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Improvement of Synthetic Vectors for Gene Therapy Using Ring-Opening Cationic Polymerization

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Summary: For efficirent gene transfer, DNA has to be associated with a carrier which can be either of biological (i.e. recombinant viruses) or synthetic origin. In the domain of polymeric synthetic vectors two general types can be envisaged, namely positive polyelectrolytes or neutral amphiphilic block copolymers. Both types can be synthesized by ring-opening cationic polymerization. Several examples show that the versatility of this polymerization technique can be used for the design of structures which after transfection studies, either in vitro or in vivo, can allow conclusions on the most important parameters which govern transfection efficacy. The present study allowed to conclude on the importance of physico-chemical characteristics of these vectors, i.e. the linear macrostructure for the positive polyelectrolytes, and the amphiphilic nature for the neutral block copolymers.

Keywords:

I- Gene Therapy and its Requirements for Synthetic Vectors

Gene therapy is a modification of the genetic information in cells by the introduction of DNA fragments whose role is to induce the synthesis of a required protein, or to correct its synthesis. In order to play such roles, DNA fragments coming from outside of the cells have to cross many barriers, such as cell membranes and nuclear membranes, and most of the time they have to survive systemic conditions and intracellular traffic. It is clear that DNA cannot resist such conditions, and this is why it must be protected by an envelope of appropriate properties. Nature gives models under the form of viruses which are able to infect cells and to add their own DNA to the original DNA, so as to produce new virus. Viruses can be used in order to achieve DNA transfer, and this is the technique of gene transfer with adenovirus and related systems based on biological tools, which can be very efficient but also very difficult to secure. The second technique which presently is less efficient but safer is the use of synthetic envelopes for DNA protection and transportation. The work described in this paper is dealing with this second domain, restricted to synthetic polymeric vectors.

DNA is a water soluble polyelectrolyte with a negatively charged backbone. Since many cells to be treated have a negatively charged surface, for instance epithelium cells of lungs or liver, DNA cannot interact with their surfaces, and for this reason, the synthetic vector which is to be used is a positively charged polyelectrolyte, and related polymers of adequate properties, or a film forming positively charged amphiphilic molecule (cationic lipids). This is the situation for instance in the case of cystic fibrosis for which the lung epithelium is the main target. However, some important situations are dealing with other cell membranes and a positively charged vector can be no longer neither efficient nor

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required. This is more particularly the case of research aiming at the transfection of muscle tissues in the context of myopathy (1). Obviously, the two types of polymeric vectors, i.e. the positively charged polyelectrolytes on the one hand and the neutral (amphiphilic) copolymers on the other hand, are not internalized in the cells by the same mechanism. The former vectors are using the way called endocytosis, receptor mediated or not, and the latter are using pores or holes induced by the interaction of the polymer with the lipidic bilayer of the target cell.

Besides polylysine or polyaspartic acid, the most studied polyelectrolyte for DNA transfection on mammalian cells with is polyethylenimine (PEI) (1–7). While the effects of the mixing sequence during formulation are not completely understood, some characteristics of the behavior of synthetic vectors begin to be known. DNA and the polymer make a complex called polyplex, in such conditions that it can be used either for studies in vitro or in vivo. Of course, in vivo studies are the most interesting because they are closer to the final goal, i.e. therapeutic treatments. The final therapeutic use governs the nature and structure of the polyplex, as well as the administration route. For instance, when the target is the lung epithelium, the introduction of the polyplex will most likely be done by nebulization. However, the main means of administration are systemic injection, which implies that the polyplex must survive long enough to finally reach its target. This second technique thus addresses two different problems, protection of DNA during transfer and targeting the right cells. Whatever the administration way, the polymer-DNA macromolecular assembly must then cross the cell membrane, most probably by endocytosis, then escape the endosome to finally reach the nucleus through the nuclear membrane. The requirements at each step are not necessarily identical and even may look somewhat contradictory.

