reviews

molecular pharmaceutics

PAMAM Dendrimer Based Macromolecules as Improved Contrast Agents

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Received April 1, 2005

Abstract: Dendrimers are an attractive platform for macromolecular imaging due to the presence of multiple terminal groups on the exterior of the molecule. Through application of appropriate metal ion chelates, large numbers of metal ions for imaging (paramagnetic or radioopaque) and therapy (radioactive particle emitters) may be conjugated to the dendrimer in combination with a targeting vector, through classic organic synthetic techniques. Thus, a large amount of these metal ions potentially may be site specifically delivered directly into the body with the dendrimer as the vehicle with the targeting vector directing the modified dendrimer. The development of targeted macromolecular agents with acceptable blood retention times and selective organ uptake then has the potential for various biological applications. A review of comparative studies of dendrimers with various externally appended imaging and targeting agents is presented herein.

Keywords: PAMAM dendrimers; macromolecular contrast agents; targeted imaging; magnetic resonance imaging; molecular relaxivity; biodistribution

I. Introduction

Cancer, congenital heart disease, and a variety of vascular abnormalities are often fatal in patients who are not fortunate enough to receive proper diagnosis and detection at an early stage. Imaging the compression of airways and vasculature has shown to be an effective diagnosis in patients suffering from these potentially fatal defects.[1] However, currently approved contrast agents are unable to detect anomalies smaller than a few centimeters using magnetic resonance imaging (MRI). While advances in magnet strength, sensitivity, and scanning techniques have aided in enhancing images, it is imperative that contrast agents also improve to maximize the potential of this imaging modality.

MRI has the possibility to be the most accurate imaging modality to date; however, despite an apparent maturity, the technique currently lacks sensitivity, and the pursuit of improvements is a burgeoning field of activity. While the sensitivity of the instrumentation continues to be improved and refined, the abundance of water molecules in tissues makes it a worthwhile modality for exploitation. When a water molecule or any other molecule with a magnetic moment is placed within a magnetic field, it aligns itself in the direction of the field. The dipole moment is expressed as a vector, which, upon excitation using a RF pulse, moves to an excited state, where it precesses about the axis of the magnetic field. Signals are then obtained as the vector realigns to the static magnetic field, its ground state. The amount of time the vector takes to realign itself back to the original magnetic field is associated with relaxation times, T1 and T2. T1 relaxation corresponds to the energy given off as the vector returns to its original amplitude aligned within the magnetic field. T2 relaxation, however, corresponds to the incoherence in magnetic field as the spin vector precesses about the plane perpendicular to the ground state, which is directly affected by the mobility of the proton. The signals obtained give correlated images of the spin vectors in the magnetic field.

MRI may further be exploited by increasing disparity of the images of different tissues through the use of contrast

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10.1021/mp050019e
Published on Web 07/08/2005

agents (CAs). Correlated images show differences in tissues of interest versus surrounding tissues dependent on the amount of water present and the relaxation times of the water (protons) associated with the tissues. CAs increase contrast by increasing differences in relaxation times. However, obtaining quality images using CAs in MRI is dependent on, among many other factors, the clearance rate and the molecular relaxivity of the CA.[2−4] Clearance rates refer to the amount of time it takes for a specific amount of a substance in the body, such as a CA, to be removed or excreted, and are dependent on, among many other things, size, shape, and chemical makeup of the molecule. Relaxivity, however, is a measure of the relaxation rate and a function of dipole−dipole interactions of the metal ion in the contrast agent with coordinated water molecules and water in the bulk solvent, known as inner and outer shell interactions, respectively.[5] Signals are obtained as a fast exchange occurs between inner- and outer-sphere water molecules on the metal ion of the CA, due to tumbling.[6] With the assumption that larger molecules rotate slower than smaller molecules because of size, the fast exchange will most likely decrease in macromolecular contrast agents, causing longer T2 times for more enhanced imaging.

Currently approved low molecular weight CAs filter rapidly through the glomerulus of the kidney and clear quickly from the body.[7] The most commonly used CA is gadolinium diethylenetriamine pentaacetic acid dimeglumine salt (Gd[DTPA]), also known as Magnevist. This agent has a molecular weight of 0.8 kDa, and 90% of the injected dose clears from the body in less than an hour, primarily by kidney filtration and leakage from the vessels.[8] With this rapid clearance rate, the physician has limited ability to complete time-dependent imaging studies or obtain highly resolved images of patients. The numbers of water molecules coordinated to Gd^{3+}, Gd[EDTA], and Gd[DTPA] are approximately 8−9, 2−3, and 1, respectively, with corresponding relaxivity values of 7.0, 4.6, and 2.0, respectively, at 37 °C and 20 MHz (0.5 T).[6] Chelation is required to obviate the inherent toxicity properties of Gd^{3+}. Introduction of a macromolecular aspect to a CA would not only decrease the rotational correlation time, τ_R, but also increase blood pool retention time. Also, with attachment of a large number of chelates and metal ions, local concentration is increased and a lower local concentration is required for image acquisition. Various macromolecular structures are currently being investigated as platforms for multiple chelates to be conjugated including superparamagnetic iron oxides (SPIO) and other nanoparticles.[9−11]

