Radiolabeled Dendrimers as Potential PET Agents for Molecular Imaging of Tumor Angiogenesis

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Abstract

Introduction: Angiogenesis is a normal physiological process that plays an imperative role during tumor development. We believe that the development of a non-invasive imaging technique targeting angiogenesis can provide a better understanding of this important process. Positron emission tomography (PET) – a highly sensitive imaging technique can offer accurate degree of disease quantification. The phenomenon of enhanced permeability and retention effect (EPR effect) is now becoming the gold standard in cancer targeting drug designing. Dendrimers have the ability to exhibit EPR effect for targeted therapeutic/drug delivery approach. Therefore, molecular imaging of tumor angiogenesis using radio-labeled dendrimers is expected to broaden the possibilities for drug development.

Body: In the present chapter, the significance of performing conjugation chemistry of bifunctional chelators quality control parameters of the radiolabeled dendrimer conjugates in vitro stability, animal biodistribution, radiation dosimetry and molecular imaging of animal tumor model after injecting radiotracer have also been discussed in detail.

Conclusion: Conjugation of the radio-metal complexes to larger molecules like dendrimers has created a new domain of research in the field of biomedical applications. Therefore, it has been proposed to develop new effective targeting moieties suitable for radiolabeling with PET tracers so as to perform molecular imaging studies.

Keywords: dendrimers, radiolabeling, PET imaging, tumor angiogenesis

1. Introduction

The present chapter highlights the importance of dendrimer based radio imaging of angiogenesis as a novel approach for molecular imaging of carcinogenesis because of their topology,
functionality and dimensions. The high loading capacity of dendrimers enables them to deliver chemotherapeutic agents as well. The dendrimer-drug complexes designed for either targeted or non-targeted drug delivery successfully penetrate through the leaky vasculature of tumor and accumulate in the cancer tissue. However, the added advantage of using dendrimer-drug complexes specifically designed for targeted delivery is that they bind to specific receptors over-expressed on the surface of cancer cells, thereby, increasing their residence time over the cell surface. Thus, the development of dendrimer complexes that incorporate the targeting ligands, anticancer drugs and positron emission tomography (PET)/β emitting radionuclides may provide the way for combinational anticancer therapies along with the in vivo imaging of the targeted tumor.

2. Tumor angiogenesis

Angiogenesis is an essential physiological process that involves formation of new blood vessels from the pre-existing ones and is one of the fundamental processes required for normal growth and development [1]. It has been recognized long ago that most solid tumors are perfused by large number of blood vessels [2]. The ability of a tumor to stimulate the formation of new blood vessels enables expansion of tumors, local invasion and dissemination. Thus, angiogenesis is also considered as one of the key requirements during tumor development as it provides oxygen and nutrients to the otherwise dormant tumors and without which the tumor cannot grow beyond 2.0–3.0 mm in diameter [3, 4]. Tumor comprises of cells that proliferate aberrantly and have lost the ability to regulate growth. Thus, tumor cells like normal cells require adequate supply of oxygen and nutrients and also needs an effective mean to remove wastes in order for metabolic processes to occur [5]. In order to fulfill these requirements, tumor cells can establish their own blood supply and this ability of tumors to promote the formation of new capillaries from the pre-existing ones is called the angiogenic switch. Tumor induced blood vessels are abnormal and leaky in nature. Since, the leaky vasculature of tumor blood vessels provides an efficient route of exit for tumor cells to leave the primary site and enter the main circulation, angiogenesis, thus has been considered as the critical component of metastasis [6].

3. Imaging of tumor angiogenesis

Angiogenesis is considered as an important therapeutic target in both cardiovascular and malignant diseases [7]. In cardiovascular diseases, the therapeutic goal is to perfuse the ischemic tissue and to promote recovery from ischemic injury by inducing angiogenesis [8, 9]. On the other hand, in tumor angiogenesis, the angiogenic therapeutic approach is based on inhibiting angiogenesis, which is responsible for tumor growth and metastasis [10]. This fact has led to an increased search for new anti-angiogenic molecules and to design targeted anti-angiogenic strategies for cancer treatment and the prevention of cancer recurrence or metastasis [11, 12]. Further, with the increasing use of anti-angiogenic drugs in the field of oncology,
the importance of imaging the angiogenic process has also increased. Various candidates for diagnosing angiogenesis include vascular endothelial growth factor (VEGF), circulating endothelial progenitor cells and biopsy specimens.

Though, biopsy specimens are extremely rich in information but they suffer a limitation from sampling bias and inherent invasiveness. The traditional gold standard measure of angiogenesis is the histological estimate of microvascular density (MVD) which quantifies the average number of micro vessels within a selected microscopic field. However, this method is invasive and will vary according to the location from which the biopsy is taken and may cause under or overestimation of the degree of angiogenesis.

Thus, the development of non-invasive imaging technique, specifically targeting angiogenesis among other biological processes would provide a better understanding of this important process and would also enable the evaluation of anti-angiogenic effect of new drugs administered as adjuvant therapy to reduce tumor growth.

4. Imaging techniques

Certain imaging modalities like computed tomography (CT) angiography, high resolution magnetic resonance angiography and contrast enhanced ultrasound are available for structural visualization of microvasculature. Multi detector (MDCT) angiography has better spatial and temporal resolution and is considered as the most useful modality in evaluating vascular structures [13]. The spatial resolution of MDCT is about 1.0 mm and the size of a human capillary is about 7.0–10.0 μm. Thus, MDCT can still not adequately visualize microvasculature [14]. Several molecules like growth factor receptors, tyrosine kinase receptors and G-protein coupled receptors can be used as specific targets for angiogenesis imaging [7].

