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Validation of ^{68}Ge/^{68}Ga generator processing by chemical purification for routine clinical application of ^{68}Ga-DOTATOC

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Abstract

Introduction: Imaging of somatostatin receptor expressing tumours has been greatly enhanced by the use of ^{68}Ga-DOTATOC and PET/CT.
Methods: In this work, a purification method for the ^{68}Ge/^{68}Ga generator eluate and a method to produce ^{68}Ga-DOTATOC suitable for clinical use were evaluated. The generator eluate was purified and concentrated on a cation-exchange cartridge in HCl/acetone media. The efficacy of this procedure in eliminating metal impurities from the ^{68}Ga solution was investigated by ICP-MS. The radiotracer quality was evaluated by radio-TLC, GC and γ-ray spectrometry.
Results: ^{68}Ga-DOTATOC preparations (n=33) were carried out with a mean synthesis yield of 59.3±2.8% (not corrected for decay) and a batch activity ranging from 555 to 296 MBq. The radiochemical and radionuclidic purity were >98% and 99.9999%, respectively. With this purification process, >95% of the Fe(III), Zn(II) and Mn(II) were eliminated from the solution.
Conclusions: ^{68}Ga-DOTATOC produced with this method can be efficiently used in nuclear medicine departments for PET evaluations.

Keywords: ^{68}Ge/^{68}Ga generator; Postprocessing of eluates; Cation-exchange chromatography; DOTATOC; PET

1. Introduction

Radionuclide therapy of tumours expressing somatostatin receptors is currently being performed with DOTA-conjugated peptides such as DOTA-D-Phe^1-Tyr^3-octreotide and DOTA-D-Phe^1-Tyr^3-Thr^8-octreotide labelled with high- and medium-energy β$^-$ emitters such as ^{90}Y or ^{177}Lu, respectively [1]. The use of ^{68}Ga-DOTATOC and positron emission tomography (PET and PET/CT) to visualize this kind of tumours allows a better planning of the treatment and a time-effective assessment of the therapeutic outcomes [2,3]. DOTATOC labelled with trivalent Ga showed high binding affinity for the human somatostatin receptor subtype 2 and improved tumour imaging capabilities in respect to the conventional tracer ^{111}In-Octreoscan and ^{90}Y surrogate SPECT radiotracer ^{111}In-DOTATOC [4–6].

The ^{68}Ge/^{68}Ga generator is a useful source of positron emitting radionuclides for institutions without a cyclotron on site. The long half-life of the parent radionuclide ^{68}Ge ($T_{1/2}$= 270.8 days) grants ^{68}Ga availability for long periods of time. Furthermore, the ^{68}Ga decay characteristics (89% β$^+$, 1.92 MeV maximum energy) and its short half-life ($T_{1/2}$=68 min) are suitable for PET application in a clinical setting.

The trivalent cation ^{68}Ga^{3+} is eluted from the generator with a 0.1 M HCl solution. Its chemical form, theoretically, allows universal application in radiopharmaceutical preparations when an appropriate coordinating agent, such as 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (DOTA), is used.

However, the presence of competing metal ions in the eluate represents a major obstacle in the complexation chemistry of ^{68}Ga [7]. The breakthrough of the long-lived

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parent ^{68}Ge from the generator TiO_2 column is $\sim 10^{-3}$% of the eluted ^{68}Ga activity, and, in addition, the eluate generally contains critical impurities such as Fe(III), Mn(II) and Zn(II). Furthermore, Ti(IV) can be eluted from the generator in relatively high concentration [7]. Therefore, the primary eluate fails to provide sufficiently pure ^{68}Ga for both high labelling yield and high specific activity when low amounts of peptide precursor are used [7,8]. Also, the rather large volume (up to 10 ml for complete elution) and high proton concentration of the generator eluate require preconcentration of the activity for labelling nanomolar amounts of peptides. For all these reasons, purification and concentration of the ^{68}Ga-eluate before labelling are mandatory.

The ^{68}Ge/^{68}Ga generator eluate can be concentrated by increasing the HCl concentration to ~6 M and passing the mixture through an anion exchange column where the anionic $[GaCl_6]^{3-}$ and $[GaCl_4]^{-}$ complexes are strongly absorbed, whilst ^{68}Ge(IV), Al(III), Ti(IV) and In(III) are practically not retained. ^{68}Ga is then eluted with <200 μl of pure water [7–9]. However, this strategy is not efficient in removing Fe(III) and Zn(II) as these cations also form strong anionic chloro-complexes [10].

Another way to purify the generator-produced ^{68}Ga is described by Breeman et al. [11] and requires an eluate fractionation. Although this approach does not eliminate but only decrease the metallic impurities, it has been successfully employed for ^{68}Ga-DOTATOC synthesis [11,12].

