Influence of Surface Functionality of Poly(propylene imine) Dendrimers on Protease Resistance and Propagation of the Scrapie Prion Protein

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Received January 27, 2010; Revised Manuscript Received March 23, 2010

Accumulation of PrP^{Sc}, an insoluble and protease-resistant pathogenic isoform of the cellular prion protein (PrP^{C}), is a hallmark in prion diseases. Branched polyamines, including PPI (poly(propylene imine)) dendrimers, are able to remove protease resistant PrP^{Sc} and abolish infectivity, offering possible applications for therapy. These dendrimer types are thought to act through their positively charged amino surface groups. In the present study, the molecular basis of the antiprion activity of dendrimers was further investigated, employing modified PPI dendrimers in which the positively charged amino surface groups were substituted with neutral carbohydrate units of maltose (mPPI) or maltotriose (m3PPI). Modification of surface groups greatly reduced the toxicity associated with unmodified PPI but did not abolish its antiprion activity, suggesting that the presence of cationic surface groups is not essential for dendrimer action. PPI and mPPI dendrimers of generation 5 were equally effective in reducing levels of protease-resistant PrP^{Sc} (PrP^{res}) in a dose- and time-dependent manner in ScN2a cells and in pre-existing aggregates in homogenates from infected brain. Solubility assays revealed that total levels of PrP^{Sc} in scrapie-infected mouse neuroblastoma (ScN2a) cells were reduced by mPPI. Coupled with the known ability of polyamino dendrimers to render protease-resistant PrP^{Sc} in pre-existing aggregates of PrP^{Sc} susceptible to proteolysis, these findings strongly suggest that within infected cells dendrimers reduce total amounts of PrP^{Sc} by mediating its denaturation and subsequent elimination.

Introduction

Prion diseases are a group of fatal neurodegenerative diseases that can have sporadic, genetic, and infectious origin. They include bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep, and, in humans, fatal familial insomnia (FFI), Gerstmann-Sträussler-Scheinker disease (GSS), and Creutzfeldt-Jakob disease (CJD) of various etiologies, such as iatrogenic (iCJD), sporadic (sCJD), familial (fCJD), and variant (vCJD), which emerged following transmission from BSE to humans.[1,2] Prion diseases are caused by aberrant metabolism of the cellular prion protein, PrP^{C}. PrP^{Sc}, the disease-associated abnormally folded isoform of PrP^{C}, accumulates in the CNS as a hallmark of prion diseases and is the major constituent in preparations of the infectious agent (termed prion). PrP^{Sc} is derived from PrP^{C} in a post-translational conversion process[3,4] in which it acquires increased resistance to proteases and reduced solubility in nonionic detergents,[5,6] accompanied by increased β-sheet content.[7,8] Coding mutations in the human prion protein gene result in hereditary forms of human prion diseases.[9–13] Expression of PrP^{C} is a prerequisite for propagating prions and developing the disease.[14,15] The intriguing characteristics of prion diseases are conveyed in the prevalent hypothesis (protein only hypothesis[16]), defining prions as infectious proteinaceous pathogens,[6] which propagate in the absence of nucleic acid by an autocatalytic process in which PrP^{C} is converted into the disease associated isoform.[17] In agreement with the protein-only hypothesis, spontaneous conversions and hereditary mutations favoring a conversion from PrP^{C} to PrP^{Sc} account for sporadic and familial cases, respectively, in addition to the infectious nature of prion diseases.[18–21]

Accumulation of PrP^{Sc} as amyloid fibrils, often in the form of amyloid plaques,[13,22–24] is accompanied by neuropathological changes primarily in the CNS, spongiform vacuolation in the (cerebral) gray matter of the brain, neuronal loss, and astrocytic proliferation (astrocytic gliosis).[9,25] Presently, no cure is available for these invariably fatal diseases. The development of therapeutic and prophylactic approaches in human prion diseases is problematic as they are characterized by a long incubation period and short clinical phase, and preclinical diagnosis is difficult.[23,25,26] A potential antiprion agent was identified in polyamino dendrimers when they were demonstrated to reduce levels of protease resistant PrP^{Sc} (PrP^{res}) in scrapie-infected cells and pre-existing aggregates of prions and purging PrP^{Sc} from chronically infected cells by these compounds abolished infectivity in mice bioassays.[27,28]

Dendrimers are synthetic polymers with a highly structured layered architecture, consisting of multiple branched monomers radiating from a central core. Layers of monomers are attached stepwise during synthesis, with the number of branch points defining the generation of dendrimer.[29–32] Their wide variety of biological applications include bioimaging, drug and gene delivery, drugs, carriers for vaccines, and scaffold for tissue repair.[33–37] The various dendrimer compounds for which an antiprion activity has been demonstrated includes the branched

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10.1021/bm100101s
Published on Web 04/20/2010

Biochimica et Biophysica Acta 1811 (2011) 221–226

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Interactions of phosphorus-containing dendrimers with liposomes

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ARTICLE INFO

Article history:
Received 22 July 2010
Received in revised form 18 November 2010
Accepted 19 November 2010
Available online 1 December 2010

Keywords:
Cationic phosphorus-containing dendrimers
Liposomes
DSC
Anisotropy
Membrane fluidity

ABSTRACT

The influence of cationic phosphorus-containing dendrimers generation 3 and 4 on model DMPC or DPPC lipid membranes was studied. Measurements of fluorescence anisotropy and differential scanning calorimetry (DSC) were applied to assess changes in lipid bilayer parameters, including fluidity, anisotropy, and phase-transition temperature. Interaction with both hydrophobic and hydrophilic regions of the bilayer was followed by these methods. Dendrimers of both generations influence lipid bilayers by decreasing membrane fluidity. The results suggest that dendrimers can interact both with the hydrophobic part and the polar head-group region of the phospholipid bilayer. Higher generation dendrimers interact more strongly with model membranes, and the concentration, as well as the generation, is of similar importance.

1. Introduction

The history of dendrimers started about 30 years ago, and today there are many kinds that all share a similar structure. Dendrimers are different from traditional linear polymers. Generally, their construction consists of branches emanating from a core, along with a well-defined number of active groups on their surface. Their unique structural features hold out promise of many applications in the fields of nanoelectronics and (electro)chemical (bio)sensors, or they can be used as carriers of active substances, anti-prion agents, insulating materials, and transfecting agents [1–9]. A relatively new promising class contains cationic phosphorus-containing dendrimers. These molecules have a hydrophilic surface and a hydrophobic backbone that allows for very efficient membrane penetration. They have protonated terminary amine end groups on their surfaces and their number is related to their generation [10]. How these carriers behave during interaction with biological membranes is worth considering when synthesizing new drug carriers. Many drugs have not been effective due to their inability to reach the appropriate tissue. It is difficult to design and develop drug carriers that behave as biocolloidal systems in the body. To use any new materials as drug carriers, one needs to understand how they interact with biological structures like cell membranes. Biological membranes are very complicated structures so it is difficult to understand their complexed interactions with dendrimers. To simplify the experimental model, lipid membranes were used for these studies instead of biological membranes. Lipid vesicles are simple structures that provide a lot of information about interactions between drugs and biological membranes. When interactions between model lipid membranes and dendrimers are examined, conclusions can be drawn about biological processes such as membrane fusion and transport of this new material for drug or gene delivery [11].

Results of interactions between cationic phosphorus-containing dendrimers of generation 3 (G3) and generation 4 (G4) with model lipid membranes composed of DMPC or DPPC phospholipids have been explored.

Because of water solubility most of the potential applications of phosphorus-containing dendrimers are related to biology. Phosphorus dendrimers are more stable then PAMAM dendrimers but less than PPI dendrimers. They possess hydrophobic interior and the hydrophilic end groups. The number of cationic end groups, molecular weights, and the structures of cationic phosphorus-containing dendrimers of generations 3 ($C_{624}H_{1104}N_{183}Cl_{48}O_{42}P_{45}S_{42}$) and 4 ($C_{1296}H_{2256}N_{375}Cl_{96}O_{90}P_{93}S_{90}$) are shown in Fig. 1 [10,12].

2. Materials and methods

2.1. Materials

Lipids: 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC); 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC); fluorescent probes: 1,6-diphenyl-1,3,5-hexatriene (DPH); *N,N,N*-trimethyl-4-(6-

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1388-1981/$ – see front matter
doi:10.1016/j.bbalip.2010.11.007

Neurons and Stromal Stem Cells as Targets for Polycation-Mediated Transfection

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Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 1, pp. 22-25, January, 2011
Original article submitted May 26, 2010

Expression of transgenes in neurons and stromal/mesenchymal stem cells (MSC) can greatly enhance their therapeutic potential. In transfection experiments, we studied properties of linear and branched (dendrimers) polycations as transgene delivery vehicles. Linear polyethyleneimine transfected neurons, but was ineffective in MSC. Polyamidoamine dendrimers showed greater transfection efficiency and mean GFP fluorescence intensity compared to phosphorus dendrimers of the same (4th) generation. Expression of neurotrophic factor BDNF in MSC transfected with polyamidoamine dendrimers was also by more than 10 times higher.

Key Words: *neurons; stromal/mesenchymal stem cells; transfection; polyethylenimine; dendrimers; brain-derived neurotrophic factor*

In vitro culturing of cells and tissues is indispensable for studying the fundamental laws of their functioning in terms of genetics, biochemistry, and physiology, as well as for applied research, such as expansion to subsequent transplantation, forming of artificial organs, *etc*. [3,6]. The development of molecular genetic methods gave rise to a new trend: gene engineering of primary animal and human cells and tissues. In particular, expression of transgenes in stem cells can compensate for the lack of necessary body proteins, provide differentiation in a given direction, increase production of trophic factors, *etc*. [2,8,13]. In situations that do not require long-term functioning of the transgene and its integration into the cellular genome, transient transfection is optimal. In contrast to the transplanted immortalized cultures, transfection of primary cell populations is not always successful.

The purpose of research was to consider options for transfection with various cationic polymers capable of condensing vector DNA due to electrostatic interaction and delivering it through the cell membrane. The objects of research were neurons and stromal stem cells of the bone marrow also known as MSC.

MATERIALS AND METHODS

For isolation of human stromal stem cells, bone marrow aspirate from healthy donors was centrifuged in Ficoll density dragient, the mononuclear fraction was collected, washed twice with PBS, and seeded in α-MEM with 10% PBS (HyClone) [6]. MSC selected by adhesion to plastic were immunotyped using antibodies to CD90, CD105, CD45, and CD34. Primary neurons isolated from sympathetic ganglia of newborn rats were purified and cultured on collagen substrate in medium IMDM (Gibco) with 10% FCS as previously described [1]. All cell cultures were cultured in a CO_2

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0007-4888/11/1511-0126

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In vivo toxicity of poly(propyleneimine) dendrimers

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Received 20 January 2011; revised 19 May 2011; accepted 27 May 2011
Published online 16 August 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.33196

Abstract: Dendrimers are highly branched macromolecules with the potential to be used for biomedical applications. Several dendrimers are toxic owing to their positively charged surfaces. However, this toxicity can be reduced by coating these peripheral cationic groups with carbohydrate residues. In this study, the toxicity of three types of 4th generation poly (propyleneimine) dendrimers were investigated *in vivo*; uncoated (PPI-g4) dendrimers, and dendrimers in which 25% or 100% of surface amino groups were coated with maltotriose (PPI-g4-25%m or PPI-g4-100%m), were administered to Wistar rats. Body weight, food and water consumption, and urine excretion were monitored daily. Blood was collected to investigate biochemical and hematological parameters, and the general condition and behavior of the animals were analyzed. Unmodified PPI dendrimers caused changes in the behavior of rats, a decrease in food and water consumption, and lower body weight gain. In the case of PPI-g4 and PPI-g4-25%m dendrimers, disturbances in urine and hematological and biochemical profiles returned to normal during the recovery period. PPI-g4-100%m was harmless to rats. The PPI dendrimers demonstrated dose- and sugar-modification-degree dependent toxicity. A higher dose of uncoated PPI dendrimers caused toxicity, but surface modification almost completely abolished this toxic effect. © 2011 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 99A: 261–268, 2011.

Key Words: poly(propyleneimine), PPI, dendrimer, glycodendrimer, *in vivo* toxicity, rat

How to cite this article: Ziemba B, Janaszewska A, Ciepluch K, Krotewicz M, Fogel WA, Appelhans D, Voit B, Bryszewska M, Klajnert B. 2011. *In vivo* toxicity of poly(propyleneimine) dendrimers. J Biomed Mater Res Part A 2011:99A:261–268.

INTRODUCTION

Dendrimers are highly branched, perfectly monodisperse macromolecules with a precisely controlled chemical structure that were first synthesized by Tomalia et al.[1] and Newkome et al.[2] Specific properties of dendrimers have attracted great interest in terms of exploring their potential in biomedical applications including as drug carriers,[3] vectors for gene transfection,[4] and as MRI agents.[5] In addition, they have contributed significantly to the fields of metal complexation,[6] host-guest chemistry,[7] and glycomics.[8] Dendrimer architecture, based on multiplied branches, offers advantages including narrow polydispersity, low viscosity compared with equivalent molecular weight linear polymers, and a high density of surface functionalities.[9] Polyamidoamine (PAMAM), poly-L-lysine (PLL), and poly(propyleneimine) (PPI) dendrimers are commercially available and have been widely investigated from a biomedical view point.

Toxicity studies are essential for proving the safety of dendrimer therapeutic applications but several general facts are already known: dendrimer toxicity is generation-dependent with higher generations being more toxic as the number of surface groups increases (usually it is doubled) with each generation.[10] The nature of the surface groups is particularly important. Cationic dendrimers are more cytotoxic and hemolytic than neutral or anionic dendrimers.[10,11] This is predominantly due to their binding to negatively charged cell membranes.[12] Most studies concerning the toxicity of dendrimers have been performed *in vitro* with few studies being carried out *in vivo*.[12–14] Generally, it is believed that dendrimers do not exhibit properties that would preclude their use in biological applications, although higher generations can produce potential biological complications.[12] However, melamine dendrimers in acute toxic studies caused 100% mortality in 6–12 h postinjection at a dose of 160 mg/kg.[14] Jain et al., in their review from 2010[15] proposed that the administration of dendrimers to biological systems required detailed study concerning their *in vivo* disposition.

The toxic properties of dendrimers depend on their structural components including the core, interior

Additional Supporting Information may be found in the online version of this article.
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Contract grant sponsor: EU European Regional Development Fund

Biochimica et Biophysica Acta 1808 (2011) 2714–2723

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

The influence of PAMAM-OH dendrimers on the activity of human erythrocytes ATPases

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ARTICLE INFO

Article history:
Received 10 May 2011
Received in revised form 11 July 2011
Accepted 15 July 2011
Available online 23 July 2011

Keywords:
PAMAM-OH dendrimers
ATPase
Erythrocyte ghosts
Liposomes
Enzyme inhibition
Red blood cell

ABSTRACT

Dendrimers are a relatively new and still not fully examined group of polybranched polymers. In this study polyamidoamine dendrimers with hydroxyl surface groups (PAMAM-OH) of third, fourth and fifth generation (G3, G4 and G5) were examined for their ability to influence the activity of human erythrocyte plasma membrane adenosinetriphosphatases (ATPases). Plasma membrane ATPases are a group of enzymes related, among others, to the maintenance of ionic balance inside the cell. An inhibition of their activity may result in a disturbance of cell functioning. Two of examined dendrimers (G4 and G5) were found to inhibit the activity of Na^+/K^+ ATPase and Ca^{2+} ATPase by 20–30%. The observed effect was diminished when higher concentrations of dendrimers were used. The experiment with the use of pyrene as fluorescent probe sensitive to the changes in microenvironment's polarity revealed that it was an effect of dendrimers' self-aggregation. Additional studies showed that PAMAM-OH dendrimers were able to decrease the fluidity of human erythrocytes plasma membrane. Obtained results suggest that change in plasma membrane fluidity was not caused by the dendrimer–lipid interaction, but dendrimer–protein interaction. Different pattern of influence of dendrimers on ATPases activity and erythrocyte membrane fluidity suggests that observed change in ATPases activity is not a result of dendrimer–lipid interaction, but may be related to direct interaction between dendrimers and ATPases.

