BOOK OF ABSTRACT

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AN OVERVIEW ON THE DEVELOPMENT OF CURCUMIN-BASED ^{68}Ga-RADIOTRACES: FROM DIRECT LABELING TO THE BIFUNCTIONAL CHELATOR APPROACH

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Introduction:

Curcumin (CUR), a natural occurring polyphenol extracted from the dried rhizomes of *Curcuma Longa* L., is by far known for its therapeutic properties. Among these, colorectal cancer represents one of its best targets, as reported in cell cultures studies [1,2]. In addition, Curcumin metal complexes are promising metal-based drugs that demonstrated widespread applications in medicine [3] and can be exploited for developing new radiotracers [4]. Herein, an overview on curcumin-based radiotracers studied by our group throughout the last decade is reported, focusing on the synthesis, chemical characterization, radiolabelling, *in vitro* and *in vivo* studies.

Materials and Methods:

Curcuminoid precursors were synthesized as reported in literature [5,6]. ^{68}Ge/^{68}Ga generator was eluted with 5 mL of 0.05 M HCl. The eluted activity was purified through a Bio-Rad AG50W-X8 cartridge. After subsequent elution, aliquots containing 50-80 MBq of [^{68}Ga]Ga^{3+} were added to the precursors (10 nmol) and the mixtures were incubated for different times and temperatures in a pH 4 buffered environment. To estimate stability, radiotracers were incubated with PBS (0.2 M, pH = 7.2), human serum (HS), and human blood (HB) at 37 °C for different time points and analyzed by radio-TLC. Lipophilicity was measured by HPLC-based method. Cellular uptake, internalization, blocking and efflux were studied in HT29 colon carcinoma cells line. PET imaging of the most promising radiotracer were determined in mice bearing HT29 subcutaneous tumour model after *i.v.* or *i.p.* injection with 3.7 MBq (1 mL, 0.2 µM solution) of radiopharmaceutical in sterile PBS.

Results:

Radiolabeling generally afforded complete (> 95%) and rapid incorporation under a range of mild and harsh conditions (from RT to 95°C) depending on the chelator. Stability of metal complexes in physiological media (PBS, HS, HB) resulted greatly enhanced when the bifunctional approached was followed rather than the direct radiolabeling of the derivatives, and it was generally > 60% after 2 h. The higher stability was also confirmed by mass spectra. Lipophilicity value is strongly affected by the chemical structure, and tends to decrease when hydrophilic chelators are used. In vitro uptake of the best derivative increased continuously in the first 60 min of incubation up to 12 KBq of radiotracer per mg of protein. At 60 min post incubation, 83% of the total radioactivity was internalized. *In vivo*, xenograft tumours could be clearly visualized after 1 h *ip* of the radiotracer (2.27% ID/cc) although high accumulation could be also recorded in kidneys, intestine and lungs.

Discussion and conclusions:

Curcumin was used both as direct chelator and as targeting vector following the bifunctional chelator approach. According to this latter, curcumin was linked to a series of chelators such as DOTA, NODAGA or AAZTA to improve the stability and potentially the biodistribution of their complexes with gallium-68. Both DOTA- and NODAGA-CUR were successfully labelled and showed interesting uptake in colorectal cancer cells (HT29), almost comparable stability in physiological media and promising lipophilicity. Particularly, [^{68}Ga]Ga-NODAGA-CUR showed improved labelling kinetics.

Acknowledgements:

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References:

[1] Basile V, Ferrari E, Lazzari S, Belluti S, Pignedoli F, Imbriano C [2009] Biochem Pharmacol 78: 1305-1315.

[2] Rubagotti S, Croci S, Ferrari E, Orteca G, Iori M, Capponi PC, Versari A, Asti M [2017] J Inorg Biochem 173: 113-119.

[3] Wanninger S, Lorenz V, Subhan A, Edelmann FT [2015] Chem Soc Rev 44: 4986-5002.

[4] Mari M, Carrozza D, Ferrari E, Asti M. [2021] Int J Mol Sci 22: 7410-7430.

[5] Asti M, Ferrari E, Croci S, Atti G, Rubagotti S, Iori M, Capponi PC, Zerbini A, Saladini M, Versari A [2014] Inorg Chem 53: 4922-4933.

[6] Orteca G, Tavanti F, Bednarikova Z, Gazova Z, Rigillo G, Imbriano G, Basile V, Asti M, Rigamonti L, Saladini M, Ferrari E, Menziani MC [2018] Bioorg Med Chem 26: 4288–4300.

CAN NUTRITIONAL REGIMENS AFFECT RADIOTRACERS UPTAKE? KETOGENIC DIET: THE POSSIBLE KEY TO ENHANCE [^{18}F]FDG PERFORMANCES

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Introduction:

Bronchioloalveolar cancer (BAC) is a subtype of non-small cell lung carcinomas (NSCLC) whose diffusion has recently largely increased worldwide. Differently from other NSCLCs, its early diagnosis using [^{18}F]FDG-PET examination, still remains unpredictable due to its lower glucidic metabolism [1,2]. In this study, the effects of a dedicated nutritional regimen, designed to trigger the glucidic metabolism, was investigated *in vitro*. Consequently, the correspondent regimen (ketogenic diet) was applied to murine models carrying xenograft BAC with the purpose to increase the [^{18}F]FDG uptake respect to mice normally nourished.

Materials and Methods:

[^{18}F]FDG was produced by using standard methods described in literature [3]. Cells isolated from a primary BAC (NCI-H358) and other cancer lung cell lines (A549 and NCI- H1299) was grown both in a glucose-rich or a glucose-depleted medium (RPMI + 10% FBS). After several days of both nutritional regimens (3, 5, 7 days) around $2 \cdot 10^5$ cells of every lines were seeded in multiwell plates (6-well). [^{18}F]FDG aliquots (10 microL, 3.7 MBq) were added to each culture well and incubated at

37°C for 60 minutes. Uptake was stopped after 60 minutes and cells were washed twice with ice-cold PBS. Finally, cells were detached with 2 mL of 0.25% trypsin/EDTA solution and the samples were centrifuged. The radioactivity associated to the pellets was measured in a □-spectrometer and data were normalized for activity injected and corrected for decay. All experiments were performed in triplicate.

For *in vivo* experiment NOD-SCID mice (n=16) were implanted with around $5 \cdot 10^6$ NCI-H358 cells and tumors were grown up to 100 mm^3 size. Three groups of mice (n=4/groups) were fed with a ketogenic regimen (PF4390 feed) for 3, 5 and 7 days, respectively while a control groups (n=4) underwent normal diet (4RF18 feed). The day after the end of the nutritional regimen mice were injected with 20-40 □Ci of [^{18}F]FDG and PET/CT tomographic acquisition were performed 2 hour p.i. Subsequently, mice were euthanized, tumor and main organs were extracted, weighted and measured in a □-counter for assessing the biodistribution. Results were corrected for decay and computed as mean ID% per grams of tissue.

Results:

As expected, when incubated with glucose-rich medium, NCI-H358 (BAC lines) cells have a really low [^{18}F]FDG uptake (up to 4-fold less) compared to A549 and NCI- H1299 cells. On the other hand, when a glucose-depleted medium is used, a significantly enhanced uptake in NCI-H358 cells respect to the other two lines (up to 3-fold higher after 5 days) was obtained. In the PET/CT images, tumors are clearly better visualized in mice subjected to ketogenic diet respect to control group already after 3 days. On the other hand, biodistribution attested the highest tumor uptake after 3 days of dietary regimen (1.2 ID/g vs 0.5 ID/g).

Discussion/Conclusions:

The study attested how [^{18}F]FDG uptake in a low glucose dependent tumor, usually not detected by PET/CT, can by controlled *in vitro* and in murine models by a dietary regimen. The results have potentially a strong translation impact since a short ketogenic diet can be easily applied to patients suspected of BAC before PET/CT examination with negligible effect on their life quality but improving the tumor early detection.

References:

[1] Barsky SH et al. [1994] Cancer. 73(4): 1163-70
[2] Balogova S et al. [2010] Nucl Med Commun. 31(5): 389-97
[3] Yu S. [2006] Biomed Imaging Interv J. 2(4): e57

SPARK PLASMA SINTERING TECHNIQUE FOR CYCLOTRON SOLID TARGET MANUFACTURING

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Introduction:

In the last years, the evidence that radiopharmaceutical labelled with emerging radionuclides (e.g., ^{67}Cu, ^{155}Tb, ^{89}Zr and ^{52g}Mn) have shown great potentialities in nuclear medicine for implementing the modern imaging procedures, as well as the Targeted Radionuclide Therapy (TRT). However, their routine supply to carry out preclinical/clinical studies is limited mainly due to limitations in targets manufacturing technology. The LARAMED group at INFN-LNL has put a lot of efforts in R&D activity on targetry [1], and now the cyclotron manufacturing technique under the spotlight is the Spark Plasma Sintering (SPS) also known as Field Assisted Sintering Technique (FAST) or Pulsed Electric Current Sintering (PECS). The recent results have shown great potentiality of this technique leading to the manufacturing of cyclotron solid targets that meet all the necessary requirements to improve the radionuliclide yield.

Materials and methods:

The SPS technique is a well-known method used in different application fields. High-quality sintered pellets, starting from powdered material are obtained by using the simultaneous application of uniaxial pressure and temperature, which is generated by a high-intensity electrical current passing through the sample compressed in a graphite die. In addition, different material can be joined or welded together (i.e., pellet on backing material) [2]. The negligible loss of the starting material makes it attractive for the target composed of expensive isotopically enriched materials usually employed for medical radionuclide production without contaminants.

A new custom TT_Sinter machine was developed by the University of Pavia for the LARAMED project with the aim to acquire the know-how to be able to supply the solid targets based on the needs of research projects on specific radionuclides.

Results:

The manufacturing technology for ^{89}Y and ^{52}Cr SPS targets was established with the new TT_Sinter machine following 1 step to bond Y onto Nb disc [3] and 3 step for Cr target (i.e., 1-Cr pellet realization starting from powder, 2-bonding of thin inert Au layer onto Nb disc, 3-bonding of Cr pellet to backing, Au/Nb). Scanning Electron Microscopy analyses have shown good adhesion between the materials that led to an improved heat dissipation capacity during the irradiation. Indeed, they withstood a heat deposited power density, during the irradiation, of about 1 kW/cm^2 (TR19 Cyclotron at Sacro Cuore Don Calabria Hospital was used). In addition, the target system (target material + backing) presents an easier handling after the irradiation process, by using a dedicated dissolution reactor which, in turn, limits the operator radiation exposure during the operations [4].

Discussione / Conclusione

The realization of other solid targets for the production of ^{67}Cu and ^{155}Tb starting from ZnO and Gd_2O_3 targets, respectively, is under investigation.

The R&D activity on SPS technology for cyclotron solid targets of different materials, might pave the way to a systematic supply of targets for further studies in radiochemistry and radiopharmaceutical fields. This topic is already under investigation in the framework of the LARAMED project.

Bibliografia

[1] Skliarova H, Cisternino S, Cicoria G, Cazzola E, Gorgoni G, Marengo M, Esposito J, [2020], J. Phys.: Conf. Ser. 1548: 012022

[2] Hu Z. et al, [2020], Materials & Design 191: 108662

[3] Cisternino S, Cazzola E, Skliarova H, Amico J, Malachini M, Gorgoni G, Anselmi-Tamburini U, Esposito J, [2022] Nucl. Med. Biol. 104-105: 38-46

[4] Sciacca G, Martini P, Cisternino S, Mou L, Amico J, Esposito J, Gorgoni G, Cazzola E, [2021] Molecules, 26: 6255

AN ATTEMPT TO HAMPER THE REDOX-INITIATED DEMETALLATION IN COPPER-BASED RADIOPHARMACEUTICALS USING A SULFUR-CONTAINING DOTA DERIVATIVE: COORDINATION CHEMISTRY, RADIOLABELING AND *IN VIVO* EVALUATION

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Introduction:

Copper-64 has shown great potential for PET imaging and cancer therapy thanks to its decay profile which combines β^+, β^- and electron capture emissions. The delivery of copper-64 to tumour cells is feasible through its stable complexation with a chelator covalently tethered to a tumour-targeting vector. Unfortunately, the *in vivo* integrity of [$^{64}Cu^{2+}$]-complexes can be constrained by the biologically triggered copper reduction that may bring to demetallation processes. In attempt to stabilize both copper oxidation states, two of the carboxylate-containing pendants in DOTA were replaced with softer S-containing arms in DO2A2S (**Figure 1**). To evaluate its potential as chelator for Cu-based radiopharmaceuticals, the coordination properties, stability and *in vivo* behaviour of DO2A2S with Cu^{2+}/Cu^{+} and copper-64 were explored *in vitro* and in animal models.