Radionuclide based imaging techniques like single photon emission computed tomography (SPECT) and PET can offer accurate degree of disease quantification (progression/regression) in view of the high sensitivity of these imaging modalities [15]. In PET imaging, the annihilation of an electron-positron pair gives rise to two 511 KeV gamma ray photons being emitted at 180°. These high energy photons are then detected by a detector ring which is made up of crystals like bismuth germanium oxide (BGO), gadolinium oxyorthosilicate (GSO) or lutetium oxyorthosilicate (LSO).

Undoubtedly, the success of such this modality lies with the development of specific molecular imaging probes for the accurate diagnosis of angiogenesis and for identifying the responders/non-responders at an early stage after the initiation of treatment [7]. Over the last decade, PET imaging has emerged as a very powerful imaging technique for use in the direct imaging of angiogenesis in animal (eventually human) models, an early diagnosis, complete staging and early treatment response assessment [16]. The ability of PET imaging to detect picomolar concentration of tracer accumulation makes it several times more sensitive technique than the SPECT imaging [17]. PET imaging not only enables in vivo visualization of physiological processes at molecular level but also helps in its quantification. PET may be considered as a
functional or a targeted imaging modality. The staging can be done more accurately using PET when compared to any other conventional diagnostic means and thus it might be used independently as the first diagnostic choice [18].

5. Role of dendrimers in angiogenesis imaging

Most of the low molecular weight anticancer drugs have a characteristic to move in and out of blood vessels freely, unless the drug is linked with a tumor specific molecular ligand like anti-VEGF antibodies or receptor-specific ligands having high binding constant [19]. However, despite their high selectivity, the slow clearance rate of antibodies limits their clinical application. Recent studies have revealed that tumor cells have diverse epitopes because of great magnitude of mutation frequency even among the same cancer patient. Thus, the specific antibodies and ligands show inefficient binding with each of the diverse epitopic targets [20, 21]. In order to overcome this problem, the phenomenon of enhanced permeability and retention effect (EPR effect) is now becoming the gold standard in cancer targeting drug designing. The EPR effect is based on macromolecular, polymeric and micellar particles including nanoparticles [22]. The leaky blood vessels of tumors enables the molecules of size greater than 40 kDa to escape out of the tumor blood vessels and accumulate into the tumor tissues whereas this EPR effect is not present in normal tissues [23]. Thus, this unique phenomenon of EPR effect is being exploited increasingly for anticancer drug development.

The concept of nanomedicine has been used extensively to develop biocompatible products for targeted drug delivery and sustained drug release at the targeted sites [24]. Nanomedicine is an emerging field that deals with interactions between molecules, cells and engineered substances like molecular fragments, atoms and molecules They have a high available surface area per unit of volume and they can be engineered to have different sizes, shapes and chemical compositions, hollow or solid structures [25]. Nanodelivery systems are believed to allow for more specific targeting. Nanotechnology products like fullerenes or dendrimers, macromolecular, micellar and polymeric particles have the ability to exhibit EPR effect for targeted therapeutic/drug delivery approach [22, 24].

Dendrimers are highly branched; 3-dimensional polymeric structures which are usually classified by the number of repeated branching cycles formed during synthesis and are reported to have an emerging role in a variety of biomedical applications [26]. They can be used as biomimetic catalysts, drug carriers, gene delivery and can also be used in boron neutron capture therapy [27–30]. The dendrimers are considered potentially advantageous due to their numerous surface functional groups, relatively low immunogenicity and also their size, which is very close to various important biological polymers. Lower generation dendrimers are asymmetric in nature and are considered as more open structures when compared to dendrimers of higher generation. With the increasing generation, dendrimers acquire a globular structure [31] and forms a closed membrane like structure due to the dense packing of branches. Dendrimers are monodisperse molecules and their solubility is influenced by the nature of functional groups present on the surface of dendrimers, that is, dendrimers with hydrophilic
terminal groups are soluble in polar solvents whereas dendrimers possessing hydrophobic end groups are soluble in non-polar solvents [32]. The need to study the biological properties of dendrimers is very important because of their increasing use in biomedical applications. The molecular dimensions of dendrimers are comparable to medium sized proteins [33].

Though, dendrimers have been extensively used as magnetic resonance imaging (MRI) contrast agents and drug delivery carriers, however, the complex of radionuclides with polyamidoamine (PAMAM) dendrimers in order to perform molecular imaging of tumor angiogenesis is a new field. In the past, dendrimers have been extensively studied as prospective carriers for drug delivery, gene delivery and moieties for modifying the drug solubility and absorption [34, 35]. The geometry of the molecule and the charges present on the surface of dendrimers influence the microvascular extravasation of polymers across the endothelial barrier.

EPR effect has been widely used for passive targeting of macromolecular anticancer agents to angiogenic solid tumors. Dendrimers with hydrophilic surfaces and molecular weights above 25–30 kDa are usually retained in the circulation for longer periods and provide an enhanced opportunity for passive targeting via EPR effect.

Many studies describing the process of dendrimer-chelator conjugation and subsequent complexation with metal ions and the potential use of dendrimers as probes in MRI and fluorescent imaging have been cited previously [36, 37]. However, studies with regard to radiolabeling of these dendrimer-chelator conjugates as PET imaging probes are very few.