The introduction of bifunctional chelates such as 2-(4-isothiocyanatobenzyl)-6-methyl-diethylenetriamine pentaacetic acid (1B4M-DTPA), *N*-[2-amino-3-(4-isothiocyanatobenzyl)propyl]-*cis*-cyclohexyl-1,2-diamine-*N*,*N*′,*N*′,*N*″,*N*″-pentaacetic acid (CHX-A-DTPA), *N*-[2-amino-3-(4-isothiocyanatobenzyl)propyl]-*trans*-cyclohexyl-1,2-diamine-*N*,*N*′,-*N*′,*N*″,*N*″-pentaacetic acid (CHX-B-DTPA), and 2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-*N*,*N*′,-*N*″,*N*‴-tetraacetic acid (p-SCN-Bz-DOTA) (Figure 1) has enabled conjugation of multiple chelates to a single macromolecular architecture. Ogan and co-workers have shown that, by attaching approximately 19 DTPA ligands to human serum albumin (HSA), molecular relaxivity increased to 273 mM^{-1} s^{-1} from 4.9 mM^{-1} s^{-1} versus ∼5 mM^{-1} s^{-1} for Magnevist.[12] Due to various factors of this agent, including size and charge, blood retention time was also increased compared to Gd[DTPA], providing improved image enhancement.

An alternative to direct protein conjugates is dendrimer conjugates: a specific class of macromolecular architectures possessing great potential for use in the development of contrast agents. Dendrimers are macromolecular polymeric compounds synthesized through a sequence of iterative reactions from an initiator core outward through branch points producing a three-dimensional organization of terminal groups.[13] They were first proposed by Tomalia as potential agents for biomedical applications due to their ability to

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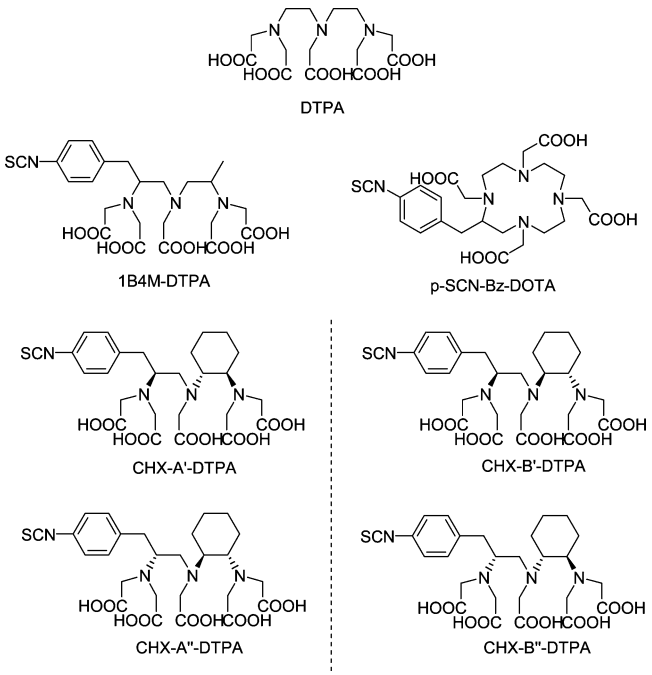

Figure 1. BifunctionalDTPA derivatives synthesized for increased stability of metals compared to the parent molecule. The vertical line between the two sets of CHX-DTPA denotes diastereoisomers CHX-A-DTPA and CHX-B-DTPA.

mimic biomolecules from simple micelles to complicated highly organized building blocks of biological systems.[13] Polymerization of dendrimers occurs in controlled, defined, and discrete steps, with each complete cycle of steps producing a product associated with a generation number. Polyamidoamine (PAMAM) dendrimers comprise a specific class of dendrimers, which have interesting characteristics and a potential for biomedical applications. This class of dendrimers are synthesized through Michael addition of methyl acrylate and the amine core. This affords the ester, which is then saponified and reacted with a diamine, such as ethylenediamine. Thus, using the chemistry of the PAMAM dendrimers of Michael addition followed by aminolysis for two complete cycles produces a generation 2 (G2) dendrimer. This process then also results in controlled growth of the dendrimer and exponential increase in the number of terminal groups from the core. The number of terminal groups and size of the dendrimers are shown in Table 1. With control over both molecular size and weight, dendrimers may be tailored for specific applications and with specific functionality for a variety of applications. PAMAM dendrimers were first presented as monodisperse molecules;[13] however, after closer examination and improved analytical techniques it has been shown that these dendrimers possess a small percentage of defects.