Materials and Methods:

Kinetic, thermodynamic, and structural properties of the Cu^{2+}/Cu^{+} complexes of DO2A2S were investigated in aqueous solution at 25°C by spectroscopic and electrochemical techniques. Concentration-dependent radiolabeling were performed with [^{64}Cu]Cu^{2+} at different temperatures and pH. Incorporation yield was assessed by radio-TLC and UHPLC. Competitions and stability assays with DOTA (1:1 and 1:1000 DO2A2S-to-DOTA ratio, respectively), stability in PBS and human serum were executed as well. [^{64}Cu][Cu(DO2A2S)] biodistribution in BALB/c nude mice and its stability in urine and blood at different time points post-injection were evaluated. Organs uptake and clearance pathways were assessed by PET/CT.

Results:

DO2A2S formed a [4N]2O octahedral cupric complexes which displayed a thermodynamic stability (pCu^{2+} = 19.4) superior to the correspondent DOTA complex at physiological pH (pCu^{2+} = 17.4). The

stability remained remarkable upon reduction to Cu^{+} due to the coordination of one S atom to the metal center (pCu^{+} = 14.1). At pH 7, DO2A2S was able to quantitatively label $[^{64}Cu]Cu^{2+}$ at 50 MBq/nmol after 10 minutes at RT whilst heating at 90°C was needed to obtain the same results at pH 4.5. In the competition studies, $[^{64}Cu]Cu^{2+}$ revealed to form complexes with DO2A2S with superior kinetics respect to DOTA resulting in a $[^{64}Cu][Cu(DO2A2S)]/[^{64}Cu][Cu(DOTA)]^{2-}$ ratio of 10:1 after 10 minutes at RT. Stability tests demonstrated that ~87% of $[^{64}Cu][Cu(DO2A2S)]$ remained intact after 24 h of incubation with DOTA. Stability in PBS and human serum was excellent (> 95%). *In vivo*, $[^{64}Cu][Cu(DO2A2S)]$ displayed a metabolic trend similar to other $[^{64}Cu]$-labelled cyclen derivatives. At 24 hours post-injection, most of the radioactivity was associated with liver and kidneys (11.5 and 10.3 %ID/g), but relatively high accumulation was found in lungs, spleen, and pancreas as well (6.0, 5.3, and 5.5%ID/g). High level of intact complex (>95%) was found in mice blood up to 24 hours post-injection while the presence of 50% of free-$[^{64}Cu]Cu^{2+/+}$ was detected in urine already at 4 hours post-injection.

Discussion/Conclusions:

DO2A2S forms highly stable complexes in presence of both Cu^{2+}/Cu^{+} thanks to the simultaneous presence of hard and soft donors that match the coordination requirement of the two oxidation states. Radiolabeling of $[^{64}Cu]Cu^{2+}$ was achieved at mild conditions and high yield exhibiting excellent serum stability over 24 h. *In vivo* studies performed so far are not enough for elucidating whether transchelation phenomena occurred or the high accumulation in the liver is due to metabolic derivatives of the intact complex.

References:

[1] Tosato M, Dalla Tiezza M, May NV, Isse AA, Nardella S, Orian L, Verona M, Vaccarin C, Alker A, Mäcke H, Pastore P, Di Marco V, [2021], *Inorg. Chem.*, 60: 11530-11547.

[2] Shuvaev S, Suturina EA, Rotile NJ, Astashkin A, Ziegler CJ, Ross AW, Walker TL, Caravan P, Taschner IS, [2020], *Dalton Trans.*, 49: 14088-14098.

[3] Wu N, Kang CS, Sin I, Ren S, Liu D, Ruthengael VC, Lewis MR, Chong HS, [2016], *J Biol Inorg Chem*, 21: 177-184.

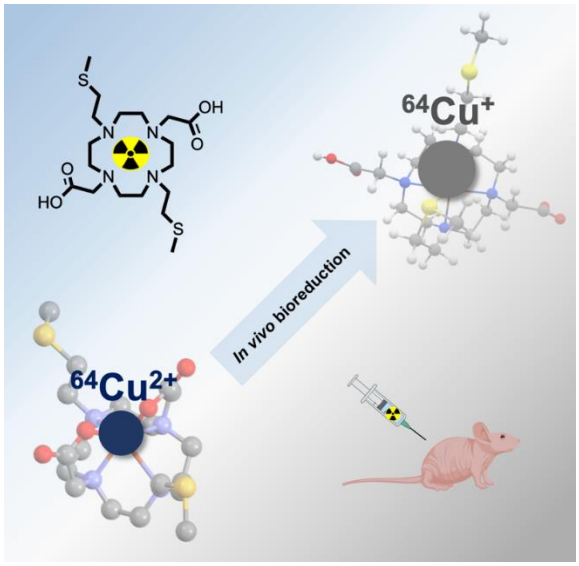

Figure 1.

ASSESSMENT OF SURVIVING FRACTION OF PROSTATE CANCER CELLS AFTER A TREATMENT WITH $^{64}CuCl_2$ OR $^{67}CuCl_2$

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Introduction

The ability of Cu^{2+} ions to selectively target cancerous cells has been assessed [1], demonstrating both diagnostic potential of $^{64}CuCl_2$ for prostate cancer [2] and therapeutic effect in patients with progressive malignant diseases [3]. In this work the surviving fraction of two prostate cell lines after administration of $^{64}CuCl_2$ or $^{67}CuCl_2$ were compared.

Materials and methods

The absorbed dose and related biological damage caused by ^{67}Cu and ^{64}Cu radionuclides were calculated with the MIRDcell software [4] using a of two concentric spheres cell model with outer and inner radius of 10 µm and 4 µm, representing the whole cell and its nucleus, the region between both spheres represents the cytoplasm.

Cellular dose factors (S-values) were obtained supposing radioactivity uniformly distributed inside the cell regions (source regions), assuming the whole cell as both the source and target region, or only the cytoplasm as the source region and the cell nucleus as the target region. Calculated S-values and the number of disintegrations in the source region per unit of activity administered, N_{source}, were used to obtain the absorbed dose to the target region:

$$D_{target \leftarrow source} = N_{source} \times S_{target \leftarrow source}$$

The cell survival fractions were estimated for LNCaP and PC3 cell lines, considering a 3D multicellular cluster with a radius of 50 µm, where 10% of the cells were labelled and uniformly distributed inside the cluster.

Results

For each radionuclide and radioactivity cellular distribution, the absorbed doses obtained for LNCaP and PC3 cell lines were equal, although higher values were obtained assuming radioactivity distributed evenly throughout the cell, compared to the more realistic approach, which considered the cytoplasm as the source region. Roughly, 1000 disintegrations of ^{67}Cu are required to reduce the surviving fraction of LNCaP cells to 50%, the number of disintegrations had to be increased to 1600 to obtain the same results with PC3 cells, due to their higher radioresistance. The number of decays must increase more than twice when ^{64}Cu-treatment was used; approximately 2100 and 3500 decays are needed to reduce to 50% the survival of LNCaP and PC3 cells respectively.

Conclusions

This work demonstrated that if ^{64}Cu remains in the cell cytoplasm the biological damage produced by its Auger electron emissions is minimal. The combination of the longer half-life and the greater number of β^- particles emitted by ^{67}Cu, results in a higher mean absorbed dose per cell. Consequently, the survival fractions of cancer cell treated with ^{67}Cu was lower than those treated with ^{64}Cu.

References

[1] Chakravarty R, *et al*, [2016], Mol Pharm. 13:3601-3612.

[2] Capasso E, *et al*, [2015] Ann Nucl Med. 29:482-488.
[3]Capasso E, *et al*,[2015] Eur J Nucl Med Mol Imaging. 42:S12–S13.
[4]Vaziri B, *et al*, [2014] J Nucl Med. 55:1557-1564.

A PRELIMINARY STUDY FOR USE IN PET/CT OF ^{18}F-FLUOROMETHYLCHOLINE, ^{18}F-FLUOROETILTYROSINE AND ^{18}F-FLUORODIHYDROXYPHENYLALANINE IN THE DIAGNOSIS OF PRIMARY AND SECONDARY BRAIN TUMORS

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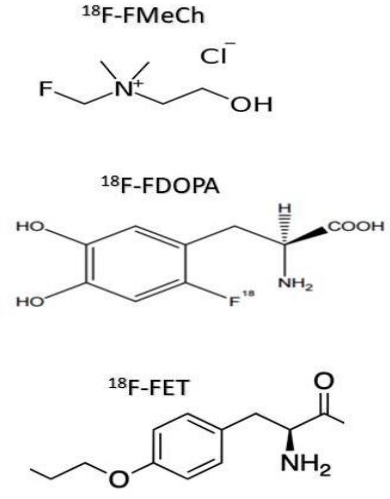
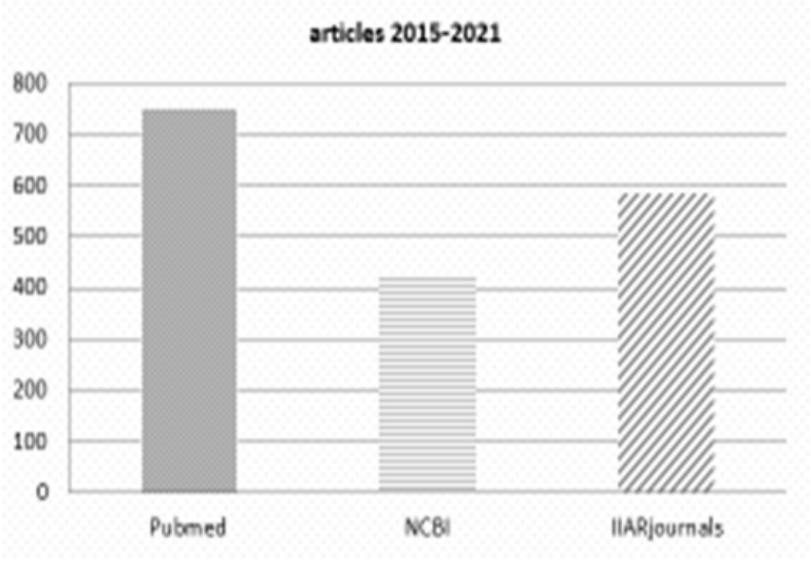
Introduction

Malignant gliomas are the most common forms of brain tumors. They are resistant to various treatments, often associated with short patient survival and in some cases difficult to diagnose. Although MRI represents the clinical gold standard for the morphological characterization of brain tumors, PET / CT with radiopharmaceuticals has acquired an increasing role in recent years in identifying the viable metabolic tumor. The work is a preliminary study to evaluate which radiopharmaceutical can be used in our Hospital in the diagnosis of glioma.

Materials and methods

From a first literature survey in the period 2015 - 2021, comprising a total of 1758 articles (751 Pubmed, 423 NCBI and 584 IIARjournals), 8 clinical studies were selected to compare the efficacy of IASOglio® (^{18}F-FET), Fluorodopa IASON® (^{18}F -FDOPA), or ^{18}F-FMCH produced at the radiopharmacy of the Hospital of Perugia, as officinal galenic, according to the European Pharmacopoeia. Costs of radiopharmaceutical were subsequently analyzed.





Results

The studies evaluated confirm that ^{18}F-FDG is not tumor specific, tends to concentrate in the cortex and is not useful in distinguishing the tumor from surrounding normal tissues.

As can be seen from the table, ^{18}F-FDOPA and ^{18}F-FET show selective absorption at the tumor site allowing diagnostic imaging of neoplastic lesions versus post-treatment inflammatory regions.

^{18}F-FDOPA is useful for classifying the primary diagnosis of gliomas, planning radiotherapy and subsequent follow-up; plays a key role in distinguishing radiation necrosis from glioblastoma recurrence; in relapsing glioma HGG allows to evaluate the response to radiotherapy treatment or anti-angiogenic therapy (es bevacizumab).

Physiological uptake of ^{18}F-FDOPA into the basal ganglia could interfere with the clear delineation of neighboring gliomas.