6. Radionuclides used for molecular imaging of dendrimers

More than 80.0% of the radiopharmaceuticals used in nuclear medicine imaging are technetium –99 m ($^{99m}$Tc) based tracers. However, a dramatic shift toward the development of PET-based novel tracers using fluorine-18 ($^{18}$F), carbon-11 ($^{11}$C), gallium-68 ($^{68}$Ga) and copper 64 ($^{64}$Cu) positron emitters have been witnessed over the last few years. Majority of these radiopharmaceuticals are administered intravenously. Radiopharmaceuticals can be divided into diagnostics and therapeutics depending on their medical applications. Diagnostic radiopharmaceuticals are predominantly metal complexes with an organic chelator for metal-essential agents or a chelator-biomolecule conjugate for target-specific radiopharmaceuticals. In general, a target-specific radiopharmaceutical can be divided into four parts: targeting biomolecule (BM), pharmacokinetic modifying (PKM) linker, bifunctional coupling or chelating agent (BFC) and radionuclide.

7. Bifunctional chelating agents (BFC)

Chelators used for labeling with PET radionuclides are usually dominated by polydentate chelators like 1,4,7-triazacyclononane-triacetic acid (NOTA), 1,4,7,10-tetraazacyclododecane-tetraacetic acid (DOTA), diethylenetriaminepentaacetic acid (DTPA), DTPA-monoamide and 4-(4,7-bis(2-(tert-butoxy)-2-oxoethyl)-1,4,7-triazacyclononan-1-yl)-5-(tert-butoxy)-
5-oxopentanoic acid (NODA-GA (tBu)₃) etc. These chelators are also called as “bifunctional chelating agents” as they possess a metal binding moiety as well as a chemically reactive functional group. The radionuclide of interest can bind to the metal binding moiety and the chemically reactive functional group provides the requisite chemistry for covalent attachment to the targeting vector/carrier of interest like proteins [38], peptides [39] or nanoparticles. BFC is covalently attached to the targeting molecule and strongly coordinates the radio-metal. The design of BFC depends upon number of fundamental criteria. Foremost seems the metal complex stability followed by other coordination chemistry criteria such as charge, chelator cavity size compatibility with the ionic radius of the radionuclide, chelate denticity and availability of donor binding groups of appropriate chemical character. Two additional properties are also critical to consider: the rate at which the metal complex forms and the rate of dissociation. All of these criteria are interrelated. Cavity size must accommodate the ionic radius of the radionuclide such that all of required donor groups can be properly aligned for optimal binding to the metal ion in such a way to adequately encapsulate the ion thereby providing high stability and limiting dissociation. The suitable radio-metals are diverse in their properties and coordination chemistry, so, unfortunately there is no bifunctional chelating agent suitable for all radionuclides. The selection of BFC depends upon the oxidation state of the radio-metal that makes it imperative to understand the coordination chemistry of chelators with any given radionuclide to be labeled. Any BFC that forms a thermodynamically stable radio-metal chelate with high kinetic inertness is considered as an ideal BFC.

8. Chelating groups/techniques

There are several conjugation groups that can be used for the attachment of a BFC to the biomolecule like anhydride, isothiocyanate, bromoacetamide, iodoacetamide, N-hydroxysuccinimide (NHS) ester and maleimide. All of these conjugation groups are electrophiles, which require a nucleophile functionality in the biomolecule but in some cases, the biomolecule of interest contains groups like carboxylic acid only which are electrophilic in nature. In these cases, a nucleophile like ethylenediamine, is used to convert the electrophilic group into a nucleophilic group. These reactive groups, can serve as “linkers” for conjugation of a BFC. Selection of conjugation group depends largely on the “linker” in biomolecules. Very often the “linker” is a primary amine or a thiol group. The functional groups reactive toward primary amines include DTPA dianhydride, NHS-activated esters and isothiocyanates while maleimide is very reactive to thiols.

**DTPA anhydride** – DTPA dianhydride is commercially available and reacts readily with primary amines to form the DTPA-biomolecule conjugate in both aqueous and non-aqueous media [40, 41]. For small biomolecules, the cross-linking may result in the improved receptor binding kinetics and proves to be beneficial because simultaneous binding of two biomolecules on adjacent receptor sites will result in a slow dissociation of the receptor ligand. Asymmetric anhydrides of DTPA and DOTA have also been used to prepare their bio conjugates [42–44].
**NHS ester** – The NHS esters have intermediate reactivity toward amines and are highly selective for aliphatic amines at an optimum pH of 8.0–9.0 in aqueous systems. Molecules containing a carboxylic group can be converted into its NHS ester, making NHS-activated ester groups among the most powerful and the most commonly used conjugation groups for large (antibodies) and small biomolecules [45, 46].

**Isothiocyanates** – Isothiocyanates are also reactive to amine groups, and form thiourea bonds with primary amines from proteins or small biomolecules. They show intermediate reactivity toward amines at pH 9.0–9.5 in aqueous solutions and are more stable in water than NHS esters. Aromatic isothiocyanates are often used to conjugate biomolecules onto DTPA and DOTA analogs [45, 46].

**Maleimide** – Maleimide reacts with a thiol group at a pH of 7.0 and leads to the formation of a thioether bond [47]. Maleimides can hydrolyze at higher pH (>8.0) to form non-reactive maleimic acids. The only limitation of using maleimide as a conjugation group is that not many biomolecules contain thiol groups thereby limiting the use of maleimide as a chelator.

Radiolabeling of DTPA and DOTA-biomolecule conjugates – DTPA analogs have a major advantage of being used as BFCs as they can be radiolabeled with high labeling efficiency even under mild conditions, but the kinetic instability of these metal chelates results in dissociation of the radio-metal from the chelate.

