



The role of additives in moderating the influence of Fe(III) and Cu(II) on the radiochemical yield of [⁶⁸Ga(DOTATATE)]



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HIGHLIGHTS

- Additives reduce the negative effect of metal ions on the RC yield of [⁶⁸GaDOTATATE].
- Addition of phosphate results in excellent RC yields for a Fe³⁺/ligand ratio of 2:1.
- Ascorbic acid was more effective with high RC yields for both Fe³⁺ and Cu²⁺.
- The effect of ascorbic acid is highly pH-dependant with optimal results at pH3.

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ABSTRACT

[⁶⁸Ga(DOTATATE)] has demonstrated its clinical usefulness. Both Fe³⁺ and Cu²⁺, potential contaminants in Gallium-68 generator eluent, substantially reduce the radiochemical (RC) yield of [⁶⁸Ga(DOTATATE)] if the metal/ligand ratio of 1:1 is exceeded. A variety of compounds were examined for their potential ability to reduce this effect. Most had no effect on RC yield. However, addition of phosphate diminished the influence of Fe³⁺ by likely forming an insoluble iron salt. Addition of ascorbic acid reduced Cu²⁺ and Fe³⁺ to Cu⁺ and Fe²⁺ respectively, both of which have limited impact on RC yields. At low ligand amounts (5 nmol DOTATATE), the addition of 30 nmol phosphate (0.19 mM) increased the tolerance of Fe³⁺ from 4 nmol to 10 nmol (0.06 mM), while the addition of ascorbic acid allowed high RC yields (> 95%) in the presence of 40 nmol Fe³⁺ (0.25 mM) and 100 nmol Cu²⁺ (0.63 mM). The effect of ascorbic acid was highly pH-dependant, and gave optimal results at pH 3.

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1. Introduction

The effectiveness of the PET radionuclide Gallium-68 (⁶⁸Ga) as ⁶⁸Ga-DOTA-D-Phe¹-Tyr³-octreotate ([⁶⁸Ga(DOTATATE)]) in the clinical situation, its suitable imaging properties ($t_{1/2}$ =68 min, 89% positron yield), 24/7 availability from a long-lived ⁶⁸Ge/⁶⁸Ga generator and relatively straight forward radiolabelling chemistry has spurred the tremendous growth of the use of this radionuclide

(Treglia et al., 2012). Numerous chelators are available today to coordinate ⁶⁸Ga³⁺ and thereby bind this radioisotope to a variety of biomolecules such as peptides, antibodies, etc. (Breeman et al., 2011; Maecke and André, 2007; Velikyan, 2011). However, the promiscuity of most chelators and the similarity between the coordination chemistry of Ga³⁺ and many transition metals, particularly Fe³⁺, can reduce the radiochemical (RC) labelling yield and effective specific activity if these metals are present in the reaction solutions (Šimeček et al., 2013). We recently studied the influence of various metal ions on the formation and stability of [⁶⁸Ga(DOTATATE)] (Oehlke et al., 2013). During the course of these experiments we asked if the negative influence of metal ions could be mitigated by an alternative to reducing metal ion contamination in the generator eluate or in solutions used in reactions. The incorporation of a suitable additive to [⁶⁸Ga(DOTATATE)]

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radiolabelling mixtures could make the reactions more robust with respect to key metal ion contaminants.

In the present study we chose to focus our investigation on Fe^{3+} and Cu^{2+} as these have the most dramatic effect on ^{68}Ga radiolabelling yields. Both metal ions hinder the formation of the ^{68}Ga complex as soon as the metal/ligand ratio is higher than 1:1 (Oehlke et al., 2013). The additives studied were chosen for their ready availability and potential ability to interact in a variety of ways with these metal ions, such as reduction to the less competitive Fe^{2+} and Cu^+ , selective coordination or precipitation.

2. Materials and methods

2.1. Materials

All chemicals and solvents were of analytical or pharmaceutical grade unless otherwise specified. Metal salts (ultrapure grade; trace metal content) were obtained from Sigma-Aldrich. DOTATATE acetate (GMP-grade) was purchased from ABX Chemicals.

Gallium-68 was obtained as solution of $[\text{}^{68}\text{Ga}(\text{OH})_4]^-$ in 0.5 M KOH from an in-house $^{68}\text{Ge}/^{68}\text{Ga}$ generator system (ANSTO, Australia) (Cutler et al., 2013; Le, 2013a, 2013b). Details on the specific elution and purification process and characteristics of the eluate used in these experiments have been published (Oehlke et al., 2013). The activity used for each reaction varied between 1.5 and 5 MBq.

All solutions were analysed before use for metal contaminants (Al, Ba, Bi, Ca, Cd, Co, Cu, Fe, Lu, Ni, Pb, Ti, Y, Yb, Zn, Zr) using inductive-coupled plasma optical-emission spectrometry (ICP-OES). Excluding Al and Ca, the total background metal ion content in each reaction solution (calculated by adding the measured metal ion content in generator eluate and reagent solutions) was less than 0.5 nmol. We made an exception for the Al and Ca content, since their interference level in our experimental set-up was insignificant as has been shown previously (Oehlke et al., 2013).

Reactions were performed in 500 μL microcentrifuge tubes (Eppendorf PCR). All solvents and chemicals were stored in plastic bottles. All labware and reaction vials used were washed with 4 M HCl followed by ultrapure H_2O before use.

2.2. Radioactivity measurements and chemical analysis

Radioactivity was measured using an ionisation chamber (Carpinter Radioisotope Calibrator CRC 15R). Trace metal content was determined by ICP-OES using a Perkin Elmer OPTIMA 5300DV instrument. Calibration was performed with single standard elements (high purity) obtained from Choice Analytical Pty Ltd.