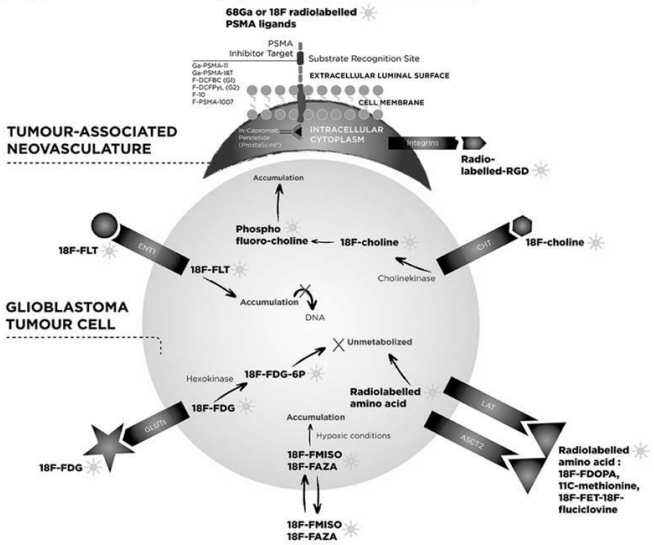
^{18}F-FET shows high uptake in primary brain tumors, particularly gliomas and is able to discriminate between glioblastoma and radiation necrosis. ^{18}F-FMC selectively accumulates in the neoplastic tissues allowing to evaluate the extent of the disease and the clinical outcome.

The cost of the radiopharmaceutical in euro/patient is: Fluorodopa IASON® 1150 euros; IASOglio® 890 euros; ^{18}F-FMCH galenic AO-PG 300 euros.

	primary diagnosis	LLG	HGG	discriminates glioblastoma from necrosis by R.I.	Cost (euro/patient)*
^{18}F-FMC galenic AO-PG	•	•		•	300
IASOglio®	•	•	•	•	890
Fluorodopa IASON®	•	•	•		1150

*The cost refers exclusively to the radiopharmaceutical. An off-label use of ^{18}F-FMCH would also involve the costs of a clinical trial

Pathophysiological mechanisms of main radiotracers used in glioblastomas investigation in functional nuclear imaging. (Front. Oncol. 01 Nov 2019 https://doi.org/10.3389/fonc.2019.01134)



Discussion/Conclusion

^{18}F-FET ^{18}F-FDOPA and ^{18}F-FMCH show good results in PET/CT imaging of brain tumors but IASOglio® and Fluorodopa IASON® are preferred comparing with ^{18}F-FMCH as they allow primary diagnoses, LLG and HGG.

As for the economic aspect, if we consider only the cost of the radiopharmaceutical, our production of the galenic ^{18}F-FMCH in Hospital seem the most advantageous solution.

However, as the use of ^{18}F-FMCH is authorized for prostate cancer and in some cases for parathyroid adenomas, the use in the diagnosis of gliomas would be off-label, so it could be carried out only in clinical trial, thus resulting in increased costs. As the cost of the different brain radiopharmaceuticals are equivalent, we believe that the most suitable choice is to use industrial radiopharmaceuticals.

Bibliografy

- Hodolic M. et al, 18F-FET or 18F-FCH PET/CT in the primary diagnosis of low-grade glioma: a pilot study, 2020 EMJ Radiol. [1]:35-37
- Evangelista L et al, Comparison Between 18F-DOPA and 18F-FET PET/CT in patients with suspicious recurrent High Grade Glioma: A Literature Review and Our Experience, Curr Radiopharm 2019;12(3):220-228
- Bolcaen J et Al, (18)F-fluoromethylcholine (FCho), (18)F-fluoroethyltyrosine (FET), and (18)F-fluorodeoxyglucose (FDG) for the discrimination between high-grade glioma and radiation necrosis in rats: a PET study, Nucl Med Biol, 2015;42(1):38-45.
- Verburg N et al, Direct comparison of [11 C] choline and [18 F] FET PET to detect glioma infiltration: a diagnostic accuracy study in eight patients;. EJNMMI Res, 2019 Jun 28;9(1):57.
- Nelli M.- thesis Master II livello gestione dei radiofarmaci –University of Study of Salerno-2021

A NEW EFFICIENT SEPARATION PROCEDURE OF CYCLOTRON PRODUCED MANGANESE-52

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Introduction

A perfect molecular matching between PET and MRI techniques can be achieved by using both paramagnetic as well as radioactive properties shown by manganese isotopes, which might allow for an unprecedented PET/MRI hybrid imaging modality. Manganese-52 may be produced starting from natural chromium target, with medium-low energy protons (10-20 MeV) mainly by the ^{52}Cr(p,n)$^{52m/g}$Mn and ^{53}Cr (p,2n)$^{52m/g}$Mn nuclear reaction routes. The radiochemical separation of manganese from the chromium target is a key step to isolate the desired product in a form suitable for its intended application.

Within the METRICS project supported by the CSN5 INFN, aiming to develop all the technology needed to get a cyclotron-driven $^{52/51}$Mn radionuclide production [1] we developed a new efficient Cr/Mn separation procedure based on column chromatography.

Material and Methods

^{nat}Cr metal pellets have been produced and bonded to Cu+Au or Nb baking materials by Spark Plasma Sintering technique [2]. The target design fits the solid target station size available at the ACSI TR19/300 cyclotron located at Sacro Cuore Don Calabria Hospital (Verona).

A solid target dissolution system has been manufactured by making some modifications to an automatic Ekert&Ziegler module which allows for performing the online target dissolution and purification processes with a cassette-based system. Different purification procedures, based on the combination of anionic (AG1-X8) and cationic (AG50W-X4) resins, have been applied to the purification process with the cassette-based system. The determination of Mn % recovery has been performed by γ-spectrometry analysis, whereas the amount of ^{nat}Cr in the final ^{52}Mn solution by ICP-OES analysis.

Results and Discussion

Ion exchange chromatography-based procedure has been selected as the most performing for the automation of two Cr/Mn separation procedures. The first reproducing a known process based on double anion exchange resin with Cr elution with a hydroalcoholic solution and Mn with HCl 0.1M [3-4]. The second approach is based on a first anion and a second cation exchange resins arranged in series, where the manganese eluted from the first resin is directly loaded onto the second one, thus avoiding time-consuming evaporation steps and large waste volume. The manganese is finally eluted in HCl 1.5M. The automatic prototype was assembled by using Eckert&Ziegler cassette-based modular units. Irradiation runs were carried out for purification process optimization. Preliminary results from bench tests, confirmed the efficiency of the selected separation processes, allowing a recovery yield of Mn of 88±5 %. Cr content in the manganese eluate after the first anion exchange resin is about 356±39 ppm. After the second anionic resin, Cr content in the final product is drastically reduced to ppb level. Whereas, if using a cationic as second resin, the time of the procedure is drastically reduced keeping Cr content in the Mn eluate at ppm-level.

Conclusion

A cassette-based automatic module together with a solid target dissolution system has been developed and implemented with two Cr/Mn separation procedures. The AG1-X8 and AG50W-X4 resin-based procedure appears to be advantageous in terms of process time and volume of radioactive waste produced. Further irradiation runs and results are currently under processing.

References

[1] Esposito J et al. [2019], Molecules, 24,20
[2] Sciacca G et al. [2021], Molecules, 26, 6255
[3] Pyles JM et al. [2021], Appl Radiat Isotopes,174,109741
[4] Fonslet J et al. [2017], Appl Radiat Isotopes121,38–43

SYNTHESIS OF [^{68}Ga]DOTA-TRASTUZUMAB: A PRELIMINARY RADIOCHEMICAL EVALUATION

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Introduction

Human epidermal growth factor receptor 2 (HER2) is a transmembrane receptor overexpressed in 25-30% of breast cancers, with effects on the regulation of cell growth, survival, differentiation, angiogenesis, and DNA repair [1,2]. Trastuzumab is a humanized IgG1 monoclonal antibody (mAb) recognizing an epitope in the extracellular domain of the receptor and is used for immunotherapy for HER2 positive tumors [3]. Although the use of trastuzumab is associated with a survival benefit, tumors will often develop resistance; thus, new approaches for targeting this pathway are being actively investigated [3]. If HER2 status can be accurately assessed noninvasively, immuno-PET imaging might be used to stratify the number and distribution of HER2-positive tumors. For this future purpose, a preliminary study of radiochemistry was undertaken. Trastuzumab was conjugated to DOTA chelator using three different excesses of DOTA-NHS, each precursor obtained was purified from not reacted DOTA and subsequently radiolabeled with Ga-68. Molar activities of 100 MBq/nmol were necessary to achieve quantitative radiolabeling. Quality controls on [68Ga]DOTA-Trastuzumab showed a radiochemical purity of about 95% requiring no further purification.

Methods

Trastuzumab (Trazimera, Pfizer) was purified from low molecular weight excipients through a size exclusion column (PD-10 desalting columns, Cytiva) by eluting with carbonate buffer (pH 9,2). Purified Trastuzumab was incubated with 5, 20 and 100 fold excess of DOTA-NHS (Chematech) at 4°C for 24 hours. Afterwards, for each batch, the reaction mixture was purified using a size exclusion column and acetate buffer 0,1 M (pH 5,5) as eluent. [68Ga]Gallium chloride was obtained by eluting a 68Ge/68Ga generator (GalliaPharm Eckert & Ziegler) using 5mL of HCl 0,1 N (Rotem). To 0,5 mL of [68Ga]GaCl3 eluate, a corresponding stoichiometric quantity of sodium acetate 1 M solution was added in order to reach a pH value of 4. Finally, 70 μg of DOTA-Trastuzumab was collected into the reaction vessel. Radiolabeling was carried-out at 40°C and at three different time points 30, 60, 90 minutes. Quality controls on precursor DOTA-Trastuzumab and on the radiotracer [68Ga]DOTA-Trastuzumab were executed using Radio-LC-MS and radio TLC.

Discussion and conclusion

The precursors DOTA-TRASTUZUMAB obtained by reaction of Trastuzumab with 5 (A), 20 (B) and 100 (C) fold excess of DOTA-NHS showed very different ESI-MS spectra. The spectrum of the precursor A was very close to that of not-modified Trastuzumab, whereas spectra of B and C deviated from it, indicating that the population of modified antibodies became more heterogeneous (figure 1).

The radiolabeling experiments conducted on precursors A, B and C showed that a molar activity of 100 MBq/nmol was necessary to achieve quantitative radiolabeling, with precursor B showing the better radiolabeling yield (almost 95%), hence requiring no further purification (figure 2).

In conclusion, conjugation with increasing amounts of chelator leads to a large heterogeneity in precursor composition, potentially compromising the affinity of trastuzumab for the epitope of HER-2. In vitro binding studies are necessary to establish what is hypothesized.

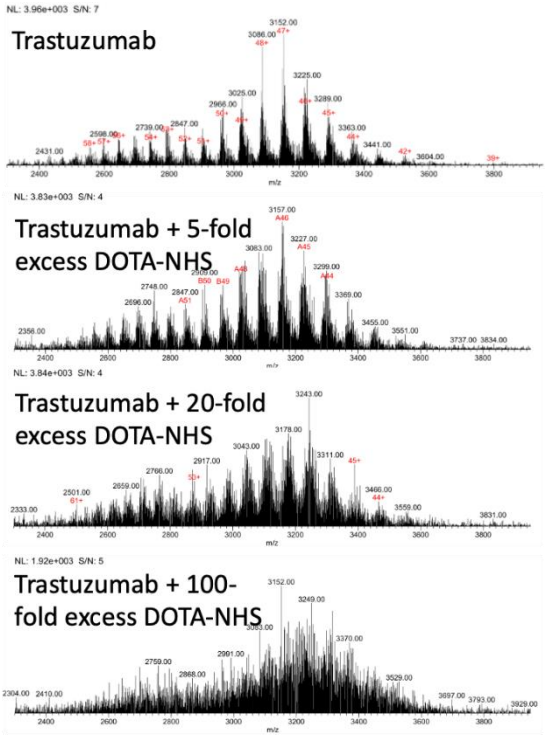

Figure 1. ESI-MS spectra comparison

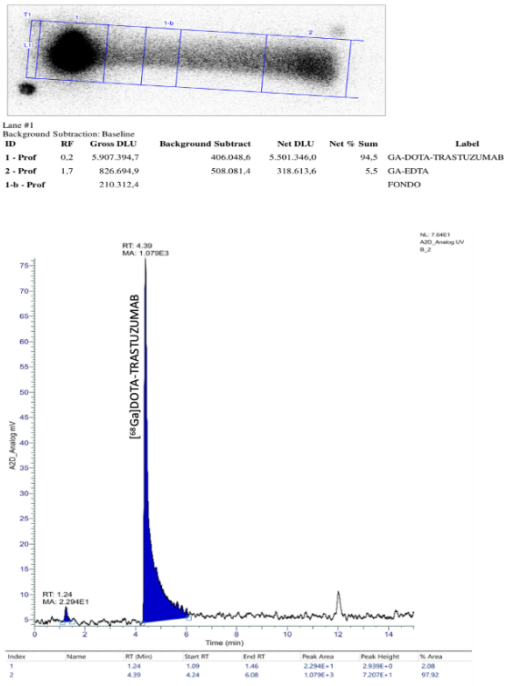

Figure 2. Radio TLC and Radio HPLC of radiolabeled precursor B

<u>References</u>

[1] Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, Mcguire WL, 1987, Science, 235:177–182.
[2] Hudis CA, 2007, N Engl J Med, 357:39–51.
[3] Leonard DS, Hill ADK, Kelly L, Dijkstra B, McDermott E and O'Higgins, 2002, NJ. Br. J. Surg, 89: 262-71.

