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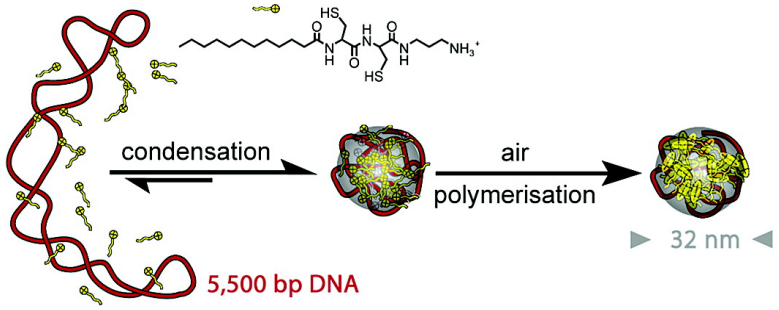

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Monomolecular DNA Nanoparticles for Intravenous Delivery of Genes

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Abstract: Delivery is the major obstacle to success of nucleic-acid-based therapies. We have neutralized DNA with a cationic detergent (C_{12}CCP) obtained by amide bond formation between dodecanoic acid, cysteinyl-cysteine, and diaminopropane. Subsequent detergent polymerization by formation of intermolecular disulfide bonds within the condensed plasmid DNA leads to 32-nm-large neutral particles. $(C_{12}CCP)_n$/DNA complexes are more stable than those formed with other gene delivery agents toward exchange with extracellular polyanions such as glycosaminoglycans. Yet exposure to phosphatidylserine, an ubiquitous intracellular anionic lipid, still releases DNA from the complexes for transcription of the carried gene. Pharmacokinetics and biodistribution in mice showed that 25% of the complexes were still circulating after 30 min (2% for other cationic lipid vectors) in a form essentially not bound to blood cells. Altogether, straightforward control over size and surface charge, stability toward aggregation or exchange, and favorable pharmacokinetics make these complexes attractive vehicles for reaching tumor metastases after injection in the blood circulation.

Introduction

Delivery of nucleic acids to cells in an animal remains a challenging problem. It is the major obstacle to success of experimental therapies using genes and oligonucleotides, including small interfering RNAs (siRNA).

Chemist's approaches to solve this problem are numerous (for reviews, see refs 1−4). They are based on metal ions, on cationic lipids, or on polymers that form ionic complexes with nucleic acids. Such condensed polymolecular aggregates are efficient enough for transfection of most animal cells in culture. However, in vivo delivery is very poor, except where large amounts of complexes can physically reach a large cell surface.[5−7] In this particular context, like a gleam of hope, a "chemical" vector was recently shown to successfully deliver a therapeutic gene in patients.[8] In general, delivery is hindered by the lack of diffusion of the DNA particles within the tissue or through the blood vessel walls. Yet another potentially favorable situation can be encountered in cancer therapy when fast-growing tumor metastases are irrigated by leaky blood vessels: blood-injected complexes can passively accumulate within the tumor mass.[9] For this to occur, however, complexes must be small, remain stable in the blood stream, and circulate for some time. Recent approaches to solve these specific extracellular problems have used chemistry either by design[10−12] or by screening.[13,14]

We have used DNA template-driven conversion of cationic detergents into gemini-lipids[15,16] for the formation of nanometric complexes reminiscent of viruses.[17] Here we extend this technique to formation of polymeric lipid complexes[18] that display increased stability and extended circulation times in the blood stream.

Results and Discussion

Design and Synthesis of a Polymerizable Cationic Detergent. Monomolecular condensation of DNA into stable nanometric particles can be achieved according to the two-step procedure depicted in Figure 1.

