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Development of a prelabeling approach for a targeted nanochelator

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Abstract The polymer polyethyleneimine with primary and secondary chelators can retain ^{225}Ac and its daughters. In this study we optimize a prelabeling approach, followed by addition of secondary chelators and explore crosslinking approaches to add a targeting molecule. A (N-Succinimidyl 3-(2-pyridyldithio)-propionate crosslinking approach was used to obtain a ∼1 to 1 ratio of the modified PEI to Trastuzumab. This approach coupled with the prelabeling approach would allow the synthesis of the radiolabeled targeted polymer for ^{225}Ac radioimmunotherapy.

Keywords ^{225}Ac · ^{149}Pm · Prelabeling · PEI · Radioimmunotherapy · Polyethyleneimine

Introduction

One of the alpha emitting ^{225}Ac daughters; ^{213}Bismuth, has been shown to shrink tumors in environments where beta therapy with ^{90}Y and ^{177}Lu was unsuccessful [1]. The ^{225}Actinium (^{225}Ac) decay chain produces four alpha particles and can be used as an in vivo alpha generator for radiotherapy [2]. For a Phase one trial to treat patients with advanced myeloid malignances the estimated patient dose is

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0.5 μCi/Kg for ^{225}Ac-HuM195.[1] These properties of ^{225}Ac and the decay chain make it a superb therapeutic isotope. However, the inability to retain the daughters of ^{225}Ac by the target utilizing either traditional methods with a single chelator [3], or passive retention approaches with liposomes have resulted in radioactive toxicity to the kidney [4]. The use of chelating agents such as 2,3-dimercapto-1-propanesulfonic acid or meso-2,3-dimercaptosuccinic acid with either chlorothiazide or furosemide were able to reduce some of the ^{213}Bi kidney dose, but the approached did increase amount of ^{213}Bi in the blood [3]. The development of an approach that would retain the daughters of ^{225}Ac at the target will allow better patient outcomes for alpha radiotherapy and result in more wide spread use of ^{225}Ac.

A nanochelator approach has shown potential to retain the daughters of ^{225}Ac. The approach uses a polymer system containing a 60,000 MW polyethyleneimine (PEI) molecule, with a primary chelator (DOTA) for ^{225}Ac and secondary chelators containing acid groups to capture the daughters of the decay chain [5]. The approach retained 50 % or greater of the daughters at 6 days and utilized a post labeling strategy to make the ^{225}Ac labeled DOTA-PEI followed by addition of acid groups to generate the ^{225}Ac nanochelator. This system showed promise at retaining the daughters, but ^{225}Ac was lost over time which could be the result of the post labeling approach. When a post labeling approach was used with a DOTA-antibody conjugate the retention of ^{225}Ac was less than 50 % at 48 h and less than 30 % was retained at 96 h [6]. In the study less than 30 % of ^{213}Bi and ^{211}Tl were retained by the

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DOTA-antibody conjugate at all-time points. An ^{225}Ac DOTA complex has been shown to be stable leading to 90 % retention of the ^{225}Ac at 10 days [7]. For the study the researchers performed the labeling at 90 °C, and the high stability is likely the result of the ^{225}Ac moving into the cavity of the chelator.[2] The high temperature of the labeling is not suitable for some biotargeting molecules and the authors looked at developing a prelabeling approach where the ^{225}Ac would be labeled with DOTA-NCS and conjugated to the targeting molecule [7]. The stability of the NCS group of DOTA-NCS during the prelabeling procedure and conjugation ability of the ^{225}Ac DOTA-NCS was reduced resulting in only 9 % of the ^{225}Ac DOTA-NCS conjugated to the antibody [7].

Approximately 30 % of breast cancer patients have tumors that have amplification and over expression of the Her2/neu receptor [8]. The expression of the receptor is highly correlated with aggressive forms of the disease and expression of HER2 is similar between the primary tumor and the corresponding secondary metastases [9, 10] The humanized monoclonal antibody, Trastuzumab, has been used in combination with radiation and chemotherapy for treatment of Her2/neu positive breast cancer. Trastuzumab binds to the HER2 protein and modulates multiple signaling targets and pathways which lead to cell cycle G1 arrest and growth inhibition [11]. The expression of the HER2/neu receptor is an attractive target for alpha radiotherapy. An ^{225}Ac labeled molecule that had affinity for the receptor would deliver its radiotherapy to the primary tumor and the corresponding metastases.