It was proposed that the high gene transfer efficiency of PEI and related polymers is due to their capacity to buffer endosomes (8–10). This hypothesis is based on the chemical structure of PEIs: they differ from other polymers such as polylysine in that only a fraction of the amino groups are protonated at physiological pH (8,11). When the pH in the endo-lysosomal compartment becomes acidic, the capacity of PEIs to capture protons causes osmotic swelling and subsequent endosome disruption (''proton sponge effect''), thus allowing to release the endocytosed material into the cytosol (10). These findings have led to the design of new polymers that, like PEIs, exploit the acidification of the endocytic vesicles (12,13). Supporting this conclusion is the fact that polylysine, partially substituted with histidyl residues which become cationic upon protonation of the imidazole groups at pH below 6.0, has a transfection efficiency that is significantly higher compared to non-modified polylysine (14). These and other results support the idea that the buffering capacities of PEIs play an important role during transfection. However, since the exact mechanism of PEI-mediated transfection remains to be elucidated, it is possible that additional properties are required to obtain high transfection efficiencies (13). For instance, the size of the formed polyplexes also was a parameter which deserved to be addressed (8). One thing is clear, there will not be a universal formulation allowing DNA transfection in all case.

A preliminary effort was directed in the lab to understand the behaviour of PEI. Two parameters had to be investigated, either the structure of the amine functions borne by the polymer, or the macrostructure of the polymer itself, branched (or hyperbranched) or linear. PEI can be obtained by two ways, either by direct ring opening cationic polymerization of the monomer ethyleneimine, or by cationic ring opening polymerization of 2-substituted-2-oxazoline (15,16) followed by hydrolysis of the resulting polymer. It is well known that the first synthesis gives a highly branched polymer (bPEI) containing approximately 25 % of primary amine, 50 % of secondary amine and 25 % of tertiary

amine functions. The most popular version of this PEI has an average number molar mass of 25 kDa and is extensively used for in vitro and in vivo studies. The second synthesis which makes use of 2-substituted-2-oxazolines, as monomers in ring opening polymerization, gives after polymerization and subsequent hydrolysis a linear polyethyleneimine (lPEI) closely corresponding to a composition of 100 % secondary amine monomer unit. It has been shown that the most efficient LPEI has an average number molar mass of 22 kDa. It is more efficient than the bPEI for in vivo DNA transfection.

II-1 Positive Polyelectrolytes as Synthetic Vectors: Synthesis of Polyamine of Various Amine Structures

A first investigation was dealing with the study of the effect of change of the structure of the amine functions borne by the polymer. This was achieved by polymer modifications starting from poly(2-alkyl-2-oxazoline)s. Suitable hydrogenation can give access to tertiary amine units. Starting from poly(2-ethyl-2-oxazoline), poly(N-propylethylenimine) can be obtained, and of course from poly(2-methyl-2-oxazoline), poly(N-ethylethylenimine). Of note is the fact that partial hydrogenation gives access to copolymers containing N-alkylethyleneimine and 2-alkyl-2-oxazoline units. This aspect is important for the modification of the solubility of some vectors (17). Similarly, partial acid hydrolysis gives access to copolymers of ethyleneimine and 2-alkyl-2-oxazolines. The combination of hydrolysis and of hydrogenation gives the way to terpolymers between the above functions.

This work showed that it is possible to include a fraction of EtOXZ units, which are not positively charged, without decreasing importantly the activity of linear PEIs (17). It was also demonstrated that it is possible to synthesize linear copolymers having tertiary and secondary aziridine units. Polymers containing tertiary amines like LPNPEIs were significantly less efficient in mediating in vitro gene transfer than PEIs, although these polymers contain more amino nitrogens which are not protonated at neutral pH. This result indicated that the capacity to buffer endo-lysosomes is not sufficient to make a molecule an efficient transfection agent (17). A similar finding was made by Tang and Szoka with starburst dendrimers (18,19). They found that in contrast to fractured dendrimers, intact dendrimers were not very efficient in gene transfer although they present groups that can be protonated during acidification of endosomes. Taken together, our results show that the high transfection efficiency of PEIs is not solely based on their capacity to buffer endosomes. Other parameters such as flexibility of the polymer may be of importance.

The introduction of primary amine functions on the PEI backbone was more difficult. Grafting N-(2-chloroethyl)acetamide on PEI was the first step. Secondary reactions leading to homopoly(2-methyl-2-oxazoline) had to be minimized and final hydrolysis gave poly(N-2-aminoethylethyleneimine-co-ethyleneimine) (17).

