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Radiosynthesis and clinical evaluation of ^{68}Ga-DOTANOC as somatostatin receptor positive benign sub-dural meningioma PET imaging agent

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Abstract: Nuclear medicine technique is a unique technique to diagnose and treat infectious and cancerous diseases at cellular or even at molecular level with great sensitivity and accuracy. Receptor positive tumors are diagnosed and treated with positron/beta-particle emitter radionuclide labeled somatostatin peptides with awesome outcome and the patient get relief in short time. DOTANOC is somatostatin hormone analogue of eight amino acid having promising affinity for somatostatin receptor positive malignancies. This study was to follow up neuroendocrine tumor suppression which also indicated the up-taking of gallium-68 (^{68}Ga) labeled DOTANOC by benign sub-dural meningioma. Regarding labeling yield, high performance liquid chromatography analysis showed 99.17 ± 0.34% radiochemical purity. Radiochemical stability in reaction mixture and blood serum was found quite stable up to 4 h. Biodistribution study revealed that radiochemical does not accumulate at non-target sites. Kidneys showed 8.17 ± 0.76 % ID/g-organ accumulation. PET/CT scintigraphy showed in addition to follow up regular tumors therapy, the agent can also accumulate at SStR positive benign sub-dural meningioma.

Keywords: Meningioma, radiopharmaceuticals, ^{68}Gallium-DOTANOC, PET-CT imaging, NETs.

INTRODUCTION

Nuclear medicine technique (NMT) in current era has gained ample attention in diagnostic field. The imaging is carried out either using single photon emission computed tomography (SPECT) or positron emission tomography (PET) (Tariq *et al*., 2020). In former case radiopharmaceuticals labeled with gamma emitter radionuclide - technetium-99m (^{99m}Tc) or indium-111 (^{111}In) (Naqvi *et al*., 2018), while in later the radiopharmaceuticals are labeled with be positron emitter – gallium-68 (^{68}Ga). PET scan is preferred over SPECT scan due to its 3D-detailed imaging ability of disease. ^{68}Ga is one of the best PET radionuclide with suitable half-life, cost and compatibility to make strong bond with DOTA chelating agent. Currently, ^{68}Ga or lutetium-177 (^{177}Lu)-labeled DOTA-coupled bio-analogues are in clinical practices to diagnose and treat neuroendocrine tumors (NETs) (Rizvi *et al*., 2018).

Neuroendocrine tumors (NETs) originate in the cells that are specific to produce hormone in the body. These cells are specific to perform or to regulate key body functions including regulating air and blood flow through the lungs and controlling the movement of food through the gastrointestinal tract. The malignancy of these cells grows in the form of pheochromocytoma, a rare NET; merkel cell cancer, a highly aggressive NET and neuroendocrine carcinoma (~60% of NET) in addition to common NETs such as pituitary gland tumors, thyroid cancer, pancreatic neuroendocrine tumors, and adrenal gland tumors (Becker *et al*., 2017; Moriyama *et al*., 2016).

The prevalence of NETs has been slowly increasing over a period of time, possibly due to the advent of new molecular imaging modalities. NET are associated with over-expression of somatostatin receptors (SStR). Variety of neuroendocrine hormone analogues of varied length of amino acid sequence, were developed and labeled with radionuclides to target SStR positive NETs – especially ^{68}Ga-DOTA-TOC/TATE and ^{68}Ga-DOTANOC. The later has been widely tested due to its affinity to broad range of SStR i.e. SStR2, SStR3 and SStR5 than other analogues. DOTA-TATE shows affinity for SStR2 while DOTA-TOC shows affinity for SStR2 and SStR5 (Reubi, 2003). This study is reported to explore the potential of 68Ga-DOTANOC to diagnose benign sub-dural meningioma in clinical setup in addition to NETs diagnosis and therapeutic follow-up.

MATERIALS AND METHODS

All the chemicals used in this study were of analytical grade and purchased from Sigma-Aldrich (Germany). The ^{68}Ga-generator and DOTANOC freeze dried compound was obtained from Germany. The required amount of

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^{68}Ga activity was eluted by rinsing the generator with 0.05M hydrochloric acid in a sterile single-use cassette. Conditioning of C-18 purification cartridge is done using 1.5 mL 70% (v/v) ethanol, purged with 5 mL air and thereafter rinse with 5 mL 0.9% sodium chloride aqueous solution to make it ready for labeling with DOTANOC.