(1) Niidome, T.; Huang, L. *Gene Ther.* **2002**, *9*, 1647−52.
(2) Merdan, T.; Kopecek, J.; Kissel, T. *Adv. Drug Deliv. Rev.* **2002**, *54*, 715−58.
(3) Zuber, G.; Dauty, E.; Nothisen, M.; Belguise, P.; Behr, J. P. *Adv. Drug Deliv. Rev.* **2001**, *52*, 245−53.
(4) Mahato, R. I.; Smith, L. C.; Rolland, A. *Adv. Genet.* **1999**, *41*, 95−156.
(5) Zou, S. M.; Erbacher, P.; Remy, J. S.; Behr, J. P. *J. Gene Med.* **2000**, *2*, 128−34.
(6) Lemkine, G. F.; Mantero, S.; Migne, C.; Raji, A.; Goula, D.; Normandie, P.; Levi, G.; Demeneix, B. A. *Mol. Cell Neurosci.* **2002**, *19*, 165−74.
(7) Jia, S. F.; Worth, L. L.; Densmore, C. L.; Xu, B.; Duan, X.; Kleinerman, E. S. *Clin. Cancer Res.* **2003**, *9*, 3462−8.
(8) Ohana, P.; Gofrit, O.; Ayesh, S.; Al-Sharef, W.; Mizrahi, A.; Birman, T.; Schneider, T.; Matouk1, I.; Groot, N. d.; Tavdy, E.; Sidi, A. A.; Hochberg, A. *Gene Ther. Mol. Biol.* **2004**, *8*, 181−192.
(9) Maeda, H.; Wu, J.; Sawa, T.; Matsumura, Y.; Hori, K. *J. Controlled Release* **2000**, *65*, 271−84.
(10) Han, S.; Mahato, R. I.; Sung, Y. K.; Kim, S. W. *Mol. Ther.* **2000**, *2*, 302−17.
(11) Oupicky, D.; Ogris, M.; Seymour, L. W. *J. Drug Target* **2002**, *10*, 93−8.
(12) Wagner, E. *Pharm. Res.* **2004**, *21*, 8−14.
(13) Akinc, A.; Lynn, D. M.; Anderson, D. G.; Langer, R. *J. Am. Chem. Soc.* **2003**, *125*, 5316−23.
(14) Chen, D. J.; Majors, B. S.; Zelikin, A.; Putnam, D. *J. Controlled Release* **2005**, *103*, 273−83.
(15) Blessing, T.; Remy, J. S.; Behr, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1427−31.
(16) Dauty, E.; Remy, J. S.; Blessing, T.; Behr, J. P. *J. Am. Chem. Soc.* **2001**, *123*, 9227−34.
(17) Ouyang, M.; Remy, J. S.; Szoka, F. C., Jr. *Bioconjugate Chem.* **2000**, *11*, 104−12.
(18) Balakirev, M.; Schoehn, G.; Chroboczek, J. *Chem. Biol.* **2000**, *7*, 813−9.

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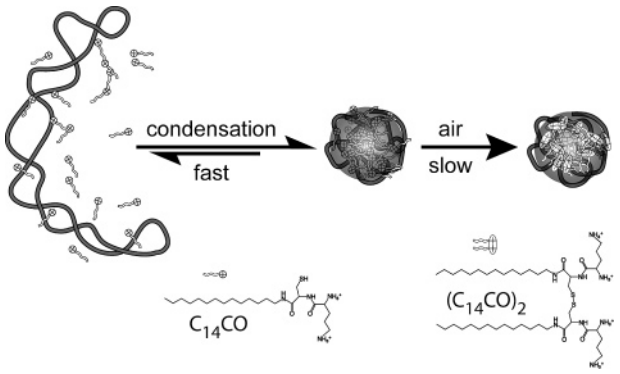

Figure 1. Plasmid DNA is condensed into monomolecular particles with a thiol-containing cationic detergent. The particles are stabilized by spontaneous oxidation of the detergent.

Plasmid DNA is reversibly condensed with a cationic thiol-containing detergent, e.g., the tetradecylamide of ornithylcysteine[16] ($C_{14}CO$), below its critical micelle-forming concentration (cmc). The formed monomolecular DNA condensates are then stabilized by oxidation of the detergent into a lipid, $(C_{14}CO)_2$. The detergent should have a high cmc to prevent cationic micelle-mediated aggregation of the anionic DNA particles during the first step, yet the resulting lipid should have a very low cmc to form stable complexes able to carry DNA to the cell nucleus. Within a series of detergents with various hydrocarbon chain length and polar headgroups, $C_{14}CO$ came out as being the best compromise in vitro.[16] Unfortunately, complexes are not very stable in vivo (see below), which calls for lower cmc's. Besides cmc, the total amount of lipid matrix present in the neutralized complexes (only one lipid molecule every two base pairs for $(C_{14}CO)_2$) may also be of importance. We therefore thought of (i) increasing the cmc gap between the starting detergent and formed lipid by using a polymerizable (instead of dimerizable) detergent and (ii) increasing the relative amount of lipid using a monocationic detergent. These considerations led us to synthesize the biscysteine detergent $C_{12}CCP$ following the scheme depicted in Figure 2.

The five-step, resin-supported synthesis gave $C_{12}CCP$ with 80% overall yield. LC-MS showed a major peak (98%) with the expected mass. Our strategy rests on the assumption that polymerization is favored over cyclization, the latter being prevented by an unfavorable cis peptide bond in an eight-membered ring.[19] Indeed, besides monomer, FAB-MS showed peaks corresponding to the linear dimer, trimer, and tetramer without evidence for cyclic compounds. An oxidation experiment was performed in 30% DMSO. Attempts to determine the degree of polymerization of the final mixture by MS were unsuccessful. However, ultrafiltration experiments showed ca. 90% of the material to be stopped following ultrafiltration through a membrane with cutoff 3500 Da. This corresponds to a degree of polymerization > 8 which should ensure a subnanomolar cmc for the final DNA carrier.