Starting from poly(2-ethyl-2-oxazoline), by this combination of hydrolysis followed by polymer modification by grafting of N-(2-chloroethyl)acetamide and of a final hydrolysis, a polymer having a linear microstructure but having a functional composition close to that of bPEI, i.e. 25 % of primary amine, 25 % of tertiary amine and 50 % of secondary amine, was obtained (Table I). This polymer was used in transfection assays in vitro on HepG2 cells (Figure 1) and in vivo on Balb C mice (Figure 2).

An interesting parameter is the ratio N/P of the nitrogen atom content of the polymer to the phosphorus content of DNA in the transfection assay. This parameter does not seem to be critical, but it is well known that it must be of the order of magnitude of 10 to give the best efficacy. Linear PEI (LPEI 22 kDa) was synthesized in the lab. These two products, LPEI and BPEI approximately

Table I.
Different PEI based polymers synthesized from various modifications of poly(2-ethyl-2-oxazoline). L-PEI is a linear PEI sample, and B-PEI is a commercial sample of branched PEI.

Polymer	Mn, kDa	Grafting (%)	Primary N (%)	Sec. N (%)	Tert.N (%)
PEI-1[a]	25	17	17	70	15
PEI-2[a]	28	30	23	54	23
PEI-3[a]	33	56	36	28	36
PEI-4[b]	29	21	0	65	35
L-PEI	22	—	0	100	0
B-PEI	25	—	25	50	25

[a] LPEI-g-CH_2-CH_2-NH_2.
[b] LPEI-g-CH_2-CH_2-$N(CH_3)_2$.

have the same efficacy. PEI-2 is the linear polymer having a polymerization degree close to that of LPEI 22kDa but having approximately the same function content, i.e. 30 % primary amine, 30 % tertiary amine and 40 % secondary amine, as the branched PEI 25 kDa (16). PEI-4 is a polymer where the primary amine functions were replaced by dimethylamino functions (tertiary amine) (20). It can be seen that all these polymers induced similar in vitro luciferase activity, independent of the nature of the amine groups borne by the polymer. The in vivo transfection efficiency was also investigated (20) (Fig. 2).

The complexes, prepared in 5 % glucose solution with the same quantity of a plasmide coding for luciferase, were injected intravenously and the luciferase activity was measured in the mice lungs (20). It is to be noted that in identical conditions the branched polymer bPEI 25 kDa induced a luciferase activity several order of magnitude lower than that of lPEI 22 kDa. It must be noticed that if our results show that the transfection efficacy seems to be rather independent of the nature of the amine functions, the toxicity may vary with the nature of the amine functions.

Altogether, our results demonstrated that the linear structure of the synthetic vector was a very important parameter leading to high transfection efficacy. It is more difficult to face toxicity problems during in vivo experiments, and of course during systemic injection than for instance during aerial administration.

II-2 Positive Polyelectrolytes as Synthetic Vectors: Synthesis of Block Copolymers with Polyethyleneimine Blocks of High Molar Mass

It is known that PEI exhibits high transfection efficiency for high polymerization

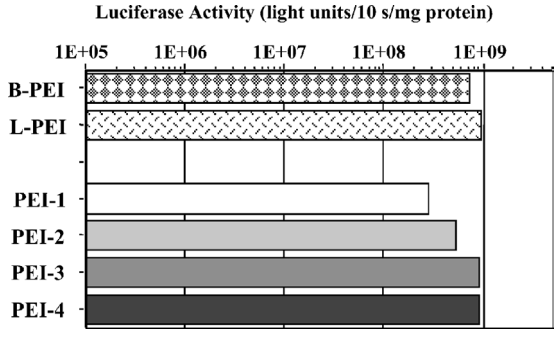

Figure 1.
In vitro transfection efficiency of various PEI based polymers (HepG2 cells). The sample characteristics are described in Table I.

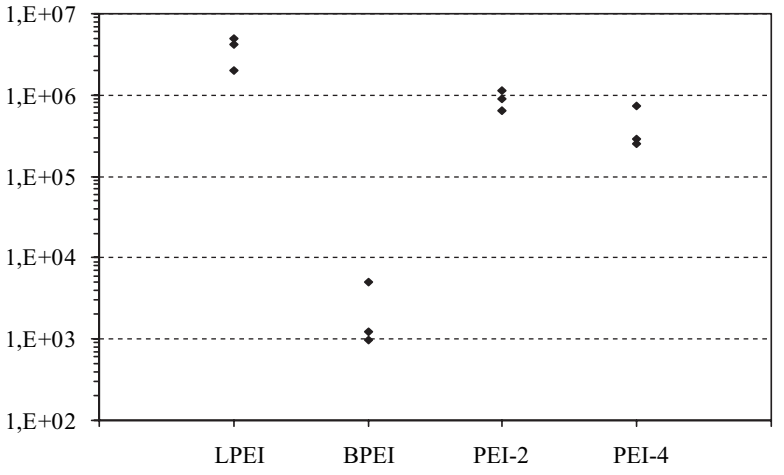

Figure 2.
In vivo transfection efficiency of modified linear poly(ethyleneimine) (Balb C mice). Transfection efficiency is measured in light units/10 sec/mg of protein.