In the first half of this paper we examine the conditions needed to complex a metal to DOTA-NCS and conjugate the metal complex to PEI. Since ^{225}Ac is in short supply ^{225}Ac, ^{149}Promethium (^{149}Pm) and ^{64}Copper (^{64}Cu) were used to develop the optimal prelabeling approach. The three isotopes share unique emission characteristics that can be used for both radiotherapy and imaging or dosimetry. ^{225}Ac has a half-life of 10 days, emits a gamma ray at 99 keV with an abundance of 1.01 %, and the decay chain is outline in Fig. 1. ^{149}Pm has a half life of 53.08 h emits a beta particle with a maximum energy of 1,071 keV and a gamma ray at 285.9 keV with an abundance of 3.1 %. The gamma rays of both ^{149}Pm and ^{225}Ac can be used to track the isotope in vivo for dosimetry. ^{64}Cu has a half-life of 12.7 h and emits a positron with an energy of 1,675 keV (61 %) and a beta particle with an maximum energy of 578.7 keV (39 %). The second part of the paper we examine the synthetic steps needed to make the targeted nanochelator. To obtain the maximum number of secondary chelators necessary for retention of the daughters of ^{225}Ac an approximate one to one
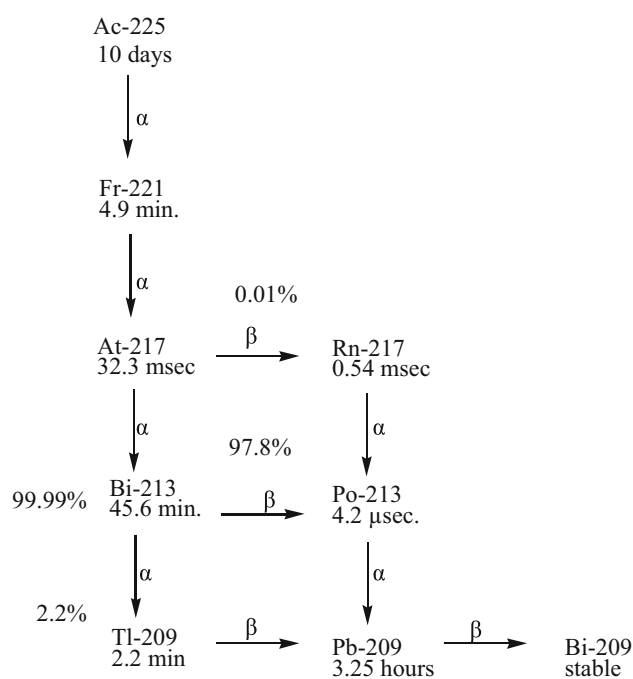
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Fig. 1 Decay scheme of ^{225}Ac

ratio of the targeting molecule to the PEI was desired in the final compound. Different synthetic approaches were explored to determine the appropriate method to synthesize Trastuzumab-PEI with PEI functionalized with acid groups and Oregon Green® 488.

Experimental

Materials

The University of Missouri Research Reactor (MURR), (Columbia, MO) provided 10 mCi of ^{149}Pm in 150 µL of 0.1 M HCl. ^{225}Ac was purchased from Isonics (Columbia, MD) and ^{64}Cu was purchased from Washington University in Saint Louis (St Louis, MO). S-2-(4-Isothiocyanatobenzyl)-1,4,7,10-tetraazacyclo-dodecane-tetraacetic acid (DOTA-NCS) was purchased from Macrocyclics (Dallas, TX). Econopack 10DG and Bio Gel 100 size exclusion columns were purchased from Bio-Rad (Bio-Rad Laboratories, Hercules, CA). Centricon 10 K centrifugal filter devices, isotemp economical dry bath incubator, Oakton Acorn pH meter, 2 mL microcentrifuge tubes and 45 µm Nalgene sterile analytical filter units were purchased form Fisher Scientific (Pittsburgh, PA). The centrifuge filter devices were centrifuged on setting 9 with a Fisher Centrific model 225 (Pittsburgh, PA). Polyethylenimine (PEI) was purchased from Acros with a MW = 60,000 in a 50 % solution of water, Oregon Green® 488 carboxylic acid succinimidyl