They are commercially available, with various initiatior cores, in full- and half-generation sizes terminating in amine or carboxylic acid groups, respectively.[14] Classical organic strategies may be applied to modify the exterior functionality of PAMAM dendrimers as well as introduction of a single attachment site for a targeting vector. The ability to create molecularly targeted macromolecular imaging and therapeutic agents is then possible.

Table 1. Some Characteristics of Both Ammonia Core (AC) and Ethylenediamine Core (EDA) PAMAM Dendrimers

core	generation	no. of terminal amines	mol wt (kDa)	hydrodynamic diameter (nm)[a]	ref
AC	0	3	0.36	1.1	17
AC	1	6	1.04	1.6	17
AC	2	12	2.41	2.2	17
AC	3	24	5.15	3.1	17
AC	4	48	10.62	4.0	17
AC	5	96	21.56	5.3	17
AC	6	192	43.45	6.7	17
AC	7	384	87.23	8.0	17
AC	8	768	174.78	9.2	17
AC	9	1536	349.88	10.5	17
AC	10	3072	700.09	12.4	17
EDA	0	4	0.52		
EDA	1	8	1.43		
EDA	2	16	3.26		
EDA	3	32	6.91		
EDA	4	64	14.21		
EDA	5	128	28.83	4.3	60
EDA	6	256	58.05	6.9	60
EDA	7	512	116.49	8.0	60
EDA	8	1024	233.38	10.2	60
EDA	9	2048	467.15	12.4	60
EDA	10	4096	934.70	14.7	60

[a] Diameter for the AC core as determined using size exclusion chromatography. Diameter for the EDA core as determined using transmission electron micrograph (TEM).

While protein-based macromolecular CAs, such as albumin, have increased resolution and blood retention time, they remain nontargeted. Direct application of a targeting moiety such as an antibody is problematic since immunoreactivity of antibodies is often compromised by addition of high numbers of ligands necessary to attain the increased concentration required for generating meaningful contrast.[15] Poly-L-lysine has been studied as a multivalent carrier for conjugation of more than 40 chelated Gd^{3+} complexes to an immunoprotein with <10% immunoreactivity loss. However, up to 100 or more paramagnetic ions per single antibody are needed for optimal visualization.[16,17] Additionally, poly-L-lysine poses challenges in clinical approval owing to the diversity of products obtained in synthesis of the conjugate

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Table 2. Molecular Relaxivity Values of Various PAMAM Dendrimers with Both Ammonia Core and Ethylenediamine Core Compared to DTPA and Albumin-Based Contrast Agents

core	generation	T1 (mM^{-1} s^{-1})	freq (MHz)	temp (°C)	Gd(III) ions	chelate	ref
DTPA		2	20	37	1	DTPA	6
albumin		273	10	37	19	cDTPA	12
AC	2	143	10	35	11	1B4M	8
AC	4	1646	200		40	1B4M	51
AC	6	5800	25	35	170	1B4M	8
AC	6	4000	10	35	170	1B4M	8
EDA	5	2880	20	23	96	DOTA	19
EDA	7	13300	20	23	380	DOTA	19
EDA	9	47520	20	23	1320	DOTA	19
EDA	10	66960	20	23	1860	DOTA	19

due to the inability to control polymerization to produce a discrete compound. The combined deficiencies associated with both low molecular weight CAs and antibody-based agents present opportunities for dendrimers to be used as novel platforms for contrast agents.

To illustrate the impact on MRI contrast potential of such agents, molecular relaxivity was reported to rise with increasing dendrimer generations (see Table 2), as illustrated by PAMAM-EDA-G10-(GdDOTA) (~1860 Gd^{3+}; 66 960 mM^{-1} s^{-1}) compared to PAMAM-EDA-G5-(GdDOTA) (~96 Gd^{3+}; 2880 mM^{-1} s^{-1}).[18] With such a large number of metal ions located on a single molecule, image contrast and resolution using PAMAM dendrimers improves from a centimeter using Gd[DTPA] to as low as ~100 μm using PAMAM-EDA-G8-(Gd-1B4M).[19] This improvement offers various possibilities for imaging anatomical structures previously undetected.

Last, the potential to incorporate site-specific targeting has been demonstrated by Wu and co-workers, and other researchers, with reports of the conjugation of antibodies or their fragments to dendrimers bearing chelating agents thereby increasing the size and density of the CAs without loss of immunoreactivity while incorporating a targeting element.[20]

With the emerging field of dendrimer-based MRI contrast agents, issues arise in the selection of target and chelating agent, the sequestration of Gd^{3+}, and the generation of dendrimer to be used in each application. This review will first discuss the chelating agents currently being conjugated to PAMAM dendrimers and a brief discourse on metal ions used for imaging and biodistribution studies. A detailed inspection into the pharmacokinetics of targeted and untargeted PAMAM dendrimer based CAs and their corresponding molar relaxivities follows. With the future of macromolecular imaging and therapeutic agents on the horizon, custom tailoring of such agents for a specific purpose may soon be possible, with the hope of eventually preparing "theranostic" agents, which will incorporate multimodal imaging and therapeutics in the same molecule.

