

Initial Clinical Experience with ^{68}Ga -DOTA-NOC Prepared Using ^{68}Ga from Nanoceria-polyacrylonitrile Composite Sorbent-based $^{68}\text{Ge}/^{68}\text{Ga}$ Generator and Freeze-dried DOTA-NOC Kits

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Abstract

Somatostatin receptor positron emission tomography–computed tomography (PET/CT) with ^{68}Ga -1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) peptides have become an indispensable part of disease assessment in patients with neuroendocrine tumors and forms the basis of personalized therapy with peptide receptor-based radionuclide therapy. With growing utilization of PET/CT in developing countries, availability of the indigenous GMP-certified $^{68}\text{Ge}/^{68}\text{Ga}$ generators is expected to further promote cost-effective molecular imaging service to the cancer patients. We present our initial clinical experience in 32 patients injected with ^{68}Ga -DOTA-NOC prepared using ^{68}Ga eluted from Bhabha Atomic Research Centre nanoceria-polyacrylonitrile sorbent-based $^{68}\text{Ge}/^{68}\text{Ga}$ generator and freeze-dried DOTA-NOC cold kits.

Keywords: 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid NOC cold kits, $^{68}\text{Ge}/^{68}\text{Ga}$ generator, Bhabha Atomic Research Centre, nanoceria, positron emission tomography-computed tomography

Introduction

Widespread availability of positron emission tomography–computed tomography (PET/CT) along with the development of many radiolabeled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) conjugated peptides for imaging and therapy of neuroendocrine tumors (NETs) has led to

significant progress in the rapidly evolving field of theranostics.^[1] In a major step toward providing a cost-effective molecular imaging service to the increasing demands of many nuclear medicine centers in India, Bhabha Atomic Research Centre (BARC), Mumbai, has developed a nanoceria-polyacrylonitrile (CeO_2 -PAN) composite sorbent-based $^{68}\text{Ge}/^{68}\text{Ga}$ Generator and single vial freeze-dried cold kits of DOTA-NOC for instantaneous labeling with ^{68}Ga from the generator without the need for automated systems.^[2,3]

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Compared to the other available commercial generators, this novel generator is reported to have consistent ⁶⁸Ga yield, minimal metal impurities, and very low ⁶⁸Ge breakthrough when long-term elution profiles were studied.^[4] Another advantage is that it provides ⁶⁸Ga of requisite quality without the need for postelution processing. Hence, such cost-effective, GMP-compliant generator could be easily used in clinical settings in hospital radio-pharmacy like the ^{99m}Tc generators.^[1-3]

We present our initial clinical experience in 32 patients injected with ⁶⁸Ga-DOTA-NOC prepared using ⁶⁸Ga eluted from BARC CeO₂-PAN sorbent-based ⁶⁸Ge/⁶⁸Ga generator and freeze-dried DOTA-NOC cold kits for various clinical indications.

Materials and Methods

Materials

DOTA-NOC was procured from ABX, Germany. Sodium acetate and Suprapure HCl were purchased from Fluka, USA, and MERCK, Darmstadt, Germany, respectively. All reagents were prepared using sterile high-performance liquid chromatography (HPLC) grade water (Merck, India). Sterility test kits were obtained from Himedia Laboratories, India, and Endosafe PTS equipment and cartridges were from Charles River Laboratories Pvt. Ltd., India. ITLC SG paper was from Agilent Technologies, USA, whereas Whatman 3 mm chromatography strips were from Whatman, UK.

Radioactivity measurements were made using a NaI (TI) counter (ECIL, India). An HPLC system (JASCO, Japan) equipped with a C-18 reversed phase column coupled to an ultraviolet/visible detector and a NaI (TI) radioactivity detector (Raytest, Germany) was used for characterization of radiolabeled peptides. Alpha 1-2 LD plus freeze dryer was purchased from Martin Christ, GmbH while 0.22 μm membrane filters (33 mm) were from M/s. Millipore Corporation, Bedford, Massachusetts, USA. PET/CT studies were acquired on Philips Medical Systems, Ohio, USA GEMINI TOF 64.