CCK2R-TARGETED MACROMOLECULES FOR DEVELOPING NOVEL PHARMACEUTICALS IN THE ISOLPHARM PROJECT

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Introduction

The cholecystokinin receptor 2 (CCK2R) is overexpressed in several cancers and it may represent an important target for tumor therapy.[1] In this context Z-360, a CCK2R antagonist with subnanomolar affinity,[2] could be exploited as a targeting agent for developing novel diagnostics or therapeutics. To this aim, Z-360 was conjugated through spacers of variable hydrophilicity to a model payload, as prototypes of innovative radiopharmaceuticals in the ISOLPHARM project. The selectivity of these carriers towards CCK2R-overexpressing cells was assessed on 2D and 3D tumour models.

Materials and methods

Z-360-L_x-Rho compounds were synthesized by conjugating Z-360[3] to Rhodamine (Rho) through linkers[4] of increasing hydrophilicity thanks to the insertion of pendant sugar Glucose < Lactose < Maltotriose, obtaining Z-360-L_G-Rho, Z-360-L_L-Rho and Z-360-L_M-Rho, respectively. Conjugates were tested in vitro on WT A431 cells (with low CCK2R expression) and A431 cell line transfected to stably express CCK2R (CCK2R$^+$-A431) to assess the receptor-targeting. Association studies were performed by incubating both cell lines with Z-360-L_x-Rho library for 30 minutes followed by flow cytometric analysis (FC). Furthermore, competition studies were carried out using identical experimental conditions but pre-incubating cells with CCK2R natural ligand Gastrin I. 3D experiments were conducted on gelatin methacryloyl-based scaffolds, where WT A431 and CCK2R$^+$-A431 cells were embedded, separately. Scaffolds were used at day 4 post-crosslinking and incubated with Z-360-L_x-Rho library solutions for 4 or 18h before images acquisition by confocal laser scanning microscope.

Results

The synthesis of Z-360-L_x-Rho library was successfully performed. FC cell association studies confirmed the ability of our compounds to interact with CCK2R-expressing cells. Notably, the increase of the linker hydrophilicity resulted in an improvement in the selectivity for CCK2R-expressing cells, as also supported by the competition studies, and an enhancement of the total cell uptake, with Z-360-L_M-Rho outperforming Z-360-L_G-Rho, Z-360-L_L-Rho. 3D scaffolds further confirmed Z-360-L_M-Rho as the best performing candidate by showing higher uptake in CCK2R$^+$-A431 cells as compared to WT A431 at both incubation times.

Conclusion

Innovative Z-360-based macromolecules targeting CCK2R overexpressed in tumor tissues were designed with increasing hydrophilicity and conjugated to a fluorescent probe as a model drug obtaining a small Z-360-L_x-Rho library. 2D and 3D in vitro cell internalization and competition studies highlighted Z-360-L_M-Rho for its selectivity towards CCK2R-expressing cells. 3D experiments confirmed the rapid diffusion of the conjugates in a 3D matrix and their boosted uptake by CCK2R$^+$ cells. These findings suggest the potential and versatility of this platform for therapeutic and diagnostic purposes upon insertion of a chelating agent and loading of a suitable radionuclide.

References

[1] Reubi, J.C.; Waser, B. 1997, *Cancer Res*, *57*, 1377–1386.
[2] Wayua, C.; Low, P.S. 2014, *Mol Pharm*, *11*, 468–476.
[3] Philip, S. 2013, U.S. Patent WO 2013126797 A1.
[4] Verona, M.; Rubagotti, S.; Croci, S.; Sarpaki, S.; Borgna, F.; Tosato, M.; Vettorato, E.; Marzaro, G.; Mastrotto, F.; Asti, M. 2021, *Molecules,* 918.

Acknowledgments

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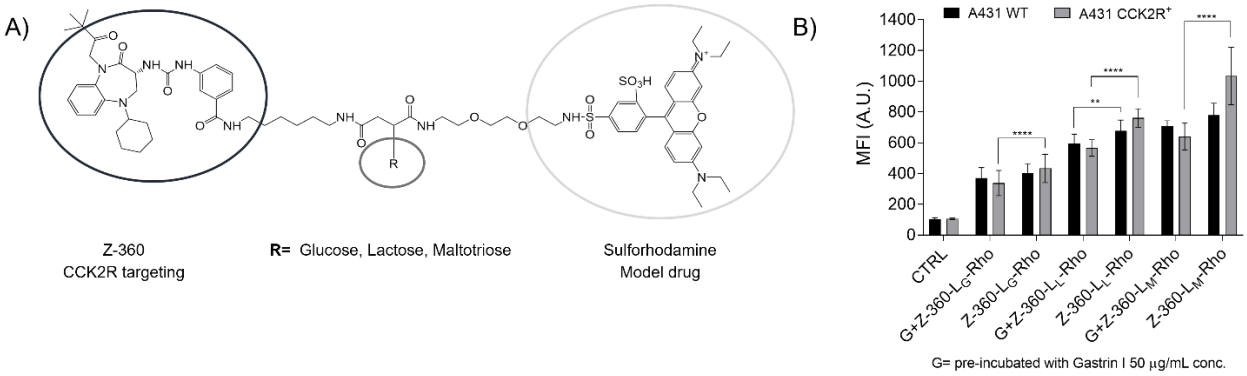
Picture 1



A) Chemical structure of Z-360-L_X-Rho conjugates at increasing hydrophilicity. B) Cell association of Z-360-L_X-Rho library after incubation on WT A431 or $CCK2R^+$-A431 cell lines in presence (competition assay) or absence of Gastrin I (G).

CHELATION OF THERANOSTIC SILVER RADIOISOTOPES WITH SULFUR-RICH POLYAZAMACROCYCLES

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Introduction:

Silver-111 ($t_{1/2}$ 7.47 d) is an attractive non-conventional radiometal both for targeted radiotherapy and for SPECT imaging due to its β^- emission ($E_{\beta,max}$ 1.04 MeV) and to its γ photons (E_γ 245.4 keV, I_γ 1.24%; E_γ 342.1 keV, I_γ 6.7%), respectively. Moreover, it forms along with the PET isotopes silver-103g ($t_{1/2}$ 65.7 min, β^+ 27%, EC 73%) and silver-104g ($t_{1/2}$ 69.2 min, β^+ 15%, EC 85%) pure theranostic pairs.

Despite its theranostic potential, the labelling chemistry of silver remains uncharted mainly because of the shortage of suitable chelators forming sufficiently stable and inert complexes in biological media. In an attempt to circumvent the current shortcomings, we have investigated a series of sulfur-rich polyazamacrocycles as potential chelators for silver (Figure 1) and evaluated their labelling properties with silver-111.

Materials and Methods:

The complexation behavior of the chelators was investigated with stable Ag^+ by pH/pAg potentiometric measurements. Mono, bidimensional and variable-temperature NMR spectroscopy combined with DFT calculations gave insights into the solution structure of the Ag^+ complexes. Silver-111 was produced by neutron irradiation of enriched palladium-110 target through the palladium-110(n, γ)palladium-111 reaction and the subsequent decay of palladium-111 to silver-111. Concentration-dependent radiolabelling at neutral pH (phosphate buffer) and different temperatures (RT and 60°C) were performed with post-processed $[^{111}Ag]Ag^+$. Incorporation yield was assessed by radio-TLC. Competition studies with Pd^{2+} and stability in PBS and human serum over time were performed as well.

Results:

The examined chelators can rapidly form 1:1 metal-to-ligand complexes with Ag^+ with a high thermodynamic stability in the order: DO4S > TRI4S > TACD3S ≈ TE4S (pAg, $_{pH\ 7.4}$ = 14.5, 12.4, 10.6 and 10.3, respectively).[1] According to DFT calculations, the most stable structures entail the coordination of a [4N]1S or [4N]2S array atoms with a distorted octahedral or square base pyramidal geometry. However, a highly fluxional behaviour due to sidearms exchange and/or macrocyclic ring turn was found in solution by NMR spectroscopy. Quantitative incorporation of $[^{111}Ag]Ag^+$ was obtained at RT with 20 nmol of both DO4S and TRI4S. A 100-fold and 10-fold highest apparent molar activity can be achieved when the reactions were heated at 60°C, respectively. Conversely, TACD3S and TE4S showed no labelling under the tested conditions. Competition experiments with a 10-fold molar ratio of Pd^{2+} respect to chelator decreased the incorporation yield to 70% and 0% for DO4S and TRI4S, respectively. $[^{111}Ag][Ag(DO4S)]^{2+}$ resulted the most stable complex with a stability > 99% and ~40% in PBS and human serum after 6 hours of incubation, respectively.

Discussion/Conclusions:

Although all the ligands are potentially suitable chelators for Ag^+ owing to their high thermodynamic stability, the size of the ring dramatically influences the labelling yield when radioactive silver-111 is handled. Sulfanyl pendant arms play a role in the coordination enhancing the stability of Ag^+-complexes respect to carboxylate containing arms of the commonly employed azamacrocycles. These premises open the way to the potential application of silver-111 as a theranostic radionuclide when bound to biological vectors.

References:

[1] Tosato M, Asti M, Dalla Tiezza M, Orian L, Häussinger D, Vogel R, Köster U, Jensen M, Andrighetto A, Pastore P, Di Marco V, [2020] *Inorg. Chem*, 59: 10907-10919.

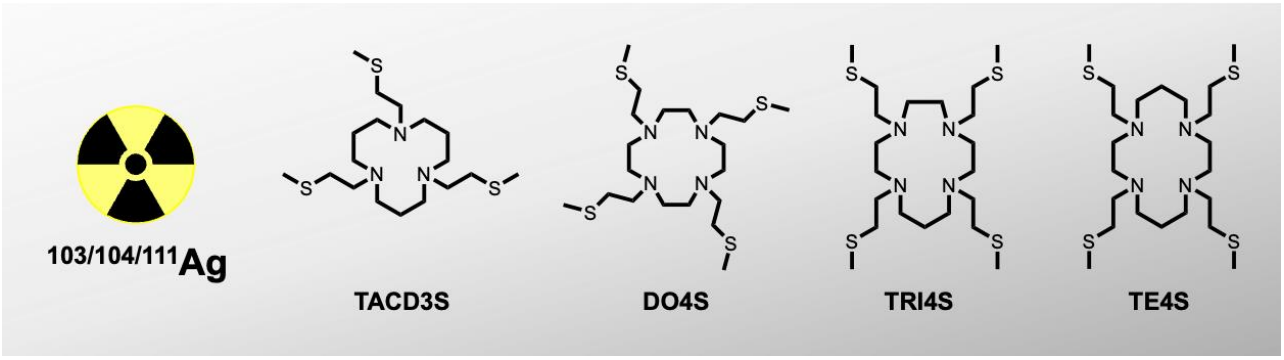

Figure 1.

EXPLORING BPCD LIGAND REACTIVITY ON ALUMINUM-[^{18}F]FLUORINE FRAMEWORK.

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Introduction

H2bpcd, N,N'-Bis(2-pyridylmethyl)-trans-1,2-diaminocyclohexane-N,N'-diacetic acid, is a symmetrical diaminodiacetic acid showing a pre-oriented chiral conformation. It is a N4O2 donor atom set featuring trans-monodentate acetate groups and cis-2-pyridylmethyl N atoms. Preorientation of the ligand, expected thanks to a restricted rotation of the C-C bonds, enhance a favorable conformation of donor atoms for metal ion complexation and provide complex stability reducing entropic penalty. N atoms linked to cyclohexane, due to trans conformation, lies on opposing orientations, this placement favors a pseudo-octahedral conformation. The proximity of nitrogens in the cyclohexane backbone of bpcd fosters the possibility of five-membered ring formation, usually more favorable for larger metal ions than for smaller metal ions. Recent review show [2] reactivity study of diaminodiacetic acid ligands on aluminum-[18F]fluoride complex preparation to overcame Gallium-68 radiopharmaceuticals limitations.
The aim of this study is the investigation of the ligand propriety for Aluminum Fluorine-18 coordination and its stability.