II. Evaluation of Ligands Used in Macromolecular Contrast Agents

Free metal ion toxicity is a major concern in biomedical applications, which may be obviated by use of highly stable metal ion chelators. Octadentate ligands such as DTPA and DOTA have been shown to be thermodynamically and kinetically stable for In(III) and Y(III) as a coordination number of 8 is favored for these specific metals.[21] Attachment of both cyclic and acyclic chelators to dendrimers is possible through application of reactive bifunctional conjugation groups, e.g., an isothiocyanato group. Inclusion of this group not only provides an attachment point but also may increase the stability of metal ion complexes.[22] While cyclic ligands have been shown to be more stable than acyclic ligands,[22] difficulties in the use of bifunctional DOTA have been encountered in both synthesis and saturation of the exterior of the dendrimer. The conjugation reaction of the dendrimer

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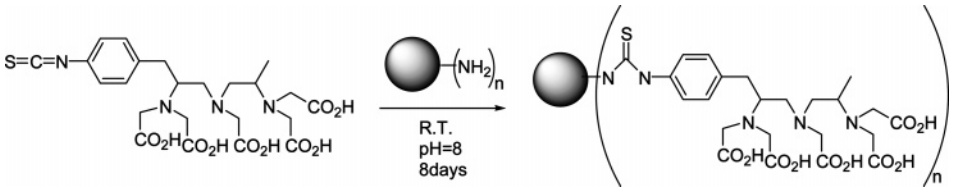

Figure 2. Scheme showing conjugation of 1B4M to a PAMAM dendrimer, depicted as a sphere.

with the bifunctional chelate is shown in Figure 2. It may be argued that the increasing steric hindrance, when conjugating the dendrimer with bifunctional chelating agent, affects the degree of saturation. For instance, in an ammonia core generation-2 (AC-G2) PAMAM, approximately 10.2 DOTA molecules are conjugated compared to 10.6 DTPA as measured using aromatic integrated signal versus alkyl integrated signal in NMR.[20] While this difference appears to not be statistically significant, further studies with higher generation dendrimers of this aspect of surface modification need to be performed.

Once chelating agents have been attached, radiometal ions or paramagnetic metal ions may be complexed for biodistribution, MRI, or therapeutic applications. For example, Camera and co-workers have reported that the biodistribution of mAb B3 conjugated with either CHX-B-DTPA or 1B4M-DTPA radiolabeled with ^{111}In resulted in comparable tumor-to-liver ratios at both 24 and 168 h, and equivalent tumor uptake at 72 h.[23]

The metal ion to be used is another major factor in the selection of the chelating agent; appropriate matching of the two is a requirement. Four of the many metal ions currently under investigation include paramagnetic Gd^{3+} for MRI, $^{111}In^{3+}$ for SPECT imaging, and $^{90}Y^{3+}$ and $^{213}Bi^{3+}$, both as therapeutics; all of these can be chelated quite successfully. While radioactive species of all four metal ions are used for biodistribution studies, imaging, and therapy applications,[24−27] Gd^{3+} is most commonly used for MRI because of its large magnetic moment.[6]

Gadolinium also seems to possess an additional level of versatility beyond its MRI application as it also has attractive properties for neutron capture therapy (NCT). NCT involves tumor uptake of a nontoxic substance containing ^{157}Gd, more commonly associated with boron, with the ability to efficiently capture neutrons and subsequently undergo a high linear energy transfer and cytotoxic radioactive decay. Gadolinium neutron activation results in production of strongly cytotoxic Auger electrons.[28,29]

III. Pharmacokinetics of Untargeted PAMAM Dendrimers

Various factors contribute to the pharmacokinetics of dendrimers due to their molecular size and chemical composition. High-generation PAMAM dendrimers have generally been accepted as spherical molecules, with the core and interior being almost completely shielded due to the densely packed terminal surface groups. Studies continue to investigate the exact shape(s) dendrimers assume in solution and the impact on the degree of mobility associated with each arm of the dendrimer.[30]

The molecular size and number of terminal groups of PAMAM dendrimers affect the biodistribution as well as the initiator core of the dendrimer, providing insight via whole body biodistribution properties. Two cores that have been used to initiate PAMAM dendrimers are the ammonia core (AC) and the ethylenediamine core (EDA), resulting in a first-generation (G1) dendrimer with 6 and 8 terminal amines, respectively. Modified dendrimers of G2 through G10 of both core types have been investigated in a battery of experiments,