Methods

Development of ⁶⁸Ge/⁶⁸Ga generator

The CeO₂-PAN sorbent was prepared by the decomposition of the cerium oxalate precursor to cerium oxide followed by incorporation in the PAN matrix, as reported earlier.^[2] To fabricate a ⁶⁸Ge/⁶⁸Ga generator, a borosilicate glass column of dimension 7 cm × 0.8 cm (id) with a sintered disk (G₂) at the bottom was packed with 0.5 g of the sorbent, in a lead shield. The column matrix was conditioned at pH 3 by passing

100 mL of 0.001 M HCl solution, at a flow rate of ~2 mL/min. The loading solution (100 mL) containing 740 MBq (20 mCi) of ⁶⁸Ge maintained at pH ~3 was allowed to percolate into the column at a flow rate of 0.5-1 mL/min. The column was then washed with 200 mL of 0.1 M HCl (pH 1) solution.

Formulation of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-NOC kits and radiolabeling with ⁶⁸Ga

Single vial kits of DOTA-NOC were formulated in 0.5 M sodium acetate, freeze dried, vacuum sealed under sterile conditions and stored at -20°C, as reported elsewhere.^[3,5] Aseptically prepared 0.1 N HCl was provided with the kits for elution of ⁶⁸Ga from the generator. Quality control of the kits was carried out by evaluating their sterility and apyrogenicity, as per the guidelines of Indian Pharmacopeia. Radiochemical yield (RCY) and radiochemical purity of ⁶⁸Ga-DOTA-NOC were determined by paper chromatography (PC) as per the reported procedure.^[3,5] For preparation of patient dose of ⁶⁸Ga-labeled DOTA-NOC, ⁶⁸GaCl₃ from the nanoceria-PAN ⁶⁸Ge/⁶⁸Ga generator was eluted using 1 mL of sterile 0.1 N HCl directly into DOTA-NOC cold kit vial. Reaction was carried out at 90°C for 10 min. RCY of ⁶⁸Ga-DOTA-NOC was estimated by PC. ⁶⁸Ga-DOTA-NOC was then filtered through 0.22 μ sterile filter, diluted with sterile saline (2 mL), and injected into the patients.

Clinical studies

We retrospectively studied 32 patients from June to July 2013 (age 16-73, mean 52) wherein ⁶⁸Ga-DOTA-NOC PET/CT was done as part of their treatment management. After obtaining a scout image, breath hold CT was acquired followed by whole body CT and then PET acquisition. PET acquisition time was 2 min/per bed position. CT parameters for breath-hold CT includes slice thickness 3 mm, pitch 1.08, field of view - 356 mm, voltage - 120 kV with automated mA correction, image matrix - 512 mL × 512. 80 mL of low osmolar nonionic intravenous contrast was administered in all eligible patients at a rate of 1.8 mL/s and scan delay was 50 s. Images were reconstructed iteratively using RAMLA algorithm. Images were viewed on display system having extended brilliance workspace software (EBW) version 4.5.3.40140 (Netherlands), Philips Healthcare. In addition to the PET/CT, all patients underwent ^{99m}Tc-hydrazinonicotinyl-Tyr3-octreotide (^{99m}Tc-HYNIC-TOC) whole body planar scan with single photon emission CT (SPECT) acquisition on Infinia, Hawkeye, GE Healthcare, as standard of care. Relative performances of PET and SPECT studies were analyzed. All studies were acquired with prior informed consent from patients.

Results

The generator was able to provide ⁶⁸Ga activity (in the form of ⁶⁸GaCl₃) in consistent yields and acceptable radionuclidic purity (<10⁻⁴% of ⁶⁸Ge breakthrough). Schematic of ⁶⁸Ge/⁶⁸Ga Generator is depicted in Figure 1. DOTA-NOC cold kits each consisting of 50 µg of the peptide conjugate in 0.5 M sodium acetate (13 mg) could be successfully formulated. The formulated kits were found to be of pharmaceutical grade as per sterility tests and BET tests. Greater than 95% radiolabeling yields could be obtained on radiolabeling with ⁶⁸Ga, as ascertained by the chromatography techniques. Specifications of DOTA-NOC kit and ⁶⁸Ga-DOTA-NOC are given in Table 1.

