evidence for severe aggregation occur-

ring upon addition of the polyplexes to the serum. Hemagglutination experiments provided evidence that positively charged complexes induce the formation of extremely large structures upon addition to erythrocytes. If formed in vivo, such large aggregates are likely to block the blood flow in the lungs. Another potential in vivo factor may be electrostatic interaction between the cationic polyplexes and the negatively charged lung cell membranes. However, incubation of polyplexes with serum albumin showed that the zeta potential of the complexes drops to negative values, making the possibility of electrostatic interactions less likely.

The 'first pass' distribution of polyplexes to the lungs severely impedes the usefulness of cationic polymers for gene delivery. Therefore, we are currently investigating the use of poly(ethylene)glycol (PEG) modified polymers. Aggregation in serum as demonstrated for non-PEGylated polymers in turbidity experiments in vitro could be prevented by coupling PEG to the polymeric structures. PEGylation also yielded a drop in the zeta potential of the complexes to almost neutral, also when incubated with serum albumin. Furthermore, severe hemagglutination was not observed when erythrocytes were incubated with the PEGylated complexes. Most importantly, initial in vivo experiments showed that gene expression in the lung using the PEGylated polyplexes is almost completely absent, which is consistent with our in vitro observations pointing to reduced interactions of PEGylated polyplexes with blood components.

References

Cherng, J.Y., van der Wetering, P., Talsma, H., Crommelin, D.J.A., Hennink, W.E., 1996. Effect of size and serum proteins on transfection efficiency of poly((2-dimethylamino)ethyl methacrylate)-plasmid nanoparticles. Pharm. Res. 13, 1038–1042.

Lehn, P., Fabrega, S., Oudrhiri, N., Navarro, J., 1998. Gene delivery systems: bridging the gap between recombinant viruses and artificial vectors. Adv. Drug Deliv. Rev. 30, 5–11.

van de Wetering, P., Chern, J.Y., Talsma, H., Hennink, W.E., 1997. Relation between transfection efficiency and cytotoxicity of poly(2-dimethylamino)ethyl methacrylate)/plasmid complexes. J. Control. Release 49, 59–69.

Zuidam, N.J., Posthuma, G., de Vries, E.T., Crommelin, D.J., Hennink, W.E., Storm, G., 2000. Effects of physicochemical characteristics of poly(2-(dimethylamino)ethyl methacrylate)-based polyplexes on cellular association and internalization. J. Drug Target. 8, 51–66.