1. Introduction

Dendrimers are a relatively new class of highly branched polymers. The first syntheses of dendrimers were reported in late 70s and early 80s of a previous century [1,2]. Important attributes of dendrimers are their high monodispersity, regular, poly-branched three dimensional organization and the presence of many terminal groups [3]. There are different types of dendrimers, depending on the building monomers, for example: PAMAM (polyamidoamine) or PPI (polypropyleneimine) dendrimers. The size of molecules and number of terminal groups depend on the generation of the dendrimer [4]. Since their first synthesis, a lot of studies related to properties of dendrimers have been performed. Dendrimers were found to be very useful in the field of pharmacy and medicine. For example they can be used as solubility enhancers for hydrophobic molecules [5] or as a part of a drug-delivery system [6]. Cationic dendrimers turned out to be good non-viral vehicles for gene delivery, that can be utilized for therapeutic applications [7–9]. There were also trials performed in order to assess the possibility of application of a dendrimer based molecule as a contrast agent in MR imaging [10,11].

The main factor that limits the possibility of the applications of dendrimers in medicine is their toxicity. Toxic activity is mainly related to cationic dendrimers. Cationic dendrimers possess surface groups that bear positive charge or groups (such as amine groups) that can be protonated under physiological conditions [12,13]. Interactions between positively charged surface of dendrimers and negatively charged lipid membranes lead to toxic effects of dendrimers [14]. This interaction may lead to nanoscale hole formation in lipid bilayers [15]. As a result of interaction between cationic dendrimers and erythrocytes, hemolysis may be observed [16]. Moreover, cationic dendrimers reveal cytotoxic activity against mammalian cells [17]. In order to reduce above-mentioned toxic effects, modifications of dendrimers' surface may be introduced. Such modifications include for example substitution of surface amine groups with: PEG [18], acetyl group [19] or saccharide moiety [20]. In the case of PAMAM dendrimers one of the possible modification is the replacement of surface amine groups for hydroxyl groups. This modification leads to the loss of surface charge. Such modified PAMAM dendrimers (called PAMAM-OH) have been shown to possess

Abbreviations: ATPase, adenosinetriphosphatase; CH, cholesterol; DLS, dynamic light scattering; DPH, 1,6-diphenyl-1,3,5-hexatriene; EDTA, ethylenediaminetetraacetic acid disodium salt dihydrate; EGTA, ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid; G3, G4, G5, third, fourth and fifth generation of dendrimer; PAMAM, polyamidoamine dendrimer; PAMAM-OH, polyamidoamine dendrimer with hydroxyl surface groups; PC, phosphatidylcholine; PMSF, phenylmethanesulfonyl fluoride; PPI, polypropyleneimine dendrimers; SM, sphingomyelin; TMA-DPH, N,N,N-trimethyl-4-(6-phenyl-1,3,5,-hexatrien-1-yl)phenylammonium p-toluenesulfonate; Tris, tris (hydroxymethyl)aminomethane

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0005-2736/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2011.07.021

Chemistry and Physics of Lipids 165 (2012) 408–413

Contents lists available at SciVerse ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Effect of phosphorus dendrimers on DMPC lipid membranes

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ARTICLE INFO

Article history:
Available online 7 December 2011

Keywords:
Phosphorus dendrimers
DMPC
DSC
ζ-Size/potential

ABSTRACT

Large unilamellar liposomes and multilamellar vesicles consisting of DMPC interacted with cationic phosphorus-containing dendrimers CPDs G3 and G4. DSC and ζ-potential measurements have shown that liposomal-dendrimeric molecular recognition probably occurs due to the interaction between the complementary surface groups. Calorimetric studies indicate that the enthalpy of the transition of the lipids that interact with CPDs is dependent on the dendrimers generation.

These results can be used in order to rationally design mixed modulatory liposomal *locked-in* dendrimeric, drug delivery nano systems.

1. Introduction

Dendrimers represent the fourth and most recent category of macromolecular architecture (Tomalia, 2005). Unlike linear polymers they have a well-defined structure that leads to low MW polydispersity index values. Dendrimers have attracted much interest since their discovery due to the specific structure which makes them suitable for a variety of biomedical applications (Klajnert et al., 2004, 2006; Smith, 2008; Svenson and Tomalia, 2005). They are small in size, while their low polydispersity can contribute to the reproducibility of their pharmacokinetic behavior (Klajnert et al., 2009). The use of dendrimers as modulators of the release rate of a drug incorporated into liposomes and the possible alterations of the drug bioavailability seems to be an attractive field for research (Gardikis et al., 2010). In the present work we especially focus on interactions between dendrimers and model lipid membranes. The findings from this study could prove helpful to rationally design new advanced liposomal drug delivery systems for bioactive molecules by combining dendrimeric and liposomal technologies.

We tested cationic phosphorus containing dendrimers for lipid membranes interaction. CPDs differ from the molecules described previously by Supattapone et al. (1999). They have a hydrophilic surface and a hydrophobic backbone which allows for efficient membrane penetration (Loup et al., 1999). Because of water-solubility most of the potential applications of phosphorus-containing dendrimers are related to biology. Phosphorus dendrimers are more stable then PAMAM dendrimers but less than PPI dendrimers (Caminade and Majoral, 2005; Solassol et al., 2004). Here we show that CPDs were able to change the properties of DMPC lipid membranes.

2. Experimental

2.1. Materials

Phosphorus dendrimers were synthesized by the Laboratoire de Chimie de Coordination du CNRS by group of Professor Majoral J.P. The main characteristics and synthesis of CPDs were described earlier (Caminade and Majoral, 2005). CPDs-G3, $C_{624}H_{1104}N_{183}Cl_{48}O_{42}P_{45}S_{42}$ (generation 3, 48 surface cationic end groups, MW: 16,280; diameter: 4.1 nm) and CPDs-G4, $C_{1296}H_{2256}N_{375}Cl_{96}O_{90}P_{93}S_{90}$ (generation 4, 96 surface cationic end groups, MW: 33,702; diameter: 5 nm), are presented in Fig. 1. Phospholipid: 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), was purchased from Avanti Polar Lipids and used without further purification. All other reagents used were of

Abbreviations: CPDs, cationic phosphorus-containing dendrimers; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; ΔH, main phase-transition enthalpy; LUVs, large unilamellar vesicles; MLVs, multilamellar vesicles; MT, endothermic main transition peak; G3, generation 3; G4, generation 4; PT, endothermic pre-transition peak; T_m, gel to liquid-crystalline phase transition temperature; $\frac{1}{2}\Delta T_p$, half width of the peak transition.

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0009-3084/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.chemphyslip.2011.11.014

Non-virally Modified Human Mesenchymal Stem Cells Produce Ciliary Neurotrophic Factor in Biodegradable Fibrin-Based 3D Scaffolds

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Received 26 October 2011; revised 4 December 2011; accepted 9 December 2011

Published online 27 December 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23033

ABSTRACT: We report the adaptation of dendrimer-based nonviral expression system for ciliary neurotrophic factor (CNTF) overproduction in human mesenchymal stem cells (hMSCs) embedded into fibrin-based three-dimensional (3D) matrix. Time-restricted neurotrophin expression enables autologous adult stem cells for additional trophic support and increases their therapeutic potential in neuroregeneration applications. Polyamidoamine (PAMAM)–NH_2 dendrimers of fourth generation effectively provided virus-free delivery and expression of CNTF–internal ribosome entry site–green fluorescent protein cassette with a transfection efficiency in hMSCs over 11%. CNTF levels in transfected cultures were 10-fold higher as compared with the control cells. Dendrimer-driven CNTF expression also persisted in hMSCs embedded into fibrin-based 3D matrix, an emerging vehicle for cell delivery or bioartificial organ formation. Nonviral modification of autologous adult stem cells with use of dendrimers is a novel tool perspective in terms of biosafety and technological availability. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:1546–1554, 2012

Keywords: CNTF; non-viral gene delivery; human mesenchymal stem cells; transfection; PAMAM dendrimers; gene therapy; polymeric biomaterials; fibrin scaffold

INTRODUCTION

Human mesenchymal stem cells (hMSCs) are pluripotent adult stem cells with a potential for *in vitro* expansion and differentiation into a number of tissue lineages such as chondrocytes, osteoblasts, adipocytes, and so on. Genetic modification of hMSCs may enable trophic, differentiation, antitumor, or other factors to be expressed, providing a powerful tool for cell-based gene therapy. With emerging attempts to use hMSCs as readily available source of autologous stem cells in the therapy of neural injuries and disorders,[1,2] engineered expression of neurotrophic factors can facilitate trophic support, increased axon outgrowth, neuroprotection, and regeneration. Biosafety considerations appeal to the use of nonviral carriers, which include polycations, lipophilic compounds, magnetic particles, dendrimers, and so on.[3–5]

Dendrimers are macromolecules characterized by highly branched three-dimensional (3D) structure, providing a high degree of surface functionality and versatility. Their branching architecture, multivalency, and well-defined size and molecular weight clearly distinguish these nanoparticles as unique carriers in biomedical applications such as gene transfection, drug delivery, cell culture, and so on.[5–7] Use of dendrimers as vectors for gene delivery is based

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Journal of Pharmaceutical Sciences, Vol. 101, 1546–1554 (2012)

Journal of
Biomedical Nanotechnology
Vol. 8, 57–73, 2012

RESEARCH ARTICLE

Carbosilane Dendrimers are a Non-Viral Delivery System for Antisense Oligonucleotides: Characterization of Dendriplexes

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The success of gene therapy depends on the development of suitable carriers, and because of their architecture dendrimers are promising tools for gene delivery. This research concerns the use of second generation carbosilane dendrimers as carriers for anti-HIV oligodeoxynucleotides (ODNs). The aim was to characterize complexes formed by positively charged dendrimers and negatively charged oligonucleotides using a fluorescence method, laser Doppler electrophoresis, dynamic light scattering (DLS), atomic force microscopy (AFM), transmission electron microscopy (TEM) and molecular modeling. The zeta-potential of ODNs increased from −25 mV to positive values after the addition of dendrimers. DLS and TEM revealed that the diameters of dendriplexes ranged from 75 to 240 nm and from 50 to 260 nm, respectively, and this was dependent on the type of dendrimer and the molar ratios of the complexes formed; complexes were stable for between 100 and 300 minutes. AFM measurements and molecular modeling studies were carried out to determine the structure and size of dendriplexes. The physicochemical properties of the dendriplexes studied and data from previous research suggest that carbosilane dendrimers are good candidates for nucleic acid delivery.

Keywords: Carbosilane Dendrimer, Drug Delivery, Antisense Oligonucleotide, Dendriplex, Zeta Potential, Hydrodynamic Diameter, Atomic Force Microscopy, Molecular Modeling.

1. INTRODUCTION

Antisense oligonucleotides (ONs) can inhibit gene expression at the level of transcription. Phosphodiester oligonucleotides are nuclease-sensitive, and nuclease-resistant phosphorothioate oligonucleotides are the subject of clinical trials. However, owing to their anionic charge, ONs cannot cross cell membranes and they interact with serum albumins.[1] Therefore, several delivery systems (viral and non-viral) for oligonucleotides have been developed.[2] Viral delivery systems can be highly efficient, but large-scale production, immunogenicity, regulatory constraints and recombination with wild type viruses restrict their use and application. Non-viral vectors include peptides, cationic lipids, liposomes, polymers and dendrimers. The delivery performances of these non-viral vectors differ greatly and depend on the physicochemical and colloidal properties of the complexes that they form with nucleic acids. Highly branched and symmetrical dendrimers have been widely studied and are promising tools for biomedical

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 1550-7033/2012/8/057/017 doi:10.1166/jbn.2012.1369

NJC

View Online / Journal Homepage / Table of Contents for this issue
Dynamic Article Links

Cite this: *New J. Chem.*, 2012, **36**, 350–353

www.rsc.org/njc

LETTER

The biodistribution of maltotriose modified poly(propylene imine) (PPI) dendrimers conjugated with fluorescein—proofs of crossing blood–brain–barrier†

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Received (in Montpellier, France) 26th May 2011, Accepted 1st August 2011
DOI: 10.1039/c1nj20444k

Oligosaccharide-modified poly(propylene imine) dendrimers are promising candidates as drug carriers and as anti-prion agents. Here, we report the biodistribution of maltotriose-modified 4th generation poly(propylene imine) (PPI) dendrimers and their ability to cross the blood–brain–barrier that is important if these glycodendrimers are considered as potential therapeutic agents in the central nervous system (CNS).

The globular/spherical and high-surface-group containing dendritic structures, including dendrimers and hyperbranched polymers, have been explored as the next outstanding polymer architecture in the last three decades.[1] Since mid 1990's dendrimers have been developed as polymeric therapeutics and diagnostics in many fields of biomedical applications.[2] Moreover, dendrimeric agents have attracted growing interest in the treatment for many diseases of the brain.[3] However, a selective blood–brain–barrier (BBB), composed principally of specialized capillary endothelial cells fitted with highly restrictive tight junctions, prevents the passage of some therapeutic agents from blood to the central nervous system (CNS).[4]

Different physiological factors are responsible for affecting the drug delivery to CNS.[5] Various potential transport mechanisms differing for nutrients, drugs, and larger nanoscopic systems such as liposomes, polymeric particles, micelles and dendrimers were discussed by Denora *et al.*[5] Most nanoscopic systems need either lipophilic properties or coupling of antibodies and molecular recognition sequences or ligands to enhance going across BBB.[5] In the case of dendritic therapeutic agents, only few examples are known, where transferrin-conjugated PEG-modified PAMAM dendrimers have been successfully used as gene delivery systems in brain.[6,7] Furthermore, the decoration of prodrug–dendrimer conjugates with D-glucosamine has enhanced not only crossing the BBB, but also the uptake to tumors, due to facilitative glucose metabolism by the glucose transporters in the tumor.[8] Inspired by the example of the D-glucosamine-decorated dendrimer[8] characterized by enhanced permeability across BBB and the potential use of dendritic architectures as anti-prion[3a,9,10] and anti-amyloid[9] agents in Alzheimer's disease, we investigated the biodistribution of maltotriose-modified 4th generation PPI glycodendrimers in a living organism, in order to check potential BBB crossing properties. Unmodified amino-terminated PPI dendrimers are not ideal candidates for ongoing projects in the field of biomedical applications due their high toxicity,[11–13] even though they possess interesting biological properties as anti-prion and anti-amyloid agents.[9] Therefore, amino-terminated PPI dendrimers modified by attaching various sugar moieties on the surface were synthesized and such glycodendrimers were checked as potential therapeutic agents[3a,10] and drug carrier systems.[3a,14,15] Sugar decoration caused significantly lower cytotoxicity of the PPI dendrimers. The same was observed for other types of sugar modified dendrimers.[15a] Klajnert *et al.* demonstrated that PPI dendrimers modified with maltose moieties exhibited good solubility under physiological conditions[3a] and showed almost complete loss of haemolytic activity in comparison to unmodified PPI dendrimers.[9] Most available data concerning the toxicity of PPI dendrimers were determined by *in vitro* studies.[12,13] The first biodistribution experiment for mannose- and lactose-coated 5th generation PPI dendrimers in mice showed the preferred uptake of dendritic glycostructures in liver.[16]

It has been shown that oligosaccharide-modified 4th and 5th generation PPI dendrimers perturb the aggregation of a prion protein PrP^{Sc10} and a prion peptide (PrP 185–208),[3a] which is an important part of prion protein involved in the genesis of spongiform encephalopathies, but also the aggregation of Aβ 1–28 involved in Alzheimer's disease (data not published). This fact was the main motivation to check whether these dendrimers are able to cross BBB. The combination of successful crossing of BBB and being potential therapeutic agents[3a,10] would give us the chance to develop the next generation of therapeutics for brain diseases.