degree. Thus it was anticipated that in the case of block copolymers, it was necessary to use polymers for which the PEI blocks are of a high molar mass, for instance of the order of magnitude of 10 kDa or more.

During transportation in the blood, polyplexes meet aggressive proteins, and it has been shown that one way to prevent their destruction is to protect them by the use of a layer of polyethylene oxide in which proteins cannot penetrate. In other words, a better synthetic vector would be a block copolymer poly(ethyleneimine-b-ethylene oxide). This block copolymer interacts with DNA by the polyethyleneimine segment which is partly quaternized at the physiological pH and protects the polyplex by a hair of poly(ethylene oxide) (PEO) segments which prevent contact with enzymes. During research investigating the potential of PEI grafted with PEO, it was shown that even with a low grafting density of PEO, P(EI-g-EO) was not an efficient transfection agent, probably for the reason already mentioned above that PEI segments between two PEO branches had a too low polymerization degree to efficiently cover DNA. In other words, PEO branches could prevent an efficient complexation of DNA by the vector because of steric hindrance.

Various diblock copolymers poly (ethylene oxide-b-2-alkyl-2-oxazoline) were synthesized (21). Since it is known that a high polymerization degree is required for the PEI segment, poly(2-ethyl-2-oxazoline) should be used. However, it is to be kept in mind that the copolymer must be hydrolysed to allow the transformation of the poly(2-alkyl-2-oxazoline) units into poly(ethyleneimine) units. Because of the presence of the PEO segment, acid hydrolysis leading to ether cleavage is to be avoided. From these requirements it was clear that 2-methyl-2-oxazoline monomer could not be used, not giving access to high DPn. On the other hand poly(2-ethyl-2-oxazoline), while allowing high DPn, is not soluble in the basic medium required for basic hydrolysis. Thus, the copolymerization of 2-methyl-2-oxazoline with 2-ethyl-2-oxazoline was studied, hoping to avoid this last drawback. The reactivity ratios were determined. In acetonitrile in the conditions of this synthesis (18), $r_{1MeOXZ} = 1.24$, $r_{2EtOXZ} = 0.41$, showing that MeOXZ was more reactive than EtOXZ. Hydrolysis of the random copolymers MeOXZ/EtOXZ showed that a MeOXZ content of 75 % was necessary in order to allow complete hydrolysis. Finally, the best conditions for copolymerization were used with α-methoxy-ω-tosylate-PEO

as initiator. The terpolymer poly(EO-b-(methyloxazoline-co-ethyloxazoline)) was synthesized and hydrolyzed without cleavage with an average molar mass around 50 kDa. It must be noticed that this diblock copolymer poly(EO-b-ethyleneimine) gave good transfection results when used for in vitro experiments on HepG2 and HEK293 cells (21), and was at high concentration approximately of the same efficacy as LPEI. However, this diblock copolymer was clearly found less toxic than LPEI (Fig. 3). It can be seen that at high concentration the protein content is higher for the diblock P(EO-b-EI) showing a lower toxicity.

III- Neutral Polymers as Synthetic Vectors for DNA Transfection

It has recently been discovered that muscular cells can be transfected by naked DNA, but only at a very low efficiency so that a therapeutic treatment of genetic diseases such as myopathy cannot be carried out by this way. However, keeping in mind that these cells have not the same surface charge characteristics as lung epithelium, positive polyelectrolytes are not to be necessarily used as synthetic vectors, and conditioning DNA with amphiphilic neutral block copolymers such as the well known pluronics, block copolymers of poly(ethylene oxide) and poly(propylene oxide), revealed to be much more efficient in transfection assays than with naked DNA (22-24). These experiments raised the question to know the nature of the interactions between DNA and these amphiphilic block copolymers, and to know how DNA can penetrate inside cells in these conditions. It was first recognized that interactions between plasmids or DNA could be due to the development of hydrophobic interactions with the macromolecular vector which do not induce condensation. Thus, the transfection increase was assigned at least partly to a protection from nuclease degradation. It was recognized that the amphiphilic nature of the vector was important, and this aspect raised the question of the role of the hydrophobic