DOTA analogs can be used as BFCs because if the kinetic inertness of their radio-metal chelates. However, the radiolabeling of DOTA chelates depends upon various factors like chelate concentration, pH, reaction temperature and incubation time, buffer concentration and presence of metal ions such as Zn (II) and Fe (III) [48–51]. In spite of the high solution stability of their radio-metal chelates, slow radiolabeling kinetics remains a major obstacle for the wide use of DOTA analogs as BFCs in target-specific radiopharmaceuticals. Coordination chemistry plays an imperative role in designing BFCs, radiolabeling, solution stability, modification of pharmacokinetics and formulation development.

### 9. Characterization and purification techniques

Certain mass spectroscopic techniques are utilized to determine the yields of bio-conjugation reactions. Liquid chromatography-mass spectrometry (LC–MS) technique is used for the characterization of dendrimers with mass below 3000 Da. Electrospray ionization-mass spectrometry (ESI-MS) is used for dendrimers which are able to form stable multiple charged species. Matrix assisted laser desorption ionization: time of flight mass spectrometry technique (MALDI-tof) is used to characterize the chelate dendrimer conjugates with high molecular weight. These mass spectrometric techniques are used to analyze and compare the mass spectra of unmodified dendrimers and dendrimer-chelate conjugates in order to confirm the degree of conjugation. The average number of chelate molecules conjugated at the surface of dendrimer molecule is calculated using the formula:
Number of DOTA molecules conjugated = \left[ \frac{\text{Increase in molecular weight relative to unmodified dendrimer}}{\text{Molecular weight of BFC}} \right]. \hspace{1cm} (1)

Conjugation of BFCs with dendrimer can also be confirmed by Fourier transform-infrared spectroscopy (FT-IR). Chromatography techniques like size exclusion chromatography (SEC) helps in the purification and separation of dendrimer-chelate conjugates from free BFC’s according to their sizes.

10. Dendrimer-chelate cytotoxicity

“Cationic” dendrimers (e.g., amine terminated PAMAM dendrimers) are generally hemolytic and cytotoxic [52]. The toxicity of dendrimers depends upon the generation number and increases with the increasing number of surface groups. Anionic dendrimers that bear carboxylate surface groups are not cytotoxic even at a broad concentration range [53]. Amine terminated PAMAM dendrimers are believed to have been showing more cytotoxicity because of the interaction between positively charged dendrimers and the negatively charged cell membranes [54]. Thus, the cytotoxicity of these cationic dendrimers can be decreased by either shielding or decreasing the positive charge on their surface. Thus, the positively charged groups are usually capped with neutral molecules [55, 56]. Similarly, the surface amino groups/carboxylate groups of dendrimers are modified with BFCs resulting in a significant decrease in the number of positive charges on their surface. The toxicity of dendrimers on cells is concentration, time and generation dependent. Previous studies have demonstrated that the dendrimers with surface modifications are less toxic and more biocompatible when compared with the unmodified dendrimers [52, 54].

11. Radiolabeling of dendrimer-chelate conjugates

The radiolabeling of the purified and characterized dendrimer-chelate conjugates depends upon various factors and needs to be standardized. The ability of these bifunctional chelators to coordinate with a variety of metals makes them more sensitive to metallic impurities and contaminants en route the reaction process. Also, considerable attention has to be given for optimizing the reaction conditions so as to achieve best results for all conjugation and radiolabeling experiments [57]. Factors like buffer pH, buffer volume, concentration of conjugate and incubation time has to be optimized in order to achieve best radiolabeling efficiency. The lead for setting up the range of test conditions for optimizing the radiolabeling of dendrimers with different radio-metals was taken from the previous studies [58].

12. \textsuperscript{68}Ga containing dendrimers for PET imaging

More than 80.0% of the radiopharmaceuticals used in nuclear medicine imaging are \textsuperscript{99m}Tc based tracers. Among, several PET radionuclides, there has been a renewed interest in \textsuperscript{68}Ga for many
reasons. $^{68}$Ga is well suited for use as a radiolabel for PET because of its comparatively shorter half-life of 68 min. The emission of two divergent photons per decay allows the construction of three-dimensional images. Also, the advances in generator technology for $^{68}$Ga production, favorable chemistry of $^{68}$Ga for radio-complexation have paved the way for emerging applications of $^{68}$Ga radiopharmaceuticals [59]. The most stable oxidation state of gallium in aqueous solution is +3 and its coordination number is 6. The coordination chemistry of Ga$^{3+}$ is very similar to high spin Fe$^{3+}$ ion. Both the ions have oxidation state of +3 and have almost same ionic radii (62 pm for Ga$^{3+}$ and 65 pm for Fe$^{3+}$). Ga(III) can undergo ligand exchange with protein transferrin when injected into the biological system. Transferrin contains two iron binding sites with high affinity for this metal ion. At physiological conditions, the human transferrin has a high binding affinity for Ga$^{3+}$ given by log $K_{ST} = 20.3$ [60]. Thus, radiolabeling of $^{68}$Ga is best achieved by using bifunctional chelators which can strongly chelate the gallium ion and are covalently bound to targeting vectors [61, 62]. The most widely used bifunctional chelator for $^{68}$Ga radionuclide labeling is DOTA [63]. Ga(DOTA) complex is stable enough to be used in clinical practice. These macrocyclic chelators display high conformational and size selectivity toward metal ions. This category of chelators can encapsulate the metal ions with high efficiency keeping it away from the competing species like blood transferrin [64].

