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In vivo toxicity of poly(propyleneimine) dendrimers

Barbara Ziemba,[1] Anna Janaszewska,[1] Karol Ciepluch,[1] Maria Krotewicz,[2] Wiesława A. Fogel,[3] Dietmar Appelhans,[4] Brigitte Voit,[4] Maria Bryszewska,[1] Barbara Klajnert[1]

[1]Department of General Biophysics, University of Lodz, 141/143 Pomorska St., Lodz 90-236, Poland
[2]Department of Neurophysiology, University of Lodz, 141/143 Pomorska St., Lodz 90-236, Poland
[3]Department of Hormone Biochemistry, Medical University of Lodz, 7/9 Zeligowskiego St., Lodz 90-752, Poland
[4]Leibniz Institute of Polymer Research Dresden, Hohe Strasse 6, Dresden D-01069, Germany

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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

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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

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Correspondence to: B. Ziemba; e-mail: barzie@biol.uni.lodz.pl
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LETTER

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

A. Janaszewska,[a] B. Ziemba,[a] K. Ciepluch,[a] D. Appelhans,*[b] B. Voit,[b] B. Klajnert*[a] and M. Bryszewska[a]

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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^{Sc}[10] 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.

[a] *Department of General Biophysics, University of Lodz, 141/143 Pomorska Street, 90-236 Lodz, Poland. E-mail: aklajn@biol.uni.lodz.pl; Fax: +48 42 635-4474*
[b] *Leibniz Institute of Polymer Research Dresden, Hohe Strasse 6, 01069 Dresden, Germany. E-mail: applhans@ipfdd.de; Fax: +49 351-465-8565*

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Mini review

INFLUENCE OF DENDRIMERS ON RED BLOOD CELLS

BARBARA ZIEMBA*, GABRIELA MATUSZKO, MARIA BRYSZEWSKA and BARBARA KLAJNERT
Department of General Biophysics, University of Łódź, 141/143 Pomorska St., 90-236 Łódź, Poland

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.

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* Author for correspondence. e-mail: barzie@biol.uni.lodz.pl, tel.: (+48 42) 635 41 44, fax: (+48 42) 635 44 74

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

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ORIGINAL PAPER

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

Karol Ciepluch · Barbara Ziemba · Anna Janaszewska · Dietmar Appelhans · Barbara Klajnert · Maria Bryszewska · Wiesława Agnieszka Fogel

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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

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

K. Ciepluch (✉) · B. Ziemba · A. Janaszewska · B. Klajnert · M. Bryszewska
Department of General Biophysics, University of Lodz,
141/143 Pomorska St.,
90-236 Lodz, Poland
e-mail: ciepluch@biol.uni.lodz.pl

D. Appelhans
Leibniz Institute of Polymer Research Dresden,
Hohe Strasse 6,
D-01069 Dresden, Germany

W. A. Fogel
Department of Hormone Biochemistry,
Medical University of Lodz,
7/9 Zeligowskiego St.,
90-752 Lodz, Poland

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Genotoxicity of Poly(propylene imine) Dendrimers

Barbara Ziemba,[1] Gabriela Matuszko,[1] Dietmar Appelhans,[2] Brigitte Voit,[2] Maria Bryszewska,[1] Barbara Klajnert[1]

[1] *Department of General Biophysics, University of Lodz, Lodz, Poland*

[2] *Leibniz Institute of Polymer Research Dresden, Dresden, Germany*

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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

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

Barbara Ziemba,[1] Inessa Halets,[2] Dzmitry Shcharbin,[2] Dietmar Appelhans,[3] Brigitte Voit,[3] Ireneusz Pieszynski,[4] Maria Bryszewska,[1] Barbara Klajnert[1]

[1]Department of General Biophysics, University of Lodz, 141/143 Pomorska St., Lodz 90-236, Poland
[2]Institute of Biophysics and Cell Engineering of Natl. Acad. Sci. Bel., Akademicheskaya 27, Minsk 220072, Belarus
[3]Leibniz Institute of Polymer Research Dresden, Hohe Strasse 6, Dresden D-01069, Germany
[4]Department of Medical Rehabilitation, Medical University of Lodz, 75 Drewnowska St., Lodz 91-002, Poland

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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: 00A:000–000, 2012.

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

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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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