Applied Radiation and Isotopes 105 (2015) 40–46

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

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

Radiolabeling optimization and characterization of ^{68}Ga labeled DOTA–polyamido-amine dendrimer conjugate – Animal biodistribution and PET imaging results

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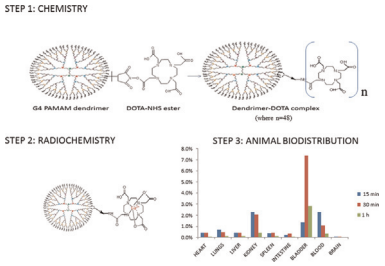
HIGHLIGHTS

- Chemical conjugation of G-4 PAMAM dendrimers with DOTA-NHS carried out successfully.
- Purification and characterization of the conjugate was done by SEC and MALDI-TOF.
- Radiolabeling of PAMAM-DOTA-conjugate with 68Ga yielded high radiolabeling efficiency.
- [^{68}Ga] DOTA–PAMAM-Dhas rapid blood clearance and excreted mainly through the kidneys.
- No significant retention of the radiotracer was seen in any other organ.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 April 2015
Received in revised form
21 July 2015
Accepted 21 July 2015
Available online 22 July 2015

Keywords:
Gallium-68 complexes
PAMAM conjugates
DOTA-NHS
Radiolabeling
Nanoparticles
Angiogenesis

ABSTRACT

The present study describes the optimization of ^{68}Ga radiolabeling with PAMAM dendrimer–DOTA conjugate. A conjugate (PAMAM–DOTA) concentration of 11.69 μM, provided best radiolabeling efficiency of more than 93.0% at pH 4.0, incubation time of 30.0 min and reaction temperature ranging between 90 and 100 °C. The decay corrected radiochemical yield was found to be 79.4 ± 0.01%. The radiolabeled preparation ([^{68}Ga]-DOTA–PAMAM-D) remained stable (radiolabeling efficiency of 96.0%) at room temperature and in serum for up to 4-h. The plasma protein binding was observed to be 21.0%. After intravenous administration, 50.0% of the tracer cleared from the blood circulation by 30-min and less than 1.0% of the injected activity remained in blood by 1.0 h. The animal biodistribution studies demonstrated that the tracer excretes through the kidneys and about 0.33% of the %ID/g accumulated in the tumor at 1 h post injection. The animal organ's biodistribution data was supported by animal PET imaging showing good 'non-specific' tracer uptake in tumor and excretion is primarily through kidneys.

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http://dx.doi.org/10.1016/j.apradiso.2015.07.021

Additionally, DOTA–PAMAM-D conjugation with $\alpha_V\beta_3$ receptors targeting peptides and drug loading on the dendrimers may improve the specificity of the ^{68}Ga labeled product for imaging and treating angiogenesis respectively.

1. Introduction

Dendrimers are highly branched, 3-dimensional polymeric structures which and are often 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 (Tomalia and Frechet, 2002). These applications range from their use as biomimetic catalysts, drug carriers, gene delivery and boron neutron capture therapy (Huck et al., 1998; Peppas et al., 1994; Bielinska et al., 1996; Hawthorne, 1993). The dendrimer platform is considered potentially advantageous due to their relatively low immunogenicity, numerous surface functional groups and also their size, which is very close to various important biological polymers (Holister et al., 2010). Of the multitude of dendrimers that have been examined for biomedical applications, polyamidoamine (PAMAM) is the family of dendrimers that has been most extensively studied for their biomedical applications. These compounds are synthesized with polyamide branches functionalized with tertiary amines as focal points and an ethylene diamine core (Hawker and Frechet, 1990).

The current approach of linking the drugs with tumor specific molecular ligands like antibodies or receptor-specific ligands/peptides has inherent problem which lead to an inefficient binding due to the presence (of mutations) of diverse epitopic targets (Tobias et al., 2006; Wood et al., 2007). The concept of an enhanced permeability and retention (EPR) effect is now becoming the 'gold standard approach' for cancer targeting and drug designing. A wide range of nanotechnology products like fullerenes or dendrimers, macromolecular, polymeric and micellar particles including nanoparticles can exhibit 'EPR' effect for targeted therapeutic/drug delivery approach (Bharali et al., 2009, Greish, 2010). PAMAM dendrimers due to their enhanced permeability and retention properties may be used effectively both for drug loading and precise delivery to the angiogenic sites.