Materials and Methods

The initial phase of the study was to standardizing [^{18}F]AlF production on Neptis module. The [^{18}F]AlF solution was left to react with different H_2bpcd forms. We assessed the reaction in the three variables: pH, reaction temperature and the ligand concentration. Further, a kinetic study for all the sample were performed acquiring TLC at T0, 3, 5, 8, 10, 15, 20, 25, 30 minutes to evaluating reactivity and stability after purification. HPLC were perform at 15' and 30' time points. TLC analysis were performed with Itlc-SG mobile phase NH_4Ac (77g/L; H_2O/MeOH).
HPLC luna OMEGA 5 µm 100A Polar C18 250x4,6 mm FM A= (H_2O +0.1TFA); B=(ACN+0.1%TFA, Flow 1 ml/min gradient 0-3 min 0% B 3-25 min 0-100% B.

Results

The study shows a fast reactivity at 80 °C with a conversion of free [^{18}F]AlF^{2+} in to the final complex in less than 15 minutes. To confirm the identity of the complex a complete characterization was perform using HPLC-mass spectroscopy using, [^{18}F]AlFbpcd standard complex. Complex stability was performed after Aluminum SPE purification at 4h reaching RCY > 95%. Transchelation with EDTA, competitions study with $FeCl_3$ stability on PBS and Human serum at 37°C was assessed.

Discussion and Conclusion

The preliminary results of the reactivity show a complete conversion of [^{18}F]fluorine in to the [^{18}F]AlFbpcd stable complex in weak reaction condition. The final complex can by purify to increasing the RCY by different SPE. The obtained product was assessed on stability for four hours and on different competitive condition showing relatively good stability. In vitro studies were planned in the next future.

Bibliography

[1] Florian J, McLauchlan C, Kissel D, Eichman C, Herlinger A: "Metal Ion Complexes of N,N'-Bis(2-Pyridylmethyl)-trans-1,2-Diaminocyclohexane-N,N'-Diacetic Acid, H2bpcd: Cis/trans Isomerization Equilibria" Inorganic Chemistry 2015, 54, 10361-10370.

[2] Archibald S, Allott A,: “The aluminium-[18F]fluoride revolution: simple radiochemistry with a big impact for radiolabeled biomolecules” EJNMMI Radiopharmacy and Chemistry 2020; 17:, 2245-59;

EFFICIENT LABELING, HIGH YIELD AND HIGH SPECIFIC ACTIVITY USING A HPLC PURIFICATION OF 18F-RDG-ECHI

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Abstract
Various techniques have been developed which allow efficient labeling of peptides with 18F without affecting their receptor-binding properties. Moreover, the development of a variety of prosthetic groups has facilitated efficient site-specific labeling of peptides with 18F. 18F-labeled peptides hold enormous clinical potential due to their ability to quantitatively detect and characterize a wide variety of human diseases using PET. Recently, a number of 18F-labelled bioactive peptides have shown great promise as diagnostic imaging agents [1]. High effective specific activity N-succinimidyl-4-[18F]-fluorobenzoate was prepared using a reversed phase HPLC procedure. Reversed phase HPLC removed several additional impurities further increasing the effective specific activity. Small quantities (200micrograms) of peptides can be labeled with this reagent in high yield without aggregation. We studied the labeling synthesis of RGD-Echi-hCit with fluorine-18 and an HPLC purification of this radiocompound, and we tested it in mice. Conclusions We used the N-succinimidyl-4-[18F]-fluorobenzoate to label the amino group of a lysine peptide and achieved radiolabeling yields of 73% at pH=7 at 40°C. A proline does not contribute to helix formation because it has no amide hydrogen therefore it tends to break a helix and, the hydrogen bonding that leads to helix formation is absent for proline. To avoid labeling Lys15, the RGDechi sequence was modified by substituting the aminoacid with homocitrulline residue (RGDechi-hCit). The RGDechi-hCit was synthesized replacing the lysine at the C-terminus with homocitrulline to increase the labeling yield. In fact, only one lysine (Lys1) will react with 18F-N-succinimidyl-4-fluorobenzoate. The radioligand was easily purified with an acetonitrile gradient in HPLC and purified by passing it through a C18 cartridge. The C18 cartridge was eluted with 1mL of ethanol collected in 5 eppendorf vials of about 200 microliters each. We chose the more active fractions and we diluted with physiological saline for direct injection in animals. The yield of the HPLC fraction was sufficient for carrying out experiments in a large number of mice.
Key Words: fluorine-18; labeling synthesis of peptides; N-succinimidyl-4-[18F]-fluorobenzoate; RGDechi; angiogenesis; integrin αvβ3
References
Okarvi SM. Recent progress in fluorine-18 labelled peptide radiopharmaceuticals. Eur J Nucl Med (2001) 28:929–938.

SITE-SPECIFIC [99mTc][Tc(CO)3(OH2)3]-LABELED scFvD2B-HysTag FOR SPECT IMAGING OF PSMA IN PROSTATE CANCER

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Introduction

scFvD2B is a single chain variable fragment (29 kDa) of the monoclonal antibody IgGD2B specific to the extracellular domain of PSMA, especially overexpressed in prostate cancer (PCa) [1,2]. scFvD2B has shown promising properties, especially in terms of high stability, favorable pharmacokinetics and specificity, efficiently accumulating in PSMA-expressing PCa tumors. Moreover, the small size of scFvD2B allows both faster penetration into tumor tissue and rapid clearance from nontarget organs, thus permitting the achievement of good contrast and sensitivity on the day of injection or the day after injection. This allows the use of relatively short-lived radionuclides such as 99mTc or 64Cu with the benefit of an appreciable reduction in the dose absorbed by patients compared to other radionuclides. Hexahistidine tags (His-tags), incorporated into recombinant proteins to facilitate purification using metalaffinity chromatography, are useful binding sites for radiolabeling with $[^{99m}Tc(CO)_3]$ + framework. Thus, in order to obtain a radioimmunoconjugate (RIC) suitable for PCa SPECT imaging, a His-Tag sequence was engineered at the C-terminal portion of scFvD2B and labeled using the $[^{99m}Tc(CO)_3]$ + approach [3].

Methods

scFvD2B-HisTag was synthesized by cloning the IgGD2B VH and VL chain genes into an E. Coli vector5 . [99mTc][Tc$(CO)_3(OH_2)_3$] + was produced through the commercial IsoLink® kit and the radiolabeling reaction was carried out at 37°C for 2 hours using 100-150 µg of scFvD2B-HysTag in a final volume of 250 µL. RIC was characterized by RP-HPLC, purified by gel filtration, and evaluated for stability in PBS, human serum, and transchelation (His, Cys, GSH, EDTA 10 mM). In vitro, uptake and internalization was assessed in PSMA + (LNCaP and PC3-PIP) and PSMA – (PC3) cell lines.

Results

Under labeling conditions, the [99mTc][Tc(CO)3(scFvD2B-HisTag] returned a RCY in the range 28-43%; after purification the RCP > 98%. The RIC showed a steady stability profile over time. According to cell studies, the uptake and internalization values were encouraging for future in vivo biodistribution insights: in LNCaP cells 6% and 4% respectively, in PC3-PIP 30% and 15% and in PC3 3% and 0,7%. Furthermore, blocking studies, with an excess of native scFvD2B-HisTag, confirmed the specificity of [99mTc][Tc(CO)3(scFvD2B-HisTag] for PSMA receptor.

Conclusion

$[^{99m}Tc][Tc(CO)_3(OH_2)_3]$-labeled scFvD2B-HisTag, was easily produced with a high RCP. The reaction was site-specific and RIC was stable in vitro and possessed a high cellular uptake in PSMA(+) cells. The process was receptor mediated. Studies are in progress to determine the in vivo performance of RIC.

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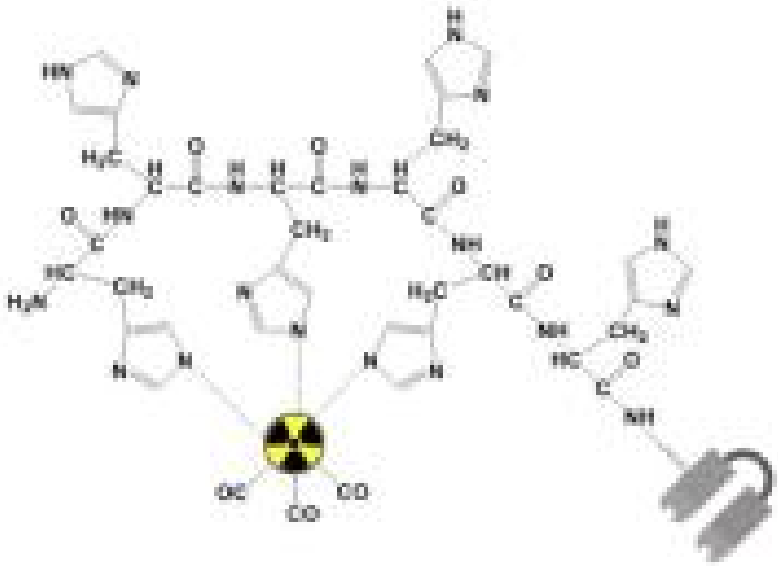
References

[1] Carpanese D, Zorz A, Evangelista L., Salvarese N. Targeting prostate cancer with the anti-PSMA scFvD2B: a theranostic promise for nuclear medicine. Clinical and Translational Imaging 2019 7:4 7, 295–301 (2019).

[2]Carpanese D. et al. Development of 177Lu-scFvD2B as a Potential Immunotheranostic Agent for Tumors Overexpressing the Prostate Specific Membrane Antigen. Scientific Reports 10, (2020).

[3] Williams, JD et al. Optimal His-Tag Design for Efficient [99mTc(CO)3] + and [188Re(CO)3] + Labeling of Proteins for Molecular Imaging and Radionuclide Therapy by Analysis of Peptide Arrays. Bioconjugate Chemistry 32, 1242–1254, 2000.



BPCD LIGAND FOR THERANOSTIC RADIOPHARMACEUTICAL APPLICATION

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Introduction

In the last fifteen years Gallium-68 have assumed more and more an important role inside a Nuclear Medicine due to the favorable half-live, easy access to the nuclide and important theranostic pair in combination with Lutetium-177. Bpcd N,N'-Bis(2-pyridylmethyl)-trans-1,2.diaminocyclohexane-N,N'-diacetic acid is an interest diaminoacetic acids ligand that can be potentially apply to coordinating a group IIIB nuclides like Gallium-68. The H2bpcd employs two acetic acid function, as well as four Nitrogen, present inside of two 2-pyridylmethyl donor groups for the final hexa metal coordination. The rotation restriction given by the rigid C-C bond inside to the cyclohexane ring constrains the amine nitrogen atoms in to a pre-oriented configuration.

The aim of this study is the investigation of the ligand property for Gallium-68 coordination and its stability.

Materials & Methods

The [^{68}Ga]$GaCl_3$ solution, originated from the elution of E&Z generator with 0.1M HCl were used like Gallium-68 source. We performed different protocols, using bpcd, in order to test several ligand concentrations at different pHs and reaction temperatures. The bpcd were used on R,R; R,S and racemic mixtures.

A complete screening of reaction conditions was performed to find the optimal one. A Kinetic study for all the sample were performed acquiring TLC at T0,3 ,5 ,8 ,10 ,15 ,20 ,25 ,30 minutes to study reactivity and stability in reaction condition. HPLC were perform at 15' and 30' time points.

TLC analysis were performed with iTLC -SG mobile phase NH_4Ac (77g/L; H_2O/MeOH).

HPLC luna OMEGA 5 µm 100A Polar C18 250x4,6 mm FM A= (H_2O +0.1TFA); B=(ACN+0.1%TFA, Flow 1 ml/min gradient 0-3 min 0% B 3-25 min 0-100% B.

Results

The study shows a fast reactivity at 80 °C with a conversion of free [^{68}Ga]GaCl3 in to the final complex in less than 15 minute. To confirm the identity of the complex a complete characterization of [Gapbdc]$^+$ was performed using NMR and HPLC-mass spectroscopy. Radio-HPLC comparison between [^{68}Ga]Ga/Ga complexes was carry out.

Discussion / Conclusion

The preliminary results of the reactivity show a complete conversion of [^{68}Ga]Gallium in to the [[^{68}Ga]Gapbdc]$^+$ stable complex in the reaction condition. Citotoxicity test was performed and shows complexes low toxicity. We are planning to performing the in vitro stability test in the next future.