Physicochemical Properties of the Detergent and of Its Complexes with DNA. As stated above, the critical micelle-forming concentration of the detergent is important, as no micelles should be present during DNA condensation. The cmc was measured by tracking the fluorescence enhancement of a lipophilic aromatic dye with increasing detergent concentrations (Figure 3). The cmc of $C_{12}CCP$ was found to be ca. 90 μM, i.e., above the typical plasmid DNA concentration used for transfection (1 μg/mL, 60 μM phosphate residues). This means that mixing $C_{12}CCP$ with plasmid DNA, both at 60 μM, should result in the encounter of individual molecules and lead to clean monomolecular collapse of DNA. Titration of residual thiol groups vs time using Ellman's reagent showed that it takes 24 h for the oxidation reaction to come to completion (not shown).

Complex formation of DNA with $C_{12}CCP$ at various *N*/*P* (detergent ammonium over DNA phosphate) charge ratio

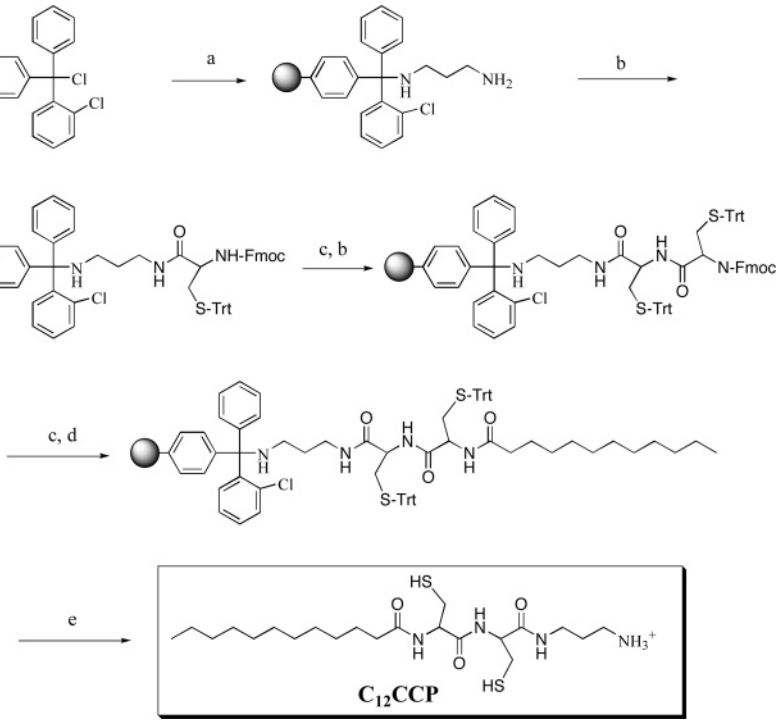

Figure 2. Resin-supported synthesis of the polymerizable detergent $C_{12}CCP$. (a) 1,3-Diaminopropane, (b) Fmoc-Cys-(Trt)-OH, PyBOP, DIEA, (c) 25% piperidine/DMF, (d) $C_{11}H_{23}COOH$, PyBOP, DIEA, (e) TFA/TIS/H_2O (95:4:1% v/v/v).

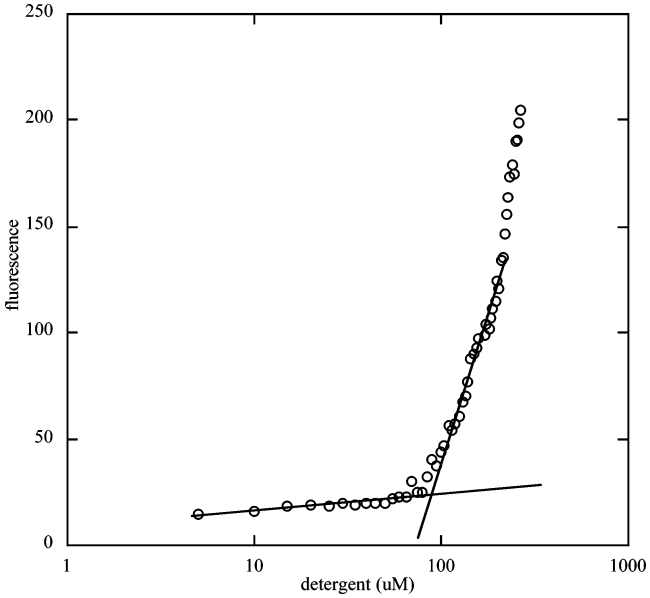

Figure 3. Determination of the detergent cmc. The fluorescence of *N*-phenyl-1-naphthylamine was measured in the presence of increasing amounts of C_{12}CCP in a neutral and reducing buffer solution (HEPES/DTT pH 7.4).