Clinical studies

⁶⁸Ga-DOTA-NOC PET/CT was positive in 22/32 patients (12 - initial characterization/staging/diagnosis, 10 - restaging) [Figures 2 and 3]. PET/CT was negative in 10/32 patients (4 - medullary Ca thyroid, 1 - suspected Insulinoma, 3 metastatic NET with MiB index, 1 - Ectopic Cushing's syndrome, 1 - thymoma) [Tables 2a and b]. Number of lesions identified on ⁶⁸Ga-DOTA-NOC PET/CT was 142 and on the whole body ^{99m}Tc-HYNIC-TOC scintigraphy was 85 [Table 3].

Discussion

Molecular imaging with ⁶⁸Ga radiopharmaceuticals is rapidly evolving. Somatostatin receptor (SSTR) targeting

Table 1: Generator and cold kit performance parameters

DOTANOC kit	DOTANOC kit provides a pre-dispensed sterile formulation in freeze dried form (Vial 1) suitable for preparation of ready-to-use ⁶⁸ Ga-DOTANOC Each kit contains three vials Vial-1: ~50 µg DOTANOC and ~14 mg Sodium acetate in freeze dried form Vial-2: 1 mL of sterile HPLC grade water Vial-3: 1 mL of sterile 0.1 N HCl
Expiry	8 months from the date of manufacturing
Storage	-20°C After preparation, ⁶⁸ Ga-DOTANOC has the following specifications
Appearance	Clear solution
pH	~5.0 (after appropriate dilution with sterile saline)
Radionuclidic purity	⁶⁸ Ge levels less than 10-4%
Radiochemical purity of ⁶⁸ Ga-DOTANOC	>95%
Average elution to dose preparation time (Including quality control)	35 min
Expiry	1 hour from the time of formulation
Storage	At room temperature with adequate lead shielding

⁶⁸Ga-DOTA peptides have been successfully investigated in multiple clinical indications such as neuro-endocrine tumors, para-gangliomas, neuroblastomas, meningiomas,

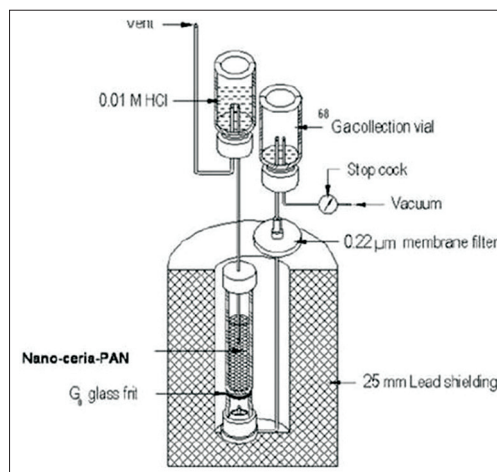


Figure 1: Schematic diagram of Bhabha Atomic Research Centre ⁶⁸Ge-⁶⁸Ga generator

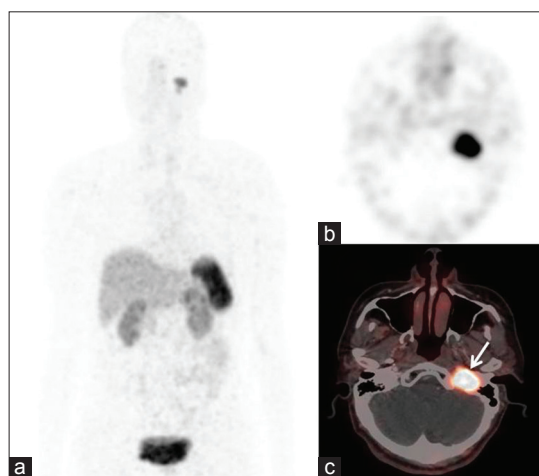


Figure 2: ⁶⁸Ga-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-NOC positron emission tomography-computed tomography of a 54-year-old patient, showing maximum intensity projection (a), transaxial positron emission tomography (b) and fused positron emission tomography-computed tomography (c) showing focal increased tracer uptake in the left glomus jugulare (white arrow)