ester was purchased from Invitrogen Corporation Carlsbad, CA. Traut's reagent, DMF, (N-Succinimidyl 3-(2-pyridyldithio)-propionate (SPDP), Sulfosuccinimidyl 4-[N-maleimidomethyl] cyclohexane-1-carboxylate (Sulfo-SMCC), BupH phosphate buffered saline packs, Slide-A-Lyzer 10 K MWCO dialysis cassettes were purchased from Pierce (Rockford, IL). Trastuzumab was obtained from the hospital pharmacy at the University of New Mexico Health Sciences Center, Albuquerque, NM. All other reagents were purchased from Fisher Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO). All aqueous solutions were made with 18 M Ω water. The buffers were eluted from a column of Chelex 100, the pH measured, and then filtered. Radiolabeling was performed in acid washed 2 mL microcentrifuge tubes.

Prelabeling studies

General procedure prelabeling studies with ^{225}Ac

To a vial was added 0.09 mg of (1.29×10^{-7} mol) DOTA-NCS and the solid was dissolved with 0.060 mL of a 1 M HEPES buffer (pH 6.8). ^{225}Ac (0.030 mL) was added to the vial and the solution was incubated at 56 °C. A 0.030 mL portion of the Actinium labeled DOTA-NCS (4.30×10^{-8} mol) was removed, placed in a vial, 0.020 mL of 1×10^{-3} M DTPA was added and the vial was left at room temperature for 5 min. Then 0.045 mL of a 0.003 M stock solution of (1.35×10^{-8} mol) PEI was added followed by 0.405 mL of 1 M sodium carbonate. In all prelabeling studies reactions were setup with a 3 to 1 ratio of DOTA-NCS to PEI. The vial was capped and mixed. The activity in the 0.5 or 1 mL samples was determined with either an automated gamma counter or a high purity germanium detector. Then the samples were added to 10 KDa Centricon® centrifugal filter device, 1 mL of 10× PBS buffer was added and concentrated at 3,100 rpm. To the 0.3 mL sample was added 1 mL of 10× PBS and the concentration step was repeated for a total of 4 times. After purification the samples were diluted to the original volume and the activity was determined again.

Labeling time

Briefly, the general procedure was followed with DOTA-NCS (0.177 mg, 2.53×10^{-7} mol) 0.150 mL of HEPES buffer, and 0.030 mL ^{225}Ac. The sample was incubated at 56 °C and a 0.030 mL portion of the ^{225}AC-DOTA-NCS (4.2×10^{-8} mol) solution was removed at 5, 10, 20, 30, 45, and 60 min, added to DTPA and the conjugation was performed with sodium carbonate 1 M pH 8.9 for 1 h. The study was repeated with ^{149}Pm ($N = 3$).

pH of conjugation

To a vial was added 0.00321 g of DOTA-NCS (4.60×10^{-6} mol) which was dissolved in 0.54 mL of 1 M HEPES buffer (pH 6.8), then 0.020 mL of ^{149}Pm was added and the solution was heated at 55 °C for 45 min. To a second vial was added 0.03 mL of ^{149}Pm-DOTA-NCS (2.46×10^{-7} mol), 0.02 mL of a 1×10^{-3} M DTPA (2×10^{-8} mol) and the solution was incubated for 5 min. Next 0.045 mL of PEI (9.9×10^{-8} mol) and 0.405 mL of 1 M sodium carbonate (from pH 8.0–10.0) was added. This procedure was repeated with the remainder of the ^{149}Pm-DOTA-NCS solution, and a series of conjugation reactions were prepared in triplicate at pH values of 8.0, 8.5, 9.0, 9.5, and 10.0. The samples were incubated for 1 h and the general procedure described above was followed to purify the reaction. The sample was repeated with ^{225}Ac with the exception that the conjugation incubation time was either 105 or 180 min and the conjugation pH was either 8.0, 8.5, 9.0, 9.5, 10.0, 10.5. The experiment was repeated for ^{64}Cu with the exception that only 0.060 mL of 1 M sodium bicarbonate was used and the total volume during conjugation was 0.130 mL ($N = 3$).