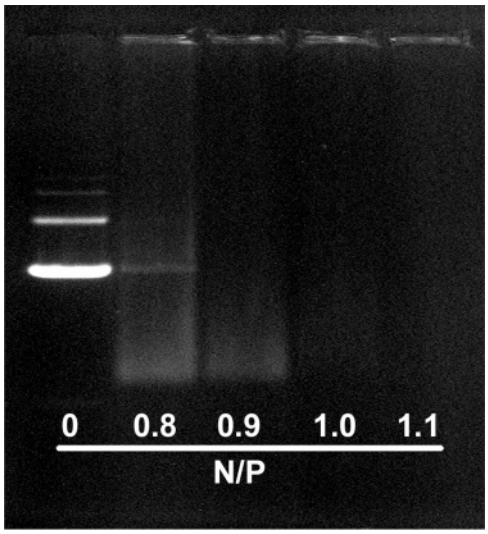

Figure 4. Agarose gel electrophoresis of $(C_{12}CCP)_n$/DNA complexes show complete retention in the wells for detergent to DNA charge ratio $N/P > 0.9$.

followed by oxidation was monitored by agarose gel electrophoresis (Figure 4). The band corresponding to free DNA was transiently replaced by a faint, faster moving band ($N/P = 0.8$). As shown previously, this is evidence for the formation of stable anionic DNA nanoparticles able to diffuse through the gel. Indeed, unstable particles do not withstand electrophoresis conditions,[15] and large polymolecular DNA aggregates remain in the wells of the gel.[16] However, in contrast to $(C_{14}CO)_2$/DNA, $(C_{12}CCP)_n$/DNA complexes no longer migrate for $N/P = 1$ and above. The question was whether this was due to the absence of electric charge, to aggregation, or to both?

We measured the zeta potential of the complexes and found a value (-5 ± 3 mV, $N/P = 1.0$) very close to neutrality. This again contrasts with $(C_{14}CO)_2$/DNA complexes which display highly negative zeta potentials (-50 to -20 mV), irrespective of N/P.[16] $(C_{14}CO)_2$/DNA complexes thus obey Manning's counterion condensation theory,[20] leading to about 90% neutralization of DNA charges in the condensates, while the polymerized detergent displays quasi-irreversible binding, hence a zeta potential governed by the N/P charge ratio.

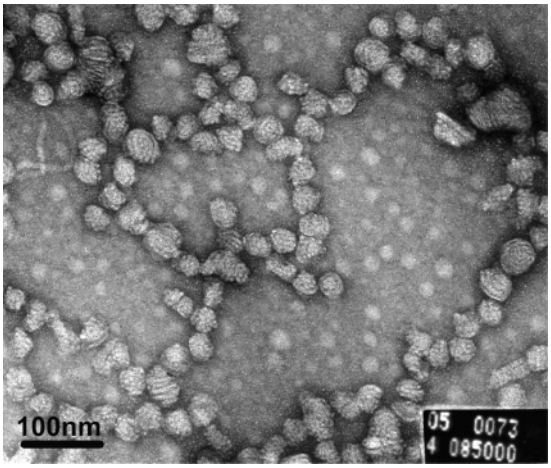

Figure 5. Transmission electron microscopy picture of neutral $(C_{12}CCP)_n$/DNA complexes. The 30-nm-large particles contain a single molecule of plasmid DNA entangled in a cationic tubular lipid phase.

Neutral particles composed of cationic lipid/DNA particles are unstable colloids that tend to aggregate.[21] We observed the $(C_{12}CCP)_n$/DNA complexes after 48 h by transmission electron microscopy (Figure 5): particles were homogeneous with respect to size (32 ± 3 nm for 90% of the population, $n = 50$). As found previously for the $(C_{14}CO)_2$/DNA particles, their internal structure appeared as a tubular phase with a ca. 6 nm repeating distance. Assuming hexagonal packing of condensed DNA, the volume of a 5.5 Kbp plasmid is equal to 11 800 nm^3. Hexagonal packing of a tubular lipid phase containing 5500 molecules ($N/P = 1$), assuming a surface area of 0.7 nm^2/molecule, gives an additional 6400 nm^3. Adding the volumes of DNA and amphiphile to each other as an approximation for the volume of the complex corresponds to a sphere with a diameter of 34 nm. Thus, the size of the complexes is good evidence for monomolecular condensation of plasmid DNA.

Stability of the Complexes. Interaction between oppositely charged polyelectrolytes is very strong, and falling apart is unlikely in normal salt conditions. Yet reduction back to the monomeric detergent could lead to fast DNA release. DNA can also be released by competitive exchange with other polyanions. For transfection to be effective, this should not happen outside the cell yet must occur somewhere inside to allow binding of the transcription machinery. We therefore tested various compounds for their ability to release plasmid DNA from the polymerized complexes. After increasing incubation times, aliquots were subjected to agarose gel electrophoresis and the presence of free plasmid was estimated visually ($-$ when $<5\%$, $+$ when $>30\%$; see Table 1).