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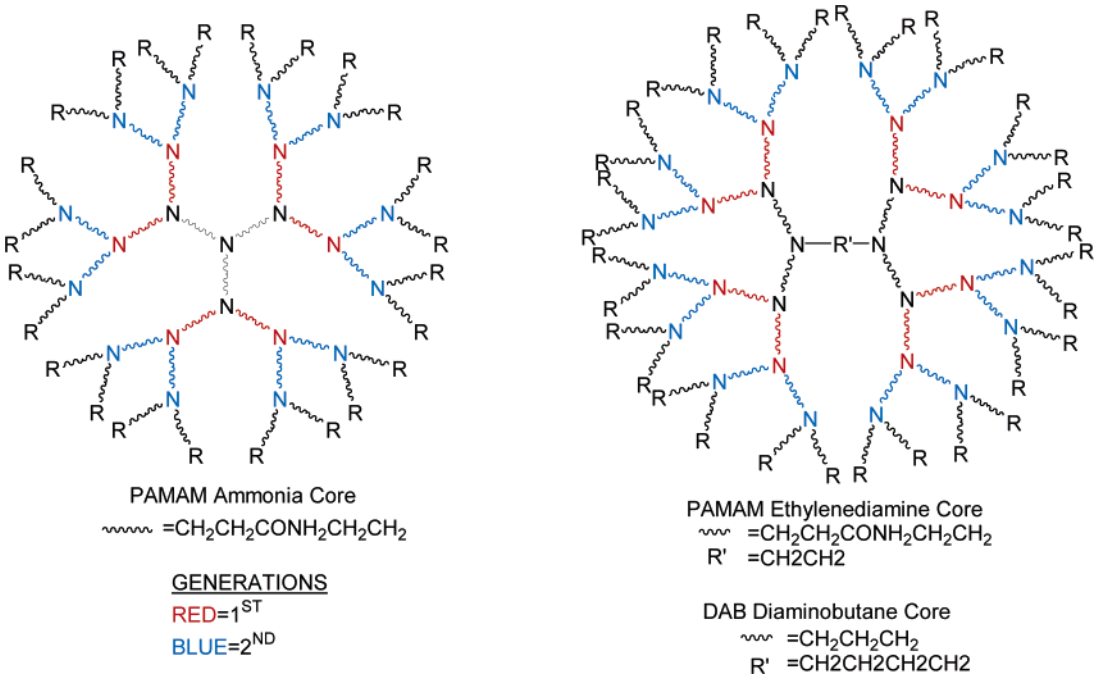

Figure 3. Scheme depicting structural differences between cores and scaffolding in both PAMAM and DAB dendrimers. Generation 1 is denoted in red while generation 2 is in blue.

providing insight into the pharmacokinetics as both targeted and untargeted imaging agents.[2,8,31−33]

PAMAM dendrimers contain a scaffold of repeating amine and amide units unlike the DAB (diaminobutane) dendrimer, which only contains amines linked through a diaminobutane core with a propyleneamine scaffold, as illustrated in Figure 3. While a great deal of this review deals with modified PAMAM dendrimers, it is important to compare the biodistribution of these dendrimers to other types of similarly modified dendrimers. Through comparisons of PAMAM and DAB dendrimers of similar size and charge, it is possible to begin to develop an understanding of how each dendrimer is processed, distributed, and cleared from the body. Studies of the pharmacokinetics of modified PAMAM and DAB dendrimer series have revealed that the interior scaffold has a direct affect on the body's uptake.[34,35] In these preliminary studies that compared PAMAM-EDA to DAB propyleneimine dendrimers, distinct biological deposition differences were reported as attributed to differences in overall hydrophobicity between these two classes of dendrimers.[35,36] DAB-G2-$(Gd\text{-}1B4M)_{12}$ was observed to behave similarly to the larger PAMAM-AC-G6-$(Gd\text{-}1B4M)_{170}$. This result was in contrast to its generational counterpart PAMAM-AC-G2-$(Gd\text{-}1B4M)_{11}$ as discerned by the corresponding biological half-lives of 3 h and 3.3 h versus 40 min, respectively.[36] Proton relaxivities also increase for the DAB dendrimers bearing chelated Gd^{3+} compared to the corresponding PAMAM as shown in Table 3.[36] Furthermore, DAB-G4-$(Gd\text{-}1B4M)_{64}$ was seen to accumulate greater than twice as much in the liver compared to PAMAM-EDA-G4-$(Gd\text{-}1B4M)_{64}$, and ~30% less in the kidney.[35] The study has also shown that the DAB-G2-$(Gd\text{-}1B4M)_{16}$ has an 8% retention 48 h postinjection equating to a 13 h half-life.[35] While there is some disparity of data for the modified DAB-G2, both values demonstrate a longer half-life than its PAMAM counterpart does.