Bibliography

[1] Florian J, McLauchlan C, Kissel D, Eichman C, Herlinger A: "Metal Ion Complexes of N,N'-Bis(2-Pyridylmethyl)-trans-1,2-Diaminocyclohexane-N,N'-Diacetic Acid, H2bpcd: Cis/trans Isomerization Equilibria" Inorganic Chemistry 2015, 54, 10361-10370; DOI:10.1021/acsInorgchem.5b01586.

THE PET TRACER ^{52G}MN: FROM CROSS-SECTION MODELING TO DOSIMETRIC SIMULATIONS

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Introduction

Manganese is a unique element to obtain magnetic resonance imaging (MRI) of neuronal function because it can be transported along the axons cross synapses. However, high quantity of Mn^{2+} can cause neurological disorders, therefore, MRI has not been widely used in clinics[1]. Positron emission tomography (PET) allows obtaining all information derived from MRI using trace levels of Mn^{2+} and minimizing its toxic effects[2]. Among Mn-radionuclides, ^{52g}Mn appears the most suitable thanks to its decay properties (β+ = 29.4 %, E(β+) avg = 242 keV) and its quite long half-life ($t_{1/2}$ = 5.6 day)[3]. Recently the reaction $^{nat}V(\alpha,x)^{52g}Mn$ has been proposed as a possible alternative to the standard $^{nat}Cr(p,x)^{52g}Mn$ one[4]. Focus of this work is to develop precise simulations and models

useful to compare the quantity and quality of both production routes, in terms of radionuclidic purity (RNP) and dose increase (DI) due to the radioisotopic contaminants co-produced.

Materials and Methods

The nuclear code Talys has been employed to optimize the evaluation of the excitation function of the $^{nat}V(\alpha,x)$ reaction, by performing a tuning of the parameters relevant for the nuclear level densities[5]. Dosimetric assessments after the injection of $[^{xx}Mn]Cl_2$ in female and male phantoms have been accomplished with the OLINDA software[6,7]. Finally, the yield of Mn radioisotopes estimated for the different production routes have been combined with the dosimetric results, to obtain the DI due to the presence of contaminants, at different times after the end of the irradiation.

Results

The comparison of the cross sections suggests that α on natural vanadium targets leads to higher yield and higher purity than the ones obtained from the proton interaction with natural chromium targets. The assessment of the DI shows a less harmful impact on patients' health in the α–^{nat}V case due to a reduced contamination by other Mn radioisotopes.

Conclusions

Both $^{nat}V(\alpha,x)^{52g}Mn$ and $^{nat}Cr(p,x)^{52g}Mn$ production routes provide $^{52g}MnCl_2$ for PET imaging with a low DI due to contaminants, for the entire range of time where the RNP is acceptable (i.e. greater than 99%). However, the $^{nat}V(\alpha,x)^{52g}Mn$ reaction provides a DI systematically lower than the one obtainable with $^{nat}Cr(p,x)^{52g}Mn$ and a longer time with RNP higher than 99%.

References

[1] Yang J and Li QQ, [2020], Front. Neurol. 11:16.
[2]Graves SA *et al.*, [2017], Sci. Rep. 7.
[3] Wooten AL *et al.*, [2017], Plos One. 12(3).
[4] Colombi A *et al.*, [2019], Nucl. Technol. doi:10.1080/00295450.2021.1947122
[5] Goriely S, Hilaire S, Koning AJ, [2008], Astron. Astrophys., 487:767.
[6]Stabin MG, Sparks RB, Crowe E, [2005], J. Nucl. Med. 46:1023–1027.
[7]De Nardo L *et al.,* [2019], Appl. Radiat.Isot., 153:108805.

NOVEL PURIFICATION METHOD OF L-[^{11}C]METHYL-METHIONINE FOR ROUTINE CLINICAL PRACTICE

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Introduction

L-[^{11}C]methyl-methionine is an essential clinical tool for the PET imaging of different cancers. Although its radiosynthesis is a well-established procedure, generally reported with high chemical and radiochemical yields,[1] many are the side reactions which can take place during the preparation of methionine. The eventual side products (i.e. oxidized forms of the sulfide function) can be challenging to remove, reducing the overall purity of the tracer, and potentially resulting in a non-conformity of the production batch and its consequent rejection from clinical applications.
That happened in our production site, where a series of radiolabeled impurities caused inconsistent synthetic results, which presented high radiochemical yields but out of the

specifications required (RCP > 95%). Thus, we attempted to reduce the side products without drastically altering both the process and the final formulation, resulting in a new standardized method for the clinical production of L-[^{11}C]methyl-methionine.

Materials and methods

The precursor L-homocysteine thiolactone hydrochloride (3 mg) is dissolved in 200 µL of a solution of NaOH 0.4 M in 40% EtOH and loaded on a tC18 plus short cartridge, connected in series with two ion-exchange cartridges (Chromafix PS-H+, small). This precursor is reacted for 30 seconds at room temperature with [^{11}C]CH_3I formed by standard procedure from cyclotron-produced [^{11}C]CO_2. Then, using 5 mL of H_2O, the product is transferred to the ionic cartridges and separated from the polar, non-ionic side-products. Purified L-[^{11}C]methyl-methionine is finally eluted with NaOH 0.5 M (4 mL) and collected in a vial containing NaH_2PO_4 0.05 M (7.2 mL). The cartridges are further washed with NaCl 0.9% (3.8 mL) for a complete recovery of the activity.

Results

The introduction of an additional purification step using ion-exchange cartridges allowed us to increase the overall radiochemical purity of the process, obtaining a stable RCP = 98.25 ± 1.23 over almost 20 syntheses. Indeed, the PS-H+ cartridge was able to capture the produced methionine and separate it from most of the unwanted side products. Moreover, from the analysis of the waste, it was possible to determine that the use of two consecutive ion cartridges was sufficient to trap almost all the product, which was then easily recovered with sodium hydroxide and sodium chloride washings. The yield of the radiolabeling reaction calculated on the [^{11}C]CH_3I activity was 42.74 ± 16.37. Overall, we were able to obtain approximately 6 GBq of labelled methionine starting from an average of 30 GBq of activity from the cyclotron.

Conclusion

With this work, we present a new method for the preparation of L-[^{11}C]methyl-methionine. By introducing a simple purification step on cartridge we were able to meet the specific purity requirements without substantially altering the preparation operations, time consumption and the final solution composition.

References

[1]Pascali C, et al., [1999], J. Labelled Compd. Rad., 42: 715-724

[^{68}Ga]Ga-DOTATOC/PSMA-11 AUTOMATED SYNTHESIS WITH FRACTIONAL PRE-PURIFICATION BY TRASIS ALLINONE SYNTHESIZER

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Introduction

Since 2015, [^{68}Ga]Ga-DOTATOC/PSMA-11synthesis has been performed by Eazy synthesis module. In order to have a new production line, we have evaluated to transfer our production method to AllinOne synthesis module. The special feature of our layout is to use the Ga-68 eluate from one or two in parallel-generators, depending on the number of schedulated patients, and the possibility of connecting the Ge-68/Ga-68 generators in an easily interchangeable way between the modules mentioned above. Currently the method used by AllInOne module for a double generators configuration is based on the well-known cationic pre-purification method. The aim of

the work was to create a variation of the standard TRASIS method, using fractional pre-purification and optimizing some aspects such as: the overall synthesis time reduction and the absence of activity loss usually due to the cartridge used in cationic pre-purification processes.

Materials and methods

For the initial set-up of the new synthesis process, it was necessary to create the connection between the synthesis module and the two generators, that are located below the hotcell worktop. Therefore, the two output lines of the generators were connected together, by a Tee fitting, to a ventilated filter that allows the suction of the dead volume. The process as structured required a second syringe pre-filled with hydrochloric acid. The new theoretical synthesis sequence with fractional pre-purification was tested on siteto verify its functionality in both DOTATOC and PSMA-11 labelling and to optimize it experimentally.

Results

The first [68Ga]Ga-PSMA-11 synthesis provided a very low yield (47.3%). At this point it was necessary to optimize the elution volumes, the fractional elution "cutting" method and the conditions for transferring of the Gallium-68 eluate into the synthesis reactor. Reached the final set-up, the next two syntheses of PSMA-11 and DOTATOC have been succesfully carried out, with an in specification yield , i.e. 73.3 % and 70.4 % respectively.

	Starting activity (MBq)	**Final activity (MBq)**	**Synthesis Yeald (ndc)**	**Radiochemical purity (HPLC) (EP Monograph)**	**Radiochemical purity (TLC) (EP Monograph)**
N1 [68Ga]Ga-PSMA11	1184	560	47,3%	98,8%	99,9%
N2 [68Ga]Ga-PSMA11	970	711	73,3%	99,3%	100%
N3 [68Ga]Ga-Dotatoc	774	545	70,4%	99,1%	100%

Discussion/Conclusions

All the optimizations resulted in a significant increase in synthesis yield that is now over 70%; this confirms the possibility and the oppurtunity of transferring the synthesis process to the AllinOne module according to our configuration. The radiochemical purity did not change, always remaining above 98% (EP Monograph). The data must be confirmed by subsequent validation runs.

THERAPEUTIC APPLICATION OF A $^{64/67}$Cu-MIXTURE

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Introduction

^{64}Cu and ^{67}Cu radioisotopes could be useful tools for diagnosis and therapy of cancers, due to the increased accumulation of Cu^{2+} ions in tumor cells. ^{64}Cu can be produced with high specific

activity by using low energy biomedical cyclotrons and it is already commercially available. High yield production of ^{67}Cu is instead difficult, due to the co-production of other Cu-isotopes, especially ^{64}Cu. As both $^{64/67}Cu$ radioisotopes have favorable decay characteristics, in this work the possibility of using a mixture of them for therapeutic purposes has been evaluated.

Materials and methods

Copper radioisotopes production yields were calculated by considering proton beam irradiation of both ^{70}Zn and ^{68}Zn targets under different energy ranges. The contribution of each copper radioisotope to the human absorbed dose was estimated with the OLINDA software [1] using the biokinetic model for $CuCl_2$ published by ICRP 53 [2]. The total absorbed dose generated by the $^{67/64}CuCl_2$ mixture, obtained through different production routes, was calculated at different times after the end of the bombardment (EOB). A simple spherical model, representing tumours of different sizes, was used to calculate the expected absorbed dose due to the self-irradiation for a uniformly distributed $^{67/64}Cu$ mixture.

Results

Healthy organs absorbed dose, as well as the effective dose factors are higher for $^{67}CuCl_2$ than for $^{64}CuCl_2$. Absorbed dose factors for $^{67/64}CuCl_2$ mixture increase with time after the EOB, due to the increasing amount of ^{67}Cu nuclide fraction in the mixture. Using the sphere model it was found that ^{64}Cu administered activity must be about five times higher than that of ^{67}Cu to obtain the same absorbed dose for tumour mass 0.01-10 g and about ten times higher for smaller ones. The supplemental activity of the $^{67/64}CuCl_2$ mixture, required to get the same tumour absorbed dose produced by $^{67}CuCl_2$, triggers a dose increment in healthy organs which decreases with time post-EOB.

Conclusions

A mixture of cyclotron produced $^{67/64}Cu$ radioisotopes proved to be an alternative solution for the therapeutic use of $CuCl_2$ with minimal dose increment to healthy organs as compared to pure ^{67}Cu. Among all the production routes investigated, 185 h irradiation of a $^{70}Zn+^{68}Zn$ target in the 70-35 MeV proton energy range provides the maximum amount of activity, the shortest waiting time necessary to keep the healthy organ dose increment below 10% and less than 1% of ^{61}Cu and ^{60}Cu impurities, consequently it is the best option [3].

References

[1] Stabin M, Farmer A [2012] J Nucl Med, 53 (1): 585
[2]ICRP Publication 53 [1988] Ann. ICRP 18 (1-4)
[3]De Nardo L *et al*. [2022] Med Phys, https://doi.org/10.1002/mp.15524

HOW TO DEVELOP YOUR "VIRTUAL CYCLOTRON&RADIOPHARMACY FACILITY": A SOFTWARE OPTIMIZATION FOR TRACEABILITY APPLICATION IN A NUCLEAR MEDICINE

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Introduction

During the last decade several software have been designed for supporting the management and traceability in Nuclear Medicine, especially for radiopharmacy activities. These applications have

been also developed to facilitate operational improvements in the production and use of radiopharmaceuticals, reducing errors and increasing efficiency and compliance. In this framework, the radiopharmaceutical manufacturing within a cyclotron-radiopharmacy facility introduces the highest level of complexity. The different PET-production phases involve personnel and a range of equipment in a short time, generating multiple output and reports. An ideal software is required to trace all operations, personnel, instrumentation and used materials from the radioactive precursor production to the synthesizer run, until the creation of completed batch records. It is also expected to perform reliable and interconnected calculations, converting the produced radiopharmaceutical activity into a detailed dose planning for single patients. This work is an example of software customization for a cyclotron-radiopharmacy facility use, both for daily clinical activities, typically 30-35 patients with 2 or more in-house produced PET-tracers, as well as for academic research programs.