Radiolabeling of DOTANOC with ^{68}Ga

Radiolabeling was carried out by reported protocol (Vis *et al*., 2015). Briefly, 100 µL DOTANOC solution (75µg DOTANOC acetate/150µL 0.25M sodium acetate buffer) was added into pre heated vial (in a dry heating block) containing 1mL sodium acetate buffer (0.25M). After 5min heating, direct elution of ^{68}Ga was carried out with 4mL HCl (0.05M) from ^{68}Ge/^{68}Ga generator/sterile single-use cassette into the reaction vessel. The labeling reaction was allowed to complete at 90°C – 95°C for 10 min. Subsequently, the reaction mixture was eluted through C-18 cartridge followed by rinsing with 5mL saline solution to purify the ^{68}Ga-DOTANOC. Finally, the C18 cartridge was eluted with 1mL 60% (V/V) ethanol and rinsed the cartridge with 5mL saline solution through the on-line 0.2µm filter into the labeled product vial. The labeling percentage was determined by HPLC equipped with ray-detector.

High-performance liquid chromatographic analysis

Radiochemical purity was assessed with high performance liquid chromatography (HPLC) coupled with interconnected NaI gamma ray and UV-visible detectors. The HPLC analysis was carried out by injecting 200µL sample having C-18 column as stationary phase. The gradient solvent phase comprises of acetonitrile and ultrapure deionized water (80:20 V/V for 5 min; 20:80 V/V up to 15 min and then 80:20 V/V up to 20 min). The flow rate of mobile phase was maintained to 0.8 ml/min and UV-visible detection was made at 220 nm wavelength.

Self-life and serum stability study of ^{68}Ga-DOTANOC

The labeled ^{68}Ga-DOTANOC radiopharmaceutical after radiolabeling was studied for stability and shelf-life. The labeled compounds were periodically pipit out and injected to HPLC for analyzing at an interval of 30 min up to 2h for intact percentage. The stability in human blood serum was studied using freshly harvested healthy human blood serum. To a 0.8mL blood serum added 0.2mL radiolabeled compound, vortexed and incubated at 37ºC in CO_2-incubator. At different time intervals, typically 0.5, 1, 2 and 4h time points, took 5µL incubated mixture and analyzed with HPLC to note percent value of intact ^{68}Ga-DOTANOC radiopharmaceuticals.

Biodistribution of ^{68}Ga-DOTANOC in rabbit model

The biodistribution behavior of freshly synthesized ^{68}Ga-DOTANOC was studied in three normal rabbits. About 200µL radiochemical was injected intravenously through rear ear vein of three rabbits. After 4h the rabbits were given chloroform anesthesia – then the radioactive counts in emitted from each organ were recorded using gamma camera. The distribution was expressed in term of percent injected dose per gram organ (%ID/g organ).

SStR affinity on benign sub-dural meningioma

Typically, DOTANOC is well known for its good binding affinity at SStR which overexpressed at the cell surface of NETs. Under the hospital guidelines after ethical approval from Medical Board, the regular follow up procedure was in process of 71-year-old male with histopathologically proved case of carcinoid of appendix in nuclear medicine department. The subject was intravenously administrated with 2.3mCi of freshly prepared ^{68}Ga-DOTANOC radiopharmaceutical. For scintigraphy, after an initial uptake phase of about 101 min, a CT-Scan without oral and IV contrast, and without breath holding at low mA level was acquired (arms were held up), only for attenuation correction and localization purposes. Subsequently PET images from the vertex to mid-thigh were obtained. CT, PET and PET-CT fused images were reconstructed in trans-axial, coronal and sagittal projections and interpreted from a workstation which also indicated the accumulation in benign sub-dural meningioma. Reconfirmation imaging study indicated the positive results for benign sub-dural meningioma.

STATISTICAL ANALYSIS

The data was analyzed using CoSTAT V 6.3 software (Cohort software, Berkeley, California, USA).

RESULTS

^{68}Ga-DOTANOC yield and HPLC analysis

The radiolabeling was carried out under the guidelines of good manufacturing practice (GMP) with 99% radiolabeling yield. The results of the HPLC analysis of ^{68}Ga-DOTANOC showed only one peak at 9.45 min that indicate the purity of the radiochemical as shown in fig. 1.

