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Note

Intrapericardial administration of novel DNA formulations based on thermosensitive Poloxamer 407 gel

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Abstract

Inherited cardiopathies are leading to life-threatening conditions such as heart failure. Moreover, treatments currently available fail in altering the cardiac phenotype. Thus, gene therapy appears as an attracting alternative to conventional treatments. However, gene delivery remains a major hurdle in achieving this goal. To obtain regional delivery of plasmid DNA, intrapericardial administration seems to be an interesting approach. In order to improve retention time at the site of injection, formulations based on a thermosensitive gel of Poloxamer 407 were assessed. Protection and condensation of plasmid DNA was initially performed through complexation with polyethyleneimine (PEI), a widely used polymer. Characterization of the size and zeta potential of the complexes suggested interactions between the polyplexes and the Poloxamer gel through significant increase of the size of the polyplexes and shielding of the surface charges. *In vivo* evaluation has highlighted the toxicity of PEI/DNA polyplexes toward the myocardium. However, feasibility of intrapericardial injection of Poloxamer based formulations as well as their very low toxicity has been established.
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Keywords: Gene therapy; Plasmid DNA; Intrapericardial administration; Thermosensitive gel; Polyethyleneimine

When considering familial cardiopathies related to a genetic defect, treatments currently available mainly affect the biochemical and hemodynamic environment of the myocardium but fail in modifying the cardiac muscle cells by themselves. In such context, a therapeutic approach based on gene transfer seems to be interesting in order to modify the cardiac phenotype (Nayak and Rosengart, 2005; Sun, 2006). However, the major hurdle remains the design of safe and efficient gene carriers.

Our work aims at designing a new delivery system to achieve gene transfer to the myocardium. In order to obtain regional expression of the therapeutic gene, various routes of administration have been considered (Jones and Koch, 2005; Quarck and Holvoet, 2004). Direct injections into the myocardium can be performed, nevertheless leading for each injection site to a restricted area of transfection. Since cardiomyopathies are displaying diffuse impairments of the myocardium, more global treatment of the heart is required. To improve diffusion, advantage could be taken from coronary infusion (Donahue et al., 1997; Logeart et al., 2001). However, the residence time at the site of injection is very brief due to coronary blood flow and could hamper the efficiency of the treatment. In the past years, administration in the pericardial sac has been described and could be an interesting alternative to administer nucleic acids (Fromes et al., 1999). The closed cavity of the pericardium will indeed allow a prolonged retention time of the formulations that might further be improved by inclusion of the gene carrier in a gel matrix. Poloxamer 407, a copolymer of ethylene oxide and propylene oxide, is able to undergo a sol to gel transition at high concentration and at body temperature (Edsman et al., 1998). Such a system was shown to allow retaining colloidal particles at a local site and to induce a more controlled and prolonged delivery of nucleic acids (Bochot et al., 1998; Fattal et al., 2004). Furthermore, the formulation should ensure condensation and protection of plasmid DNA. This will be achieved through the association of DNA with polyethyleneimine (PEI), a polymer displaying a high density of positive charges, widely used as

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gene carrier (Boussif et al., 1995; Demeneix and Behr, 2005; Godbey et al., 1999; Lungwitz et al., 2005).

In this work, the feasibility of intrapericardial administration of PEI/DNA complexes (polyplexes) introduced into a thermosensitive gel was assessed. The polyplexes, included or not into the Poloxamer gel, were characterized in terms of size and zeta potential. *In vivo* evaluation of the systems was also performed after intrapericardial injections.

The plasmid coding for the bacterial β-galactosidase (pCMV-LacZ, Clontech, France) was used as a reporter system in this study. The plasmid was amplified in JM109 *E. coli* and purified using the Endofree Plasmid Giga Kit (Qiagen S.A., France). DNA was condensed with branched polyethylenimine (average MW: 25 kDa; Sigma–Aldrich, France) at a nitrogen to phosphate ratio (N/P ratio) of 10. Equal volumes of suitably diluted solutions of DNA and PEI were gently mixed by pipetting and allowed to equilibrate at least 30 min at room temperature prior to use. Poloxamer 407 (Lutrol® F127; BASF, France) gels at 35% (w/v) were prepared according to the cold process described by Schmolka (1972): an appropriate amount of Poloxamer was slowly added to sterile water maintained at 4 °C under constant stirring. Afterwards, gels are stored overnight at 4 °C prior to use. Hydrodynamic diameter and zeta potential of the polyplexes were assessed by dynamic light scattering (DLS) and laser Doppler velocimetry (Zetasizer Nano ZS, Malvern), respectively. Experiments were carried out on polyplexes displaying a DNA concentration ranging from 0.1 to 0.8 mg/ml. Evaluations were performed either on polyplexes diluted with a 1 mM NaCl solution or dispersed within Poloxamer gel (20%; final concentration). Gel samples were allowed to equilibrate at 4 °C for 5 h before use. Afterwards, 200 μl of Poloxamer formulations were diluted in 4 ml of 1 mM NaCl. Solutions obtained are considered to be Newtonian. Each formulation was evaluated on three independent samples, and three measurements were performed on each sample. Results were averaged for each formulation (mean ± standard deviation). The same samples were used for both DLS measurements and zeta potential analysis. *In vivo* evaluation was performed on wild type Syrian hamsters ($n = 3$ for each formulation). Each animal was injected with 200 μg of DNA, either naked or condensed with PEI (N/P = 10). Both preparations were included in 600 μl (final volume) of an aqueous solution or the Poloxamer gel at 20% (w/v; final concentration). All formulations and materials employed were kept at 4 °C. Administration through the intrapericardial route was achieved as previously described (Fromes et al., 1999). Briefly, animals were anaesthetized and a small laparotomy was performed below the xyphoid appendix. A transdiaphragmatic approach was used to achieve injections into the pericardial sac. At settled intervals (1, 3 and 5 weeks), one animal of each group is sacrificed with an overdose of anaesthetics and heart as well as lung, liver, kidney and spleen were harvested. Serial sections were performed on frozen organs, assayed for β-galactosidase activity by X-Gal (Promega, France) incubation and counterstained by haematein and eosin.