Recently, a third method to purify Ga(III) by means of a cation-exchange column eluted with HCl/acetone mixtures was reported [13]. Zhernosekov et al. [14] demonstrated that ^{68}Ga in 0.1 M HCl can be quantitatively absorbed on a cation-exchange resin and further purified from Ge, Ti, Fe, Zn and Al impurities by adjusting the HCl concentration and HCl/acetone ratio of the eluent. Finally, ^{68}Ga can be released from the resin in a chemical form suitable for labelling by increasing the acetone content.

Herein, the abovementioned approach was used to process the ^{68}Ge/^{68}Ga generator eluate in order to obtain pure ^{68}Ga for direct labelling of DOTATOC at high specific activity and radiochemical purity for clinical purposes. The labelling process was evaluated systematically and optimized by using a manual synthesizer. Particular attention was paid at reducing the personnel radiation exposure during the manual operation steps.

2. Materials and methods

A 1100-MBq ^{68}Ge/^{68}Ga generator (Cyclotron Co., Obninsk, Russian Federation) was evaluated for 7 months, and 33 ^{68}Ga-DOTATOC syntheses were performed adopting the chemical purification of the eluate on an AG 50W-X8 (400 mesh) cation-exchange resin (Biorad, Hercules, CA, USA) [14]. A manual postprocessing module, installed in a shielded laminar flow cell, was used for the preparations. Chemicals of the highest purity grade and Milli-Q water (18.2 MΩ cm, Millipore Corp., Milan, Italy) were used for all the reaction steps. HCl (metal ions free), ethanol and acetone were purchased from Fluka (Buch, SG, Sweden). Anhydrous sodium citrate and DOTATOC were purchased from Merck (Whitehouse Station, NJ, USA) and piCHEM (Graz, Austria), respectively.

The ^{68}Ga solution was eluted from the generator with 7.5 ml of 0.1 M HCl at a flow rate of ~3 ml/min. The generator eluate was directly passed through a cartridge containing a cation-exchange resin (~50 mg), where >99% of ^{68}Ga was retained. The cartridge was then rinsed with 1 ml of a mixture of 0.15 M HCl/acetone (20/80) and ^{68}Ga was eluted with 0.4 ml of 0.05 M HCl/acetone (2:98). To improve ^{68}Ga recovery, the cartridge was first filled with 0.15 ml of HCl/acetone mixture, and after 2 min, with the rest of the eluent. From the cartridge, the ^{68}Ga solution was transferred directly into the reaction vial containing 14 nmol DOTATOC (in 5 ml of water) and preheated to ~100°C. After a 10-min incubation, the reaction mixture was passed through a Sep-Pack light C-18 cartridge (Waters, Milan, Italy). Unreacted ^{68}Ga was eliminated by washing the cartridge with 4 ml of water. Then, ^{68}Ga-DOTATOC was eluted with 0.45 ml of ethanol into the final vial, containing 5 ml of saline, and the activity was measured in an Aktivimeter ISOMED 2000 dose calibrator (MED Nuklear-Medizintechnik, Dresden, Germany).

During these procedures, each finger of the operators was equipped with plastic thimbles containing TLD detectors (Technorad, Verona, Italy) in order to evaluate the hand exposition.

2.1. Quality control

The radiochemical purity of each ^{68}Ga-DOTATOC solution (n=33) was assessed by two thin layer chromatography (TLC) systems on (1) RP-18F (Merck, Whitehouse Station, NJ, USA) and (2) ITLC-SG plates (Pall Corporation, East Hills, NY, USA). A 0.1 M sodium citrate solution (pH=5) and a 1 M ammonium acetate/methanol (1:1) mixture were used to elute Systems 1 and 2, respectively. The plates were analyzed on an AR 2000 Imaging Scanner (Bioscan, Washington, DC, USA). TLC system 1: ^{68}Ga-DOTATOC: R_f=0.0; free $^{68}Ga^{3+}$ R_f=0.9. TLC system 2: hydrolyzed ^{68}Ga: R_f=0.1 ^{68}Ga-DOTATOC: R_f=0.9. Residual acetone in the samples (n=6) was measured using a FOCUS GC gas chromatography system (Thermo Scientific, Inc., Waltham, MA, USA) equipped with a flame ionization detector and an AI/AS 3000 series autosampler. The sterility of the preparations was also assessed (n=6), according to the European Pharmacopeia standard procedures.