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† This article is part of the themed issue Dendrimers II, guest-edited by Jean-Pierre Majoral.

NJC

Dynamic Article Links

Cite this: *New J. Chem.*, 2012, **36**, 428–437

www.rsc.org/njc

PAPER

Cytotoxicity of PAMAM, PPI and maltose modified PPI dendrimers in Chinese hamster ovary (CHO) and human ovarian carcinoma (SKOV3) cells†

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Received (in Montpellier, France) 6th June 2011, Accepted 21st September 2011
DOI: 10.1039/c1nj20489k

Characterization of dendrimers as potential therapeutics or drug carriers is complete only when toxicity is assessed. There are numerous studies on the influence of surface modification of PAMAM and PPI dendrimers on their cytotoxic properties but without proposing a mechanism for their toxic effect. In this study cytotoxicity profiles of acid-terminated PAMAM G3.5 and amino-terminated PAMAM G4 in comparison to unmodified amino-terminated PPI-G4 and maltotriose modified PPI-G4 dendrimers were checked. Also the mechanism of cell death in Chinese hamster ovary (CHO) and human ovarian carcinoma (SKOV3) cell lines was investigated. The anionic PAMAM G3.5 dendrimers seem to be the most suitable dendrimers for therapeutic applications, because of their high biocompatibility and low cytotoxicity. Cationic PPI-G4 and PAMAM G4 were the most harmful for both CHO and SKOV3 cell lines, especially in high doses. Maltotriose modification has significantly reduced toxicity within the series of PPI-G4 dendrimers. The moderately doxorubicin and cisplatin resistant human ovarian carcinoma SKOV3 cell line was more vulnerable to modified PPI dendrimers than Chinese hamster ovary CHO cell line which does not show resistance to majority of anticancer agents. This unique property makes these dendrimers potentially interesting for an anticancer therapy.

Introduction

Dendrimers are nanoparticles with a well-defined structure, shape and size. They have found many applications not only in a biomedical field but also in chemistry, biological science, material science, engineering and electronics. The most promising biomedical area of dendrimers application is using dendrimers as carriers of drugs and bioactives.[1] Dendrimers can improve solubility and bioavailability of therapeutics.[2] Moreover they could govern the release of drug.[3] These globular molecules which, for example, possess hydrophobic interior and hydrophilic surface can improve the solubility and bioavailability of poorly water-soluble drugs.[4] Generation, size, pH, core, polymeric architecture and functional groups—all these have an influence on dendrimer mediated solubilization of drugs.[5] Other important properties of dendrimers as drug carriers are extended time of the drug residence, increased stability of bioactives, protection from biological environment and biodegradability.[6a,b] Positively charged poly(propylene imine) (PPI), carbosilane (CBS) and poly(amido amine) (PAMAM) dendrimers can form stable complexes with nucleic acids and transport them inside the cell. The transfection efficiency and specificity can be enhanced by the surface modification of dendrimers.[7] Dendrimers are promising molecules in anticancer therapy not only as anticancer agents' carriers[8a,b] but also as contrast agents[9] or as agents in cancer gene therapy.[10] Baker *et al.* demonstrated that acetylated PAMAM dendrimers may be coupled to methotrexate or tritium and conjugated to folic acid as a targeting agent. Such a modification increased the antitumor activity of methotrexate and markedly decreased its toxicity, allowing therapeutic responses not possible with a free drug.[8c] Those biomedical applications encouraged scientists to develop a new kind of dendrimers and to exhibit tunable properties of well-known molecules.

Thanks to that many kinds of dendrimers exist: polyamido amine (PAMAM), poly(propylene imine) (PPI), phosphorus, carbosilane, peptide, polyester. Besides, many modified dendrimers were synthesized, such as PAMAM with lauroyl or propranolol molecules on the surface,[11] carbohydrate-coated PPI dendrimers,[12] PAMAM with dodecyl or cholesteryl moieties[13] as

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† This article is part of the themed issue Dendrimers II, guest-edited by Jean-Pierre Majoral.

J Physiol Biochem (2012) 68:447–454
DOI 10.1007/s13105-012-0158-y

ORIGINAL PAPER

Modulation of biogenic amines content by poly(propylene imine) dendrimers in rats

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Received: 5 September 2011 / Accepted: 15 February 2012 / Published online: 28 February 2012

Abstract Biogenic amines and polyamines participate in all vital organism functions, their levels being important function determinants. Studies were performed to check whether repeated administration of poly(propylene imine) (PPI) dendrimers, synthetic macromolecules with diaminobutane core, and peripheral primary amine groups, may influence the endogenous level of amines, as represented by the two of them: spermidine, a natural derivative of diaminobutane, and histamine. The experiment was carried out on Wistar rats. Fourth generation PPI dendrimer, as well as maltotriose-modified fourth generation PPI dendrimers with (a) cationic open sugar shell and (b) neutral dense sugar shell that possess a higher biocompatibility, was used. Applying the combination of column chromatography on Cellex P and spectrofluorimetric assays of *o*-phthaldialdehyde, the final amine condensation products were employed to analyze tissue spermidine and histamine outside the central nervous system. Furthermore, radioenzymatic assay was used to measure histamine levels in the brain. The obtained results indicate that in some tissues, the endogenous concentrations of histamine and spermidine may be affected by dendrimers depending on their dose and type of dendrimers.

Keywords Biogenic amines · Histamine · Spermidine · Polyamines · Poly(propylene imine) dendrimers · In vivo study · Immune response · Rats

Abbreviations

Fourth generation PPI	Poly(propylene imine) dendrimers
BA	Biogenic amines
PA	Polyamines
OPT	*o*-Phtalaldehyde

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Introduction

There are many publications on dendrimers, their biophysical properties and biological activity [19]. These macromolecules can become new promising pharmaceuticals used in biomedicine as drug carriers, in gene transfection, and in other applications. The important key issue is that a drug carrier should be of nanometer scale, easily crossing the cell membrane, and furthermore

New Drug Delivery Nanosystem Combining Liposomal and Dendrimeric Technology (Liposomal *Locked-In* Dendrimers) for Cancer Therapy

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Received 12 October 2009; revised 11 December 2009; accepted 15 January 2010

Published online 29 March 2010 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.22121

ABSTRACT: Liposomal locked-in dendrimers (LLDs), the combination of liposomes and dendrimers in one formulation, represents a relatively new term in the drug carrier technology. LLDs undergone appropriate physicochemical investigation can merge the benefits of liposomal and dendrimeric nanocarriers. In this study generation 1 and 2 hydroxy-terminated dendrimers were synthesized and were then "locked" in liposomes consisting of DOPC/DPPG. The anticancer drug doxorubicin (Dox) was loaded into pure liposomes or LLDs and the final products were subjected to lyophilization. The loading of Dox as well as its *in vitro* release rate from all systems was determined and the interaction of liposomes with dendrimers was assessed by thermal analysis and fluorescence spectroscopy. The results were very promising in terms of drug encapsulation and release rate, factors that can alter the therapeutic profile of a drug with low therapeutic index such as Dox. Physicochemical methods revealed a strong, generation dependent, interaction between liposomes and dendrimers that probably is the basis for the higher loading and slower drug release from the LLDs comparing to pure liposomes. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:3561–3571, 2010

Keywords: liposome; dendrimer; doxorubicin; *in vitro* release; differential scanning calorimetry; fluorescence spectroscopy

INTRODUCTION

Since the late 1960s, interest in methods of drug delivery has focused on the creation of new modifications of established drugs with the objective of getting a drug into the patient in the simplest possible way. The proper choice of delivery system can overcome problems relating to solubility, can regulate bioavailability and can therefore improve the overall ADME profile (*A*bsorption, bio*D*istribution, *M*etabolism, and *E*xcretion) of a candidate drug.[1]

The effectiveness of a drug can generally be improved in cases where there is need of controlled release in the bloodstream. This is particularly important in the case of the treatment of certain diseases, cancer therapy, for example, in which the administration of low molecular weight cytostatic drugs by themselves can cause severe side effects due to their poor biodistribution, whereas controlled delivery can greatly improve their therapeutic profile. In this respect, drug delivery systems based on nanoscale materials have the potential for minimum release prior to reaching the target site and selective accumulation at the desired locations *in vivo* due to the enhanced permeation and retention (EPR) effect.[2]

Polymers and liposomes represent two of the most thoroughly studied categories of nanoparticles with

Additional Supporting Information may be found in the online version of this article.

This work is a part of PhD thesis of Konstantinos Gardikis MSc.

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Journal of Pharmaceutical Sciences, Vol. 99, 3561–3571 (2010)

International Journal of Biological Macromolecules 50 (2012) 1138–1143

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Phosphorus-containing dendrimers against α-synuclein fibril formation

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ARTICLE INFO

Article history:
Received 7 December 2011
Received in revised form 3 February 2012
Accepted 6 February 2012
Available online 13 February 2012

Keywords:
Phosphorus-containing dendrimers
α-Synuclein
Aggregation
Neurodegenerative diseases
Circular dichroism

ABSTRACT

The aim of this work was to study the effect of phosphorus-containing dendrimers (generations G3 and G4) on the fibrillation of α-synuclein (ASN). The inhibition of fibril formation (filamentous and aggregates) is a potential therapeutic strategy for neurodegenerative disorders such as Parkinson's and other motor disorder neurodegenerative diseases. The interaction between phosphorus-containing dendrimers and ASN was studied by fluorescence spectroscopy. The decrease in the fluorescence intensity of intrisinic tyrosine was the most marked change in the fluorescence intensity observed upon addition of dendrimers. Furthermore, the effect of dendrimers on ASN fibril formation was studied using circular dichroism (CD) spectroscopy and CD studies were complemented by fluorescence assays using the dye thioflavin T (ThT). The results showed that phosphorus-containing dendrimers G3 and G4 inhibited fibril formation, when they were used in the ASN/dendrimer ratios 1:0.1 and 1:0.5. However, the higher concentrations of dendrimers did not show this effect.

1. Introduction

Dendrimers are highly branched polymers synthesized for the first time in the mid-1980s. They are built of a series of branches around an inner core, providing products of different generations and offer intriguing possibilities. These compounds can be synthesized from almost any core molecules and the branches constructed similarly to any bi-functional molecules, while the terminal groups can be modified chemically to achieve charged, hydrophilic, or hydrophobic surfaces [1–3].

Dendrimers have unique properties due to their terminal surface functional groups and specific shapes compared with those of the conventional linear polymers. The specific structure of these compounds significantly affects their properties, which is important for their potential use in medicine. The presence of internal cavities allows encapsulating guest molecules in the macromolecule interior and transferring them. The presence of numerous functional groups on the surface enables connection and transport of small particles as well. Therefore, due to their structure, dendrimers are ideal as carriers of such molecules as anticancer drugs and gene vectors in gene therapy [4,5]. Dendrimer surface modifications can provide the drug directly to the cancer cells and ensure its release only in the place where the cancer develops. An additional effect is also slow release of the drugs, which is particularly important, because it reduces their toxicity toward to healthy cells [5].

Dendrimers may not only be carriers of drugs, but they also have therapeutic properties, which are related to their high affinity to the protein structures. They can block receptors on the cell surface, where the virus attaches, and thus block the development of infection [6].

The first dendrimer's drug VivaGel®, which prevents HIV infection, has already been developed by Starpharma. The product has an inhibitory effect on human deficiency virus (HIV), herpes simplex virus (HSV) and may be used for treatment of bacterial vaginosis [6–8].

This fact caused increased interest in dendrimers and more laboratories are working on the synthesis of new types of dendrimers. These efforts are aimed to obtain dendrimers with the best biological properties and the lowest toxicity.

The wide spectrum of dendrimers properties requires studying the effects of these polymers with chemical compounds (*e.g.*, drugs) as well as on the cell components. Another promising activity of dendrimers involves inhibition of fibril assembly as a potential therapeutic strategy in neurodegenerative disorders such as prion and Alzheimer's diseases.

For the first time it was observed that dendrimers can be used against prion diseases after incubation of infected nervous system cells with dendrimers. Cells of brain homogenate from an organism infected by prion disease were characterized by the presence of proteins resistant to digestion by the enzyme protein. These forms of protein deprived of their natural functions cause the formation of

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0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijbiomac.2012.02.003

VERSITA

CELLULAR & MOLECULAR BIOLOGY LETTERS
http://www.cmbl.org.pl

Received: 29 June 2011
Final form accepted: 09 November 2011
Published online: 16 November 2011

Volume 17 (2012) pp 21-35
DOI: 10.2478/s11658-011-0033-9

Mini review

INFLUENCE OF DENDRIMERS ON RED BLOOD CELLS [#]

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Abstract: Dendrimers, highly branched macromolecules with a specific size and shape, provide many exciting opportunities for biomedical applications. However, most dendrimers demonstrate toxic and haemolytic activity because of their positively charged surface. Masking the peripheral cationic groups by coating them with biocompatible molecules is a method to reduce it. It was proven that modified dendrimers can even diminish haemolytic activity of encapsulated drugs. Experiments confirmed that anionic dendrimers are less haemotoxic than cationic ones. Due to the high affinity of dendrimers for serum proteins, presence of these components in an incubation buffer might also influence red blood cell (RBC)-dendrimer interactions and decrease the haemolysis level. Generally, haemotoxicity of dendrimers is concentration-, generation-, and time-dependent. Various changes in the RBCs' shape in response to interactions with dendrimers have been observed, from echinocytic transformations through cell aggregation to cluster formation, depending on the dendrimer's type and concentration. Understanding the physical and chemical origins of dendrimers' influences on RBCs might advance scientists' ability to construct dendrimers more suitable for medical applications.

[#] Paper authored by participants of the international conference: 18th Meeting, European Association for Red Cell Research, Wrocław – Piechowice, Poland, May 12-15th, 2011. Publication cost was covered by the organizers of this meeting.

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Abbreviations used: AFM – atomic force microscopy; CSi – carbosilane dendrimers; DOX – doxorubicin; FA – folic acid; HSA – human serum albumin; MRI – magnetic resonance imaging; PAD-PPI – dextran conjugated PPI dendrimers; PAMAM – polyamidoamine dendrimers; PEG – poly(ethylene glycol); PEO – poly(ethylene oxide); PPI – poly(propyleneimine) dendrimers; PPI-DAB – PPI dendrimers with diaminobutane core; PPI-DAE – PPI dendrimers with diaminoethane core; RBCs – red blood cells; Rms – AFM roughness values

Journal of Luminescence 132 (2012) 1553–1563

Contents lists available at SciVerse ScienceDirect

Journal of Luminescence

journal homepage: www.elsevier.com/locate/jlumin

Photo-physical and structural interactions between viologen phosphorus-based dendrimers and human serum albumin

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ARTICLE INFO

Article history:
Received 20 November 2011
Received in revised form
15 January 2012
Accepted 23 January 2012
Available online 4 February 2012

Keywords:
Dendrimers
Viologen
Phosphorus
Human serum albumin
Fluorescence quenching
Circular dichroism

ABSTRACT

This work deals with photo-physical and structural interactions between viologen phosphorus dendrimers and human serum albumin (HSA). Viologens are derivatives of 4,4'-bipyridinium salts. Aiming to rationalize the parameters governing such interactions eight types of these polycationic dendrimers in which the generation, the number of charges, the nature of the core and of the terminal groups vary from one to another, were designed and used. The influence of viologen-based dendrimers' on human serum albumin has been investigated. The photo-physical interactions of the two systems have been monitored by fluorescence quenching of free L-tryptophan and of HSA tryptophan residue. Additionally, using circular dichroism (CD) the effect of dendrimers on the secondary structure of albumin was measured. The obtained results show that viologen dendrimers interact with human serum albumin quenching its fluorescence either by collisional (dynamic) way or by forming complexes in a ground state (static quenching). In some cases the quenching is accompanied by changes of the secondary structure of HSA.