The pH of buffer plays an important role in radiolabeling procedures especially with $^{68}$Ga. In aqueous solution, free hydrated gallium, that is, [Ga(H$_2$O)$_6$]$^{3+}$ is stable under acidic conditions (pH < 3). At slightly higher pH, the aqueous solution chemistry is determined by the hydrolysis of the aqua ion leading to the formation of insoluble trihydroxide, that is, Ga(OH)$_3$. At physiological pH, the solubility of gallium is high due to the exclusive formation of [Ga(OH)$_4$]$^{4-}$ ions [61]. Formation of Ga(OH)$_3$ due to the hydrolysis can be avoided by using stabilizing weak ligands like acetate, citrate or HEPES as conjugating as well as radiolabeling buffers. For use as a radiopharmaceutical, a gallium compound must be either thermodynamically stable toward hydrolysis at physiological pH or be kinetically stable in the time frame of an imaging procedure. The reaction kinetics for the incorporation of Ga$^{3+}$ is inversely related to pH [65]. The complexation of Ga$^{3+}$ by DOTA shows slow kinetics because of its cavity size and eight donor atoms. Therefore, the radiolabeling procedures were carried at an elevated temperature of 90–100°C.

13. Quality control

ITLC – The radiolabeling efficiency is estimated chromatographically using ITLC-silica gel strips as the stationary phase and solvents such as ammonium acetate: methanol as mobile phase. The radiolabeled preparation is spotted at the origin of ITLC strips, dried and introduced into the solvent chamber containing mobile solvent. The mobile solvent is allowed to reach the top of the ITLC strip; the strip is removed, dried in air, cut into two halves and measured for its radioactivity in order to calculate its radiolabeling efficiency. The retention factor (R$_f$) can also be calculated, the strips are marked from origin and divided into 10 equal sections each of 1.0 cm. The strips are cut, put into the test tubes and counts can be recorded in sodium iodide (NaI) well counter. The observed counts from each segment can be plotted as a linear graph and the R$_f$ value for radiolabeled dendrimers can be evaluated.
**In vitro stability assay** – The *in vitro* stability of the radiolabeled formulation can be determined by radio-chromatography using ITLC. The radiolabeling efficiency of the formulation is calculated at various time intervals. This assay depicts the pattern of degradation of radiolabeled formulation as a function of time.

**In vitro serum stability assay** – The stability of radiolabeled dendrimers in systemic circulation is usually evaluated by performing serum stability assay. The radiolabeled formulation is measured for any degree of degradation, if any due to enzymatic or other factors present in the blood/serum.

**Lipophilicity assay** – Affinity of radiolabeled formulation toward the organic phase (octanol) and aqueous phase, phosphate buffered saline (PBS) can be determined by calculating the organic/aqueous partition coefficient. Log P value is considered as the measure of lipophilicity and can be calculated by using the formula:

\[
\text{Log } P = \log \left( \frac{\text{Counts in organic phase (octanol)}}{\text{Counts in aqueous phase (PBS)}} \right)
\]  

(2)

14. Biodistribution and dosimetry studies

It is important to perform biodistribution and dosimetry studies with any new radiopharmaceutical to study the dose absorbed by various “critical organs” and also to study the pharmacokinetics of these newer tracers [66]. The documentation of pharmacokinetics and dosimetry data and submission of the same to the ‘regulatory authorities’ provide a robust evidence for seeking permission to carry out first ‘human studies’ and thus, has a translational relevance. Whenever new or experimental radiopharmaceuticals are administered to patients, it becomes mandatory to get information on the patient’s radiation exposure by performing a dosimetry study. The procedure used to assess the organ’s absorbed doses has been summarized in MIRD pamphlet number 21 [67] and includes two major steps. Firstly, the quantification of the time integrated activity for each tissue localizing the radiopharmaceutical and secondly, determination of the S values, that is, the absorbed dose to target tissues per decay, in each source tissue were carried out. The S values for a radionuclide as required for the internal dosimetry must be based upon the internationally accepted reference anatomic phantoms as defined by the International Commission on Radiological Protection [68]. Further, the effective dose can be calculated by using the tissue weighing factors and the absorbed dose values to the organs. The critical organs of interest in humans for the use of a new radiopharmaceutical clinically are generally evaluated in preclinical studies in rodents and other mammalian species. However, estimates derived from the animal studies are usually considered sufficient for the purpose of grant of regulatory permissions for human trials [69].

15. Molecular imaging with radiolabeled dendrimers

Dendrimers can be used to target the tumor vasculature by modifying their surface through covalent conjugation. Such a modification increases the targeting potential of
dendrimers toward cancer cells [70]. A combination of imaging modalities and several biocompatible and biodegradable dendrimers over the decade has been used to develop bio-imaging probes that have prolonged plasma half-lives, enhanced stability, reduced toxicity and improved target specificity. However, the application of dendrimers in nuclear medicine and radiochemistry is still at its infancy. With the escalating knowledge of science and research in the field of oncology, the development of new drug delivery systems has attained great heights. It is believed that rapid technological and scientific progresses in the development of bio-imaging dendrimers and their role as drug delivery agents will provide new research opportunities for use of dendrimers in the preclinical and clinical development of new therapies. Due to the 3-dimensional structure and presence of numerous functional groups on the surface, dendrimers have generated huge interest and attention as drug delivery systems. They provide a platform for attaching drugs or genes and further releasing them through several mechanisms which include either in vivo degradation of drug dendrimer covalent bonding due to the presence of certain enzymes or drug release due to changes in physical environment such as pH and temperature.