The chelate-modified DAB dendrimer was also reported to be a good liver imaging agent by virtue of its biodistri-

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Table 3. Proton Relaxivity Values of Various PAMAM Dendrimers Compared to DAB Dendrimers and DTPA

core	generation	chelate	R1 (mM^{-1} s^{-1})	temp (°C)	freq (MHz)	ref
DTPA			5.5	20	64	44
AC	2	1B4M	21	20	25	8
AC	2	1B4M	17	20	10	8
AC	2	1B4M	13	35	10	8
AC	2	1B4M	16	35	20	6
AC	4	DTPA	30	35	20	6
AC	4	DO3A	17	37	20	6
AC	5	DO3A	19	37	25	6
AC	5	DOTA	30	23	20	6
AC	6	1B4M	34	20	25	8
AC	6	1B4M	34	35	25	8
AC	6	DOTA	31	35	25	6
AC	6	1B4M	23	20	10	8
AC	6	1B4M	23	35	10	8
AC	7	DOTA	34	23	25	6
AC	9	DOTA	36	23	25	6
AC	10	DOTA	36	23	20	6
EDA	2	1B4M	20	20	64	44
EDA	3	1B4M	25	20	64	44
EDA	4	1B4M	28	20	64	44
EDA	5	DOTA	30	23	20	19
EDA	7	DOTA	35	23	20	19
EDA	8	1B4M	35	20	64	38
EDA	9	DOTA	36	23	20	19
EDA	10	DOTA	36	23	20	19
DAB	2	1B4M	12	20	64	44
DAB	2	1B4M	31	20	25	6
DAB	3	1B4M	17	20	64	44
DAB	4	1B4M	29	20	64	44
DAB	5	1B4M	39	20	64	38

bution. However, long-term imaging using the DAB-G4-(Gd-1B4M)$_{64}$ seemed to result in decreased signal-to-noise in the blood pool due to high liver accumulation after 2 min.[37,34] DAB-G4-(Gd-1B4M)$_{64}$ did, however, give an enhanced signal-to-noise ratio in lymph nodes, while the PAMAM-EDA-G4-(Gd-1B4M)$_{64}$ was reported to qualitatively perform better in lymph nodes around the abdominal region.[38] Results of the initial biodistribution evaluation proved the PAMAM dendrimers unfavorable for targeting the liver when attaching Gd-1B4M to the exterior.

Adaptation of the biodistribution and clearance results obtained from the successful targeting of the liver using a 1B4M-modified DAB without a targeting vector were applied to creation of renal imaging through exploitation of the glomerular filtration size as well as other effects. While both AC and EDA core PAMAM dendrimers of generation 6 are reported to have comparable diameters (see Table 1), the glomerular filtration rate for the PAMAM-EDA-G6-1B4M with ~242 Gd^{3+} complexes, as compared to ~180 Gd^{3+} complexes on the exterior for the PAMAM-AC-G6-1B4M, is lower.[39] This tendency may be due to the number of metal ions on the exterior of the molecule, which theoretically may cause a large charge modification relative to the unmodified dendrimer. When comparing the metal ion unsaturated and saturated versions of the PAMAM-EDA-G2-1B4M, the unsaturated dendrimer has been found to have a slower clearance with higher liver, kidney, spleen, and bone accumulation as opposed to the metal ion saturated dendrimer.[26,40] The indium complex saturated dendrimer showed uptake of 26.5 ± 1.8, 6.8 ± 1.0, and 12.9 ± 0.8 % ID/g at 1 h postinjection for liver, kidney, and spleen, respectively, compared to 32.0 ± 1.8, 8.5 ± 0.3, and 37.5 ± 5.3 % ID/g for the unsaturated preparation.[26] This tendency may be due, among other things, to the charge present on the exterior of the dendrimer; however, further experiments must be completed.

Untargeted PAMAM dendrimers conjugated with ^{153}Gd-labeled 1B4M of both ammonia and EDA cores were compared in various imaging experiments. Generation 6 modified dendrimers of both core types demonstrated enhanced visualization of blood vessels at ~100 μm size. However, modified PAMAM EDA dendrimers enabled longer visualization time due to a greater overall retention time in the blood.[41] Generations larger than 6 appear to have interesting potential as highly sensitive imaging agents due to their increased size; however, generations ranging between G6 and G8 seem to be optimal as the Gd-1B4M modified G10 precipitates at physiological pH, preventing use in biological systems.[42] The Gd-1B4M modified PAMAM-EDA-G9 and PAMAM-EDA-G8 showed prolonged blood pool retention times, due to low kidney filtration, while the former also exhibited high liver and spleen accumulation. Modified PAMAM-EDA-G8 and PAMAM-EDA-G7 had the longest blood retentions, while the former showed increased hepatic, renal, and splenic uptake compared to the latter.[42] The results from this study indicated PAMAM-EDA-G7-

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$(Gd\text{-}1B4M)_{512}$ to be the most promising generation for blood pool imaging agents with the least amount of uptake in other organs.