Proteins bearing various polyanionic osides circulate in biological fluids. As an extreme case, we tested polysialic acid, with a charge density of 1 carboxylate per ose, i.e., comparable

(19) Kim, B. M.; Schultz, L. W.; Raines, R. T. *Protein Sci.* **1999**, *8*, 430−4.
(20) Manning, G. S. *Q. Rev. Biophys.* **1978**, *11*, 179−246.
(21) Pitard, B.; Oudrhiri, N.; Vigneron, J. P.; Hauchecorne, M.; Aguerre, O.; Toury, R.; Airiau, M.; Ramasawmy, R.; Scherman, D.; Crouzet, J.; Lehn, J. M.; Lehn, P. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 2621−6.

Table 1. Release of DNA from $(C_{12}CCP)_n$/DNA Complexes by Polyanions or Reducing Agents as Assessed by Agarose Gel Electrophoresis

external agent	incubation time 0.3 hrs	4 hrs	24 hrs	concentration of external agent
polysialic acid	−	−	−	1 mg/mL
heparin	−	+	+	1 mg/mL
chondroitin sulfate	−	−	−	1 mg/mL
sodium dodecyl sulfate	+	+	+	0.2 mM
phosphatidylserine	−	−	+	1 mg/mL
dithiothreitol (DTT)	+	+	+	1 mM
glutathione	−	−	−	10 mM

Table 2. Polyanion-Mediated Release of Plasmid DNA from Complexes with Transfection Agents

	$(C_{12}CCP)_n$	$(C_{14}CO)_2$	DOGS	PEI
heparin (1 mg/mL, 30 min)	−	+	+	+
phosphatidylserine (1 mg/mL, 1 h)	−	−	+	+

to a single stranded nucleic acid, and heparin with a charge density of 2.3 per ose. Polysialic acid did not release DNA, and it took at least 4 h in our conditions for heparin to release DNA. Anionic proteoglycans are ubiquitous in extracellular matrixes and on adherent cell surfaces. They are even the receptors for cell entry of cationic nucleic acid complexes.[22] Fortunately, chondroitin sulfate up to 1 mg/mL did not release DNA.

Incubation with an excess of a strong anionic detergent (SDS) instantaneously dissociated the $(C_{12}CCP)_n$/DNA complexes, while phosphatidylserine, an endogeneous anionic lipid, slowly dissociated the complexes (Table 1).

Similarly on the reducing side, a strong reducing agent (DTT) led to fast formation of the $C_{12}CCP$ monomer with release of DNA in the electrophoresis experiment, while glutathione, the natural reducing cofactor at intracellular concentration, was unable to do so.

Finally we compared several putative in vivo DNA delivery vehicles for their ability to hold DNA in the presence of a highly charged polyanion or an anionic lipid (Table 2). Phosphatidylserine (PS) indeed is a major component of the intracellular leaflet of the cell membrane, and therefore of the external leaflet of endosomes that result from the cell entry of cationic DNA complexes (for the mechanism of cell entry, see ref 22). Following rupture of the endosomal membrane in contact with the complexes, PS is ideally positioned to neutralize the cationic vehicle, especially when it is a lipid, and release DNA into the cytoplasm.[23] Indeed DOGS (dioctadecylglycyl spermine) but also nonamphiphilic PEI (polyethylenimine) loose DNA in the presence of excess PS after 1 h. Nanometric amphiphilic particles withstand these conditions for 1 h, presumably because they are not cationic. With heparin as discriminative competitor, the polymeric lipid $(C_{12}CCP)_n$ formed the most stable complexes with DNA.

Taken together, these results show that $(C_{12}CCP)_n$/DNA complexes are the best candidates for carrying genes in vivo: they are small, neutral, and stable. Prolonged exposure to polyanions can still release DNA for gene expression.

Pharmacokinetics and Biodistribution in Mice. The favorable stability studies encouraged us to inject the complexes into the blood circulation of mice. As controls, we used DOGS, another amphiphilic vehicle previously tested in vivo, and PEI, the most used in vivo delivery reagent. Plasmid DNA (pCMVluc) was labeled with ^{32}P and complexed to the desired vehicle. Conditions previously optimized for PEI were used (20 μg of DNA in 100 μL of isotonic glucose solution) for all complexes. The complexes were injected into the jugular vein, blood samples were collected from the tail, and animals were sacrificed after 10 or 30 min. After perfusion, major organs were dissected. Blood and organ samples were processed for scintillation counting of radioactivity.Cationic DOGS and PEI complexes behaved closely as described in the litterature.[24−27] Fast removal from the blood circulation (10−20% remaining after 10 min, falling to 2% after 30 min) was observed. More than half the injected dose ended as expected in the liver, presumably captured by macrophages. The other major organ of the reticuloendothelial system, the spleen, as well as the highly vascularised lungs, took up most of the remaining dose. $(C_{14}CO)_2$ complexes displayed a better pharmacokinetics than DOGS or PEI, with 2-to-3-fold more DNA remaining in the blood.