PAMAM dendrimers associated with 36 mer-anionic oligomers for delivering angiostatin and TIMP-2 genes have been reported to inhibit both tumor proliferation and angiogenesis (Vincet et al., 2003). The gadolinium complexes of PAMAM dendrimers have been frequently studied as MRI contrast agents (Nwe et al., 2010; Bumb et al., 2010). However, complex of radio-metals with dendrimers in order to perform molecular imaging of angiogenesis has not been attempted.

The ability of PET imaging to detect higher percentage of positron emissions makes it two to three times more sensitive technique than the SPECT imaging (Rahmim and Zaidi, 2008). Of the radionuclides used in the clinical practice for PET, the use of Gallium-68 (half-life $t_{1/2}$=68-min; positron emission intensity 87%) is on the rise (Schultz et al., 2013b). Several identifiable properties of this radionuclide include superior image quality compared to SPECT radionuclides (*e.g.*, indium-111), the potential for an on-demand production *via* generator technologies that provide reliable and high-purity ^{68}Ga in sufficient quantities for routine radiopharmaceutical production without the need for cyclotron operations (Buchmann et al., 2007; Roesch, 2012). Generator technologies for ^{68}Ga production, chemistry of gallium, and emerging applications for ^{68}Ga radiopharmaceuticals have been reviewed in detail (Prata, 2012; Roesch, 2012). These physiochemical properties provide a strong basis to use PAMAM dendrimers with ^{68}Ga as a future PET tracer for imaging angiogenesis.

In the present study, tetraazacyclododecane tetraacetic acid mono (N-hydroxysuccinimide ester) (DOTA-NHS active ester) was conjugated to G4 PAMAM dendrimers. Purification and characterization of the conjugate was achieved and radiolabeling with ^{68}Ga was optimized. ^{68}Ga labeled product was subjected to preclinical evaluation through *in vitro* testing, cell toxicity, animal biodistribution and imaging studies.

2. Material and methods

2.1. Reagents

Reagents and chemicals of analytical grade used in the study were obtained commercially. Deionized ammonium acetate (1.0 M): methanol (1:1) was used as a mobile solvent for performing instant thin layer chromatography (ITLC). Sodium acetate trihydrate (Sigma-Aldrich, USA) was dissolved in 100.0 mL of water (Deionized Milli-Q) to prepare 0.2 M sodium acetate (NaOAc). To prepare buffer of the desired pH of 4.0, 9.0 mL of NaOAc (0.2 M) was mixed with 41.0 mL of 0.2 M acetic acid (HOAc). For cytotoxicity studies (MTT assay), the various reagents used in the study *e.g.* trypsin (0.25%, EDTA, 1.0 mM in PBS), -(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (50.0 mg/ml, filter sterilized) and dimethyl sulphoxide (DMSO) were procured from Sigma-Aldrich (USA). DOTA-NHS ester (M_w=761.48 g/mole) and G4 PAMAM dendrimer (M_w=14,215 g/mole) used in the present study were procured from Chematech, France and Sigma-Aldrich, USA respectively.

Matrix-assisted laser desorption/ionization (MALDI) (Ultraflexi, Bruker system) analysis was used for characterization of the conjugate and 2, 5-dihydroxybenzoic acid (2, 5-DHB) was used as a matrix for this technique. Sephadex G-25 medium (Sigma-Aldrich, USA) was used for the purification of DOTA conjugated G4-PAMAM dendrimer. ^{68}Ga was eluted using 0.05 M hydrochloric acid (HCl) from ^{68}Ge–^{68}Ga generator (ITG, Munich, Germany). The starting activity for all the radiolabeling reactions was between 20.0 and 25.0 MBq. Animal studies were carried out in adult male balb/c mice. The mice were maintained in the Central Animal House facility, PGIMER, Chandigarh, India and the study protocol was approved by the Institutional Animal Ethics and Bio-safety committees.

3. Methods

3.1. Conjugation

10.0 mg of G4 PAMAM dendrimers (703.0 nmoles) was added to 1.0 mL of NaOAc buffer (pH: 8.5) in a round bottom flask. DOTA-NHS ester (8.5 mg, 11,248.0 nmoles) was added in the flask and pH was adjusted to 7.5–8.0 with 1.0 M sodium hydroxide (NaOH). The mixture was stirred at room temperature for 48-h. The dendrimer–DOTA chelate was purified in Sephadex® G-25 (medium) using water as an eluent. The fractions were collected, lyophilized and dissolved in sodium acetate buffer for radiolabeling. The conjugate was diluted to 2.0 mg/mL in 0.1% trifluoro-acetic acid (TFA) for matrix-assisted laser desorption ionization time of flight-