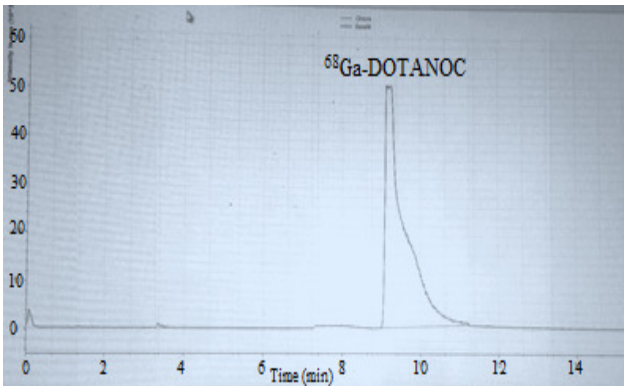

Fig. 1: HPLC analysis of ^{68}Ga-DOTANOC.

Self-life and serum stability study of ^{68}Ga-DOTANOC

Shelf life of radiochemical was studied up to 2h while stability in blood serum was studied up to 4h. The results of the studies are shown in fig. 2.

Biodistribution of ^{68}Ga-DOTANOC in rabbit model

Fig. 3 shows the biodistribution of ^{68}Ga-DOTANOC in a set of three normal rabbit model prior to human patient administration to record if there is any uneven event or toxicity appears as part of quality control procedure.

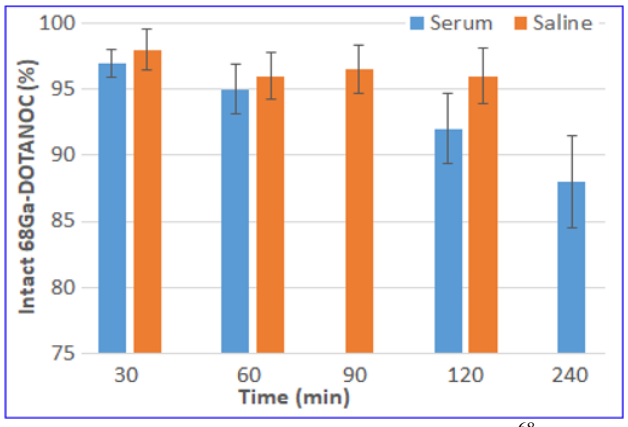

Fig. 2: Shelf-life and serum stability analysis of ^{68}Ga-DOTANOC

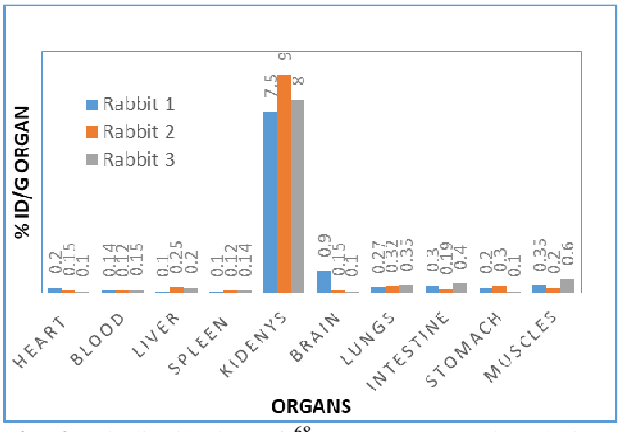

Fig. 3: Biodistribution of ^{68}Ga-DOTANOC in rabbits at 4h post administration time point

SStR affinity on benign sub-dural meningioma

The Body Mass Index of patient administrated ^{68}Ga-DOTANOC intravenously, was 35.7 and study was performed on a PET/CT scanner (GE Discovery 710 PET/CT camera using 2.5 min/bed acquisition). The dosimetry values showed a CT tube voltage 120Kv, CTDI/Vol=5.1mGy and DLP=707.9mGy-cm for entire study. The results of PET/CT scan are shown in fig. 4.

DISCUSSION

The radiolabeling yield is a key step for successful theranostic results. Typically, more than 95% labeled radiochemical is preferred to administrate into patient's body to avoid any radiotoxicity. For imaging study, the DOTANOC was labeled with positron emitter ^{68}Ga radionuclide with 99.17 ± 0.34% radiochemical yield which omit the chance of radiotoxicity.

Quality control parameters i.e. radiochemical stability in reaction mixture and in blood serum revealed promising results. The former study showed more than 96.67 ± 2.09% intact ^{68}Ga-DOTANOC while the later study showed 92.54 ± 2.67% intact radiochemical at 2 h post-labeling period. The stability profile is also in good agreement with other clinically approved radiopharmaceutical (Mueller *et al*., 2016). Biodistribution data showed that ^{68}Ga-DOTANOC completely clear from blood within 2h without degradation as revealed from serum stability results. The body organs of rabbits showed negligible uptake which mainly the blood circulation carrying the tracer. Only kidneys showed significant uptake which filter blood as a secretory organ. However, the kidney uptake is in good agreement with reported data (Leisser *et al*., 2019).