Materials Methods

The Fenix software (EL.CO. S.r.l., Cairo Montenotte, Savona) was developed and compiled in the js and java programming language based on oracle database. The Fenix suite was developed using open-source tools combined with a proprietary framework. It is W3C validated and certified as a medical device.

Results:

The software was optimized to manage the full production workflow, from the preliminary laboratory operations to the dispensing of the personalized PET-doses, maximizing the adherence to the personnel activities in our cyclotron and radiopharmacy facility.

Discussion and conclusions

The Fenix software is now implemented with daily checks for cyclotron, quality control instrumentation and environment monitoring, to be easily performed and electronically reported prior to each day's production. Fenix keeps track of each cyclotron run data and Ge68/Ga68 generator's status, reporting the available activities for batch planning. The software allows for manual data entry of key radiosynthesis and quality controls, including the hardware and consumables, adaptable for the different produced radiopharmaceuticals. The status of the manufacturing and QC process is available to view at any moment and from any workstation of the facility. When all production steps and QC tests are completed, the radiopharmaceutical can be released for patient injection and all the reports, including the batch record, are generated and signed by the responsible persons, using a password-protected signature. All reports are consolidated in a secure database and the data can be extracted and elaborated for statistical analysis.

Conclusion

In this work we report an example of software customization for the management of a comprehensive range of activities of a cyclotron-radiopharmacy facility. The optimization of this software has strengthened our Quality System, promoting the standardization of existing processes and rising our operational level at the best possible efficiency.

FULLY AUTOMATED, COMMERCIAL CASSETTE-BASED PRODUCTION OF HIGH-PURITY [^{64}Cu]$CuCl_2$ FROM SOLID Ni TARGET

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Objectives

Copper-64 (^{64}Cu) is an attractive radionuclide for nuclear medical imaging. This radionuclide decays with low energy positron emission (β^+ 17.6%, E_{avg} = 653 keV) and intermediate half-life ($t_{1/2}$ = 12.7 h). ^{64}Cu is amenable for labelling both short (minutes) and long (hours/days) incubating biological vectors and is exemplified by the many reports of ^{64}Cu labelled small molecule, peptides, proteins and nanoparticles. Clinical studies with ^{64}Cu have also progressed significantly in the past two decades. This work focuses on obtaining high-purity [^{64}Cu]$CuCl_2$ via a cassette-based automated separation method using a one column approach implemented on the Taddeo-PRF chemistry platform.

Methods

^{64}Ni is electroplated onto a target shuttle and irradiated with 14.4 MeV protons to produce ^{64}Cu. The irradiated target is dissolved and purified to obtain [^{64}Cu]$CuCl_2$. Two purification methods are tested on the ALCEO system (COMECER); 1. AG 1-X8 resin (Biorad); 2. TK201 resin (Triskem). ICP-MS has been performed to monitor the quality of [^{64}Cu]$CuCl_2$.

Results

^{64}Cu is produced using the AG 1-X8 method with a yield of 2.49 ± 0.67 MBq/µAh/mg (N=21), electroplating 53.93 ± 20.42 mg of ^{64}Ni and bombarding at 35 ± 0.2 µA for 247.95 ± 74.74 minutes with a proton beam. For the TK201 method, the yield is 2.30 ± 0.83 MBq/µAh/mg (N=61), electroplating 69.50 ± 17.61 mg of ^{64}Ni and bombarding at 35 ± 0.2 µA for 201.13 ± 58.10 minutes. ICP-MS shows for the AG 1-X8 method (N=12): [Ni-64] 70728 ± 81643 ppb, [Cu] 1125 ± 657 ppb, [Fe] 8040 ± 4287 ppb, [Al] 3303 ± 1925 ppb, [Zn] 2336 ± 2602 ppb, [Co] 33 ± 35 ppb. Instead, for the TK201 method shows (N=30): [Ni-64] 8966 ± 11045 ppb, [Cu] 1244 ± 1636 ppb, [Fe] 426 ± 408 ppb, [Al] 1499 ± 2042 ppb, [Zn] 2717 ± 7196 ppb, [Co] 66 ± 84 ppb.

Conclusions

An automated method capable of producing high purity ^{64}Cu from ^{64}Ni targets has been developed. In summary, the entire process can be achieved in ~ 120 min (60 min for dissolution and resin loading, 30 min for resin elution and 30 min for evaporation of the 0.5 M acid) min from EoB to EoS, with average yields of 2.54 ± 0.8 MBq/µAh/mg when irradiating with 35 µA for 2.5 to 3.5 h. The quality of the final product has been tested and it is suitable for all labelling processes.

IMPACT OF DIFFERENT [Tc(N)PNP]-FRAMEWORKS ON THE PHARMACOKINETICS OF THE SMALL RGDFK PEPTIDE

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Introduction
The [99mTc][Tc(N)(PNP)]-system, where PNP is a bisphosphinoamine, is an interesting platform for the development of tumor 'receptor-specific' agents. Here, we compared the reactivity and impact of three [Tc(N)(PNP)]-frameworks on the stability, receptor targeting properties, biodistribution, and metabolism of the corresponding [99mTc][Tc(N)(PNP)]-tagged RGDfK peptide to determine the best-performing agent and to select the framework useful for the preparation of [99mTc][Tc(N)(PNP)]-housing molecular targeting agents.
Methods
RGDfK pentapeptide was conjugated to Cys and labeled with the [Tc(N)(PNP)]- frameworks (see figure). Radioconjugates were evaluated for their lipophilicity, stability, in vitro and in vivo targeting properties, and performance.
Results
All compounds were equally easy to synthesize and purify (RCY ≥ 95%). Remarkably, the use of PNP3OH allows to label the peptide at room temperature without significantly reducing the labeling efficiency or stability of the final compound. The main influences of the synthon on the radioconjugate were observed in the in vitro cell binding and in vivo performances. In healthy and xenograft animal models, different pharmacokinetics and tumor accumulation were observed as a function of lipophilicity and sterical hindrance of the synthon. By considering the overall data, the ws[99mTc][Tc(N)(PNP3OH)]− and [99mTc][Tc(N)(PNP3)]− synthons perform better in terms of efficiency and biological profile than the [99mTc][Tc(N)(PNP43)]− one.
Conclusions
In this study, we assessed and compared the effects of the chemical-physical properties of three different [99mTc][Tc(N)(PNP)]-synthons on the synthesis and biological behavior of a small [99mTc][Tc(N)(PNP)]- labeled peptide (i.e. RGDfK) to identify the best performing synthon useful in preparation of a target specific compound. All compounds are equally easy to synthesize and purify. Indeed, the good labeling properties at room temperature of ws[99mTc][Tc(N)(PNP3OH)]−framework can be exploited to extend this platform to 99mTc labeling of temperature-sensitive biomolecules. Variation in the nature of the PNP has a profound impact on the overall chemical and physical properties of the radioconjugate. This has a great influence on their biological properties (in vitro cell binding and in vivo biodistribution and pharmacokinetics). Basically, ws[99mTc][Tc(N)(PNP3OH)]− and [99mTc][Tc(N)(PNP3)]−tagged RGDfK are more performing, thus more suitable for further radiopharmaceutical applications (1).

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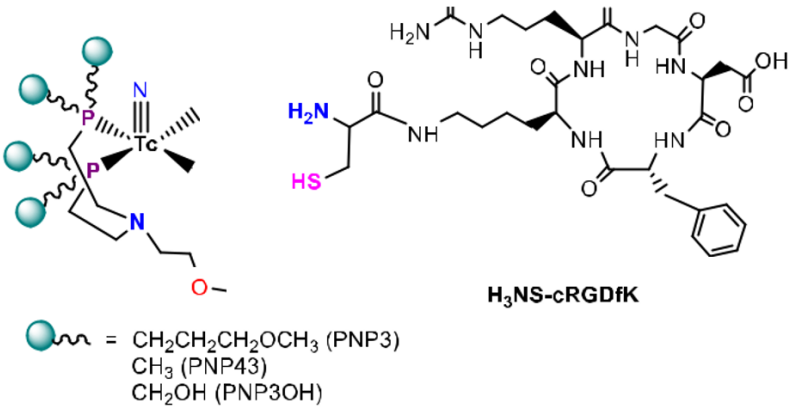
References

[1] Bolzati C, Salvarese N, Spolaore B, Vittadini A, Forrer D, Brunello S, Ghiani S, Maiocchi A. Water-Soluble [Tc(N)(PNP)] Moiety for Room-Temperature 99mTc Labeling of Sensitive Target Vectors. Mol Pharmaceutics (2022) doi: 10.1021/acs.molpharmaceut.1c00816.



Schematic drawing of the synthons and of the penta-peptide used in our experiments.

A COMPARISON OF DIFFERENT APPROACHES AIMED TO INTRODUCE FLUORINE-18 IN BIOLOGICALLY ACTIVE MOLECULES

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Introduction

Due to its favourable physical characteristics and prompt availability, fluorine-18 is the most frequently used PET radionuclide.

Radiolabelling of biologically active molecules is becoming increasingly important, but there are still challenging aspects to be considered, with particular emphasis for the need to prevent their denaturation and subsequent loss of biological properties, which require a careful selection of mild radiosynthetic conditions.

Aim of this study was the comparison of three different fluorine-18 radiolabelling approaches, applied to a modified version of the popular “theranostic” radiopharmaceutical PSMA-617: i) via a “click chemistry” reaction type [3+2] Huisgen cycloaddition Cu(I) catalyzed between a terminal alkyne and an azide; ii) using aluminum-fluoride complex ($[^{18}F](AlF)^{2+}$) together with suitable chelators previously conjugated with the PSMA derivative (e.g. NODA and RESCA); iii) ”classic” fluorine introduction via a nucleophilic substitution reaction.

Materials and methods

All the compounds (precursors and reference standards) were fully home-made synthetized, and their structure and purity have been confirmed by NMR, MS-ESI and HPLC.

Radiolabelling tests have been performed using a commercially available automated radiosynthesis system (Trasis-AllinOne) located in a suitably shielded hot cell.

Results

Selected molecules and radiosynthetic procedures are depicted in fig. 1. Compounds $[^{18}F]$ 1, 2 and 3 were obtained with yields and purity shown in Table 1.

Compounds	RCY (not corrected)	Reaction time (min)	Whole radiosynthesis time (min)	Radiolabelling Temperature (°C)	Radiochemical purity	Molar activity (GBq/µmol)
[^{18}F] 1	~6%	20	112	25	>95%	31,4
[^{18}F] 2	~30%	15	59	110	>95%	147,73
[^{18}F] 3	~20%	10	63	105	>95%	1000