16. Conclusion

The high loading capacity of dendrimers enables them to deliver chemotherapeutic agents as well. The dendrimer-drug complexes designed for either targeted or non-targeted drug delivery successfully penetrate through the leaky vasculature of tumor and accumulate in the cancer tissue. However, the added advantage of using dendrimer-drug complexes specifically designed for targeted delivery is that they bind to specific receptors overexpressed on the surface of cancer cells, thereby, increasing their residence time over the cell surface. Thus, the development of dendrimer complexes that incorporates the targeting ligands, anticancer drugs and PET/β emitting radionuclides may provide the way for combinational anticancer therapies along with the in vivo imaging of the targeted tumor.

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References

[1] Greenblatt M, Shubi P. Tumor angiogenesis: Transfilter diffusion studies in the hamster by the transparent chamber technique. Journal of the National Cancer Institute. 1968;41:111-124

[2] Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. Seminars in Cancer Biology. 2009;19:329-337

[3] Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nature Medicine. 1995;1:27-31

[4] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57-70

[5] Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. Journal of Clinical Oncology. 2005;23:1011-1027

[6] Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. The American Journal of Pathology. 1995;146:1029-1039

[7] Lee HY, Jeong JM. Multimodality imaging of angiogenesis. The Open Nuclear Medicine Journal. 2010;2:166-170

[8] Stegmann TJ. New approaches to coronary heart disease: Induction of neovascularization by growth factors. BioDrugs. 1999;11:301-308

[9] Deveza L, Choi J, Yang F. Therapeutic angiogenesis for treating cardiovascular diseases. Theranostics. 2012;2:801-814

[10] Brown JM, Giaccia AJ. The unique physiology of solid tumors: Opportunities (and problems) for cancer therapy. Cancer Research. 1998;58:1408-1416

[11] Kerbel R, Folkman J. Clinical translation of angiogenesis inhibitors. Nature Reviews. Cancer. 2002;2:727-739

[12] Cai W, Chen X. Anti-angiogenic cancer therapy based on integrin alphavbeta3 antagonism. Anti-Cancer Agents in Medicinal Chemistry. 2006;6:407-428

[13] Ippolito D, Sironi S, Pozzi M, et al. Hepatocellular carcinoma in cirrhotic liver disease: Functional computed tomography with perfusion imaging in the assessment of tumor vascularization. Academic Radiology. 2008;15:919-927

[14] Engelke C, Schaefer-Prokop C, Schirg E, Freihorst J, Grubnic S, Prokop M. High-resolution CT and CT angiography of peripheral pulmonary vascular disorders. Radiographics. 2002;22:739-764

[15] Miller JC, Pien HH, Sahani D, Sorensen AG, Thrall JH. Imaging angiogenesis: Applications and potential for drug development. Journal of the National Cancer Institute. 2005;97:172-187

References
[1] Greenblatt M, Shubi P. Tumor angiogenesis: Transfilter diffusion studies in the hamster by the transparent chamber technique. Journal of the National Cancer Institute. 1968;41:111-124
[2] Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. Seminars in Cancer Biology. 2009;19:329-337
[3] Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nature Medicine. 1995;1:27-31
[4] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57-70
[5] Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. Journal of Clinical Oncology. 2005;23:1011-1027
[6] Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. The American Journal of Pathology. 1995;146:1029-1039
[7] Lee HY, Jeong JM. Multimodality imaging of angiogenesis. The Open Nuclear Medicine Journal. 2010;2:166-170
[8] Stegmann TJ. New approaches to coronary heart disease: Induction of neovascularization by growth factors. BioDrugs. 1999;11:301-308
[9] Deveza L, Choi J, Yang F. Therapeutic angiogenesis for treating cardiovascular diseases. Theranostics. 2012;2:801-814
[10] Brown JM, Giaccia AJ. The unique physiology of solid tumors: Opportunities (and problems) for cancer therapy. Cancer Research. 1998;58:1408-1416
[11] Kerbel R, Folkman J. Clinical translation of angiogenesis inhibitors. Nature Reviews. Cancer. 2002;2:727-739
[12] Cai W, Chen X. Anti-angiogenic cancer therapy based on integrin alphavbeta3 antagonism. Anti-Cancer Agents in Medicinal Chemistry. 2006;6:407-428
[13] Ippolito D, Sironi S, Pozzi M, et al. Hepatocellular carcinoma in cirrhotic liver disease: Functional computed tomography with perfusion imaging in the assessment of tumor vascularization. Academic Radiology. 2008;15:919-927
[14] Engelke C, Schaefer-Prokop C, Schirg E, Freihorst J, Grubnic S, Prokop M. High-resolution CT and CT angiography of peripheral pulmonary vascular disorders. Radiographics. 2002;22:739-764
[15] Miller JC, Pien HH, Sahani D, Sorensen AG, Thrall JH. Imaging angiogenesis: Applications and potential for drug development. Journal of the National Cancer Institute. 2005;97:172-187
[16] Townsend DW. Physical principles and technology of clinical PET imaging. Annals of the Academy of Medicine, Singapore. 2004;33:133-145

[17] Rahmim A, Zaidi H. PET versus SPECT: Strengths, limitations & challenges. Nuclear Medicine Communications. 2008;29:193-207

[18] Hicks RJ. Should positron emission tomography/computed tomography be the first rather than the last test performed in the assessment of cancer? Cancer Imaging. 2012; 12:315-323

[19] Maeda H, Bharate GY, Daruwalla J. Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect. European Journal of Pharmaceutics and Biopharmaceutics. 2009;71:409-419

[20] Tobias S, Jones S, Laura D, Wood D, Parsons W. The consensus coding sequences of human breast and colorectal cancers. Science. 2006;314:268-274

[21] Wood LD, Parsons DW, Jones S, et al. The genomic landscapes of human breast and colorectal cancers. Science. 2007;318:1108-1113