1. Introduction

Dendrimers are mono-disperse, multifunctionalized and hyperbranched polymers whose nanometer size, topology, perfectly defined structure, multivalent character and molecular weight can be controlled rigorously during their preparation. These structural features as well as the high density of chemical reactive functions on the outer shell of these macromolecules enhanced their use in biology and medicine. Biological properties of dendrimers are certainly the most active and attractive area of the research about dendrimers. Many families of dendrimers have been synthesized with various cores, and building monomers. Among the new classes of dendrimers we can envisage to prepare that constituted both with viologen units and phosphorus linkages will be of particular interest because of the expected particular properties which might be brought both by phosphorus groups and viologen entities. Indeed phosphorus dendrimers [1–3] have already proved their utility in various fields of research such as catalysis, materials science and biology. They are biocompatible and were used as vehicles to transport DNA or plasmids inside cells [4–6], as biosensors [7,8], as organic alternative to toxic inorganic quantum dots [9]. Some of them are able to clear the abnormal scrapie isoform of the prion protein PrP^{sc} involved in the spongiform encephalopathies, fatal neurodegenerative diseases [10]. Self-assembly between the carboxylic acid phosphorus dendrimers and a Galcer analog allowed to prevent the infection of cells in HIV1 events [11]. Phosphorus dendrimers with phosphonic end groups appear as new nanotools promoting an anti-inflammatory activation of human monocytes and as a consequence prove to be good candidates for innovative anti-inflammatory immunotherapies [12–14]. In marked contrast viologen dendrimers were not subjected to many investigations concerning their biological properties. Till now, only antiviral activity of these types of compounds is known: their useful activity against human immunodeficiency virus (HIV) as well as against other viruses (herpes simplex virus, Reovirus and respiratory syncytial viruses) were nicely illustrated [15]. As part of our continuing research program to develop novel extended structures based on phosphorus dendrimers, we have recently investigated the positioning of cationic viologen-based building blocks within the phosphorus-dendrimer backbone with the aim of taking benefit from their synergistic effect [16]. The use of

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0022-2313/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jlumin.2012.01.044

molecular pharmaceutics

Article

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Biological Properties of New Viologen-Phosphorus Dendrimers

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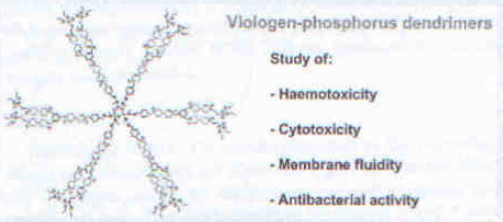
Supporting Information

ABSTRACT: Some biological properties of eight dendrimers incorporating both phosphorus linkages and viologen units within their cascade structure or at the periphery were investigated for the first time. In particular cytotoxicity, hemotoxicity, and antimicrobial and antifungal activity of these new macromolecules were examined. Even if for example all these species exhibited good antimicrobial properties, it was demonstrated that their behavior strongly depends on several parameters as their size and molecular weight, the number of viologen units and the nature of the terminal groups.



KEYWORDS: *viologen dendrimers, hemolysis, cytotoxicity, antibacterial activity, membrane fluidity*

INTRODUCTION

4,4′-Bipyridinium salts better known under the name of viologen derivatives are showing an increasing number of applications in addition to their former use as herbicides.[1] This is mainly due to their properties as photoactive and electroactive compounds, and to their ability to give strong donor−acceptor complexes with electron donating species. However viologens by themselves can present risks for human health. As an example 1,1′-4,4′-bipyridinium dichloride is known to induce formation of superoxide (O_2^{-}) and to cause damage to multiple organs.[2−4] A therapeutic protocol for the treatment of viologen poisoning based on host−guest chemistry and involving the effective inhibition of viologen toxicity by complexation of *p*-sulfonatocalix[*n*]arenes was reported.[5] In marked contrast to this isolated "dark image" of viologen monomer behavior, their introduction as building blocks for the design of polycationic dendrimers allowed De Clercq et al.[6] to point out the activity of various viologen dendrimers against human immunodeficiency virus (HIV-1, strain III_b replication in MT-4 cells), as well as to a lesser extent against herpes simplex virus (HSV), vesicular stomatitis, Punta Toro virus, Sindbis virus, Reovirus and respiratory syncytial viruses. Indeed it was demonstrated that their behavior strongly depends on the number and distance of the positive charges.

Surprisingly and to the best of our knowledge no study on other biological properties of viologen dendrimers was reported. Having these observations in mind we decided to open the field of such investigations, our first goal being to design other types of dendrimers in order to have a more precise idea on their biological activities. For such a purpose we designed new viologen monomers, dendrons, and dendrimers bearing phosphorus groups as additional units incorporated either at the focal point or at the periphery or both of these key structural positions of the dendritic backbone. This choice of strategy was aimed by the fact that we already demonstrated the key role played by phosphorus dendrimers in biology and for biomedical applications due to several specificities.[7] Briefly they have a remarkable influence on cell growth, in particular for neuronal cells,[8] and for human immune blood cells such as monocytes and Natural Killer cells,[9−12] the latter playing a key role for fighting against viral infections and cancers. The uselfulness of phosphorus dendrimers was also pointed out for elaboration of highly sensitive biosensors[13−16] and for *in vitro* drug delivery, for instance as transfecting agents[17−19] or against HIV-1[20] and the scrapie form of prions.[21] *In vivo* biological properties of phosphorus dendrimers as anti- prion agents, for ocular drug delivery,[22] and for imaging rat brain blood vessels[23,24] were

Special Issue: Biological Applications of Dendrimers

Received: October 28, 2011
Revised: January 3, 2012
Accepted: January 3, 2012
Published: January 3, 2012

dx.doi.org/10.1021/mp200549c | *Mol. Pharmaceutics* 2012, 9, 448−457

Chemistry and Physics of Lipids 165 (2012) 401–407

Contents lists available at SciVerse ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Cationic carbosilane dendrimers–lipid membrane interactions

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ARTICLE INFO

Article history:
Received 14 July 2011
Received in revised form 25 January 2012
Accepted 27 January 2012
Available online 14 February 2012

Keywords:
Carbosilane dendrimers
Liposomes
Monolayer
Anisotropy
Membrane fluidity

ABSTRACT

The aim of this work was to study interactions between cationic carbosilane dendrimers (CBS) and lipid bilayers or monolayers. Two kinds of second generation carbosilane dendrimers were used: NN16 with Si–O bonds and BDBR0011 with Si–C bonds. The results show that cationic carbosilane dendrimers interact both with liposomes and lipid monolayers. Interactions were stronger for negatively charged membranes and high concentration of dendrimers. In liposomes interactions were studied by measuring fluorescence anisotropy changes of fluorescent labels incorporated into the bilayer. An increase in fluorescence anisotropy was observed for both fluorescent probes when dendrimers were added to lipids that means the decreased membrane fluidity. Both the hydrophobic and hydrophilic parts of liposome bilayers became more rigid. This may be due to dendrimers' incorporation into liposome bilayer. For higher concentrations of both dendrimers precipitation occurred in negatively charged liposomes. NN16 dendrimer interacted stronger with hydrophilic part of bilayers whereas BDBR0011 greatly modified the hydrophobic area. Monolayers method brought similar results. Both dendrimers influenced lipid monolayers and changed surface pressure. For negatively charged lipids the monitored parameter changed stronger than for uncharged DMPC lipids. Moreover, NN16 dendrimer interacted stronger than the BDBR0011.

1. Introduction

Very often it is not a problem to create a drug but to transport it into the cell. In the bloodstream drugs are exposed to many factors like peptides or enzymes which can destroy them or interact with them. For the drug to get into the right place in the body means that it has to pass membrane barrier and interact properly inside the cell. Many publications are devoted to the problem of drug carriers formation, synthesis and way of transport (Yokoyama, 2005; Xiaopeng et al., 2007; Sahoo et al., 2008; Chen et al., 2008; Bronich, 2010; Yuan et al., 2010). Drug carriers are substances that could solve many problems in drug delivery. Those molecules should be able to improve the delivery and the effectiveness of drugs. Moreover, drug carriers are used to reduce cytotoxicity and improve drug metabolism (Petzinger and Geyer, 2006; Dong et al., 2010). They are exerted in a controlled-release technology to prolong *in vivo* drug actions (Jansen et al., 1994; Chen et al., 2008; Nanjwadea et al., 2009). Nowadays there are few systems of drug transport like liposomes, albumin microspheres, bioconjugates, virosomes or dendrimers (Bhardwaj and Burgess, 2010; Anada et al., 2009; Thakkar et al., 2005; Lua et al., 2002; Daemen et al., 2005; Najlah and D'Emanuele, 2006). Dendrimers are quite a new class of globular polymers and because of their properties they generate high interest. Among their possible biomedical applications are drug transfer or serving as DNA carriers. It is possible either by encapsulation in their interiors or by bonding to the surface groups.

For the efficient transport of a medicine a drug carrier has to pass through the cell membrane. Cell membranes are complex structures made of lipids and proteins. To understand how dendrimers can cross this barrier, first it is necessary to understand interactions between these molecules and lipid bilayers. Model membranes like liposomes are excellent research models for experiments because of their simple composition, easy preparation and a good enough time stability. In the literature there are numerous studies on interactions between mostly polyamido amine (PAMAM) dendrimers and liposomes (Ottaviani et al., 1998,1999; Castile et al., 1999; Purohit et al., 2001; Hong et al., 2004, 2006; Mecke et al., 2004, 2005a,b; Klajnert and Epand, 2005; Lee and Larson, 2006; Klajnert et al., 2006; Gardikis et al., 2006; Yan and Yu, 2009; Kelly et al., 2008, 2009; Ionov et al., 2010; Smith et al., 2010; Wrobel et al., 2011; Tiriveedhi et al., 2011; Ionov et al., 2011). Dendrimers can either pass through the lipid bilayer or dendrimers-lipids micelles are

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0009-3084/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.chemphyslip.2012.01.008

molecular pharmaceutics

Brief Article

pubs.acs.org/molecularpharmaceutics

Dendrimer-Driven Neurotrophin Expression Differs in Temporal Patterns between Rodent and Human Stem Cells

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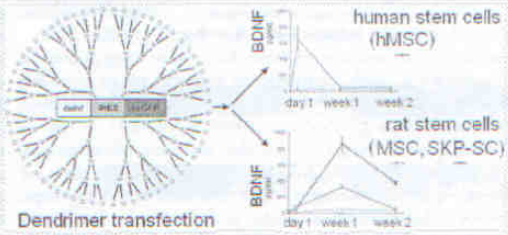
Supporting Information

ABSTRACT: This study reports the use of a nonviral expression system based on polyamidoamine dendrimers for time-restricted neurotrophin overproduction in mesenchymal stem cells and skin precursor-derived Schwann cells. The dendrimers were used to deliver plasmids for brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3) expression in both rodent and human stem cells, and the timelines of expression were studied. We have found that, despite the fact that transfection efficiencies and protein expression levels were comparable, dendrimer-driven expression in human mesenchymal stem cells was characterized by a more rapid decline compared to rodent cells. Transient expression systems can be beneficial for some neurotrophins, which were earlier reported to cause unwanted side effects in virus-based long-term expression models. Nonviral neurotrophin expression is a biologically safe and accessible alternative to increase the therapeutic potential of autologous adult stem cells and stem cell-derived functional differentiated cells.



KEYWORDS: *BDNF, NT-3, transfection, PAMAM dendrimers, mesenchymal stem cells, skin precursor-derived Schwann cells*

INTRODUCTION

Adult autologous stem cells from different sources are currently viewed as a potent tool in the emerging field of cell therapies. The expected high therapeutic potential of stem cells called for the development of numerous technologies for stem cell culture, differentiation, matrix embedding, and genetic modification.[1−3] Most of the stem cell-related techniques and protocols are first optimized in rodent models, with a perspective to translate the results to the respective human stem cells. However, a number of rodent-vs-human expression studies revealed species-specific differences with critical impact onto the pharmaceutical trials.[4−6] Understanding the differences between rodent and human stem cells is required to predict the success of the above-mentioned translation.

One of the technologies with a considerable promise to improve cell therapies is genetic engineering. In addition to their own repertoire of secreted molecules, stem cells can be modified for elevated expression and supply of trophic, differentiation, antitumor, immunomodulatory, or other factors. In particular, *ex vivo* modification for the overexpression of neurotrophic factors (NTFs) was shown to increase the effectiveness of cell therapies in some models of central[7−9] (CNS) and peripheral nervous system (PNS)[10] repair. NTFs comprise several families of proteins essential for the survival, maintenance, and regeneration of neurons.[11−15] The engineered expression of NTFs facilitates trophic support and induces axonal outgrowth, resulting in neuroprotection and improved regeneration.[10,16,17] Genetic modification eliminates the need for continuous exogenous NTF application, thus reducing the risk of additional trauma or infection. However, it was found that long-term virus-based NTF expression caused significant negative side effects in both CNS and PNS regeneration models,[18−23] such as aberrant sprouting and trapping of regenerating axons. The undesired effects of long-term expression together with biosafety considerations appeal to the use of time-restricted expression systems, which can be provided by nonviral gene carries. The latter are represented by

Received: January 22, 2012
Revised: April 2, 2012
Accepted: April 5, 2012
Published: April 5, 2012

Colloids and Surfaces B: Biointerfaces 95 (2012) 103–108

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

The influence of maltose modified poly(propylene imine) dendrimers on hen egg white lysozyme structure and thermal stability

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ARTICLE INFO

Article history:
Received 6 February 2012
Accepted 17 February 2012
Available online 27 February 2012

Keywords:
PPI dendrimers
Maltose modified dendrimers
Lysozyme
Dendrimer–protein interaction
Fluorescence quenching

ABSTRACT

In this study the influence of dendrimers' surface modification upon the strength of interaction with proteins was examined. Unmodified, cationic poly(propylene imine) dendrimer of the fourth generation (PPI G4), two PPI G4 dendrimers, partially and fully coated with maltose residues, and anionic polyamidoamine dendrimer of the third and a half generation (PAMAM G3.5 dendrimer), were used in the study. Hen egg white lysozyme, which possesses a cationic net charge under physiological conditions, was chosen as a model protein. The influence of dendrimers on the thermal stability of lysozyme was studied using differential scanning calorimetry (DSC) and circular dichroism (CD) methods. Additionally, the effect of dendrimers on the availability of lysozyme tryptophan residues to fluorescence quenchers was examined. It was shown that modification of dendrimer surface with maltose reduced its influence on lysozyme properties. However, even full surface modification, resulting in a neutral surface charge, did not deprive dendrimer of the ability to interact with the protein. It was probably caused by the introduction of a large number of hydroxyl groups from maltose residues on the surface of the dendrimer. In the study a comparable strength of influence exerted on lysozyme by cationic PPI dendrimer and anionic PAMAM G3.5 dendrimer was observed. The possible explanation of this fact is the presence of both positively and negatively charged areas on the surface of lysozyme. Such areas allow dendrimers possessing opposite surface charges to interact with lysozyme.