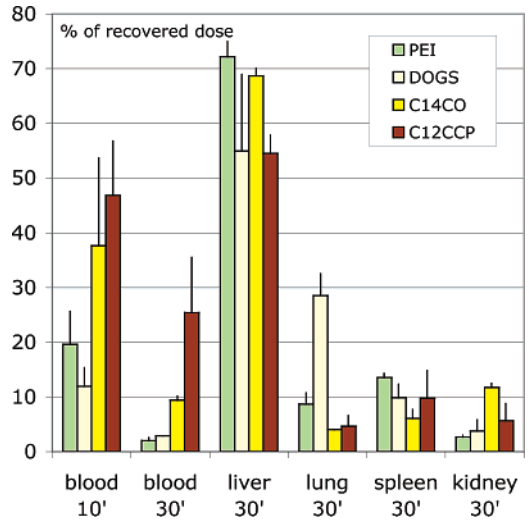

Figure 6. Pharmacokinetics and biodistribution of various DNA complexes following injection of 20 μg of plasmid into the jugular vein of mice (% of recovered dose ± sd).

The best results were obtained with $(C_{12}CCP)_n$/DNA complexes: nearly half the injected dose was remaining after 10 min and 25% was still circulating after 30 min (compare with 2% for DOGS and 10% for $(C_{14}CO)_2$). With time, a general trend of accumulation in reticuloendothelial organs at the expenses of complexes initially sieved in the lung capillaries was observed (not shown). Since only DNA radioactivity was used for the measurements, we checked for its chemical stability in conditions close to those encountered in vivo. $(C_{12}CCP)_n$/DNA complexes were incubated at 37 °C with 50% serum. Aliquots were removed, mixed with 1% SDS to release DNA and subjected to agarose gel electrophoresis. Quantitation showed that DNA (supercoiled + closed circular) remained intact for at least 1 h (not shown).

(22) Kopatz, I.; Remy, J. S.; Behr, J. P. *J. Gene Med.* **2004**, *6*, 769−76.
(23) Xu, Y.; Szoka, F. C., Jr. *Biochemistry* **1996**, *35*, 5616−23.
(24) Li, S.; Huang, L. *Gene Ther.* **1997**, *4*, 891−900.
(25) Ogris, M.; Brunner, S.; Schuller, S.; Kircheis, R.; Wagner, E. *Gene Ther.* **1999**, *6*, 595−605.
(26) Dash, P. R.; Read, M. L.; Barrett, L. B.; Wolfert, M. A.; Seymour, L. W. *Gene Ther.* **1999**, *6*, 643−50.
(27) Kunath, K.; von Harpe, A.; Petersen, H.; Fischer, D.; Voigt, K.; Kissel, T.; Bickel, U. *Pharm. Res.* **2002**, *19*, 810−7.

Finally, we asked whether complexes remaining in the blood were free or associated with blood cells. Complexes were mixed with blood and centrifuged after 10 min. Cationic DOGS and PEI complexes were found essentially in the pellet (75−90%). Indeed, such complexes bind several serum proteins, self-aggregate,[25,26,28] and aggregate highly negatively charged erythrocytes.[25] On the contrary, anionic or neutral nanoparticles formed with the thiol detergents remained mostly (55−70%) in the plasma.

Conclusions

Chemists usually test the efficiency of their molecules on cells in culture. However, crossing intracellular barriers is not everything. In vitro screening can even be misleading. For instance, large DNA complexes find cells in culture thanks to gravity but are particularly unsuited to physically reach their target cells in a patient, hence, the need for pharmacology. In solid cancer therapy, the primary tumor can often be removed surgically, eventually leaving disseminated metastases that can only be reached systemically. Unfortunately, even primoinjection of a foreign particle in the blood stream triggers complement protein tagging followed by immune cell phagocytosis. Charged particles, including anionic ones, are tagged much easier than neutral ones;[28] so are larger (100−400 nm) vs smaller (50 nm) ones.[28] The physical properties of our 30 nm neutral DNA particles lead to pharmacokinetics similar to poly(ethylene glycol)-shielded PEI/DNA particles,[25] yet with smaller, more diffusible, and more stable complexes. Stabilization can also be obtained by surface cross-linking yet raises the problem of decomplexation in the cell.[11]

Finally, neutral DNA complexes no longer bind to syndecans, their ubiquitous cell entry gate.[22] As a surrogate, folic acid receptors can be used that are overexpressed on many cancer cells, but maybe not enough to sustain cell transfection.[29,30] Successful in vivo gene delivery thus requires a delicate balance of conflicting properties that can only be tuned by animal testing.