Polyplexes prepared at low DNA concentration displayed a small size of particles (100 nm) and a strongly positive zeta potential (+44 mV). Inclusion of the polyplexes into the Poloxamer gel results in a 100% increase of the hydrodynamic diameter of the particles and a 25% decrease of their zeta potential (Fig. 1). Polyplexes prepared at higher DNA concentration displayed a major peak around 150 nm and secondary populations with hydrodynamic diameters ranging from 500 to 900 nm. Furthermore, dispersion of the polyplexes into the gel led to a significant increase in size and a reduced zeta potential.

Various formulations have been injected into the pericardial sac and toxicity and efficiency were assessed. Formulations based on polyplexes exhibited a high toxicity toward the myocardium. Five of the six animals injected with the polyplexes died within 1 h following the injection. Sections performed on the heart of the animal that did survive exhibited wide areas of inflammation and necrosis (Fig. 2a). On the contrary, animals injected with naked DNA either prepared in an aqueous solution or the Poloxamer gel, did not display any toxic effect, as confirmed by the morphological analysis of heart sections (Fig. 2b). One animal injected with the Poloxamer based formulation died at the time of the administration, due to anaesthesia. For all the formulations tested, no activity of the transgene as well as no lesions were detected on the sections performed on liver, lung, kidney and spleen (data not shown).

Characterization of the polyplexes highlighted the small size of particles at the N/P ratio used and at low DNA concentration. However, polyplexes have a high tendency to aggregate when

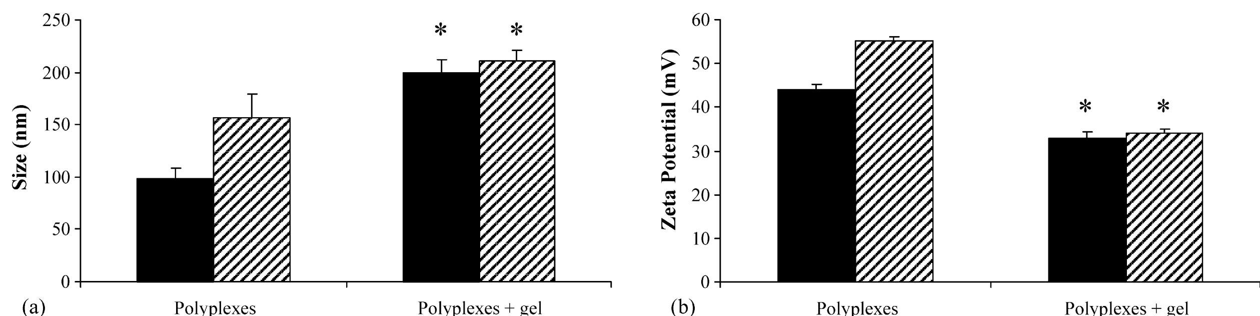

Fig. 1. Size (a) and zeta potential (b) of the polyplexes were analyzed in two distinct formulations. On one hand, polyplexes were dispersed in an aqueous solution, on the other hand in the Poloxamer 407 gel. Furthermore low DNA concentration (0.1 mg/ml; plain) and high DNA concentration (0.8 mg/ml; hatched) were evaluated. Comparisons have been made pairwise between polyplexes containing the same DNA concentration and dispersed either into the gel or in the aqueous solution. Statistical significance was assessed using Student's *t*-test. $^{*}p < 0.001$; polyplexes in aqueous solution versus polyplexes dispersed in Poloxamer gel, at the same DNA concentration.