[22] Enhanced KG. Permeability and retention (EPR) effect for anticancer nanomedicine drug targeting. Methods in Molecular Biology. 2010;624:25-37

[23] Fang J, Nakamura H, Maeda H. The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. Advanced Drug Delivery Reviews. 2011;63:136-151

[24] Bharali DJ, Khalil M, Gurbuz M, Simone TM, Mousa SA. Nanoparticles and cancer therapy: A concise review with emphasis on dendrimers. International Journal of Nanomedicine. 2009;4:1-7

[25] Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nature Nanotechnology. 2007;2:751-760

[26] Tomalia DA, Fréchet J. Discovery of dendrimers and dendritic polymers: A brief historical perspective. Journal of Polymer Science Part A: Polymer Chemistry. 2002;40:2719-2728

[27] Huck WTS, Prins LJ, Fokkens RH, Nibbering NMM, van Veggel FCJM, Reinholdt DN. Convergent and divergent noncovalent synthesis of metallodendrimers. Journal of the American Chemical Society. 1998;120:6240-6246

[28] Peppas NA, Nagai T, Miyajima M. Prospects of using star polymers and dendrimers in drug delivery and other pharmaceutical application. Pharmaceutical Technology – Japan. 1994;10:611-617

[29] Bielinska AU, Kukowska-Latallo JF, Johnson J, Tomalia DA, Baker JR. Regulation of in vitro gene expression using antisense oligonucleotides or antisense expression plasmids transfected using starburst PAMAM dendrimers. Nucleic Acids Research. 1996;24:2176-2182
[30] Hawthorne MF. The role of chemistry in the development of boron neutron capture therapy of cancer. Angewandte Chemie International Edition. 1993;32:950-984

[31] Caminati G, Turro NJ, Tomalia DA. Photophysical investigation of starburst dendrimers and their interactions with anionic and cationic surfactants. Journal of the American Chemical Society. 1990;112:8515-8522

[32] Fréchet JM J. Functional polymers and dendrimers: Reactivity, molecular architecture. Science. 1994;263:1710-1715

[33] Baig TN J, Dwivedi V, Singh A, Srivastava A, Tripathi PK. A review about dendrimers: Synthesis, types, characterization and applications. International Journal of Advances in Pharmacy, Biology and Chemistry. 2015;4:2277-4688

[34] Dong ZQ, Katsumi H, Sakane T, Yamamoto A. Effects of polyamidoamine (PAMAM) dendrimers on the nasal absorption of poorly absorbable drugs in rats. International Journal of Pharmaceutics. 2010;393:244-252

[35] Borowska K, Laskowska B, Magon A, Mysliwiec B, Pyda M, Wołowiec S. PAMAM dendrimers as solubilizers and hosts for 8-methoxypsoralene enabling transdermal diffusion of the guest. International Journal of Pharmaceutics. 2010;398:185-189

[36] Talanov VS, Regino CAS, Kobayashi H, Bernardo M, Choyke PL, Brechbiel MW. Dendrimer-based nanoprobe for dual modality magnetic resonance and fluorescence imaging. Nano Letters. 2006;6:1459-1463

[37] Xu RZ, Wang YL, Wang XL, Jeong EK, Parker DL, Lu ZR. In vivo evaluation of a PAMAM-Cystamine-(Gd-D03A) conjugate as a biodegradable macromolecular MRI contrast agent. Experimental Biology and Medicine. 2007;232:1081-1089

[38] Chinn P, Braslavsky G, White C, Hanna N. Antibody therapy of non-Hodgkin’s B-cell lymphoma. Cancer Immunology, Immunotherapy. 2003;52:257-280

[39] Norenberg JP, Krenning BJ, Konings IRHM, et al. Bi-213-[DOTA(0),Tyr(3)]octreotide peptide receptor radionuclide therapy of pancreatic tumors in a preclinical animal model. Clinical Cancer Research. 2006;12:897-903

[40] Eisenwiener KP, Powell P, Macke HR. A convenient synthesis of novel bifunctional prochelators for coupling to bioactive peptides for radiometal labelling. Bioorganic & Medicinal Chemistry Letters. 2000;10:2133-2135

[41] Liu S, Edwards DS. Synthesis and characterization of two (111)In-labeled DTPA-peptide conjugates. Bioconjugate Chemistry. 2001;12:630-634

[42] Pippin CG, Parker TA, McMurry TJ, Brechbiel MW. Spectrophotometric method for the determination of a bifunctional DTPA ligand in DTPA-monoclonal antibody conjugates. Bioconjugate Chemistry. 1992;3:342-345
[43] Sherry AD, Brown RD, Geraldes CFG, Koenig SH, Kuan KT, Spiller M. Synthesis and characterization of the gadolinium (3+) complex of DOTA-propylamide – a model DOTA-protein conjugate. Inorganic Chemistry. 1989;28:620-622

[44] Sieving PF, Watson AD, Rocklage SM. Preparation and characterization of paramagnetic polychelates and their protein conjugates. Bioconjugate Chemistry. 1990;1:65-71

[45] Lewis MR, Raubitschek A, Shively JE. A facile, water-soluble method for modification of proteins with DOTA. Use of elevated temperature and optimized pH to achieve high specific activity and high chelate stability in radiolabeled immunoconjugates. Bioconjugate Chemistry. 1994;5:565-576

[46] Lewis MR, Shively JE. Maleimidocysteineamido-DOTA derivatives: New reagents for radiometal chelate conjugation to antibody sulfhydryl groups undergo pH-dependent cleavage reactions. Bioconjugate Chemistry. 1998;9:72-86