High-performance liquid chromatography (HPLC) was performed using a Waters 600 system consisting of a quaternary pump, a UV detector (Linear UVIS Activon, tuned to 254 nm) and a radiation detector (Carroll & Ramsey Associates Model 105S) coupled in series. The system was controlled and data acquired using Empower 2. Separations were achieved on an analytical Phenomenex Synergi Hydro-RP C-18 column (4.6 mm \times 250 mm, 4 μm , 80 Å) employing a gradient consisting of acetonitrile (A), H_2O (B) and 1% trifluoroacetic acid in H_2O (C) and a flow rate of 1.0 mL/min. Method: 0 min: 5% A, 85% B, 10% C; 1 min: 5% A, 85% B, 10% C; 9 min: 90% A, 10% C; 10 min: 5% A, 85% B, 10% C; 17 min: 5% A, 85% B, 10% C.

2.3. Radiolabeling procedures

Most experiments were repeated at least 3 and up to 10 times if significant deviations were observed.

$[\text{}^{68}\text{Ga}(\text{DOTATATE})]$. The conditions for the synthesis of $[\text{}^{68}\text{Ga}$

(DOTATATE)] were a modification of well-established methods (de Blois et al., 2011): The pH of a solution of 50 μL of 1 M HEPES and 100 μL of $[\text{}^{68}\text{Ga}(\text{OH})_4]^-$ in 0.5 M KOH was adjusted with aliquots of 4 M HCl to pH \sim 4.0 (as measured by PANPEHA[®] (pH range 0–14) and Acilit[®] (pH range 0–6) indicator strips). A 5 μL aliquot of 1 mM DOTATATE in 0.1 M HCl (5 nmol) was added, the pH tested again and the mixture was heated to 95 $^\circ\text{C}$ for 10 min. Under these radiolabelling conditions a single radioactivity peak was observed at $R_t=11.5$ min using the aforementioned HPLC conditions and was assigned to the $[\text{}^{68}\text{Ga}(\text{DOTATATE})]$ complex. Radioactivity peaks occurring between 2 and 4 min were assigned to ^{68}Ga species that are not coordinated by DOTATATE. Unless otherwise indicated, all reactions were performed using 5 nmol DOTATATE in a final volume of approximately 160 to 190 μL (approximate concentration of DOTATATE: $2.6\text{--}3.1 \times 10^{-5}$ M).

Effect of additives on RC yield in the presence of Cu^{2+} and Fe^{3+} ions. To establish a baseline, 20 nmol (1 mM solutions in H_2O , 20 μL) and 100 nmol (10 mM solutions in H_2O , 10 μL) of a variety of compounds (ammonium sulphate, ascorbic acid, potassium fluoride, sodium phosphate, sodium acetate, sodium citrate, sodium hydrogen carbonate, sodium potassium tartrate, or potassium oxalate) were added to the standard radiosynthesis of $[\text{}^{68}\text{Ga}(\text{DOTATATE})]$ described before and the RC yield determined. Then, these anions were tested to investigate their potential to reduce the effect of metal contamination on RC yield. The compounds (20 and 100 nmol) were added to pH-adjusted reaction solutions which also contained CuCl_2 (5 nmol, 5 μL of a 1 mM solution in 0.1 M HCl) or FeCl_3 (7 nmol, 7 μL of a 1 mM solution in 0.1 M HCl) [$n=1$]. Two minutes after this addition the ligand was added, the mixture was heated to 95 $^\circ\text{C}$ for 10 min and RC yield determined. The influence of phosphate and ascorbic acid was studied in greater detail. In these experiments, the amounts of phosphate or ascorbic acid (1–10 μL of 10 mM or 100 mM aqueous solutions) as well as the amount of the added metal ion (1–10 μL of 1, 10 or 100 mM solutions in 0.1 M HCl) and the pH (pH 3–5) were varied while the other standard conditions were maintained. To test if colloids were formed under certain reaction conditions, the solutions were spun at 16,000xg (14,000 rpm) for 15 min using Microcon[®] Centrifugal Filters (Ultracel YM-30) in an Eppendorf 5414 C centrifuge fitted with a F-45-18-11 rotor, and the amounts of activity associated with the filter and the filtrate were measured and compared.

3. Results and discussion

Initial experiments performed in the absence of either Cu^{2+} or Fe^{3+} with a variety of additives demonstrated that only potassium oxalate resulted in decreased RC yields of $[\text{}^{68}\text{Ga}(\text{DOTATATE})]$ (reduction to 60% RC yield at 20 nmol). Next, the effect of these compounds was tested to determine if any of these could improve the RC yields when either Cu^{2+} or Fe^{3+} was present in the reaction solution (Fig. 1). It is clear in this preliminary experiment that three compounds stood out. Potassium oxalate further increased the negative effect of the metal ions, from 40% in the absence of this anion to zero RC yield of $[\text{}^{68}\text{Ga}(\text{DOTATATE})]$ for Cu^{2+} . For Fe^{3+} , the RC yield was reduced from 40% to 8%. In contrast, the addition of sodium phosphate completely abolished the effect of Fe^{3+} giving a RC yield of 100%. Similarly, ascorbic acid was most effective in abolishing the effect of both Cu^{2+} and Fe^{3+} .

To further explore the effect of phosphate, the amount of Fe^{3+} added to the radiosynthesis of $[\text{}^{68}\text{Ga}(\text{DOTATATE})]$ was increased up to 10 nmol Fe^{3+} ($\sim 5.7 \times 10^{-5}$ M). The addition of a 3-fold molar equivalent of phosphate reversed the effect of Fe^{3+} and gave RC yields $> 90\%$ (Fig. 2). Part of this effect could be the chemical

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