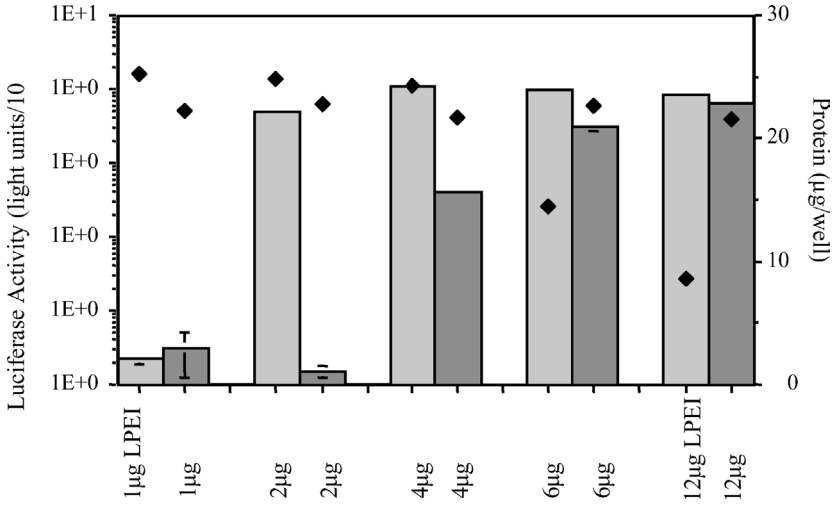

Figure 3.
In vitro transfection efficiency of poly(ethylene oxide-b-ethyleneimine) Increasing amounts of the synthetic vector P(EO-b-EI) (black bars)or LPEI (light gray bars) were mixed with a constant amount of reporter gene (4 µg per duplicate). The mixtures were incubated in a serum-free culture medium for 3h with HEK293 cells. The luciferase activity was measured 30 h post-transfection. Transfection efficiency is expressed as total light units/10 s/well as a mean of duplicates. The protein content (black squares) was measured using the BioRad protein assay.

segment. In the same vein, the role of the hydrophilic block could be also questionned: is PEO the best choice and for what reason ? In this context the chemical nature of the segments of these triblocks was varied.

III-1. Synthesis of Triblock Copolymers Poly(tetrahydrofurane-b-ethylene oxide)

It was decided to synthesize block copolymers which could mimic the poloxamer which already revealed to be useful as a transfer agent, namely Pluronic 6400 (22). According to the structure of the latter, the targeted structure was constituted of a central block of poly(tetrahydrofurane) (pTHF) of 1700 Da molar mass, and two side blocks of poly(ethylene oxide) (pOE) of around 600 molar mass. This model compound contains around 40 % (w/w) of poly(ethylene oxide) with a number average molar mass close to 2900 Da. Thus, the goal was a triblock copolymer pOE-pTHF-pOE with a mass distribution of 600-1800-600. Recipes could be found in the literature and needed to be adapted to the present study (25).

The first step was to synthesize the living pTHF. It was carried out by direct initiation between THF and trifluoromethanesulfonic anhydride. This initiator directly produces a polymer with active species at both ends. These active species can be controlled for a sufficiently long time to allow reaction with suitable other monomers. Thus, after the obtention of pTHF, the synthesis of the triblock was done using the active species provided by the ''living polymerization'' technique. The second step consisted in the reaction of a POE monomethylether with the living bifunctional pTHF according to the overall reaction (25):

$$\begin{aligned} &\text{pTHF–CH}_2\text{–O–SO}_2\text{–CF}_3 \\ &\quad + \text{HO–pOE} \\ &\rightarrow \text{pTHF–CH}_2\text{–O–pOE} \\ &\quad + \text{CF}_3\text{–SO}_3\text{H} \end{aligned} \qquad (1)$$

The theoretical advantage of such synthesis is the possibility to obtain a polymer with a narrow distribution of the molar mass. However, due to kinetic reasons which cannot be explained here in details, the polymerization of THF does not easily provide monodisperse materials. (25).