While the higher generation dendrimers have optimal efficiency in imaging deep vasculature, the higher generations of modified dendrimers were also reported to have a decreased whole body clearance rate.[26,40] When attempting to selectively image specific organs, it is necessary to efficiently target those organs. Using smaller dendrimers that allow for filtration through the glomerulus of the kidney may facilitate imaging of the renal system. In one study using the PAMAM-EDA-G4-$(Gd\text{-}1B4M)_{64}$ dendrimer, renal function was easily observed in normal mice as well as in mice with varying degrees of renal damage.[43,44] Due to improved MR techniques and instrumentation, it is now possible to monitor renal function over time in patients recovering from surgery, injury, and medication. However, there is an increased risk in toxicity due to the uptake of large dendrimer-based CAs. While filtration was reported more rapid for smaller dendrimers, such as PAMAM-EDA-G4-$(Gd\text{-}1B4M)_{64}$, 100% excretion was not observed, and this was attributed to reabsorption of the dendrimer through the proximal tubule.[45] This process was shown to be obviated by a co-injection of lysine which increases modified dendrimer excretion by 5.4-fold.[45]

In all instances, toxicity plays an important role in determining a molecule's real potential as an imaging or therapeutic agent. While lysine aided in increasing excretion of relatively smaller dendrimers being filtered out through the kidneys, much research thus far has dealt with larger modified dendrimers due to their increased blood retention. Alternative methods for decreasing hepatic uptake and toxicity are being explored, including the use of poly(ethylene glycol) (PEG). Through PEGylation of a PAMAM-EDA-G4-(Gd-1B4M) with one or two PEG groups, prolonged blood retention was achieved, excretion rate increased, and organ uptake decreased.[33] This result demonstrates that the addition of prosthetic groups such as PEG will enable fine-tuning of macromolecular PAMAM dendrimer based CAs for specific organ uptake and excretion.

With such potential, PAMAM-based MR imaging agents may replace other agents or when used in conjunction with other modalities provide greater levels of information. Current lymphangiography imaging techniques use fluorescence,[46,47] which has higher sensitivity than MRI, but only allows for superficial imaging. MRI, on the other hand, allows for accurate deep imaging through the use of large modified dendrimers (see Table 1), which increase blood retention and resolution.[48] The PAMAM-EDA-G6$(Gd\text{-}1B4M)_{256}$ dendrimer, however, was reported to possess a long lymphatic lifetime with some leakage from the vessels, which had a negative affect on the vessel-to-tissue ratio.[49] Combining both MR and fluorescent imaging agents on a single dendrimer for lymphangiography, as well as other imaging studies, may prove to be beneficial.

IV. Pharmacokinetics of Targeted PAMAM Dendrimers

Proteins, such as albumin, have been used for direct ligand attachment due to their size and availability of multiple attachment sites. However, direct use of a monoclonal antibody (mAb) presents several problems with the primary encountered being compromised immunoreactivity. Shreve and co-workers have shown that immunoreactivity of the mAb decreased to 56% when directly conjugated with 20 Gd^{3+} DTPA complexes; immunoreactivity dropped to 36% and 17% with 100 and 250 Gd^{3+} DTPA complexes, respectively.[15] This obstacle may be circumvented by attaching a Gd^{3+} complex enriched dendrimer to a mAb to maintain the targeting vector characteristics while also expanding the scope of targeted macromolecular imaging.

One of the first dendrimer−protein constructs reported used the 2E4 mAb conjugated to a PAMAM-AC-G2 dendrimer with DOTA complexing Gd^{3+} and showed 76.6% binding to the positive control 5.1.2 cells, compared to (6.0 ± 0.4)% found in negative control RAJI cells.[20] A similar study using humanized anti-Tac IgG (HuTac) showed 83%

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immunoreactivity when directly conjugated to ^{111}In-labeled 1B4M versus 73% when attached to a dendrimer containing approximately 14.3 ^{111}In-labeled 1B4M ligands.[26]

The immunoreactivity retention in antibodies, however, was found to be dependent not only on the dendrimer and the ligand but also on the metal ion itself, as in the case of better biodistribution studies for Y^{3+} compared to In^{3+}.[26] A similar study compared Gd^{3+} with In^{3+} using mAb OST7. The PAMAM-EDA-G4-1B4M moiety was attached to OST7 through an introduced maleimidyl group on the protein and labeled with either ^{153}Gd or ^{111}In for comparison. Kobayashi and co-workers reported 91% immunoreactivity of this IgG-PAMAM-EDA-G4-1B4M$_{43}$ conjugate compared to 84% for the IgG-1B4M-DTPA conjugate, and also improved biodistribution when saturated with Gd^{3+} compared to In^{3+}.[40]