1. Introduction

Dendrimers are a relatively new group of polymers, characterized by a unique structure. Each of the monomers, that build the dendrimer, is ended with a group or groups, to which at least two new monomers can be attached. As a result a poly-branched, layered structure is obtained. Another important feature of the structure of dendrimers is the presence of a large number of surface groups. Addition of a new layer of monomers gives rise to obtaining a dendrimer of higher generation [1,2]. The properties of dendrimers make them very useful in biomedical applications. For example dendrimers can be used as contrast agents for magnetic resonance, as a part of targeted drug delivery system or as a non-viral vectors in gene therapy [3]. The main problem with the biomedical applications of dendrimers is their toxicity. Unfortunately, the most suitable dendrimers are simultaneously the most toxic ones, due to their cationic surface groups. The main source of dendrimers' toxicity is the interaction between cationic surface of dendrimer and negatively charged biological membranes [4]. One of the ways to circumvent this problem is the modification of the surface of dendrimer. Cationic surface groups can be substituted with anionic (e.g. carboxylate) or neutral (e.g. hydroxyl) groups. It is also possible to attach different moieties to the surface of dendrimers, such as polyethylene glycol (PEG) chains or carbohydrates, what results in the diminishment of the accessibility of cationic surface charge. Such modifications results in lowered dendrimers' toxicity [4]. Despite of diminished toxic activity, dendrimers with carboxylate or hydroxyl surface groups are still able to interact with the components of biological environment. For example polyamidoamine (PAMAM) dendrimers terminated with carboxylate or hydroxyl groups have been shown to possess the ability to influence the structure or function of proteins such as: acetylcholinesterase [5], bovine serum albumin (BSA) [6] and membrane adenosinetriphosphatase (ATPase) [7]. In the case of human serum albumin (HSA) and PAMAM dendrimers, the interaction of hydroxy-terminated dendrimers (which do not possess surface charge) with analyzed protein is evidently weaker than interaction of dendrimers possessing charged surface groups. More detailed

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doi:10.1016/j.colsurfb.2012.02.021

Genotoxicity of Poly(propylene imine) Dendrimers

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Received 22 December 2011; revised 21 February 2012; accepted 7 March 2012
Published online 19 March 2012 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/bip.22056

ABSTRACT:

Dendrimers are highly branched macromolecules with the potential in biomedical applications. Due to positively charged surfaces, several dendrimers reveal toxicity. Coating peripheral cationic groups with carbohydrate residues can reduce it. In this study, the cytotoxicity and genotoxicity of three types of 4th generation poly(propylene imine) dendrimers were investigated. Peripheral blood mononuclear cells (PBMCs) were treated with uncoated (PPI-g4) dendrimers, and dendrimers in which approximately 40% or 90% of peripheral amino groups were coated with maltotriose (PPI-g4-OS or PPI-g4-DS) at concentration of 0.05, 0.5, 5 mg/ml. Abbreviations OS and DS stand for open shell and dense shell respectively, that describes the structure of carbohydrate modified dendrimers. After 1 h of cell incubation at 37°C, the MTT and comet assays were performed. PPI dendrimers demonstrated surface-modification-degree dependent toxicity, although genotoxicity of PPI-g4 and PPI-g4-OS measured by the comet assay was concentration dependent up to 0.5 mg/ml and at 5 mg/ml the amount of DNA that left comet's head decreased. Results may suggest a strong interaction between dendrimers and DNA, and furthermore, that coating PPI dendrimers by maltoriose is an efficient method to reduce their genotoxicity what opens the possibilities to use them as therapeutic agents or drug carriers. © *2012 Wiley Periodicals, Inc. Biopolymers 97: 642–648, 2012.*

Keywords: dendrimer; glycodendrimers; PPI; genotoxicity; comet assay

This article was originally published online as an accepted preprint. The "Published Online" date corresponds to the preprint version. You can request a copy of the preprint by emailing the Biopolymers editorial office at biopolymers@wiley.com

INTRODUCTION

Dendrimers, firstly synthesized by Tomalia et al.[1] and Newkome et al.,[2] are perfectly branched, almost monodisperse macromolecules with a precisely controlled chemical structure. A globular shape of dendrimers is a result of their internal structure, in which all bonds emerge radially from a central core with repeat units. This generates branching points which provides the possibility to attach at least two monomers.[3,4] The development of dendrimers structure, size, and shape is of eminent interest in biomedical applications including as enhancing MRI signal,[5] transporting drugs,[6] or gene therapy.[7] Due to their nanometric size, dendrimers can interact effectively and specifically with cell components such as membranes, organelles, and proteins.[7–9] As other cationic polymers with high charge density, dendrimers can also interact with nucleic acids on the basis of ionic interaction between negatively charged backbone phosphate groups and positively charged amino groups of the polymer.[10] Molecular modelling studies indicate that the number of dendrimer–DNA contact points increases with dendrimer generation. Shape of such complexes between DNA and dendrimer (polyplexes)

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Contract grant sponsor: 'Biological Properties and Biomedical Applications of Dendrimers' operated within the Foundation for Polish Science TEAM programme cofinanced by the European Regional Development Fund

Biochimica et Biophysica Acta 1818 (2012) 2209–2216

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

siRNA carriers based on carbosilane dendrimers affect zeta potential and size of phospholipid vesicles

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ARTICLE INFO

Article history:
Received 10 February 2012
Received in revised form 24 April 2012
Accepted 26 April 2012
Available online 2 May 2012

Keywords:
Carbosilane dendrimers
siRNA
Unilamellar vesicles
Zeta potential
Surface charge

ABSTRACT

One of the major limitations in gene therapy is an inability of naked siRNA to passively diffuse through negatively charged cell membranes. Therefore, the siRNA transport into a cell requires efficient carriers. In this work we analyzed the charge-dependent interaction of the complexes of cationic carbosilane dendrimers (CBD) and anti-HIV siRNA (dendriplexes) with the model membranes — large unilamellar vesicles (LUV). We used the second generation of branched with CBD carbon–silicon bonds (CBD-CS) which are water-stable and that of oxygen–silicon bonds (CBD-OS) which are slowly hydrolyzed in aqueous solutions. The LUVs were composed of zwitterionic dimyristoylphosphatidylcholine (DMPC), negatively charged dipalmitoylphosphatidylglycerol (DPPG) and their mixture (DMPC/DPPG, molar ratio 7:3). The interaction of dendriplexes with LUVs affected both zeta potential and size of the vesicles. The changes of these values were larger for the negatively charged LUV. CBD-CS resulted in the decrease of zeta potential values to more negative ones, whereas an opposite effect took place for CBD-OS suggesting a different kind of interaction between LUVs and the dendriplexes. The results indicate that both CBD-CS and CBD-OS can be used for transport of siRNA into the cells. However, CBD-CS are preferred due to a better stability in water and improved bioavailability of siRNA on their surface.

1. Introduction

Strategies for inhibition of gene expression have been applied in several therapeutic applications including the treatment of HIV infection [1–4]. For this purpose, siRNA should be transported through cell membrane into the cytoplasm. However, the naked siRNA is unable to passively diffuse through the negatively charged cell membranes. There is also a tendency of antisense drugs to bind non-specifically to the serum proteins due to electrostatic interactions [5]. These interactions decrease a drug efficacy. In order to obtain the desired therapeutic effects higher doses of drug are necessary, which, however, can be toxic for organisms. The HIV virus contains in its genome some genes which can be considered as a potential target for siRNA [1]. Therefore, RNAi could be a promising therapeutic tool for HIV/AIDS treatment. Every drug based on RNAi should be stable, selective, non toxic and should easily penetrate through the cell membrane. Thus, a substantial effort is required to develop a proper delivery vehicle for siRNA in order to target viral mRNA inside the cell.

Dendrimers and lipid vesicles represent two of the most thoroughly studied categories of nanoparticles that could be used as carriers of bioactive molecules. With careful tailoring of their physicochemical characteristics and thermodynamic parameters these nanocarriers can alter the ADME profile (Absorption, Distribution, Metabolism and Excretion) of a candidate molecule leading to more potent therapeutic agents [6–11]. An attractive approach for delivery of siRNA to cells is based on cationic dendrimers [12–17]. The cationic dendrimers can form complexes with nucleic acids, and are widely studied as carriers for the transfection of cells with DNA and siRNAs in vitro [18]. Cationic dendrimers interact efficiently with nucleic acids, forming dendrimer/nucleic acid complexes by bonding to the surface groups. Among the cationic dendrimers the carbosilane dendrimers (CBD) are of special interest. They have been synthesized specifically for siRNA and oligonucleotide delivery system [19–21]. Interaction between dendrimers and siRNA or oligonucleotide has electrostatic nature due to negative charge of oligonucleotide backbone and positive charge of dendrimer functional groups. CBD are branched structures made of either carbon–silicon bonds (CBD-CS) (Fig. 1A) which are stable in water or oxygen–silicon bonds (CBD-OS) (Fig. 1B) which are slowly hydrolyzed in aqueous

Abbreviations: PBS, phosphate-buffered saline; CBD, carbosilane dendrimers; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DPPG, dipalmitoylphosphatidylglycerol; LUVs, large unilamellar vesicles

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0005-2736/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2012.04.019

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Cite this: *New J. Chem.*, 2012, **36**, 1992–1999

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PAPER

Impact of maltose modified poly(propylene imine) dendrimers on liver alcohol dehydrogenase (LADH) internal dynamics and structure†

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Received (in Montpellier, France) 18th May 2012, Accepted 4th July 2012
DOI: 10.1039/c2nj40406k

The toxicity of cationic dendrimers is one of their most important drawbacks. Modification of the surface of those dendrimers is a way to obtain compounds that are tolerable by living organisms. However, the sole knowledge how such modifications influence the toxicity of dendrimers is not enough. It is also important to know how such modifications influence the ability of dendrimers to interact with biomolecules, as such interactions may be responsible for dendrimers fate *in vivo*. In this study the ability of poly(propylene imine) dendrimers of the fourth generation (G4 PPI) with surface modified with maltose moieties to interact with horse liver alcohol dehydrogenase (LADH) was examined. Fluorescence, room temperature tryptophan phosphorescence (RTTP), circular dichroism (CD), dynamic light scattering (DLS) and zeta potential measurements were applied to fully investigate those interactions. As a result, an ability of all studied G4 PPI dendrimers to interact with LADH was shown. However, the differences in the strength of influence of dendrimers on different parts of protein, depending on the dendrimer surface structure, were observed. All dendrimers increased flexibility of the core part of LADH to a similar degree. However, changes in LADH secondary structures upon the interaction with dendrimers depended on the type of the dendrimer. Additionally, experiments performed allowed us to propose the most probable part of LADH that is the subject of the structural changes as the cleft between catalytic and coenzyme binding subdomains of LADH.

Introduction

Nanomedicine may be regarded as a field of science that utilizes nanoparticles in the development of new diagnostic and therapeutic agents.[1] Some of the important polymeric structures used in nanomedicine are dendrimers. Dendrimers are highly branched, monodispersed polymers, characterized by specific, tree-like spatial structure and a large number of surface groups.[2] First syntheses of dendritic molecules were reported in 1970s and 1980s.[3–7] Since their discovery, dendrimers have been extensively investigated towards their applicability in nanomedicine. Dendrimers have been shown to possess various applications in nanomedicine and related fields. They may be employed as drug carriers in anticancer therapy. Drug molecules can be either encapsulated inside the dendrimer or attached to its surface. Additionally, a targeting molecule (for example an antibody, a vitamin or a hormone molecule) may be covalently linked to the surface of the dendrimer, to maximize selectivity of the action.[8] Dendrimers may potentially be used as boron carriers in a boron neutron capture therapy.[9] Other important application fields of dendrimers are diagnostics[10] and the antiviral therapy.[11] The most prominent example of the latter one is the dendrimer based microbiocide – SPL7013 (VivaGel®). SPL7013 was shown to possess anti HIV-1 and anti HSV-2 properties. This compound is under investigation regarding its applicability as an intravaginal agent that prevents HIV infection.[12,13] Dendrimers may be also applied in diagnostic and treatment of vascular inflammatory diseases,[14] as well as non-viral transfecting agents, for example Superfect™, a poly-amidoamine (PAMAM) dendrimer based transfecting agent, is commercially available.[15]

The most important drawback of dendrimers is their toxicity that is connected to the presence of a positive charge on the surface of cationic dendrimers. This toxicity may be greatly reduced through the modification of the dendrimers' surface groups.[16] One such modification is the attachment of carbohydrate moieties to the surface groups of a dendrimer. In the case of poly(propylene imine) (PPI) dendrimers, it was previously shown that it substantially reduces dendrimers' toxicity both *in vitro* and *in vivo*.[17–19]

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2nj40406k

Time Evolution of the Aggregation Process of Peptides Involved in Neurodegenerative Diseases and Preventing Aggregation Effect of Phosphorus Dendrimers Studied by EPR

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Received July 20, 2010; Revised Manuscript Received October 1, 2010

A key pathological event of prion and Alzheimer diseases is the formation of prion and amyloid plaques generated by peptide aggregation in the form of fibrils. Dendrimers have revealed their ability to prevent fibril formation and therefore cure neurodegenerative diseases. To provide information about the kinetics and the mechanism of peptide fibril formation and about the ability of the dendrimers to prevent peptide aggregation, we performed a computer-aided EPR analysis of the selected nitroxide spin probe 4-octyl-dimethylammonium,2,2,6,6-tetramethyl-piperidine-1-oxyl bromide (CAT8) in water solutions of the β-amyloid peptide Aβ 1−28 and the prion peptide PrP 185−208, which contain the fibril nucleation sites, in the absence and in the presence of phosphorus dendrimers. After a careful selection of the experimental conditions that allow aggregation to occur and to be monitored by EPR analysis over time, it was found that the Aβ 1−28 fibrils formed in 220 min at 0.5 mM peptide, 0.05 mM CAT8, 0.04 mg/mL heparin, and pH = 5. As a consequence, the interacting sites available for cooperative interactions with CAT8 were engaged in the peptide−peptide interactions and a fraction of the probe was extracted in the fluid fibril/water interphase, while another fraction was trapped at the peptide/peptide interphase, showing a decrease in mobility. Conversely, in the presence of the dendrimer (at the selected, after several trials, peptide/dendrimer molar ratio = 50), due to dipole−dipole interactions with peptide monomers, the probe remained at the dendrimer/peptide interphase and the spectral parameters negligibly changed over time. A fraction of probes inserted in PrP 185−208 low-packed aggregates and monitored their fast formation after 90 min. However, the binding organization of the prion peptide negligibly changed upon aggregation in comparison to Aβ 1−28. It is proposed that dendrimers mainly interfere in the lag (nucleation) phase of the prion peptide.