In parallel to ^{68}Ga/^{177}Lu-DOTANOC, ^{68}Ga/^{177}Lu-DOTATATE is an approved NET theranostic agent with somatostatin analogue peptide having a sequence of eight amino acids bearing disulfide bond between 3rd and 7th position cysteine amino acids (Das et al., 2014). DOTANOC differ with DOTATATE at phenylalanine position which replaced with naphthyl alanine (Nal3) at position 3 in DOTANOC. This change enables ^{68}Ga/^{177}Lu-DOTANOC to bind other SStR subtypes i.e. SStR3 and SStR5 in addition to SStR2 which enhances its potential to fix variety of other NETs (Baum et al., 2018). Finally, freshly prepared 2.3 mCi ^{68}Ga-DOTANOC was injected intravenous into human subject who have completed the therapeutic procedure for NET at hip joint. NET follow up post 1 h administration of ^{68}Ga-DOTANOC scintigraphy using PET/CT showed complete eradication of the tumor. However, there was sub-dural lesion with intense focal tracer accumulation of ^{68}Ga-DOTANOC seen in the frontal lobe of the skull anteriorly. However, the subject had no complaints related to the skull and these finding were labeled as normal variant for uptake of ^{68}Ga-DOTANOC. There was physiological activity identified within the pituitary gland, thyroid gland, spleen, liver, uncinated process of pancreas and renal collecting system, and abnormal uptake of the tracer in the appendicular fossa was noted. Other organs of the body also showed no tracer uptake and behaved like normal organ. Based on the clinical history and normal CT scan findings, frontal lobe lesion was labeled as most likely suggestive of SStR positive benign sub-dural meningioma.

CONCLUSION

^{68}Ga-DOTANOC radio-synthesis yield is quite promising and its stability also compatible to clinical practice. High

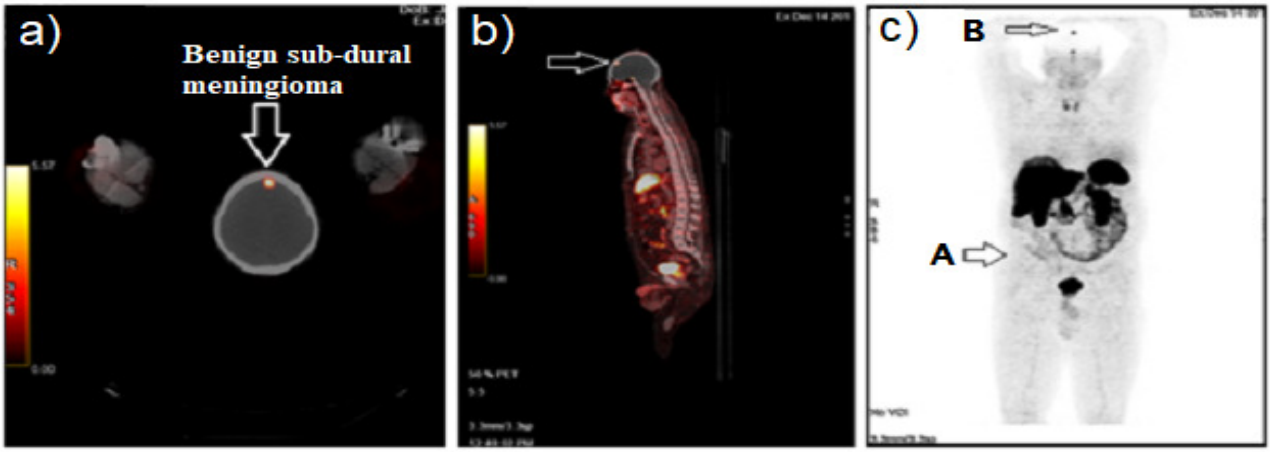

Fig. 4: ^{68}Ga-DOTANOC PET/CT showing a) axial fused PET-CT, b) sagittal view of fused PET-CT; the arrow in both scans showing the uptake by benign sub-dural meningioma, c) show the anterior view of maximum intensity projection (MIP) image with A showing the clearance of malignant tumor and B showing the benign sub-dural meningioma.

sensitivity for SStR positive targets make it the NETs theranostic agent of choice. However, clinicians must keep in view the normal variants in which ^{68}Ga-DOTANOC shows significant uptake and must not confuse with cancer metastases

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