Considering the immunogenicity conservation issues associated with mAbs, other known cancer targeting vectors, such as folate, have also been conjugated to dendrimers. The high-affinity folate receptor (hFR) has been found to be present in higher quantities on endothelial tumor cells, including breast and ovarian cancer cells, potentially making folate a better alternative to mAbs.[32,50] When folate was conjugated to a PAMAM-AC-G4-1B4M-DTPA conjugate, relaxivity of cells was found to double from MR experiments conducted in control cells to positive hFR cells.[51] In addition to folate and 1B4M-Gd, fluorescein was also conjugated to the same PAMAM-AC-G4 dendrimer, and a 650% increase in fluorescence was seen in cells containing the hFR receptor compared to those without fluorescein.[51] A similar study showed comparable in vivo results with a 33% change in MRI contrast enhancement.[52] Additionally, a competition study of folate-PAMAM-AC-G4-1B4M with free folic acid present showed a 2700% increase in binding when no folic acid was present, compared to only 260% when folic acid was present.[53] Although it is not understood why hFR levels are overexpressed in certain cancer cells,[53] folate clearly continues to be a potentially high specificity targeting vector.

Another targeting agent includes the use of the avidin for intraperitoneally disseminated tumor cell internalization.[54] Exploiting this technique opens the door to multiple forms of imaging and therapy using the avidin−biotin system. Avidin has been shown to have high uptake in tumor and liver cells through endocytosis.[55,56] The incorporation of avidin onto PAMAM-EDA-G6-(1B4M-Gd)$_{254}$ was shown to result in a high targeting capability to SHIN3 ovarian cancer cells.[28] Biodistribution studies showed a 366-fold increase of tumor cell uptake and accumulation for the avidin-conjugated PAMAM-EDA-G6-(Gd-1B4M)$_{254}$ dendrimer compared to a control PAMAM-EDA-G6-(Gd-1B4M)$_{254}$ lacking avidin, while the tumor-to-normal tissue ratio for the specific conjugate increased from 17:1 to 638:1 at 24 h postinjection.[28,57]

The biotin−avidin system is well understood,[58,59] and knowing that avidin accumulates efficiently in the liver, we may exploit this system. Injection of a biotin−dendrimer−chelate moiety into the body gives blood retention times relative to dendrimer size and makeup.[57] An injection of avidin 4 min after the dendrimer, also known as the avidin "chase", clears the blood pool of the dendrimer binding the biotin to the avidin within 2 min and causes subsequent accumulation in the liver, enabling better visualization of vascular leakage.[57] This enhanced imaging of vascular leakage is possible since the avidin−dendrimer containing Gd-1B4M is cleared from the blood pool leaving all molecules that have "leaked", i.e., not in the blood pool, unaffected by the avidin chase, and not overshadowed by the blood pool background.

Other targeting agents are currently being investigated to target contrast agents to areas of interest. With the exploitation of dendrimer technology in imaging, better tumor targeting may become possible for early detection and management of disease.

V. Summary

By correlating biodistribution results with size, molecular weight, and chemical structure of the gadolinium complex conjugated PAMAM and propyleneimine dendrimer, a better

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understanding of the potential as contrast and therapeutic agents becomes possible. In the future, prediction of the results from specific modifications might be possible in addition to being able to tailor modifications to target exact locations in the body or extend the lifetime for time-based imaging studies, while keeping molecular relaxivity at a maximum.

Changing the internal chemical structure of these dendrimer-based contrast agents from PAMAM to DAB, while modifying the exterior using the same method, has been shown to affect drastic changes in hepatic uptake.[35] Changing the core of PAMAM dendrimers from ammonia to EDA changes the number of possible chelates and metal ions on the exterior that may be bound to the surface of the dendrimer,[39] which then influences uptake by the renal and/or the reticular endothelial systems. The number of chelates and metal ions also affects the proton relaxivity of the molecule, as shown in Table 3, which can impact contrast in the image.

No studies have shown that varying the chelating agents themselves has any effect on the biodistribution of the dendrimer conjugate; however, it is apparent that the chelate has an effect on metal ion selection and the maximum number of chelates and metal ions added to the dendrimer. The chelator does allow for utilization of specific metal ions for imaging, biodistribution studies, or therapy. As stated previously, $Gd^{3+}$and Y^{3+} have shown better pharmacokinetic results compared to In^{3+}; however, Gd^{3+} is a more versatile metal ion, which may be used in imaging, biodistribution studies, and NCT.

Future studies in the field using PAMAM dendrimers are expected to focus on multimodal imaging using various techniques. By integrating fluorescence and MR modalities, a physician may more accurately excise cancerous tissue along with affected lymphatics. While fluorescence is not able to image deep intravascular tissue, it can be envisioned as being an intraoperative tool to identify targeted areas in real time while also having the MRI information as a "road map".

Understanding the pharmacokinetics of dendrimer-based conjugates also allows for fine tailoring of molecules through the implementation of targeting agents, therapeutics, and imaging agents. It may be possible soon to produce "theranostics" for specific patients and specific diseases according to their medical history, with the hope of better management of all diseases.

MP050019E