Introduction

There is a group of neurodegenerative disorders that occurred due to accumulation of wrongly folded proteins in so-called amyloid plaques constituted by fibrils of β-sheet-structured amyloid peptides. Recent literature reports about formation of prefibrillar intermediates, mainly oligomers in dimeric form, which are considered coresponsible of the toxicity of the final misfolded peptides.[1−3]

In the case of Alzheimer's disease, these plaques are built mainly of Aβ 1−40 and Aβ 1−42 peptides, whereas for prion disorders, they consist of prion peptides PrP^{Sc}. Currently, there are no efficient treatments for these diseases, which are eventually fatal. Therefore, there is a continued need to explore new therapeutic possibilities. Several compounds (e.g., porphyrines, phthalocyanines, and polycyclic aromatics) have been reported as promising therapeutic agents in prion diseases.[4,5] Unfortunately, they have demonstrated a weak capacity to avoid fibril formation and no ability to remove the pre-existing fibrils from an infected organism. Dendrimers have demonstrated to be promising materials in biomedical applications as drug carriers and transfection agents.[6−11] A very recent study using solid-state NMR nicely describes the ability of dendrimers to insert in the hydrophobic core of biomembranes, thereby monitoring hydrophilic/hydrophobic interactions.[12] This study shows that dendrimers are well suited to interact with Aβ amyloid peptides, thus, preventing their aggregation. Furthermore, previous studies have demonstrated the ability of different types of dendrimers to prevent the aggregation of amyloid peptide (Aβ 1−28) and prion peptide (PrP 185−208), which both are responsible for neurodegenerative disorders.[13−18] These two sequences where selected not only because they contain the fibrilization sites, but also because they present a structural homology, which could play an important role in the amyloidogenic process.[19] The role of dendrimers to disturb fibril formation has been mainly studied by ThT assay and by monitoring changes in the secondary structure of peptides by FTIR. Although both methods proved to be very useful, they are not free of disadvantages. ThT fluorescent intensity is not an absolute measure of the amount of amyloid fibrils formed from different proteins and is sensitive to pH.[20] Similarly, the results obtained by FTIR may not be conclusive because the occurrence of β-sheet structures indicates the presence of both amyloid fibril and intermediate products: oligomers. Therefore, there is a need to seek other experimental methods to confirm

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10.1021/bm100824z
Published on Web 10/19/2010

Nanomedicine: Nanotechnology, Biology, and Medicine
8 (2012) 815–817

nanomedicine
Nanotechnology, Biology, and Medicine

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Short Communication

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Surface modification of PAMAM dendrimer improves its biocompatibility

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Received 10 February 2012; accepted 28 March 2012

Abstract

Modification of dendrimer surface groups is one of the methods available to obtain compounds characterized by reduced toxicity. This article reports results of preliminary biocompatibility studies of a modified polyamidoamine dendrimer of the fourth generation. Reaction with dimethyl itaconate resulted in transformation of surface amine groups into pyrrolidone derivatives. Interaction of the modified dendrimer with human serum albumin (HSA) was analyzed. The influence of the dendrimer on mouse neuroblastoma cell line viability and its hemolytic properties were also investigated. The binding constant between analyzed dendrimer and HSA was found to be equal to $1.2 \times 10^5 \pm 0.2 \times 10^5\ M^{-1}$. Small changes in HSA secondary structure were observed. The analyzed dendrimer revealed minor toxic activity, as diminishment in cell viability was observed only for dendrimer concentrations higher than 2 mg/mL. Moreover, under the applied experimental conditions, no hemolytic activity was observed. Those observations point to the potential of the analyzed compound for further studies toward its applicability in nanomedicine.

From the Clinical Editor: This basic science paper explores the biocompatibility of fourth generation surface modified PAMAM dendrimers for future preclinical applications.

Key words: PAMAM dendrimer; Surface modification; Biocompatibility, Circular dichroism; Hemolysis

Dendrimers have attracted increased attention from the scientific community because of their unique spatial structure.[1] Applicability of dendrimers as drug carriers, transfection agents, or magnetic resonance imaging contrast agents has been extensively studied.[2-5] A serious drawback of dendrimers is their toxicity. However, this toxicity is mainly related to the positive charge present on the surface of the so-called cationic dendrimers.[6-8] Appropriate modifications of the dendrimers' surface can greatly reduce their toxicity.[9] Such modified dendrimers can be used in nanomedical applications, as they possess the advantage of dendrimers' spatial structure and simultaneously possess reduced toxic activity. In this study we examined the biocompatibility of a modified polyamidoamine (PAMAM) dendrimer of the fourth generation (G4). The amine surface groups of a G4 PAMAM-NH_2 dendrimer were transformed into pyrrolidone derivatives by means of reaction with dimethyl itaconate. To preliminarily assess biocompatibility of the dendrimer, we analyzed the ability of the obtained dendrimer to interact with human serum albumin (HSA), its influence on viability of mouse neuroblastoma (N2a) cell line, as well as its hemolytic activity.

Methods

Reaction of G4 PAMAM-NH_2 dendrimer with dimethyl itaconate resulted in pyrrolidone ring formation.[10] The dendrimer obtained ([*core*: 1,4-diaminobutane]; (4→2); *dendri*-{poly(-amidoamine)-(4-carbomethoxy pyrrolidone)$_{64}$}, G = 4 PAMAM; referred to as G4 PAMAM-pyrrolidone dendrimer) possessed carbomethoxypyrrolidone surface groups instead of amine surface groups. The scheme of reaction for modification of the G4 PAMAM-NH_2 surface groups is presented in Figure 1.

To assess the biocompatibility of the modified dendrimer, several experiments were performed. The ability of the G4 PAMAM-pyrrolidone dendrimer to interact with HSA was examined through the use of circular dichroism (CD) and fluorescence measurements. The influence of the dendrimer on the viability of the mouse N2a cell line was estimated with the use of the trypan blue exclusion assay. In addition, the hemolytic activity of the dendrimer was also investigated. For comparison, some of the experiments were repeated for an unmodified G4 PAMAM-NH_2 dendrimer.

This study was co-financed by project "Biological Properties and Biomedical Applications of Dendrimers" operated within the Foundation for Polish Science TEAM Programme (Project TEAM number TEAM/2008-1/5).

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1549-9634/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.nano.2012.03.009

Please cite this article as: M., Ciolkowski, et al, Surface modification of PAMAM dendrimer improves its biocompatibility. *Nanomedicine: NBM* 2012;8:815-817, doi:10.1016/j.nano.2012.03.009

Society For Biomaterials

Influence of fourth generation poly(propyleneimine) dendrimers on blood cells

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Received 26 February 2012; revised 3 April 2012; accepted 5 April 2012
Published online 24 May 2012 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.34222

Abstract: Dendrimers provide many exciting opportunities for potential biomedical applications. However, owing to their positively charged surfaces, poly(propyleneimine) (PPI) dendrimers show toxic and haemolytic activities. One of the methods for masking the peripheral cationic groups is to modify them using carbohydrate residues. In this study, three types of the fourth generation PPI dendrimers-uncoated (PPI-g4), approximately 35% maltotriose (Mal-III)-coated (PPI-g4-OS), and approximately 90% Mal-III-coated (PPI-g4-DS) were investigated by assessing their effects on red blood cell (RBC) haemolysis in samples of pure RBCs, RBCs in the presence of human serum albumin (HSA) or human plasma, and RBCs in whole blood. Lymphocyte proliferation and platelet (PLT) aggregation were also studied in the presence of various concentrations of dendrimers. Although all dendrimers examined affected all the blood cells studied, the unmodified PPI-g4 had the most damaging effect. It caused high RBC haemolysis rates and PLT aggregation and greatly inhibited lymphocyte proliferation. These effects were caused by the cationic surface of this polymer. The modification of PPI-g4 with Mal-III reduced the effect of the dendrimer on all blood cells. The presence of HSA or plasma in the buffer containing the RBCs or RBC in whole blood significantly decreased the extent of dendrimer-driven haemolysis. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 100A: 2870–2880, 2012.

Key Words: poly(propyleneimine), PPI, dendrimer, glycodendrimer, lymphocytes, platelets, red blood cells, aggregation, haemolysis, proliferation, toxicity, HSA, blood

How to cite this article: Ziemba B, Halets I, Shcharbin D, Appelhans D, Voit B, Pieszynski I, Bryszewska M, Klajnert B. 2012. Influence of fourth generation poly(propyleneimine) dendrimers on blood cells. J Biomed Mater Res Part A 2012:100A:2870–2880.

INTRODUCTION

Since their introduction,[1,2] dendrimers have attracted great interest in biomedical applications because of their unique dendritic structures and multiple surface properties. Owing to the presence of a large number of terminal groups, drug molecules can be attached to the dendrimer surface through covalent bonds,[3,4] whereas internal cavities are capable of encapsulating small molecules.[5,6] This makes the dendrimers suitable for drug delivery systems. Because they can interact with nucleic acids, cationic dendrimers can be also used as vectors for gene transfection.[7] Dendrimers can interact effectively and specifically with cell components such as membranes, organelles, and proteins.[8,9] Nevertheless, their interactions with cell compounds and compartments are nonselective, so they also have the potential to cause cytotoxicity and haemotoxicity because of their terminal cationic groups.[4,10] One of the methods of reducing dendrimer toxicity is to modify their surfaces by substitution of amino groups with neutral or anionic moieties such as polyethylene glycol (PEG),[11–13] amino acids,[14–16] or carbohydrate residues.[5,14,17] More information on dendrimer toxicity and biological properties is still needed before they can be used safely and effectively in biomedical applications. In previous studies, we have shown that glycodendrimers with open or dense oligosaccharide shells, created using maltose or maltotriose, exhibit the desired biocompatibility under in vitro and in vivo conditions.[17–21] However, from these studies, further concern was raised about the application of the drug-glycodendrimer complexes/conjugates by different administration processes and their detailed interactions with, and effects on, red blood cells (RBC). This concern arose because the circulatory system seems the best way of administering the drugs for the dendrimer-drug complexes/conjugates to reach distant, directly inaccessible

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Contract grant sponsor: Saxonian Ministry of Science and Art and Federal Ministry of Education and Research (BMBF) and the COST Action TD0802
Contract grant sponsor: The project 'Biological Properties and Biomedical Applications of Dendrimers' operated within the Foundation for Polish Science TEAM programme cofinanced by the European Regional Development Found

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Cite this: *New J. Chem.*, 2012, **36**, 1610–1615

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PAPER

Characteristics of complexes between poly(propylene imine) dendrimers and nucleotides†

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Received (in Montpellier, France) 3rd March 2012, Accepted 7th May 2012
DOI: 10.1039/c2nj40165g

The aim of our work was to study the G4 and the G5 generations of cationic poly(propylene imine) dendrimers (PPI) as potential anticancer drug carriers. PPI dendrimers with 50% of primary amino surface groups modified with maltose were applied (PPI-m). Adenosine 5′-phosphates (AMP, ADP and ATP)—structural analogues of anticancer drug (fludarabine) were chosen as model molecules. Using Fast Performance Liquid Chromatography (FPLC) it has been shown that nucleotide analogues form stable complexes with the dendrimers. The number of nucleotides complexed with PPI-m G4 and G5 dendrimers depends on (1) nucleotide form, (2) time, (3) concentration of NaCl in the solution and (4) pH of solution. Obtained results are promising enough to further study PPI-m dendrimers as nanosized carrier systems for anticancer drugs.

Introduction

Physiological nucleoside anologues (NAs) are commonly used in treatment of hematological cancers.[1–3] The most important anticancer nucleoside analogues are fludarabine, cytarabine, cladrybine, gemcitabine, fluorouracil, and 6-mercaptopurine.[1,2,4] NAs belong to a group of compounds which are still in the spotlight of scientists.[5–10] However, therapeutic efficacy of many NAs is often limited by various factors *e.g.* low specificity of interaction with cancer cells, low solubility, fast metabolism, and disadvantageous biodistribution. The use of nucleoside analogues in treatment of leukemia is also limited by drug resistance.[4,5,11]

Most of the nucleoside analogues have the same way of metabolism. They penetrate into the cell through nucleoside transporters (NTs).[12,13] Then, in a cell, NAs are phosphorylated by deoxycitidine kinase (dCK), monophosphate kinases (NMPKs) and diphosphate kinases (NDPKs) to 5′-mono-, di- and triphosphates.[1,2,4] All NAs have a similar mechanism of activity (Fig. 1). They interfere with nucleic acids during their synthesis. These molecules can incorporate into DNA or RNA or modify the physiological nucleosides metabolism leading to apoptosis of cancer cells.[1,2,4,14] Moreover, several NAs can cause apoptosis by direct activation of the apoptosome.[14]

Fludarabine is an analogue of deoxyadenosine and is used in treatment of chronic lymphocytic leukemia.[15,16] Fludarabine is used in an anticancer therapy as a monophosphate form (F-ara-AMP) because of poor solubility of a nucleoside form in water. F-ara-AMP is unstable and soon after intravenous administration it is dephosphorylated to F-ara-A and in this form it is transported into a cell, where it is metabolized into an active form of F-ara-ATP (Fig. 2).[1,2]

Currently used anticancer drugs are not always efficient enough. Moreover, they cause toxicity against normal tissues, which leads to numerous side effects, *e.g.* fludarabine causes nausea, vomiting, stomatitis, zoster or neurotoxicity.[1] Thus, drug carrier systems are studied to improve the efficiency and specificity of anticancer drugs.[17] Dendrimers might be potential drug delivery systems. Dendrimers are hyperbranched, monodispersed, and uniformly structured polymers.[18–21] These polymers possess internal cavities filled with a solvent and a large number of surface functional groups. These features allow to encapsulate drug molecules inside a polymer or bind them to surface groups.[12,19,20]

Hydrogen bonds, hydrophobic or electrostatic interaction, participate in encapsulation of drug molecules inside the structure of dendrimers,[18,20] whereas binding of drugs to surface groups of dendrimers is stabilized by electrostatic interaction or covalent bonds.[18,23] Formation of dendrimer–drug complexes increases stability, solubility and biocompatibility of anticancer drugs. Moreover, release of drugs from drug–dendrimer complexes might be controlled that results in an increase in drug specificity. Complexes of anticancer agents and dendrimers cause changes in biodistribution. Thus, accumulation of drugs can be increased in cancer cells.[12,18–20,22,23]

Based on electrostatic forces, stable complexes can be created between anionic drugs and cationic dendrimers

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2nj40165g

NJC

Cite this: *New J. Chem.*, 2012, **36**, 2215–2222

www.rsc.org/njc

PAPER

Antimicrobial activity of poly(propylene imine) dendrimers

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Received (in Montpellier, France) 23rd May 2012, Accepted 2nd August 2012
DOI: 10.1039/c2nj40421d

Poly(propylene imine) dendrimers have been investigated for their biological applications but their antibacterial activity has not been extensively explored. Thus, the fourth generation of poly(propylene imine) dendrimers (PPI-G4) and PPI dendrimers with a surface modified by attaching maltose in 25% and 100% (PPI-25%mG4 and PPI-100%mG4, respectively) was evaluated for the antibacterial activity against Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442 and yeast *Candida albicans* ATCC 10231. Cytotoxicity of all tested dendrimers was checked on a Chinese hamster fibroblast cell line (B14), human liver hepatocellular carcinoma cell line (HepG2), mouse neuroblastoma cell line (N2a) and rat liver cell line (BRL-3A). The obtained results indicate that studied nano-sized macromolecules possess the greatest antimicrobial activity against *S. aureus*. PPI G4 dendrimers modified with 25% of maltose display antibacterial activity and a striking selectivity toward *S. aureus*, at the concentrations, which are at the same time harmless for the eukaryotic cell lines. Moreover, at the higher concentrations of unmodified dendrimers efficient growth inhibition of *S. epidermidis* and *C. albicans* has been observed.