Materials and Methods

Materials. 2-Chlorotrityl chloride resin (1,13 mmol/g), *N*-fluorenylmethoxycarbonyl-*S*-trityl-[*R*]-cysteine (*N*-Fmoc-*S*-trityl-[*R*]-cysteine), and benzotriazole-1-yl oxytrispyrrolidinophosphonium hexafluorophosphate (PyBOP) were purchased from Novabiochem (Meudon, France). *N*,*N*-Diisopropylethyleneamine (DIEA), 4-dithio-DL-threitol (DTT), glutathione (GSH), piperidine, triethylamine (TEA), and trifluoroacetic acid (TFA) were from Fluka (St Quentin Fallavier, France). Dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), ethanol (EtOH), methanol (MeOH), chloroform ($CHCl_3$), acetic acid (AcOH), and tetrahydrofuran (THF) were from Carlo Erba (Val de Reuil, France). Polysialic acid sodium salt, heparin sodium salt, chondroitin sulfate B sodium salt, and phosphatidylserine were from Sigma (St Quentin, France). For solid-phase synthesis, we used, as a reaction vessel, a glass column equipped with a glass frit (porosity n°2) and a valve at one end and with a join connection at the other end. pCMV-EGFP-Luc plasmid was purchased from TebuBio (Le Perray-en-Yvelines, France) and was quantified spectrophotometrically. JetPEI (Polyplus-transfection, Illkirch, France) and DOGS[31] (Transfectam, Promega, Charbonnières, France) were used according to manufacturer protocols with the charge ratio $N/P = 5$.

Solid-Phase Synthesis. Detergents were synthesized according to slightly adapted FMoc procedures. Solid-phase reactions were carried out at room temperature, and reaction mixtures were stirred by connecting the column to a rotatory evaporator. After each step, the solvents were drained out from the vessel, and the resin beads were extensively washed with DMF (3 × 5 mL), MeOH (3 × 5 mL), and DCM (3 × 5 mL) unless indicated. The quality of the coupling was followed using the Kaiser ninhydrin test.

1-[2-Chlorotrityl-amino],3-aminopropane Resin. 2-Chlorotrityl chloride resin (250 mg, 1.13 mmol/g) was poured into the reaction vessel and extensively washed with dry dichloromethane. A solution of 1,3-diaminopropane (81 mg, 1.1 mmol) and DIEA (37 mg, 0.28 mmol) in DCM was added to the resin, and the suspension was stirred for 1 h. The solvent was drained, and the resin beads were subjected to the washing cycle and dried under reduced atmosphere.

Elongation with *N*-Fmoc-*S*-trityl-cysteine. A solution of *N*-Fmoc-*S*-trityl-cysteine (495 mg, 0.847 mmol), PyBOP (440 mg, 0.847 mmol), and DIEA (307 mg, 2.5 mmol) in DMF (5 mL) was added to the 1-[2-chlorotrityl-amino],3-aminopropane resin (250 mg, 1.13 mmol/g). The suspension was stirred at room temperature for 4 h. The resin beads were washed.

Removal of the Fmoc Protecting Group. The resin was suspended in a solution of 5% piperidine in DCM/DMF (1/1) (5 mL). After 30 min of stirring, the solvents were removed, and the resin beads were suspended in 25% piperidine in DMF (5 mL) for 2 h to ensure complete deprotection of Fmoc groups.

Elongation with Dodecanoic Acid. After repeating the elongation/deprotection steps, a solution of dodecanoic acid (294 mg, 0.678 mmol), PyBOP (352 mg, 0.678 mmol), and DIEA (250 mg, 2.03 mmol) in DCM (5 mL) was added to the resin beads (200 mg, 1.13 mmol/g) and the suspension was gently stirred for 4 h. After the washing step, the beads were dried under reduced atmosphere.

Cleavage and Deprotection of the Detergent from the Resin. The resin (100 mg) was suspended in a solution of TFA/TIS/H_2O (95/4/1 v/v/v). After stirring for 30 min at room temperature, the solution containing the detergent was collected and concentrated under reduced atmosphere. The residue was washed 3 times with cold diethyl ether (5 mL), yielding C12CCP (41.8 mg; 80% overall yield). ^{1}H NMR (MeOH-d_4) δ 0.89 (t, 3H, $J = 6$ Hz, CH_3), 1.40 (m, 20H, CH_2), 1.55−1.70 (m, 2H, CH_2 (propyl)), 1.85 (t, 2 H, $J = 7$ Hz, CH_2−CO), 2.29 (t, 2H, $J = 7$ Hz, CH_2−N), 2.81−3.02 (m, 6H, 2X CH_2−S, CH_2−NH−CO), 4.35−4.50 (m, 2H, 2X C_αH). FAB-MS (*m*/*z*, %): 463.1 $(M)^+$ (100%), 923.4 $[(2M - 2) + H]^+$ (60%), 1383 $[3(M - 2)]^+$ (20%), 1844 $[4(M - 2)]^+$ (4%). HRMS: Calculated for $C_{21}H_{43}N_4O_3S_2$ 463.277, found 463.277. LC-MS: 463 (98%).

A 100 mM ethanolic detergent stock solution was kept at −80 °C.