Conjugation time and temperature

To a vial was added 0.00393 g of DOTA-NCS (5.64×10^{-6} mol), 0.075 mL of a 1 M HEPES buffer (pH = 6.8), 0.045 mL of ^{149}Pm and the solution was heated at 55 °C for 45 min. Then 0.005 mL of the ^{149}Pm-DOTA-NCS (2.4×10^{-7} mol) was removed and 0.02 mL of a 1×10^{-3} M DTPA (2×10^{-8} mol) was added and the solution was incubated for 5 min. Next 0.045 mL of PEI (9.9×10^{-8} mol) and 0.090 mL of a 1 M sodium bicarbonate (pH 8.5) was added. This procedure was repeated so a total of 18 reactions were setup. In nine of the reactions the conjugation temperature was 55 °C and the other nine were at room temperature. After a reaction time of 15 min, 0.84 mL of 10× PBS buffer was added to 3 reactions at rt and 3 reactions at 55 °C and this was repeated at 30 and 60 min. The samples were purified as described in the general method.

Synthesis of Trastuzumab linked PEI derivatives

Synthesis of PEI-Oregon Green® 488 (PEI-OG)

Oregon Green® 488 was conjugated to PEI according to published methods [12]. A ten to one mole ratio of Oregon Green® 488 carboxylic acid succinimidyl ester (3.008×10^{-6} mol) to PEI (3.41×10^{-7} mol) was used in the reaction. The reaction was purified with a PD-10 column with fractions 3–6 pooled and concentrated with a 10 KDa

Centricon® centrifugal filter device to 1 mL and UV absorbance at 496 nm was used to determine a ratio of Oregon Green® 488 to PEI.

Synthesis of PEI-OG containing the pyridyldithiol reactive group (SPDP PEI-OG)

PEI-OG was conjugated to SPDP according to published methods [13]. A two to one mole ratio of SPDP $(1.1584 \times 10^{-7}$ mol) to PEI-OG $(5.7 \times 10^{-8}$ mol) was used in the reaction. A PD-10 desalting column was used to purify the reaction and fractions 3–5 were pooled and spun to ~ 1.2 mL with a 10 KDa Centricon® centrifugal filter device. The level of SPDP modification to PEI-OG was quantify with DTT and the absorbance at 343 and 496 nm was measured before and 15 min after the addition of DTT. The molar ratio of SPDP to Oregon Green® 488 and PEI was determined.

Linkage of Trastuzumab to PEIAC-OG (Trastuzumab PEIAC-OG)

A 1 mL sample of a 4.75×10^{-5} M SPDP PEI-OG $(4.75 \times 10^{-8}$ mol), 0.125 g of bromoacetic acid $(8.95 \times 10^{-4}$ mol) and 2 mL of 1 M sodium bicarbonate were added to a vial and the reaction was incubated for 1 h. The sample was concentrated to 0.5 mL with a 10 KDa Centricon® centrifugal filter device, purified with a 10 DG size exclusion column and concentrated to 1 mL with a 10 KDa Centricon® centrifugal filter device. A DTT solution was prepared as described above with 0.5 mL of PBS EDTA. To the SPDP PEIAC- OG solution was added 0.1 mL of the DTT solution and the sample was incubated at room temperature for 1 h. The reaction was purified with a 10 DG size exclusion column followed by concentration to 1 mL with a 10 KDa Centricon® centrifugal filter device. Trastuzumab was modified with Sulfo-SMCC according to published methods [14]. A 3.5 mL solution of maleimide modified Trastuzumab $(4.4 \times 10^{-9}$ mol) was concentrated to 0.7 mL with a10 KDa Centricon® centrifugal filter device. To the filter device was added 0.2 mL of the 4.75×10^{-5} M solution of the thiol containing PEIAC-OG $(9.5 \times 10^{-9}$ mol), 0.3 mL of PBS EDTA and the sample was concentrated to 0.2 mL. The sample was purified with Bio Gel p-100 with a bed volume of 1 mL. The ratio of PEI, Oregon Green® 488 and Trastuzumab was determined by UV–Vis.