Introduction

The common use of antibiotics causes a rise in the resistance among disease-causing microorganisms. This public health problem has increased especially for the last decade.[1] Due to this fact, the search for new and effective antimicrobial agents with novel chemical structures is a great worldwide concern. Dendrimers are a relatively new class of regularly branched macromolecules with unique structure and topological features.[2] The development of molecular nanostructures with a well-defined particle size and shape is of eminent interest in biomedical applications such as delivery of active pharmaceuticals.[3] Dendrimers with their specified 3-dimensional structure and well defined composition are an excellent material for such an application. Numerous studies have described dendrimers as encapsulating or complexing agents for antimicrobial drugs.[1,4,5] It is well documented that dendrimers can be used as drug-delivery systems, but only few reports describe the use of dendrimers as nano-drugs.[3] The antimicrobial activity of macromolecules can be obtained by functionalization of the periphery with biologically active groups such as quaternary ammonium. So far, antimicrobial activity has been described for amines and ammonium terminated carbosilane dendrimers,[6] amino-terminated G5 PAMAM nanoparticles[7] and for quaternary ammonium functionalized PPI dendrimers.[8] The mentioned compounds are potent antimicrobial agents against both Gram-positive and Gram-negative bacteria. The knowledge of PPI dendrimer properties is still weak in contrast to that of PAMAM dendrimers, which have been the most extensively studied nanopolymers. Despite the studies of PPI dendrimers for biomedical application, antibacterial activity of unmodified PPI dendrimers has not been reported. PPI dendrimers consist of propylene imine monomers as repeating units and butylenediamine is used as a core molecule (Fig. 1). To expand the biomedical application of dendrimers, we have investigated PPI dendrimers as antibacterial agents. It has been documented that amino-terminated dendrimers modified by attaching various sugar moieties on the surface cause significantly lower cytotoxicity.[9,10] Janaszewska *et al.*[9] have demonstrated that maltotriose modification significantly reduces toxicity toward Chinese hamster ovary (CHO) and human ovarian carcinoma cells (SKON3). It has been shown that oligosaccharide-modified PPI dendrimers exhibit good solubility under physiological conditions and show loss of haemolytic activity.[11,12] Also PAMAM dendrimers with surface modifications are characterised by lower toxicity to eukaryotic cells.[13,14] In this communication we report the antibacterial

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CHAPTER 14

Dendrimers as Drug Carriers

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CONTENTS

1. INTRODUCTION

Dendrimers are one of the most fascinating families of polymers based on dendritic architecture. Attempts to synthesize dendritic structures began independently in several laboratories. Vögtle's team synthesized small ones in 1978 [1]. Interestingly, even at that time, they saw the potential of these polymers as molecular containers for smaller molecules. Newkome et al. [2] synthesized what they called "cascade molecules" and showed that their properties mimicked micelles. Later, Newkome's group called their polymers "arborols," from the Latin "arbor" meaning "tree." Over the years, however, the term "dendrimer," derived from the Greek words "dendron" for tree and "meros" for part, has become best established. This term was first used by Donald Tomalia, who published the synthesis of polyamidoamine dendrimers [3]. It was a turning point, but several years still had to pass before this novel class of polymers attracted serious attention because of their unique structure and properties.

All dendrimers are characterized by a globular shape, a result of their internal structure. All bonds in dendrimers emerge radially from a central core to which monomer units are attached. Each monomer unit possesses a branch point to which at least two other units can be attached. This allows dendrimers to be built in a regular manner, layer by layer. They have two interesting molecular-level properties: a large number of terminal groups and an interior that can encapsulate small molecules. This structure makes dendrimers suitable as drug delivery systems, using either of two approaches: a drug may be covalently coupled to the dendrimer surface, or a hydrophobic drug may be complexed within the hydrophobic interior (Fig. 1).

ISBN: 1-58883-180-9

Nanoparticles: Synthesis, Characterization and Applications
Edited by R. S. Chaughule and R. V. Ramanujan
Pages: 345–357

molecular pharmaceutics

pubs.acs.org/molecularpharmaceutics

Phosphorus Dendrimers Affect Alzheimer's ($A\beta_{1-28}$) Peptide and MAP-Tau Protein Aggregation

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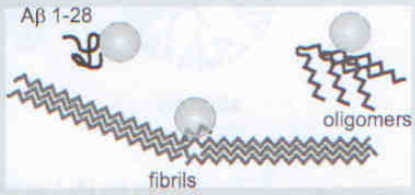
ABSTRACT: Alzheimer's disease (AD) is characterized by pathological aggregation of β-amyloid peptides and MAP-Tau protein. β-Amyloid ($A\beta$) is a peptide responsible for extracellular Alzheimer's plaque formation. Intracellular MAP-Tau aggregates appear as a result of hyperphosphorylation of this cytoskeletal protein. Small, oligomeric forms of $A\beta$ are intermediate products that appear before the amyloid plaques are formed. These forms are believed to be most neurotoxic. Dendrimers are highly branched polymers, which may find an application in regulation of amyloid fibril formation. Several biophysical and biochemical methods, like circular dichroism (CD), fluorescence intensity of thioflavin T and thioflavin S, transmission electron microscopy, spectrofluorimetry (measuring quenching of intrinsic peptide fluorescence) and MTT-cytotoxicity assay, were applied to characterize interactions of cationic phosphorus-containing dendrimers of generation 3 and generation 4 (CPDG3, CPDG4) with the fragment of amyloid peptide ($A\beta_{1-28}$) and MAP-Tau protein. We have demonstrated that CPDs are able to affect β-amyloid and MAP-Tau aggregation processes. A neuro-2a cell line (N2a) was used to test cytotoxicity of formed fibrils and intermediate products during the $A\beta_{1-28}$ aggregation. It has been shown that CPDs might have a beneficial effect by reducing the system toxicity. Presented results suggest that phosphorus dendrimers may be used in the future as agents regulating the fibrilization processes in Alzheimer's disease.



KEYWORDS: *phosphorus dendrimers, β-amyloid, MAP-Tau protein, fibril formation, amyloid peptide, aggregation, ThT assay, ThS assay, cytotoxicity*

■ INTRODUCTION

Pathological aggregation of proteins that leads to creation of amyloid fibrils is related to numerous human disorders, such as Alzheimer's and Parkinson's diseases, type II diabetes, and Creutzfeldt–Jakob disease.[1,2] In the case of neurodegenerative disorders, pathogenesis is strictly related to the accumulation of neurotoxic forms. Independently of amino acid sequence and the type of the disease there is a lot of evidence that oligomer forms are more toxic than mature fibrils.[3−7] Alzheimer's disease (AD) is the most frequent cause of memory loss and dementia in the elder human population. AD is characterized by loss of neurons and synapses in the cerebral cortex and certain subcortical regions.[8] A central role in the pathogenesis of AD is played by β-amyloid, a low molecular mass peptide (39- to 43-amino acid sequences), a proteolytic product of a high molecular mass amyloid precursor protein (APP) by enzymatic cleavage by β-secretases and γ-secretases.[9−11] β-Amyloid is released from neurons, skin and intestine cells and circulates in blood and cerebrospinal fluid.[12] In the Alzheimer's brain the transport across a blood–brain barrier is unsettled, and that results in accumulation and aggregation of the peptide. The process of aggregation can be followed by observation of changes in the secondary structure from unordered and α-helical ones to β-sheet-rich.[13,14] The mechanism of $A\beta$ toxicity may involve generation of reactive oxygen species, mitochondria damage and destabilization of intercellular calcium (Ca^{2+}) homeostasis.[15−17]

The second hallmark of AD is a manifestation of neurofibrillar tangles (NTFs) composed of hyperphosphorylated MAP-Tau protein aggregates.[18] MAP-Tau is a well soluble protein and in physiological conditions hardly shows a tendency to aggregate to insoluble fibers characteristic for the AD brain. MAP-Tau plays an important role in regulating microtubule stability and the rate of microtubule assembly, which is involved in numerous cellular processes such as maintaining a cell shape. The pathological aggregation is correlated with hyperphosphorylation of MAP-Tau in neurons and glia.[19] Hyperphosphorylated MAP-Tau is unable to bind to

Special Issue: Biological Applications of Dendrimers

Received: November 3, 2011
Revised: December 10, 2011
Accepted: December 27, 2011
Published: December 29, 2011

dx.doi.org/10.1021/mp2005627 | Mol. Pharmaceutics 2012, 9, 458–469

CHAPTER 12

Dendrimeric Polymers for Pharma Applications—Anti-Cancer Therapies

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Abstract *During the last twenty years dendrimers have attracted wide interest as potential therapeutics. These novel macromolecules differ in many ways from traditional polymers. Dendrimers are globular, possessing a core molecule to which layers of branched monomers are attached. The number of layers is described by so-called generations. The structure of dendrimers results in plenty of terminal surface groups and empty internal cavities. Both these features are important when considering dendrimers for biomedical applications. Moreover, precise methods of synthesis enable the tailoring of dendrimers to specific purposes. In this concise review, a few examples of the part dendrimers play in anti-cancer therapies will be presented. It is worth stressing that these examples of pharma applications of dendrimers do not include all areas of study that are presently being conducted, and that each year produce new ways to use dendrimers in medicine.*

1. INTRODUCTION

Delivery of drugs to tumors has been the area of constant research, because the development of safe and effective dosage is a necessity. One of the most common approaches in the fight against cancer is chemotherapy, which aims at complete elimination of tumor mass. Because of the great toxicity associated with most anti-cancer agents, however, there has been much effort devoted to the development of strategies for specifically and preferentially targeting tumors, while at the same time reducing the access of these drugs to healthy tissues. The disadvantages of conventional chemotherapy raised interest in other, innovative approaches, such as gene therapy, phototherapy and targeted radiotherapy.

The aim of this chapter is to summarize the achievements of the last few years in using dendrimers—a new group of polymers—to improve the treatment of can-

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DRUG DEVELOPMENT RESEARCH 71 : 383–394 (2010)

Research Overview

Metabolic Limitations of the Use of Nucleoside Analogs in Cancer Therapy May Be Overcome by Application of Nanoparticles as Drug Carriers: A Review

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT Purine and pyrimidine nucleoside analogs (NAs) are antimetabolites commonly used in cancer therapy. Administered as prodrugs, NAs permeate the mambrane using specialized transporters. Following phosphorylation, they interfere with multiple cellular pocessess inducing cytotoxicity. Toxic effects of NAs are dependent on metabolic conversion from a prodrug into the active form. For instance, an exceptionally high activity of deoxycytidine kinase (dCK) in lymphocytes is correlated with a good therapeutic effect of fludarabine in leukemic cells. On the other hand, several studies have shown that nucleoside-transporter (NT)-deficient-cells are highly resistant to NAs. Attempts to overcome the metabolic limitations of chemotherapeutics would result in the use of lower drug doses for effective therapy, reduce side effects, and ensure the independence of drug resistance mechanisms. To meet this need, several nanoparticles have been designed to deliver efficently NAs directly to cancer cells. Such vehicles include liposomes, albumins, and dendritic and linear polymers. Therapeutic agents encapsulated or conjugated to nanoparticles have improved pharmacokinetics, solubility, and stability. These factors improve the efficacy of commonly used drugs. Moreover, modification of nanoparticles with targeting molecules such as sugar moieties or folic acid ensures more specific delivery without affecting healthy tissues. Drug Dev Res 71:383–394, 2010.

Key words: nucleoside analogs; drug resistance; nanoparticles; gemcitabine; cytarabine

INTRODUCTION

Purine and pyrimidine nucleoside analogs (NAs) belong to a class of anti-cancer drugs that are widely used in hematological disorders, solid tumours, and antiviral infections [Galmarini et al., 2002]. Purine analogs include 6-mercaptopurine (6-MP), 6-thioguanine, cladribine, clofarabine, and fludarabine. Pyrimidine analogs include cytosine arabinoside (araC, cytarabine), 5-fluorouracil (5-FU), gemcitabine, and decitabine (Fig. 1). NAs mimic physiological nucleosides and share common metabolic pathways with normal nucleosides in terms of transport, phosphorylation, and degradation mechanisms. Administered as pro-drugs, NAs permeate the membrane using specialized transporters. Within the cell, NAs are sequentialy phosphorylated to mono-, di-, and tri-phosphates and as nucleotides become toxic [Jordheim and Dumontet, 2007]. Since NA prodrugs must go through a sequence of metabolic processes to exert their anti-cancer activity, several resistance mechanisms can potentially arise along the way. This review presents different

Grant sponsors: Co-financed by the Fund for Polish Science and the European Development Fund.

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Received 27 May 2010; Accepted 15 August 2010

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/ddr.20390

Biomaterials 31 (2010) 8749–8758

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Phenotype and functional analysis of human monocytes-derived dendritic cells loaded with a carbosilane dendrimer

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ARTICLE INFO

Article history:
Received 26 March 2010
Accepted 23 July 2010
Available online 15 September 2010

Keywords:
Dendritic cells
Carbosilane dendrimer 2 G-NN16
Immunomodulation
Nanoparticle
Peptide

ABSTRACT

Dendritic cells (DCs) play a major role in development of cell-mediated immunotherapy due to their unique role in linking innate and adaptive immunities. In spite of improvement in this area, strategies employing *ex vivo* generated DCs have shown limited efficacy in clinical trials. Dendrimers have been proposed as new carriers for drug delivery in aim to ameliorate DCs antigen loading that is a pivotal point in DCs approaches. In this study, we have investigated the phenotypic and functional characteristics of human monocytes-derived dendritic cells after HIV-derived peptides uptake *in vitro*. We have found that iDCs and mDCs were able to capture efficiently water soluble carbosilane (CBS) dendrimer 2 G-NN16 and did not induce changes in maturation markers levels at the DCs surface. Therefore, CBS 2 G-NN16-loaded mDCs migrated as efficiently as unloaded DCs towards CCL19 or CCL21. Furthermore, DCs viability, activation of allogenic naïve CD4 + T cells by mDCs and secretion of cytokines were not significantly changed by 2 G-NN16 loading. Summing up, our data indicate that CBS 2 G-NN16 has no negative effects on the pivotal properties of DCs *in vitro*. It should therefore be feasible to further develop this antigen loading strategy for clinical use in immunotherapy against viral infections.

1. Introduction

The present anti-HIV vaccine candidates have failed to protect from HIV replication in the long term and from immune system exhaustion associated to HIV infection. In the past few years a lot of difficulties have raised in development of new therapeutic vaccine candidates [1,2]. Moreover, the constraint of active antiretroviral therapy (ART) limitation has led to the development of immunotherapies (also known as immunovaccines), notably based on dendritic cells (DCs) to enhance anti-HIV immune responses during ART. The immunostimulatory potential of DCs has led to their exploration as cellular vaccines or adjuvant in immunotherapies against cancer or viral infections such as HIV infection [3–8]. Adequately activated myeloid DCs regulate important functions during HIV infection such as antibodies mediating neutralization, cytotoxicity, complement dependent lyses and other antiviral activities.

Up to now, clinical trials using these DC-based vaccines have shown that the rates of objective clinical benefit are low in vaccine recipients for cancer and in HIV trials [5,9,10]. However, several studies employing *ex vivo* generated DCs have shown a correlation between the persistence of antigen loaded in DCs and the magnitude of the immune responses [5,9,11]. Therefore, the choice of activation and mode of DCs loading are critical for the immune responses orientation. An inaccurate activation or maturation of

Abbreviations: mAbs, monoclonal antibody; ART, active antiretroviral therapy; CBS, carbosilane; CFSE, carboxyfluorescein succinimidyl ester; CCL19 or CCL21, C–C motif chemokine 19 or 21; MHC-II, major histocompatibility complex class II; iDC or mDC, immature or mature dendritic cells; GM-CSF, granulocyte-macrophage colony stimulating Factor; HIV, human immunodeficiency virus; HLA-DR, human leukocyte antigen DR; IFN, interferon; IL, interleukin; LDH, *Lactate dehydrogenase*; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; poli(I:C), polyinosinic polycytidylic acid; PI, propidium iodide; PGE2, prostaglandin 2; NMR spectroscopy, nuclear magnetic resonance spectroscopy; Th1 or Th2, T cells helper 1 or 2; TNF, *Tumor necrosis factor*.