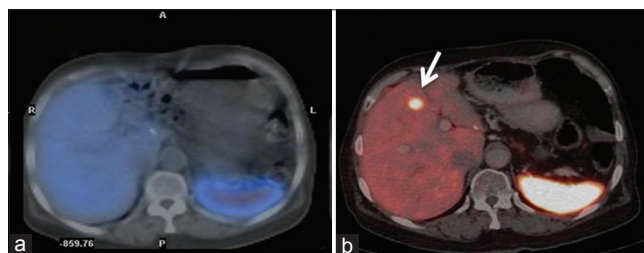


Figure 3: Case of solitary liver metastasis from an operated case of duodenal neuroendocrine tumor showing fused images of negative ^{99m}Tc-hydrazinonicotinyl-Tyr3-octreotide single photon emission computed tomography/computed tomography (a) study and positive 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-NOC positron emission tomography-computed tomography (b, white arrow) study

Table 2a: Indications and results of ⁶⁸Ga-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-NOC positron emission tomography-computed tomography for initial characterization

Indication	No	Purpose	Positive	Negative
Metastatic NET with unknown primary	4	Localization	3	1
Tumor induced osteomalacia	1	Localization	1	0
Glomus Jugulare	2	Diagnosis	2	0
NET Thymus	2	Diagnosis	1	1
Meningioma	2	Diagnosis	2	0
Insulinoma	2	Localization	1	1
Metastatic NET lung	1	Staging	0	1
NET stomach	1	Staging	1	0
NET Pancreas	1	Staging	1	0
Medullary Ca thyroid	1	Diagnosis	0	1
Ectopic Cushing's	1	Localization	1	0
Total	18		13	5

Table 2b: Indications and results of ⁶⁸Ga-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-NOC positron emission tomography-computed tomography for follow-up evaluation

Indication	No.	Purpose	Positive	Negative
Metastatic NET pancreas	3	Restaging	3	0
Metastatic NET unknown primary	2	Restaging	2	0
NET duodenum	2	Follow up	1	1
Medullary Ca thyroid	3	Follow up	0	3
Metastatic NET gall bladder	1	Restaging	1	0
NET lung	1	Restaging	1	0
Periampullary NET	1	Restaging	0	1
NET ascending colon	1	Restaging	1	0
Total	14		9	5

Table 3: Comparison of relative performance of ^{99m}Tc-hydrazinonicotinyl-Tyr3-octreotide TOC single photon emission computed tomography and ⁶⁸Ga-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-NOC positron emission tomography-computed tomography for lesions detection

Site of lesions	PET/CT	SPECT
Liver	80	46
Lymph nodes	39	20
Skeleton	7	4
Pancreas	3	2
GI tract	4	4
Cranium	6	6
Neck/chest	3	3
Total	142	85

and oncogenic osteomalacia.^[6] Beyond SSTR imaging, promising clinical studies have emerged using ⁶⁸Ga with biomolecules such as PSMA, bombesin, and macroaggregated albumin.^[7-9]

The first and foremost important clinical indication for ⁶⁸Ga-DOTA peptides have been NETs. NET represents

a subset of tumors originating from enterochromaffin cells of the foregut, midgut, or hindgut. Survivor, Epidemiology and End results database has shown rise in the incidence of NET for the past few decades, probably related to the increased detection rate.^[10] Prognosis of this disease is highly dependent on stage with 5-year survival in localized disease above 90% and 5-year survival about <30% in patients with distant metastasis.^[11] Knowing the tumor biology, its location and extent through imaging has been to improve patient's treatment outcomes.^[12] SSTR PET/CT is proven to be diagnostically superior to conventional scintigraphy and CT and used extensively for staging, restaging and assessing response to treatment in patients with NET.^[13] ⁶⁸Ga-DOTA-NOC PET/CT impacts management of NET patients by modifying treatment in over 50% of referred patients.^[14] ⁶⁸Ga-SSTR-PET/CT forms the basis of selecting patients for PRRT, a new and promising treatment for NET patients. PRRT has shown response rates of 25-30% with comparable progression-free survival and overall survival with other available medical treatments.^[15]