In order to evaluate the efficacy of the purification process, the presence of metal impurities was evaluated in some of the generator eluates (n=6) and final products (n=6) by inductively coupled plasma mass spectrometry (ICP-MS) using a 7500 Ce ICP-MS analyzer (Agilent, Santa Clara, CA, USA). In these samples, the ^{68}Ge breakthrough was also estimated, as previously described [11], using a γ-

Table 1
Concentration of cationic metallic impurities and ^{68}Ge breakthrough in generator eluates and final [^{68}Ga]-DOTATOC samples

Cations (μg/L)	Before purification	After purification
Fe(III)	2100±1300	17.6±11.4
Zn(II)	5050±147	52.7±16.2
Mn(II)	42.3±25.5	0.9±0.3
Pb(II)	8.5±5.3	9.7±4.6
Ti(IV)	14.7±6.6	3.1±4.6
Cr(III)	504±132	3.4±0.7
Al(III)	1080±125	9.1±7.1
Cd(II)	0.39±0.3	0.07±0.05
Co(II)	4.3±2.2	0.12±0.11
Cu(II)	14.5±6.2	6.5±3.1
Ni(II)	254±124	0.6±0.2
V(IV)	6.1±3.2	1.6±0.5
^{68}Ge (Bq/ml)	12,300±400	<0.1

spectrometer (Canberra DSA 1000, Canberra, Meriden, CT, USA) equipped with a coaxial HPGe detector.

3. Results and discussion

The ^{68}Ge/^{68}Ga generator was eluted three times a week for radiotracer production. In order to minimize the amount of metal impurities at the time of ^{68}Ga-DOTATOC synthesis [7], a pre-elution was carried out at the beginning of each week and the eluate was discarded.

The elution yield, the ratio of the measured ^{68}Ga activity to the calculated ^{68}Ge activity at time of elution, ranged between 82% and 69% after 7 months' use (~100 elutions). The ^{68}Ge breakthrough to the generator was measured monthly and, differently from the findings of previous studies [7], was found to be $\sim 10^{-2}$% of the eluted ^{68}Ga activity. The amount of ^{68}Ge breakthrough increased slightly over time (~15% increases per month), ranging from $1.1 \cdot 10^{-2}$% and $2.6 \cdot 10^{-2}$% of the ^{68}Ga activity within the 7 months of evaluation.

The ICP-MS analysis of the eluates confirmed the presence of metal impurities that could interfere in Ga(III) complexation chemistry. In the solutions, 10^3 μg/L for Fe (III) and Zn(II), 10^2 μg/L for Ni(II) and 10 μg/L for Co(II), Cd(II) and Cu(II) were detected, respectively. Amounts of other metal ions, such as Mn(II), Pb(II), Ti(IV), Cr(III), Al (III) and V(IV), ranged from 10 to 10^3 μg/L.

The purification step permitted a >95% reduction in Fe (III), Zn(II), Mn(II), Cr(III), Al(III), Co(II) and NI(II) impurities and >75% reduction of Cd(II), V(IV) and Ti(IV) ions, as shown in Table 1. Most importantly, the ^{68}Ge breakthrough was decreased by a factor of 10^5 compared to the nonpurified eluate achieving <0.1 Bq/ml concentration in the final product.

The amount of ^{68}Ga-DOTATOC produced with the processed eluates ranged from 555 to 296 MBq, depending on the ^{68}Ge/^{68}Ga generator age (n=33). The mean synthesis yield was 59.3±2.8% (not corrected for decay) in a total synthesis time of ~23 min. The synthesis parameters were optimized as described previously [7,11,14]. In the present study, 14 nmol DOTATOC could be labelled at pH 2.3 with a 10-min incubation at 100°C, and up to a theoretical 40 MBq/nmol specific activity was achieved. The radiochemical purity of ^{68}Ga-DOTATOC was improved by using solid-phase extraction on a C-18 cartridge (>98%, n=33). In the final product, the most abundant radiochemical impurity was residual $^{68}Ga^{3+}$ (1%) while <0.5% hydrolyzed ^{68}Ga was present, as determined by radio-TLC. Acetone concentration in the final solutions was found to be under the detection limit (<0.1 mg/L) and all the tested samples were sterile.

The mean dose absorbed by the operator fingertips during the synthetic procedure ranged from 5.53± 1.22 mSv/GBq for the left index finger to 0.43± 0.08 mSv/GBq for the left fifth finger. The 75th and 95th percentile of fingertips dose distribution were 1.78 and 4.00 mSv/GBq, respectively. Therefore, in order to adhere to the dose limits (500 mSv/year), it is recommended that the dose received be monitored and the number of procedures per operator restricted.

In conclusion, in this paper we systematically evaluated a chemical method for processing ^{68}Ga solutions eluted from commercially available ^{68}Ge/^{68}Ga generators. After eluate purification, ^{68}Ga-DOTATOC was obtained with high specific activity, yield and radiochemical purity.

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