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URLS: http://immunolab-hiv.org, http://www.retic-ris.net/

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doi:10.1016/j.biomaterials.2010.07.093

International Journal of Pharmaceutics 383 (2010) 228–235

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Transfection efficiencies of PAMAM dendrimers correlate inversely with their hydrophobicity

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ARTICLE INFO

Article history:
Received 18 June 2009
Received in revised form
10 September 2009
Accepted 11 September 2009
Available online 19 September 2009

Keywords:
Dendrimer
Gene delivery
Transfection
Dendriplex
Ethidium bromide intercalation assay
ANS fluorescence

ABSTRACT

Dendriplexes were characterized by ethidium bromide intercalation assay and their transfection efficiency was studied using HEK 293 cells and human mesenchymal stem cells. PAMAM G4 showed a higher transfection efficiency than PAMAM G3–G6, G4-OH, G4-25% or G4-50% dendrimers. Substitution of OH groups for the NH_2 surface groups rendered the dendrimer unable to form dendriplexes and to transfect cells. Partial (25%) substitution of CH_3 groups for the NH_2 groups markedly impaired transfection; 50% substitution decreased the ability of PAMAM G4 to transfect threefold. It was concluded that increased hydrophobicity decreased the ability of dendrimers to transfect. PAMAM G4-50% is highly hydrophobic and forms micelles in solution, which can transfect pGFP. The results of ethidium bromide intercalation assays, ANS fluorescence studies and transfection efficiencies of PAMAM dendrimers were correlated. Subsequently, we constructed a neurotrophin-encoding plasmid and studied its delivery to mesenchymal stem cells using PAMAM G4 dendrimer and Lipofectamine 2000. Lipofectamine 2000 was a more effective carrier (18.5%) than PAMAM G4 dendrimer (1.2%).

1. Introduction

Since they were first synthesized at the end of the 1970s (Flory, 1954; Buhleier et al., 1978; Tomalia et al., 1985; Newkome et al., 1985; Hawker and Fréchet, 1990), dendrimers have found their place in biology (Shcharbin et al., 2007a; Weber et al., 2008; Klajnert and Bryszewska, 2007). One of their promising applications is gene transfer: they have proved to be non-toxic and highly efficient carriers for delivering nucleic acids and short oligodeoxynucleotides (Shcharbin et al., 2007a; Weber et al., 2008; Klajnert and Bryszewska, 2007; Dufés et al., 2005). Such complexes have been called dendriplexes by analogy with polyplexes (polymer/nucleic acid complexes) and lipoplexes (liposome/nucleic acid complexes). Polyamidoamine (PAMAM) dendrimers (Tomalia et al., 1985) are based on an ethylenediamine core and branched units are built from methyl acrylate and ethylenediamine. The third, fourth, fifth and sixth generations of PAMAM-NH_2 dendrimers (PAMAM G3, G4, G5 and G6) possess 32, 64, 128 and 256 surface amino groups, respectively; their molecular weights are respectively 6.9, 14.2, 28.8 and 60 kDa, with corresponding molecular diameters 3.1, 4, 5.3 and 6.7 nm (Klajnert and Bryszewska, 2007). PAMAM G4-OH differs from PAMAM G4 by surface groups—it has 64 surface OH groups, which are neutral at pH 7.4. The structure of PAMAM-OH G4 dendrimer is presented in Fig. 1. PAMAM G4-25% has 75% NH_3^+ groups and 25% CH_3 groups; PAMAM G4-50% has 50% NH_3^+ groups and 50% CH_3 groups. The first attempts to analyze DNA delivery using PAMAM dendrimers with EDA cores were described in Haensler and Szoka (1993) and Kukowska-Latallo et al. (1996); PAMAM G2–G10 were shown to be effective delivery agents for transfecting different cell lines. Later, Steven Kuo and Lin (2007) showed that PAMAM G5 dendrimer was more efficient than PAMAM G2 (EDA core) for transfection with pDNA (pSG5lacZ), which encodes the lacZ gene for β-galactosidase, into human macrophage-like U937 and mouse fibroblast NIH/3T3 lines. Characterization of dendriplexes showed no clear correlation with transfection efficiency. Sometimes, a dendriplex was clearly observable by electron microscopy and gel-electrophoresis but showed low transfection efficiency (Haensler and Szoka, 1993; Kukowska-Latallo et al., 1996; Steven Kuo and Lin, 2007). The dependence of transfection efficiency on PAMAM dendrimer generation has a bell-shaped character. The transfection efficiency increases with increasing generation from 1st, reaches a maximum at 4–5th and decreases significantly by 9–10th (Haensler and Szoka, 1993; Kukowska-Latallo et al., 1996; Steven Kuo and Lin, 2007).

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doi:10.1016/j.ijpharm.2009.09.020

Colloids and Surfaces B: Biointerfaces 82 (2011) 8–12

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Interaction of cationic phosphorus dendrimers (CPD) with charged and neutral lipid membranes

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ARTICLE INFO

Article history:
Received 21 May 2010
Received in revised form 19 July 2010
Accepted 21 July 2010
Available online 12 August 2010

Keywords:
Phosphorus-containing dendrimers
Model lipid membranes
Liposome–dendrimer interactions
DSC
Surface charge

ABSTRACT

Despite the rapid development of modern pharmaceutics, delivery of drugs to sites of action is not always effective. The research on new targeting delivery systems of pharmacologically active molecules is of great importance.

Surface properties such as surface charge of drug delivery particles frequently define their pharmacokinetic profile; hence the efficiency of drugs can be increased by application of nanoparticles having appropriate surface properties.

The aim of the present work was to study the interactions of cationic phosphorus-containing dendrimers (CPD) with model lipid membranes with no charge or bearing surface charge. The interactions of two generations of phosphorus dendrimers on the thermotropic behavior of model lipid membranes composed of DMPC (uncharged) or DMPC/DPPG (negatively charged) were studied using differential scanning calorimetry (DSC). The results of this study showed that CPDs can alter the thermotropic behaviour of the bilayer by reducing the cooperativity of phospholipids and this effect strongly depends on membrane surface charge. The information resulting from this study may be applied to the rational design of new drug carriers combining liposomal and dendrimeric technology.

1. Introduction

Dendrimers represent the so-called 4th architectural class of polymers and the newest category of drug delivery carriers [1]. They have a well-defined highly branched structure suitable for pharmaceutical applications [2–7]. They are synthesized by surrounding a core molecule with layers of branching elements. They are small in size, with low polydispersity which is a crucial factor to the reproducibility of the pharmacokinetic behavior of the encapsulated drug [8]. The use of dendrimers as modulators of the release rate of a drug encapsulated into liposomes and the possible alterations of the drugs' bioavailability seems to be an attractive field for research [9]. Differential scanning calorimetry (DSC) is an important technique to project the interactions between biomaterials or biomaterials and drug [9] and to design carriers based on the findings from their thermal behaviour. Recent published works on liposomes mixed with dendrimers as modulatory and controlled release drug delivery system (MCRS) [8,10,11], have shown that the mixed biomaterials should be studied using different physical techniques [i.e., Raman, photon correlation spectroscopy (PCS)] as well as thermal analytical techniques such as DSC, in order to investigate the kind of interactions between the materials of the system. In this study we focus on the interactions between cationic phosphorus dendrimers (CPD) with model lipidic membranes composed of dimyristoylphosphatidylcholine (DMPC) and of dimyristoylphosphatidylcholine with dipalmitoylphosphatidylglycerol (DPPG) (97:3 molar ratio) in order to collect data and to evaluate the changes of the thermal parameters of the system, for producing new and advanced mixed drug delivery systems which can modulate the release of the encapsulated drug. CPDs differ from the molecules described previously by Supattapone et al. [12], principally in their protonated tertiary amine end-groups. They have a hydrophilic surface and a hydrophobic backbone which allows for very efficient membrane penetration [13]. The findings from

Abbreviations: CPDs, cationic phosphorus dendrimers; Cp, heat capacity; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPG, dipalmitoylphosphatidylglycerol; D:l, dendrimer:lipid molar ratio; DSC, differential scanning calorimetry; ΔH, main phase-transition enthalpy; MLVs, multilamellar vesicles; G3, generation 3; G4, generation 4; T_m, gel to liquid–crystalline phase-transition temperature; T_{onset}, temperature at which the thermal effect starts; $\Delta T_{1/2}$, width of the transition at half-peak height.

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0927-7765/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2010.07.046

Colloids and Surfaces B: Biointerfaces 83 (2011) 360–366

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Characterization of complexes formed by polypropylene imine dendrimers and anti-HIV oligonucleotides

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ARTICLE INFO

Article history:
Received 24 September 2010
Received in revised form
15 November 2010
Accepted 3 December 2010
Available online 9 December 2010

Keywords:
HIV/AIDS
Polypropylene imine dendrimers
Gene therapy
Antisense oligonucleotides
Dendriplex

ABSTRACT

Current anti-HIV therapies are capable of controlling viral infection but do not represent a definitive cure. They rely on the administration of antiretroviral nucleoside analogues, either alone or in combination with vectors. Dendrimers are branched, synthetic polymers with layered architectures, promising non-viral vectors in gene therapy. The aim of the paper was to study the interactions between three anti-HIV antisense oligonucleotides (ODNs): SREV, ANTI TAR, GEM91 and different generation polypropylene imine dendrimers (PPI) by monitoring changes in the fluorescence polarization of fluorescein attached to the ends of the ODNs when increasing concentrations of dendrimers were added. Laser Doppler electrophoresis, dynamic light scattering (DLS) and transmission electron microscopy (TEM) were used to characterize, respectively, zeta potential, particle size and morphology of dendriplexes formed in different molar ratios. Antisense oligonucleotides interacted with polypropylene imine dendrimers in different molar ratios depending on generation. Zeta potential of dendriplexes varied from (−25 to −21) mV to −5 mV (for PPIG3 and PPIG4 complexes) and to zero (for PPIG2 complexes). The structures presented a polydisperse size from about 50 nm to even 700–800 nm by TEM and about 250 nm by DLS. It means that besides single dendriplexes, aggregates were also present.

1. Introduction

HIV/AIDS remains a global health problem of unprecedented dimensions. Statistics for the end of 2008 indicate that around 33 million people are living with HIV, the virus that causes AIDS. Each year around 2.7 million more people become infected with HIV and 2 million die of AIDS [1]. Drug therapies have greatly improved the quality of life of many infected persons, however millions of people have no access to this treatment since it is highly expensive. Moreover, with current drugs, it is still impossible to completely eradicate the virus from the body. Gene therapy seems to be a promising approach in the therapy against HIV. In the past 25 years, a wide variety of antisense strategies have been developed. Antisense gene therapy includes oligonucleotides (ONs), small interfering RNA (siRNA) and ribozymes. The purpose of HIV gene therapy is to render cells resistant to HIV by introducing recombinant genetic material that interferes with HIV infection/replication.

Different phase I and phase II clinical trials have been completed or are on-going to determine the safety and the efficacy of HIV gene therapy [2–4]. Vitravene, designed to treat AIDS-associated retinitis, was the first antisense drug approved for use by the US FDA (it is also approved by European regulators). Side-effects and toxicities associated with Vitravene treatment include inflammation and increased eye pressure in approximately 25% of treated patients [5]. Less common side-effects include abnormal vision, cataracts, bleeding in and around the eye, reduced color vision, eye pain, retinal detachment, stomach pain, low blood count, weakness, dehydration, cough, flulike symptoms, and chest pain [6]. Many regions of the HIV genome have been targeted with antisense, including the *rev*, *tat*, *gag*, *pol*, and *env* genes, and the 5′-untranslated region and psi sequences [7]. GEM91 (Trecovirsen) is a 25-mer PS-ON (phosphorothioate oligonucleotide) that binds at the translation initiation site of the HIV *gag* mRNA, which encodes a 24 kDa viral protein responsible for membrane anchoring, *env* interactions, and is part of the core virus capsid [8]. It reached to phase III clinical trial, but it was interrupted due to secondary effects [9]. The problem is the tendency of antisense drugs to non-specific binding to serum proteins which leads to the decrease of antisense drug bioavailability. To achieve the desired therapeutic effects the higher doses of drug are necessary,

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0927-7765/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2010.12.008

Colloids and Surfaces B: Biointerfaces 83 (2011) 388–391

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Short communication

Carbosilane dendrimers NN8 and NN16 form a stable complex with siGAG1

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ARTICLE INFO

Article history:
Received 16 July 2010
Received in revised form 12 October 2010
Accepted 9 November 2010
Available online 16 November 2010

Keywords:
Carbosilane dendrimer
siGAG1
Complex
Circular dichroism
Fluorescence
Zeta-potential
Zeta-size

ABSTRACT

A new mechanism of gene expression inhibition has been discovered as RNA interference, in which the ability of double-stranded RNA to stimulate specific degradation of an mRNA target with a complementary sequence to one of the double-stranded RNA strands. Water-soluble carbosilane dendrimers containing ammonium or amine groups at their periphery are biocompatible molecules that may be good candidates as non-viral carriers of small interfering RNA. In studying the formation of complex between anti-HIV siRNA siGAG1 and carbosilane dendrimers NN8 and NN16 by circular dichroism, fluorescence, and zeta-potential, the size of nanoparticles formed has been estimated by dynamic light scattering. At a charge ratio of 1:3–4 (siGAG1:dendrimer), the dendriplexes formed were in the size range of 250–350 nm.

1. Introduction

A new mechanism of gene expression inhibition by RNA interference is the ability of double-stranded RNA to stimulate specific degradation of mRNA targets with sequences that are complementary to one of the double-strands of the RNA. Long double-stranded RNA entering a cell is cleaved by endonucleases into short 19–21 bp double-stranded fragments with two protruding nucleotides at the 3′-ends of the strands. These short duplexes, called siRNAs, form within a complex of protein catalytic structures, causing directed degradation of target complementary mRNA. RNA interference is now used for gene expression regulation and for investigating functional genomics of eukaryotes. Regarding other nucleic acids, one of the main limitations of the applicability of RNA interference for gene therapy is the problem of siRNA delivery into cells [1].

Dendrimers with positively charged groups that bind to DNA/RNA are a class of polymers that has structural advantages for gene transport. Dendrimers are monodisperse, stable, and are characterized by relatively low viscosity at high molecular mass. They also have numerous end-groups that can be ionized, which means that they can efficiently bind with a large amount of genetic material [2]. Water-soluble carbosilane dendrimers containing ammonium or amine groups at their periphery are biocompatible molecules which may make good candidates as non-viral carriers for ODN [3].

We have been investigating the formation of dendriplexes between amino-terminated carbosilane dendrimers NN8 and NN16 and anti-HIV siRNA (siGAG1).

2. Materials and methods

2.1. Structures of dendrimers and siRNA

The structures of dendrimers are presented in Ref. [3]:
siGAG1: sense: GAGAACCAAGGGGAAGUGACAdTdT;
antisense: UGUCACUUCCCCUUGGUUCUCdTdT;
which were purchased from Sigma–Aldrich (USA).

2.2. Circular dichroism

The CD spectra of NN16/siRNA and NN8/siRNA complexes were measured with a Jasco-815 spectropolarimeter. NN16 and NN8 concentrations used in the experiments increased over the range from 0 to 43.12 μM. Measurements were performed in a buffer

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doi:10.1016/j.colsurfb.2010.11.009