Linkage of Trastuzumab to PEI-OG (Trastuzumab PEI-OG)

The previous procedure to synthesis Trastuzumab–PEIAC-OG was followed to synthesis Trastuzumab PEI-OG with the exception that the step to add bromoacetic acid was
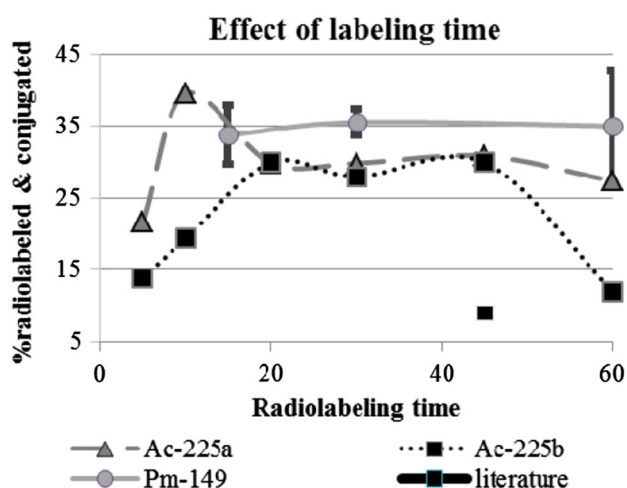

Fig. 2 The radiolabeling time was varied and the percent radioactivity labeled and conjugated to PEI was determined after purification by size exclusion centrifugation. Literature data from Reference [7] $(N = 31)$

omitted. For the reaction a three to one mole ratio of SPDP PEI-OG $(7.13 \times 10^{-9}$ mol) to Trastuzumab $(2.2 \times 10^{-9}$ mol) modified with a maleimide group was used.

Results

Pre-labeling studies

Mole ratios of metal-DOTA-NCS to PEI were 2.5–3 to 1 for all prelabeling studies. The conditions studied were labeling time, and for the conjugation step: pH, time, temperature, and buffer volume. The labeling time was examined with ²²⁵Ac and from 20 to 45 min the labeling and conjugation gave consistent results with ~ 30 % of the ²²⁵Ac DOTA-NCS conjugated to PEI (Fig. 2). The conjugation pH was studied with all three isotopes and the data is summarized in Fig. 3. For ²²⁵Ac and ¹⁴⁹Pm the amount of the isotope labeled with DOTA-NCS and conjugated to PEI was between 22 and 33 %. The ¹⁴⁹Pm study had a slight decrease in the percent of the isotope that was incorporated into DOTA-NCS and conjugated to PEI as the pH increased. There was a significant negative relationship between the amount of ¹⁴⁹Pm incorporated into DOTA-NCS and conjugated to PEI as the pH increased to 10, $(r(13) = 0.769$, $p < 0.001)$. The relationship can be represented by Eq. 1.

$$y = -2.8x + 50.8, \; R^2 = 0.92 \tag{1}$$

In contrast, the ²²⁵Ac showed no distinctive pattern over conjugation pH and time. The ⁶⁴Cu study had labeled and conjugated values of ⁶⁴Cu-DOTA-NCS to PEI of
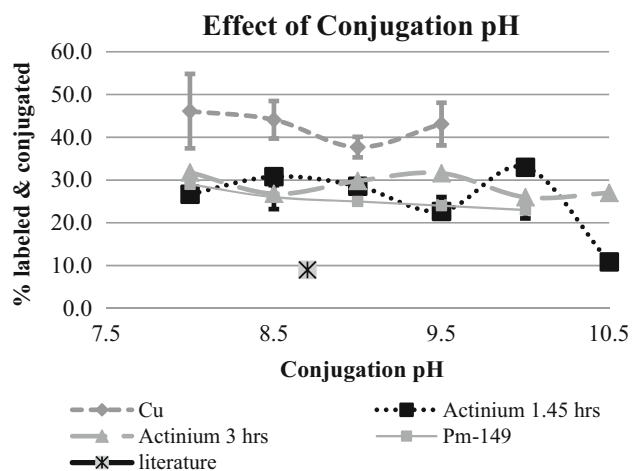
Fig. 3 The conjugation pH was varied and the percent radioactivity labeled and conjugated to PEI was determined after purification by size exclusion centrifugation. $N = 3$ for ^{149}Pm and ^{64}Cu. Literature data from Reference [7] ($N = 31$)

Fig. 4 The conjugation temperature was varied and the percent radioactivity labeled and conjugated to PEI was determined after purification by size exclusion centrifugation. Literature data from Reference [7] ($N = 31$)