The reaction (1) also shows that the synthesis of the triblock makes use of a reaction between terminal functions borne by oligomers. This situation is not favorable to high yields.

The results of a transfection study carried out on the reference product Pluronic PE6400 showed an only negligible transfection activity in vitro with 293 cells. These observations are in agreement with previously published results by Pitard et al. (22). Moreover a significant toxicity was observed even when PE6400 was used at a 0.5 % concentration. The toxicity became very important for concentrations equal or higher than 1 %.

Similar study performed with the triblock copolymer poly(ethylene oxide-b-tetrahydrofurane-b-ethylene oxide) corresponding to the formula: $p(OE_{11}\text{-b-}THF_{25}\text{-b-}OE_{11})$ (MW = 2800, % EO = 33) (26). When cells 293 were used, no transfection was detected, showing a behavior similar to that of PE6400. However, it was noticed that the polymer seemed to be much less toxic than PE6400.

A set of in vivo experiments carried out with the same polymer triblock POE-PTHF-POE: $p(OE_{11}\text{-b-}THF_{25}\text{-b-}OE_{11})$ (MW = 2800, % EO = 33) was realised with CMV-Luc (5 μg/muscle). The results are shown above (Fig. 4) (26).

It was shown that the copolymer seems to behave similarly to PE6400, and these results were encouraging since they show that the level of expression in the muscle was similar to that of PE6400. It is to be recalled that block copolymer $p(OE_{11}\text{-b-}THF_{25}\text{-b-}OE_{11})$ is less toxic than its Pluronic counterpart. Figure 4 shows that in our conditions, 0.05 % are as efficient as 0.25 % of Pluronic 6400. Since this result was not optimized, there is space for improvements in term of efficacy and toxicity. The main

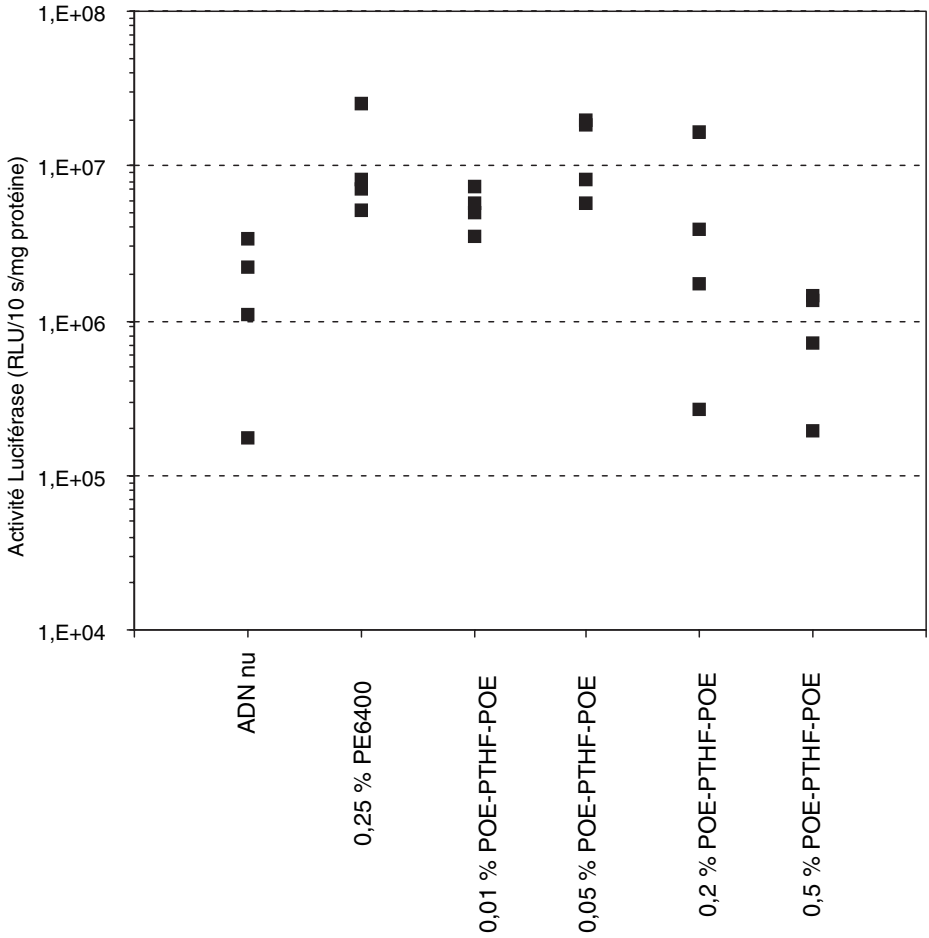

Figure 4.
In vivo DNA transfection: luciferase activity after systemic injection of mixtures of poly(OE-b-THF-b-OE) triblock with DNA.

conclusion obtained in this set of experiments was that the hydrophobic nature of the central block is important, as far as the transfection efficiency is concerned.