[47] McCall MJ, Diril H, Meares CF. Simplified method for conjugating macrocyclic bifunctional chelating agents to antibodies via 2-iminothiolane. Bioconjugate Chemistry. 1990;1:222-226

[48] Stimmel JB, Stockstill ME, Kull Jr FC. Yttrium-90 chelation properties of tetraazatetraacetic acid macrocycles, diethylenetriaminepentaacetic acid analogues, and a novel terpyridine acyclic chelator. Bioconjugate Chemistry. 1995;6:219-225

[49] Liu S, Ellars CE, Edwards DS. Ascorbic acid: Useful as a buffer agent and radiolytic stabilizer for metalloradiopharmaceuticals. Bioconjugate Chemistry. 2003;14:1052-1056

[50] Liu S, Cheung E, Ziegler MC, Rajopadhye M, Edwards DS. (90)Y and (177)Lu labeling of a DOTA-conjugated vitronectin receptor antagonist useful for tumor therapy. Bioconjugate Chemistry. 2001;12:559-568

[51] Liu S, Edwards DS. Stabilization of (90)Y-labeled DOTA-biomolecule conjugates using gentisic acid and ascorbic acid. Bioconjugate Chemistry. 2001;12:554-558

[52] Roberts JC. Preliminary biological evaluation of polyamidoamine (PAMAM) starburst dendrimers. Journal of Biomedical Materials Research. 1996;30:53-65

[53] Wolinsky JB, Grinstaff MW. Therapeutic and diagnostic applications of dendrimers for cancer treatment. Advanced Drug Delivery Reviews. 2008;60:1037-1055

[54] Malik N, Wiwattanapatapee R, Klopsch R, et al. Dendrimers: Relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of 125I-labelled polyamidoamine dendrimers in vivo. Journal of Controlled Release. 2000;65:133-148

[55] Hong SBA, Mecke A, et al. Interaction of poly(Amidoamine) dendrimers with supported lipid bilayers and cells: Hole formation and the relation to transport. Bioconjugate Chemistry. 2004;15:774-782
[56] Diall MS, Christie S, Swaminathan P, Balogh L, Shi X, Goddard WA. Dendritic chelating agents 1 cu (II) binding to ethylene diamine core 147 poly(amidoamine) dendrimers in aqueous solutions. Langmuir. 2004;20:2640-2651

[57] Pellegrini PA, Howell NR, Shepherd RK, et al. Synthesis and Radiolabelling of DOTA-linked glutamine analogues with Ga-67,Ga-68 as markers for increased glutamine metabolism in tumour cells. Molecules. 2013;18:7160-7178

[58] Bricova V, Laznickova A, Laznicek M, Polasek M, Hermann P. Radiolabeling of PAMAM dendrimers conjugated to a pyridine-N-oxide DOTA analog with 111In: Optimization of reaction conditions and biodistribution. Journal of Pharmaceutical and Biomedical Analysis. 2011;56:505-512

[59] Ghai A, Singh B, Hazari PP, Schultz MK, Parmar A, Kumar P, et al. Radiolabeling optimization and characterization of [68Ga] labeled DOTA–polyamido-amine dendrimer conjugate -animal biodistribution and PET imaging results. Applied Radiation and Isotopes. 2015;105:40-46

[60] Harris WR. Thermodynamic binding constants of the zinc human-serum transferrin complex. Biochemistry. 1983;22:3920-3926

[61] Fani M, Andre JP, Maecke HR. Ga-68-PET: A powerful generator-based alternative to cyclotron-based PET radiopharmaceuticals. Contrast Media & Molecular Imaging. 2008;3:53-63

[62] de Sa A, Matias AA, Prata MIM, Geraldes CFGC, Ferreira PMT, Andre JP. Gallium labeled NOTA-based conjugates for peptide receptor-mediated medical imaging. Bioorganic & Medicinal Chemistry Letters. 2010;20:7345-7348

[63] Roivainen A, Tolvanen T, Salomaki S, et al. Ga-68-labeled oligonucleotides for in vivo imaging with PET. Journal of Nuclear Medicine. 2004;45:347-355

[64] Harris WR, Pecoraro VL. Thermodynamic binding constants for gallium transferrin. Biochemistry. 1983;22:292-299

[65] Bartholoma MD, Louie AS, Valliant JF, Zubieta J. Technetium and gallium derived radiopharmaceuticals: Comparing and contrasting the chemistry of two important radiometals for the molecular imaging era. Chemical Reviews. 2010;110:2903-2920

[66] IAEA. Role of internal dosimetry in nuclear medicine. Report of a Consultancy Meeting [WWWDocument] Available from: /http://nucleus.iaea.Org/HHW/MedicalPhysics/NuclearMedicine/InternalDosimetry/consultantsreport/Report_of_CM_Internal_Dosimetry.pdf. 2012

[67] Bolch WE, Eckerman KF, Sgouros G, Thomas SR. MIRD pamphlet No. 21: A generalized schema for radiopharmaceutical dosimetry-standardization of nomenclature. Journal of Nuclear Medicine. 2009;50:477-484

[68] Kaul A. Radiation dose to patients from radiopharmaceuticals: Addendum 3 to ICRP Publication 53. Ann ICRP. 2008;38
[69] Kesner ALHW, Czernin J, Padgett H, Phelps ME, Silverman DH. Radiation dose estimates for [18F]5-fluorouracil derived from PET-based and tissue-based methods in rats. Molecular Imaging and Biology. 2008;10:341-348

[70] Banerjee D, Harfouche R, Sengupta S. Nanotechnology-mediated targeting of tumor angiogenesis. Vascular Cell. 2011;3:3