46.1 ± 8.7 to 37.9 ± 2.4. Studies of time and temperature associated with the ^{149}Pm DOTA-NCS conjugation reactions with PEI were more informative (Fig. 4). In the reactions at room temperature the percent labeled and conjugated to PEI were 33–36 % compared to 19–22 % when the conjugation was performed at 55 °C. The time needed for the conjugation step had very little impact on the percent labeled and conjugated to PEI and the data indicates the reaction is finished within 15 min. A reduction of the conjugation buffer volume resulted in an increase of the percent labeled and conjugated to PEI from 26.0 ± 2.8 % for reactions with a total volume of 0.5 mL to 33.8 ± 0.6–35.1 ± 2.1 % with a total volume of 0.160 mL for ^{149}Pm. There was a significant inverse relationship between the amount of ^{149}Pm incorporated into DOTA-NCS and total volume of the conjugation solution [$r(10) = 0.995$, $p < 0.001$]. The highest amount of labeled DOTA-NCS conjugated to PEI was for ^{64}Cu, and the total volume of the conjugation reaction was 0.130 mL.

Synthesis of Trastuzumab PEIAC-OG

The synthesis of Trastuzumab PEI-OG and Trastuzumab PEIAC-OG was examined using different methods and is summarized in Fig. 5 and in Table 1. The amount of Oregon Green® 488 per PEI was determined by the UV–Vis absorbance at 496 nm and assumed no loss of PEI. For PEI-OG the ratio of Oregon Green® 488 to PEI was 5.36 to 1 PEI. In the SPDP modified PEI-OG the ratio of SPDP to PEI was determined by UV–Vis absorbance at 343 and 496 nm before and after deprotection with DTT. The change in absorbance at 343 nm was calculated from Eq. 2.
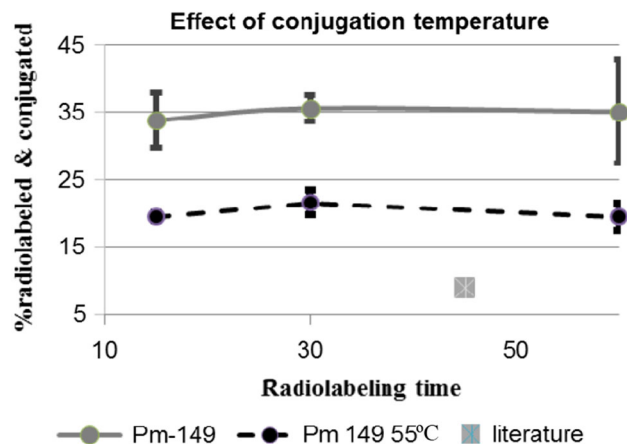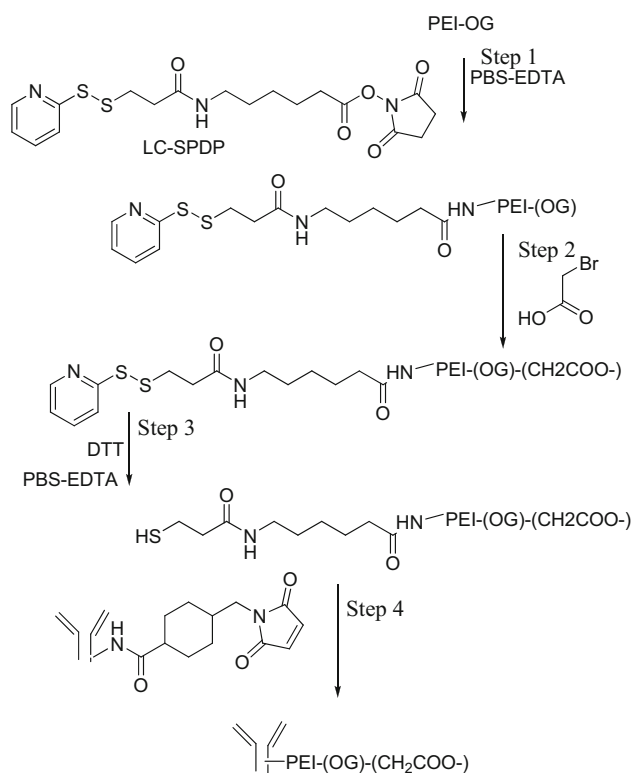

Fig. 5 The synthetic approach used to make a bioconjugate with a 1 to 1 ratio of Antibody to PEI-(CH₂COO-) Ratio PEI/OG/Ab 087/4.7/1

$$\Delta A_{343} = (\text{Ave. } A_{343} \text{ after DTT}) - (\text{Ave. } A_{343} \text{ before DTT}) \tag{2}$$

To calculate the molar ratio of SPDP to PEI Eq. 3 was used.