III-2 Synthesis of a Triblock Copolymer Poly(alkyloxazoline-b-propylene oxide-b-alkyloxazoline)

It was also of interest to determine the effect of a chemical change of the hydrophilic moiety of the amphiphilic block copolymers. The PEO blocks were replaced by poly(2-methyl-2-oxazoline) which also is a water soluble polymer. Thus, the ring opening cationic polymerization of 2-methyl-2-oxazoline was initiated by a fully tosylated poly(propylene oxide).

NMR investigation of the polymerization products showed that the initiation reaction was slow, phenomenon which was explained by the fact that the tosylate groups are bound to a secondary carbon instead of a primary carbon, contrary to the case of poly(ethylene oxide) functionalization. Consequently some efforts were devoted to the modification of the PPO chain ends in order to get primary hydroxylic functions. Three steps were involved

in this synthesis:, oxydation of hydroxylic functions of PPO by pyridinium chlorochromate (PCC), oxydation of the resulting ketone into carboxylate functions by the haloform reaction, and reduction of the resulting carboxylate groups in primary alcohol functions. Final results showed that the final PPO was far from being quantitatively functionalized.

Another strategy was used by Darcy et al. (27). for the functionalization of POE grafted β-cyclodextrines by reaction with ethylene carbonate. This synthesis used the electrophilic addition of propylene carbonate to a secondary alcohol. The analysis of the reaction products by NMR and MALDI spectroscopies showed that a large excess of propylene carbonate had to be used, but that this reactant had a tendency to polymerize as well which induced the presence of short segments of poly(ethylene oxide) on both ends of PPO. It is clear that the presence of these short chains is not a problem if they can be kept within small number of EO units.

Finally, another set of reactions was selected in order to gain a better specificity: PPO was first allylated. After subsequent hydroboration and hydroxylation, the di-primary hydroxytelechelic PPO was tosylated in the presence of triethylamine, giving in high yield and high specificity di-3-tosylpropyl telechelic PPO (28). The polymerization of 2-methyl-2-oxazoline was carried out using this functionalized PPO as initiator.

The copolymer which was synthesized was characterized by ^{1}H NMR spectroscopy and by SEC (28). As an average, the hydrophobic PPO segment had a degree of polymerization of 37, and a number of poly(2-methyl-2-oxazoline) units of 48 corresponding to an number average molar mass of 6200 Da. However, careful analysis of the chain end functions according to ref. 28 showed that the sample was composed of around 70% (mole/mole) of triblock and 30 % of diblock.

In vivo transfection experiments carried out on BalbC mice gave the following results (Fig. 5).

These experiments showed that used at 0.01% the tribloc copolymer allowed an increase of the level of expression of luciferase in the the tibialis muscle compared to that of the naked DNA by a factor of at