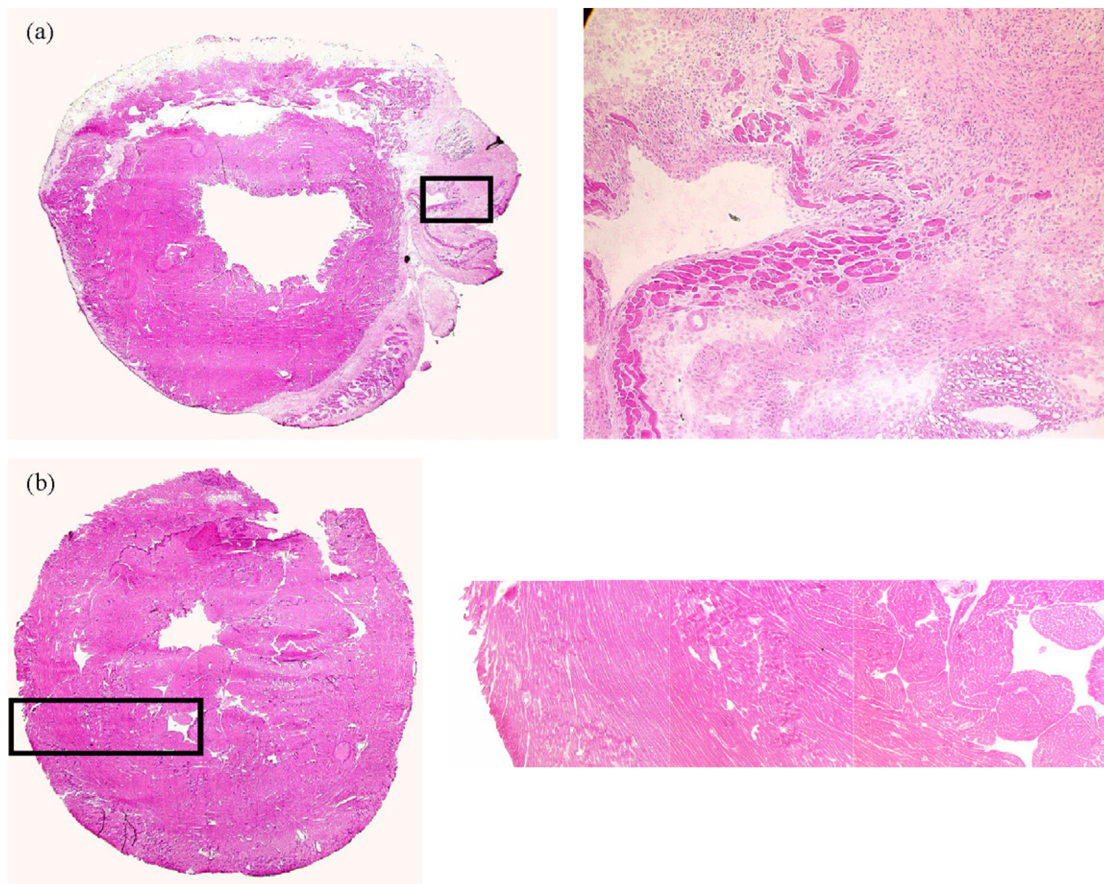

Fig. 2. Sections performed on harvested hearts injected with PEI/DNA complexes in aqueous solution (a) or naked DNA included into the Poloxamer 407 gel (b). No inflammatory lesions were observed on the myocardium or on the pericardium with Poloxamer 407 based formulations associated to naked DNA (b).

increasing DNA concentration, a result consistent with previous observations (Kircheis et al., 2001; Ogris et al., 1998). When introducing polyplexes into a Poloxamer gel at 20%, the mean hydrodynamic diameter of polyplexes is significantly increased. A significant decrease of zeta potential values is also observed. These results suggest the development of interactions between the Poloxamer and the complexes, leading to the shielding of surface charges of the polyplexes. Such observations were also reported when dispersing cationic liposomes into Poloxamer 407 gels and, at a lesser extent, with negatively charged liposomes (Bochot et al., 1998).

Intrapericardial administration of naked DNA did not result in significant transfection in the myocardium. Moreover, inclusion of DNA into the Poloxamer gel did not allow improving activity of the transgene. These observations are not surprising since the pericardium has been shown to be an anatomical barrier to gene transfer due to the tight junction of the pericardial cells (Fromes et al., 1999; Lamping et al., 1997). To increase transfection efficiency, our approach consisted in condensing DNA with PEI, one of the most effective non-viral gene carrier currently available. However, cytotoxic effects have been observed with such formulations (Chollet et al., 2002; Regnstrom et al., 2003) and seem to be at least partially related to free PEI. Achieving purification of the formulations by removal of free polycations led *in vivo* to reduced toxicity but also to a 200-fold decrease in the transgene expression (Boeckle et al., 2004). Inclusion of the polyplexes in a gel matrix was expected to allow progressive release of the polyplexes and broader diffusion of the formulations around the myocardium. However, these formulations exhibited high toxicity toward the myocardium in our experimental conditions. Due to this pronounced toxic effect, transgene activity could not be detected.

Nonetheless regarding the practical utility of Poloxamer gels, our study has shown the feasibility of the administration of formulations based on a thermosensitive gel through intrapericardial injection. The very low toxicity of the Poloxamer gel toward the myocardium was also established. Moreover, neither ectopic expression of the transgene has been observed nor extracardiac toxicity.

In summary, intrapericardial administration of gene carriers dispersed in a Poloxamer 407 gel seems a promising approach to target the myocardium. However, the optimization of the vector will be required in order to obtain safe and efficient delivery systems.

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