Table 1 UV–Vis characterization of Trastuzumab PEI-OG and Trastuzumab PEIAC-OG synthesized by different methods

	Abs 496	Abs 280	Dye/Ab	Ab/PEI
PEI-OG-Ab				
SPDP method Fr 1	1.245	0.944	4.54	0.83
SPDP method Fr 2	0.407	0.292	4.85	0.88
Traut's			5.57	0.96
PEI-OG-Acid-Ab				
SPDP method Fr 1	1.282	0.929	4.79	0.87
SPDP method Fr 2	0.403	0.322	4.26	0.78
Traut's method A	0.998	2.433	1.1	0.2
Traut's method B	0.536	1.62	2.1	0.4

moles SPDP per mole PEI $= (\Delta A343/8{,}080)$
$$* (60{,}000/\text{mg/mL of PEI}) \quad (3)$$

where 8,080 is the extinction coefficient for pyridine-2-thione at 343 nm: $(8.08 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1})$ and 60,000 is the MW of PEI. The molar ratio of SPDP to PEI was 1.5 SPDP/PEI and was determined with 3 different concentrations. In the samples Trastuzumab PEI-OG and Trastuzumab PEIAC-OG the ratio of Oregon Green® 488 dye to PEI and to Trastuzumab was determined by UV–Vis absorbance at 496 and 280 using Eq. 4.

$$A_{protein} = A_{280} - A_{max} * (CF) \quad (4)$$

A_{max} is the absorbance maximum for Oregon Green® 488 (496 nm) and CF is defined as $CF = A_{280}$ free dye/A_{max} free dye and is 0.12. Trastuzumab concentration was determined using $A_{protein}/1.4$. The amount of Oregon Green® 488 per Trastuzumab was determined using Eq. 5.

Oregon Green®488 per molecule
$$= (A_{max} * MW)/[\text{modified Trastuzumab}] * \epsilon \text{dye} \quad (5)$$

where MW is 145,000 g/mol for Trastuzumab and ϵdye is 70,000 cm^{-1} M^{-1}. The amount of Oregon Green® 488 per Trastuzumab derivative was determined using Eq. 5 where MW is 210,000 g/mol for the Trastuzumab PEI-OG and Trastuzumab PEI-OG acid and 60,000 g/mol for the PEI-OG. Trastuzumab PEIAC-OG was synthesized by different approaches and the SPDP modified PEI approach (Fig. 5) resulted in an average PEI/OG/Trastuzumab ratio of 0.82/4.52/1 for fractions 1 and 2. Synthetic approaches that used Traut's reagent to synthesis Trastuzumab PEIAC-OG are summarized in Table 1 and resulted in a ratio of PEI/OG/Trastuzumab for method A. 0.2/1.1/1 and method B. 0.4/2.1/1.[3] Synthetic approaches using either SPDP or Traut's reagent to make Trastuzumab PEI-OG resulted in similar PEI/OG/Trastuzumab ratios of 0.83–0.96/4.5–5.5/1.

Discussion

The use of the PEI polymer allows multiple locations where multiple chelating groups and targeting molecules can be attached. The toxicity of PEI has been examined in different cell models and the toxicity is dependent on the size and branching of the PEI. Fischer et al. explored the toxicity of low molecular weight branched PEI (LMW-PEI) with molecular weights of 11,900 D and high molecular weight branched PEI (HMW-PEI 1,616,000 D) [15]. Cells showed no significant toxic effects to the mitochondrial activity when incubated with LMW-PEI at concentration of 1 mg/mL. Exposure of cells to HMW-PEI caused a rapid and complete loss of cell viability independent of the incubation time and the researchers determined HMW-PEI had an $IC_{50} > 35$ μg/mL. The mechanism of cytotoxicity of HMW-PEI is caused by the interaction of the positively charged polymer with the negative charged surface of the cell. Higher molecular weight PEI has been visualized along the surface of the cell and causes massive necrosis within 30 min [15]. In contrast LMW PEI were present inside cells in endocytic vesicles which had undergone macropinocytotic uptake. In postlabeling approaches with [149]Pm or Oregon green labeled Trastuzumab-PEI we observed cell binding ($K_d = 20$ nM) of [149]Pm-DOTA-PEI-Trastuzumab in SkBr-3 cells.[4] Internalization of Trastuzumab-PEI-Oregon green (Ab-PEI-OG) into SkBr-3 cells was observed by confocal microscopy after incubation for 2 and 18 h.