The increasing need of ⁶⁸Ga-labeled molecules for the above-mentioned oncological and nononcological indications has propelled the development of many GMP-certified ⁶⁸Ga-Generators. Despite excellent advances of present-day ⁶⁸Ga-radiopharmacy, it is pertinent to point out that majority of the commercially available ⁶⁸Ge/⁶⁸Ga generators have several shortcomings and are not directly amenable for use in clinical context.^[16,17] To circumvent these limitations, "state-of-the-art" automated modules have been developed over the last few years for postelution processing of ⁶⁸Ga and subsequent radiopharmaceutical preparation.^[18,19] However, these automated modules are highly expensive and beyond the reach of most nuclear medicine departments, especially in developing countries. Therefore, we have indigenously developed a ⁶⁸Ge/⁶⁸Ga generator using CeO₂-PAN sorbent which could directly be used for the preparation of radiopharmaceuticals. Recently, Isotope Technologies Garching GmbH has launched a new GMP-certified ⁶⁸Ge/⁶⁸Ga generator in the clinical market that utilizes pyrogallol-derivatized SiO₂ as an adsorbent and is directly amenable for clinical use. A detailed performance evaluation of both these generators was recently carried out by our group, and the performances of these two generators were found to be comparable.^[20] Thus, we could ensure that our indigenously developed generator is suitable for clinical use.

To achieve the goal of making ⁶⁸Ga as the "work-horse" of PET, just as ^{99m}Tc is for SPECT, it is prudent to develop cold kits containing precursors in freeze-dried form for extemporaneous preparation of ⁶⁸Ga radiopharmaceuticals. Our group has demonstrated feasibility of preparation of ⁶⁸Ga radiopharmaceuticals

utilizing cold kits of peptide conjugates.^[3,5] Herein, we report our first clinical experience with ⁶⁸Ga-DOTA-NOC prepared using ⁶⁸Ga from the indigenous generator and freeze-dried cold kits of DOTA-NOC. ⁶⁸Ga-DOTA-NOC in consistently high yields could be prepared in 10 min and within 35 min, radiolabeled DOTA-NOC was ready for injection into the patients after quality control analysis. Moreover, availability of cold kits independent of ⁶⁸Ga generator provides more control over the reaction parameters compared to automated modules for ⁶⁸Ga radiopharmaceutical preparation.

We analyzed the application of ⁶⁸Ga-DOTA-NOC imaging for a wide variety of indications, which included pulmonary/gastro-enteropancreatic NETs, paragangliomas, ectopic ACTH syndrome, suspected meningioma, and tumor-induced osteomalacia. PET/CT showed intense uptake in two patients with paraganglioma and two patients with suspected meningioma, supporting the increasing use of PET/CT for these indications. PET/CT was successful in localizing the primary tumor in four patients (1 - tumor induced osteomalacia, 3 - metastatic NET of unknown primary). PET/CT was negative in 10 patients (4 - medullary Ca thyroid, 1 - suspected insulinoma, 3 metastatic NET with high proliferation index, 1 - ectopic Cushing's syndrome, 1 - thymoma). Compared to the standard imaging for NET, ^{99m}Tc-HYNIC TOC, significantly more number of lesions was identified on ⁶⁸Ga-DOTA-NOC PET-CT. However a change of management was noted in only one patient, which could be explained by small number of patients and heterogeneous clinical indications in our study.

Conclusion

The first clinical experience with BARC's CeO₂-PAN sorbent-based ⁶⁸Ge/⁶⁸Ga Generator and freeze-dried DOTA-NOC kits was found to be satisfactory in various clinical indications. Larger studies can be considered, to promote its widespread use as a cost-effective molecular imaging agent for the assessment of NETs.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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