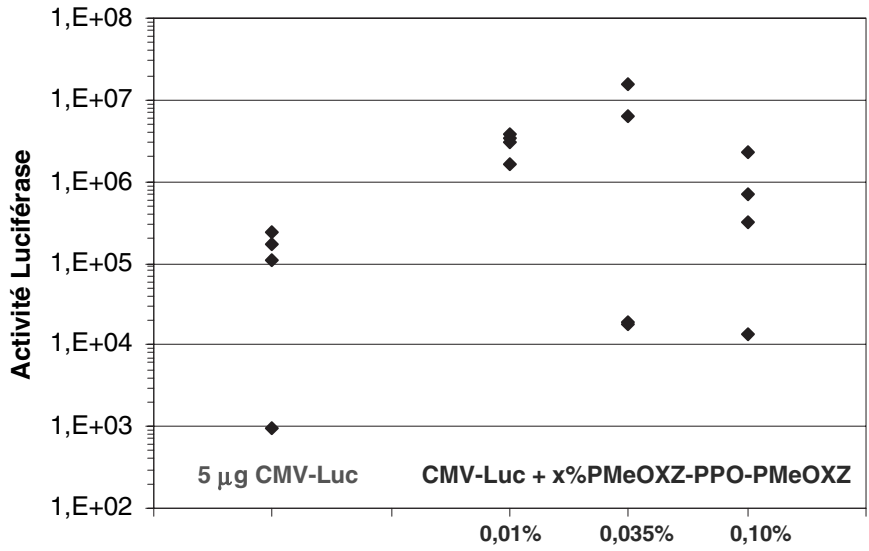

Figure 5.
In vivo transfection : luciferase activity after tibialis injection of Balb C mice of mixture of CMV-Luc + P(MeOXZ-b-PPO-b-MeOXZ).

least 10. While these preliminary results gave rather scattered values when the triblock was used at 0.035% and more, it is worth mentioning that these results are not yet optimized, but they allowed to conclude that the replacement of the PEO segments by poly(2-methyl-2-oxazoline) is possible and gives vectors which can work for DNA transfection in muscles.

IV- Discussion of the Results and Future of This Research

The design of more efficient synthetic vectors for DNA transfer requires the controlled synthesis of various hydrophilic or amphiphilic block copolymers. In this context, cationic ring-opening polymerization offers new avenues, since it allows the controlled synthesis of a large number of polymers which have not yet been tried either in vitro or in vivo. However, due to the extreme versatility of these block copolymers and their corresponding homopolymers, it is necessary to use very specific polymerization and purification techniques, in order to ascertain the conclusions which can be drawn from the transfection studies. The living character of many such polymerizations offers a way to meet further requirements which were not described above such as the fixation on the chain ends of labels for cell targeting. In the case of vectors of the positive polyelectrolyte type it was clear that a linear macrostructure had to be preferred versus highly branched or hyper branched macrostructure,. The reason of such a conclusion is probably to be found in the size of particles for the formed polyplexe. It has been estimated that the best transfection efficacy necessitates particle diameter lower than around 100 A°. This is probably linked with the mechanism of endocytosis which is probably less efficient for particles of a large diameter. It can be hypothesized that the higher flexibility of the linear vector allows a better wrapping of DNA and a more compact polyplex. The final transfection efficacy results not only from the internalization mechanism, but also from the release from endosomes, the intracellular trafficking and from the crossing of the nuclear membrane. Presently, little is known about intracellular trafficking and nuclear membrane crossing. It has been published that targeting the nuclear membrane was possible with sugar residues (29). However a clear demonstration of such an effect of the fixation of these functions is still waiting. In this context, cationic ring opening polymerization could help the synthesis by the reaction of the active species with a suitable molecule functionalized by an amine group. This type of synthesis is a particular case of labeling with markers in order to follow the fate of these vectors.

In the case of neutral vectors the mechanism which governs the efficiency is less clear. It has been shown that the amphiphilic nature of the vector is necessary for a successful transfection. The surprising fact which is not yet understood is the low efficiency noticed when these vectors are used in vitro, while they promote DNA transfection when used in vivo. Another surprising fact is the absence of strong interactions between these neutral vectors and DNA. It has been shown, for instance that mixtures of DNA with vectors of this type do not exhibit any noticeable retardation effect by electrophoresis. On the other hand it has been shown that DNA and the vectors must be present together in contact with the target tissue in order to observe transfection. It was shown in the laboratory by an electrophysiological study carried out on the model block copolymer Pluronic 6400 that this polymer can interact with bilipidic membranes, model of cell membranes, making transitory or more permanent holes (30). It could be thought that the amphiphilic nature of the vector helps to stabilize such holes in the cell membranes allowing the penetration of DNA (30).

V- Conclusion

This research showed that some properties linked with the physical structure of the synthetic vectors are as much important as

their chemical nature, if not more important: linear macrostructure for the vectors of the positive polyelectrolyte type, amphiphilic structure for the neutral vectors. Of course, toxicity depends on the chemical nature. While it is to be noted that neither the structure of these synthetic vectors for DNA transfection nor their chemical composition were optimized, research work described above opened new avenues for the understanding of transfection mechanisms. This is certainly one of the main challenges for the design of more efficient synthetic vectors for gene therapy.

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