In this paper we focused on optimizing the conjugation of a radionuclide DOTA-NCS complex to PEI and developing an antibody targeted PEI with secondary chelators and a ratio of PEI to antibody of 1 to 1. During the [225]Ac and daughter retention study the PEI approach slowly released [225]Ac and at 1 week approximately 50 % of [225]Ac remained (decay corrected) [5]. One possible explanation is the [225]Ac was weakly chelated by the DOTA-PEI in the approach. This work examines the synthetic optimization of a prelabling approach that could lead to better retention of [225]Ac.

The labeling time, and conjugation: pH, time, temperature, buffer volume for a prelabeling were explored. For [225]Ac and [149]Pm the amount of the isotope labeled with DOTA-NCS and conjugated to PEI was between 22 and 33 %. The radiolabeling step for both isotopes was finished within 30 min. As the conjugation pH increased the [149]Pm had a slight decrease in the percent of the isotope that was incorporated into DOTA-NCS and conjugated to PEI (Eq. 1). In contrast, the [225]Ac showed no distinctive pattern. This could be the result of the higher hydrolysis pH of [225]Ac (pH 10) versus the lower hydrolysis pH of

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[3] Supplemental information.

[4] Fitzsimmons J, Atcher R, unpublished results.

Lanthanides [16]. In the [149]Pm reactions where the conjugation step was performed at room temperature the percent labeled and conjugated to PEI were 33–36 % compared to 19–22 % when the conjugation was performed at 55 °C. This could be a result of the isothiocyanate group of DOTA-NCS hydrolyzing faster at the elevated temperature compared to the rate at which conjugation to PEI occurred. The time needed for the conjugation step had very little impact on the percent labeled and conjugated to PEI and the data indicates the reaction is finished within 15 min.

The synthetic approach to make Trastuzumab PEIAC-OG was explored utilizing SDPD and Traut's methods. Initially Traut's reagent was used to provide a sulfhydryl group on the antibody, and the low hydrolysis rate of Traut's reagent should allow control of the substitution on Trastuzumab [17]. The approach should allow a low molar excess of Traut's reagent to Trastuzumab compared to cross linkers with active esters. Although Traut's reagent can be used to crosslink the Trastuzumab to a maleimide containing PEI, approaches to modify the approach to add secondary chelators to PEI resulted in poor Trastuzumab to PEI ratios (<0.5 to 1). The poor ratios of Trastuzumab to PEI in the Traut's approaches are consistent with similar approaches where Trastuzumab was linked to PEI-PEG [18]. This is likely the result of either hydrolysis of the maleimide group at the basic condition needed to modify the PEI with acid groups. An alternative explanation is the acid groups on the PEI block or hinders the ability of Sulfo-SMCC to react with the polymer. This would cause a lower ratio of the Trastuzumab to PEI in the subsequent step. The SPDP approach is not base sensitive and is compatible with the addition of the secondary acid groups. During the synthesis of a radiolabeled Trastuzumab PEIAC molecule the SPDP approach outlined in Fig. 5 should be followed. The radiolabeled DOTA-NCS complex should be conjugated to SPDP-PEI prior to the addition of the secondary chelators in step 2 of Fig. 5.

Conclusions

The SPDP crosslinking approach was used to prepare Trastuzumab PEIAC with an appropriate ratio of the PEI to Trastuzumab. This approach can be easily modified with a radiolabeled DOTA-NCS complex for the synthesis of a targeted radiotherapeutic to treat tumors and metastases. This approach has great potential for use with [225]Ac and in vitro and in vivo studies should be performed.

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