Critical Micelle Concentration. Increasing amounts of detergent from an ethanolic stock solution were added to a 2 mL solution of 15 mM Hepes, pH 7.4 containing 10 mM DTT and 1μM *N*-phenyl-1-naphthylamine (NPN).[32] The fluorescence of NPN (Excitation and emission wavelengths at 356 and 410 nm, respectively) was then measured and plotted versus the logarithm of detergent concentration. The sharp break was taken as the cmc.

Surfactant/DNA Complex Formation. The detergent (1 mM in ethanol) was added in the desired ratio to plasmid DNA (20 μg/mL; 60 μM phosphate) in 15 mM Hepes buffer, pH 7.4. Oxidation was allowed to proceed at 20 °C in the dark for at least 24 h. Agarose gel electrophoresis (0.8%) was performed in Tris acetate-EDTA buffer (8 V/cm, 60 min). After electrophoresis, DNA-containing complexes were visualized by staining with 10 μM ethidium bromide in aqueous solution.

(28) Devine, D. V.; Wong, K.; Serrano, K.; Chonn, A.; Cullis, P. R. *Biochim. Biophys. Acta* **1994**, *1191*, 43−51.
(29) Dauty, E.; Remy, J. S.; Zuber, G.; Behr, J. P. *Bioconjugate Chem.* **2002**, *13*, 831−9.
(30) Zuber, G.; Zammut-Italiano, L.; Dauty, E.; Behr, J. P. *Angew Chem., Int. Ed.* **2003**, *42*, 2666−2669.
(31) Behr, J. P.; Demeneix, B.; Loeffler, J. P.; Perez-Mutul, J. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 6982−6.
(32) Brito, R. M.; Vaz, W. L. *Anal. Biochem.* **1986**, *152*, 250−5.

Light Scattering Measurements. ξ-potentials of the polymerized complexes were determined by dynamic light scattering using a Zetamaster 3000 (Malvern instruments, Paris, France) with the following specification: sampling time, 30s; 10 measurements per sample; medium viscocity, 1.054 cP; medium dielectric constant, 80; temperature, 25 °C; beam mode F(Ka) = 1.5 (Smoluchowsky).

Transmission Electron Microscopy. Electron microscopy analysis was performed on a Philips EM operated at 80 kV. The sample was transferred onto a ultrathin carbon film grid (Ted Pella, Redding, CA, Cat number 1822-F, Formvar removed) by placing the grid on top of a 10-μL drop for 1 min. The grid with adherent particles was wicked from one side, placed on 100-μL water drop for 30 s for washing, wicked, placed for 1 min on a 60-μL drop of freshly filtered 1.33% uranyle acetate, wicked again, and air-dried prior to viewing.

Competitive Displacement Experiments. $(C_{12}CCP)_n$/DNA complexes were prepared as above. They were then mixed with the anionic polymers, with reducing agents, or with sonicated phosphatidylserine vesicles at concentrations indicated in the tables. The released plasmid DNA was detected after the indicated time interval by 0.8% agarose gel electrophoresis.

Preparation of Monomolecular DNA Particles for in Vivo Injection. All experiments were performed in sterile conditions. ^{32}P-labeling of the plasmid was done by nick-translation using a nick-translation kit (Invitrogen, Cergy Pontoise), and the plasmid was purified from unincorporated nucleotides by silica gel chromatography (QIAquick, Qiagen, Courtaboeuf). The highly radioactive plasmid was diluted with cold plasmid to obtain the desired concentration for in vivo injection. The radioactive pEGFPLuc plasmid (30 μM bp, 10^5-10^6 cpm) was condensed with the detergent to neutrality ($N/P = 1$) by addition of a ca. 1 mM detergent solution in ethanol to 1 mL of DNA solution containing 20 μg of pEGFPLuc in 5 mM Tris-HCl, pH 7.5, 5% glucose and 0.5 mM EDTA. After 48 h oxidation in air at 20 °C, DNA particles were concentrated 10-fold by ultracentrifugation (Centricon-100 filter devices, Millipore) to a final DNA concentration of 200 μg/mL.

In Vivo Gene Transfer. All experiments were conducted according to French regulations on animal experimentation. 5 weeks old Balb/c mice were first anaesthetized by intraperitoneal injection of sodium pentobarbital diluted in 150 mM NaCl to a final dose of 4.2 mg/kg. Complexes (100 μL, 20 μg plasmid) were injected over 2 s through the jugular vein. After 10 or 30 min, blood was collected by cardiac puncture and mice were sacrificed by cervical dislocation. Major organs (liver, kidneys, heart, lungs, and spleen) were dissected, washed, and homogenized (Ultraturrax) in phosphate buffer saline (PBS) pH 7.4. Radioactivity content was then measured using a liquid scintillation analyzer (Packard-Biosciences). Each experiment was performed with 3−5 animals. The percentage of radioactivity per organ was calculated with respect to the overall recovered radioactivity.

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