Table 1

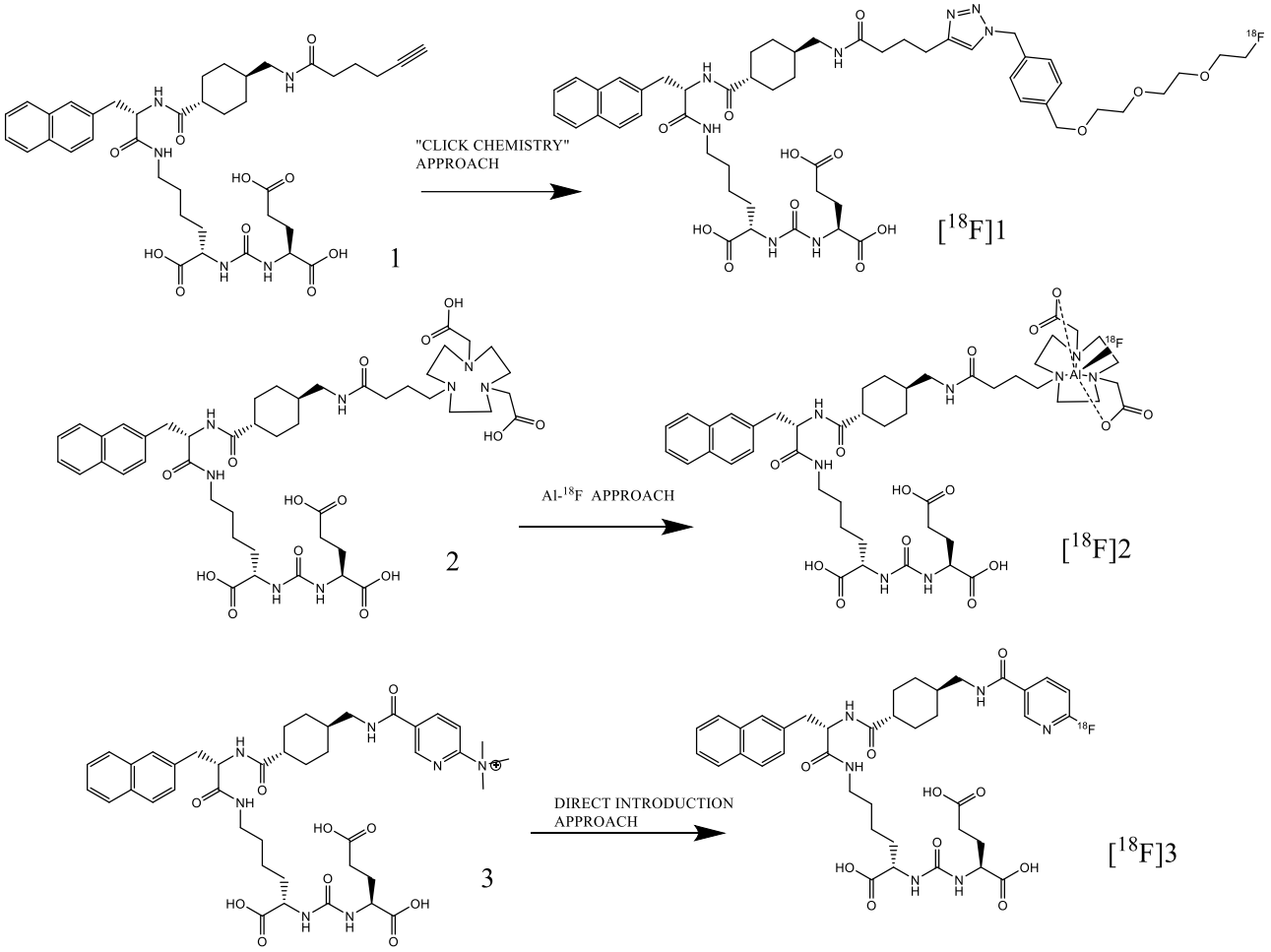

Figure 1

Discussion and conclusions

1) As for the "click reaction", the terminal alkyne-functionalised Precursor 1 was reacted with fluorine-18 azide with typical CuAAC conditions. Reaction occurred at room temperature with a modest radiochemical yield. Moreover, the whole synthetic strategy (dual-step synthesis and semi-preparative HPLC purification) took a long time and required a complicated setup of module's cassette. Finally, molar activity of **[^{18}F]1** was lower compared with other approaches.

2) In the second approach, PSMA-617 derivative functionalised with NODA chelator was reacted with [^{18}F] $(AlF)^{2+}$ complex, obtaining **[^{18}F]2** in good yield and molar activity. Reaction took place at 110°C, which is typical for reactions with NODA chelator. The whole radiosynthesis time, including HPLC purification, was < one hour.
3) Finally, also in case of direct fluorine-18 introduction reaction conditions were rather "harsh" and whole radiosynthesis time including HPLC purification was around 60 min. Satisfactory RCY and molar activity were obtained.
Stability in plasma for the three derivatives was also assessed, and [^{18}F]$(AlF)^{2+}$ resulted to be the most stable.
In conclusion, we have implemented the fully automated radiosynthesis of three [^{18}F]PSMA-617 derivatives, two of which, namely those obtained by chelation and nucleophilic substitution, were obtained with good yield and molar activity; the next step will be their preclinical testing in animal models. Despite of its suitably mild reaction conditions, "click chemistry" approach did not prove to be feasible due to an excessively troublesome preparation setup.

PRELIMINARY COMPARISON OF DOSIMETRIC RESULTS OBTAINED WITH DIFFERENT SOFTWARE FOR A ^{47}Sc-LABELLED RADIOPHARMACEUTICAL

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Introduction

In recent years, the scientific community focused on the radionuclide ^{47}Sc because of its attractive decay characteristics: half-life ($T_{1/2}$=3.3492 d) long enough for labelling molecules with slow biodistribution profile, γ-rays (E_γ=159.381 keV) suitable for single photon emission computed tomography (SPECT) imaging and β^--particles ($E_{\beta\text{-mean}}$=162.0 keV) employable in the treatment of small-medium sized tumours. However, ^{47}Sc is not clinically used yet, due to its scarce availability. The aim of this work was to assess the amount of ^{47}Sc produced by ^{nat}V(p,x)^{47}Sc nuclear reaction and of its Sc-contaminants (1) and to evaluate the patient radiation dose after administration of a DOTA-folate conjugate cm10 labelled with ^{47}Sc (^{47}Sc-cm10) using the free software MIRDOSE (2). Results were compared to the outputs obtained with the OLINDA software (3).

Material and methods

The cross-section values of the ^{nat}V(p,x) nuclear reactions were used to determine the yields of ^{47}Sc and its main contaminant ^{46}Sc ($T_{1/2}$=83.79 d). The biodistribution studies carried out on mice for the ^{47}Sc-cm10 (4), adjusted to the human organ masses through the relative mass scaling method, were used as input for the dosimetric calculations. The absorbed dose to healthy organs and effective dose following the injection of the radiopharmaceutical to a patient were determined for each Sc-radioisotope using the MIRDOSE software, based on a voxel phantom model.

Results

The preliminary results reached with the MIRDOSE software were slightly different from values obtained with OLINDA software (5). The reasons for these small discrepancies can be associated to differences in organ contours and inter-organ spacing between the phantoms implemented by both software. However, according to the general criteria of radionuclidic purity >99% and contribution of the contaminant ^{46}Sc to the effective dose <10%, the optimal irradiation conditions for the ^{nat}V(p,x)^{47}Sc nuclear reaction calculated by both software were very similar.

Conclusions

Dosimetric calculations performed with two different software showed in general similar effective dose, although several organ absorbed dose values presented some variations. Further investigations are planned to understand which aspects contribute more to the differences in the results.

References

[1] Barbaro F *et al.* [2021], PhysRevC 104 044619.
[2] Stabin MG [1996], JNuclMed 37(3): 538-546.
[3] Stabin MG *et al.* [2005], JNuclMed 46: 1023-1027.
[4] Müller C *et al.* [2014], JNuclMed 55:1658-1664.
[5] De Nardo L *et al.*[2021], PhysMedBiol 66 025003.

PREPARATION OF [^{177}Lu]Lu-PSMA-617: FOCUS ON QUALITY

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Introduction

In 2017 Italian Regulatory Agency AIFA approved our clinical trial “Radiometabolic Therapy (RMT) with [^{177}Lu]Lu-PSMA-617 in advanced castration resistant prostate cancer (CRPC): efficacy and toxicity evaluation” EudraCT 2016-002732-32. The clinical trial includes the administration of the radiopharmaceutical [^{177}Lu]Lu-PSMA-617 prepared in our Radiopharmacy according to Italian regulation.

Materials and methods

To submit the clinical study to the Competent Authority an Investigational Medicinal Product Dossier (IMPD) according to EMA guideline has been drafted. The drug substances involved were PSMA-617 supplied by Endocyte (a Novartis Compay) and lutetium-177 chloride n.c.a (non-carrier added) supplied by ITG. For [^{177}Lu]Lu-PSMA-617 we performed a manual synthesis in three steps:

- prepare the reaction vial containing: incoming lutetium-177, gentisic/ascorbic buffer, and a mass of PSMA-617 adequate to obtain the specific activity of 36-37 GBq/mg;
- heat the reaction vial to 100 °C for 8 minutes;
- dilute the product with saline solution to a final volume of 15-20 ml, through a sterile line equipped with a 0.22µm sterilizing filter.

Since a EP Monograph for [^{177}Lu]Lu-PSMA-617 is not available yet, quality controls, acceptance criteria, specifications and release timing were chosen in compliance with the general texts and monographs of the current European Pharmacopoeia. Analytical procedures have been validated in accordance with - ICH Q2 (R1)

Results

IMP consists of a solution of [^{177}Lu]Lu-PSMA-617 with an activity range of 17800-21100 MBq at End of Synthesis (EOS), which in this case is also considered Activity Reference Time (ART). The final volume is defined as 15-20 mL and the radioactive concentration is between 1050 and 1200 MBq/mL. [^{177}Lu]Lu-PSMA-617 is formulated in a multidose vial with the components described in Table 1.

Table 1

Components	Function	Amount/Activity
[^{177}Lu]$LuCl_3$	Active Pharmaceutical Ingredient (API)	18390-21800 MBq (ART)
PSMA-617	Precursor	500-600 µg (480-576 nmol)
Water for injection	For reconstitution of PSMA	0.5-1ml
Gentisic/ascorbic buffer composition:		
Gentisic acid	Radical scavenger	16.8 mg (109 µmol)
Sodium acetate	Buffer solution	32.4 mg (395 µmol)
Sodium Hydroxide	pH balance buffer	9.6 mg (240 µmol)
Ascorbic Acid	Radical scavenger	31.2 mg (177 µmol)
NaCl 0,9%	diluent	15 - 20 ml

As showed in Table 2, [^{177}Lu]Lu-PSMA-617 stability was assessed at 24 and 30 hours after end of synthesis, according with time of patient admission and discharge. Acceptance criteria for radiochemical purity was established at ≥ 97%.

Table 2

Parameter	Method	Acceptance Criteria	N1_24h	N2_24h	N3_24h	N1_30h	N2_30h	N3_30h
Appearance	Visual test	Clean and Colorless	Complies	Complies	Complies	Complies	Complies	Complies
Radiochemical Purity	TLC	[^{177}Lu]Lu colloids ≤ 3% [^{177}Lu]Lu-PSMA ≥ 97%	0.1% 99.9%	0.1% 99.9%	0.1% 99.9%	0.2% 99.8%	0.2% 99.8%	0.1% 99.9%
Radiochemical Purity	HPLC	[^{177}Lu]Lu ≤ 3% [^{177}Lu]Lu-PSMA ≥ 97%	0.04% 97.4%	0.06% 97.6%	0.3%. 97%	0.3%. 97.2%	0.3%. 97%	0.3%. 97%
pH	pH Strips	4.5–5.5	5	5	5	5	5	5

From April 2017 to December 2021 our Radiopharmacy supplied 334 patient's on the basis of our IMPD. The average labelling yield (n.d.c.) is 96%, the average radiochemical purity is 99,6% (HPLC) and the 99,7% (TLC). All the samples resulted sterile and pyrogen-free.

Discussion/Conclusions

This study demonstrates that [^{177}Lu]Lu-PSMA-617 can be prepared in a clinical radiopharmacy by applying a well-implemented quality assurance system that clearly defines acceptance criteria, validations plans and methods for quality control and stability. It is important to underline that this production is only allowed for patients enrolled in our clinical study, which will be closed at the end of 2022. After this date, it is very likely that the radiopharmaceutical with a European market authorization will be available for the patients.

PRODUCTION AND CHARACTERIZATION OF 111-Ag RADIOISOTOPE FOR MEDICAL USE IN A TRIGA MARK II NUCLEAR REACTOR

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Introduction

RadioPharmaceutical Therapy (RPT) comes forth as a promising technique to treat a wide range of tumors while ensuring low collateral damage to nearby healthy tissues. Recently, 111-Ag was proposed as a promising core of a therapeutic radiopharmaceutical for treating large tumors, given its penetrating β-emission. Moreover, it also emits two γ-rays (342 keV I γ =6.7% and 245 keV I γ =1.24%), suitable for SPECT imaging, making the therapy follow-up feasible. Finally, its half-life of 7.5 days allows for all the mandatory radiochemical procedures that lead from the radioisotopes to the actual radiopharmaceutical.

Materials & Methods

In this contribution, the production of 111-Ag via neutron activation inside a nuclear reactor was modeled using two different Monte Carlo codes (MCNPX and PHITS) and compared with experimental measurements making use of a spectroscopic system composed of an HPGe and a LaBr3 scintillator. The whole process was simulated starting from an MCNPX-based reactor model. Irradiation experiments were carried out using both natural and enriched Palladium samples irradiated inside the central thimble of a Triga Mark II Research Reactor. After the extraction, irradiated samples were spectroscopically characterized before and after the chemical separation of the produced 111-Ag from its Palladium matrix.

Discussion / Conclusion

The benchmarking of Monte Carlo simulations using experimental data allowed for a precise estimation of 111-Ag yield following irradiation of Palladium samples inside a TRIGA Mark II Reactor which will be used for producing a first batch of 111-Ag with the aim of radiolabeling an innovative radiopharmaceutical developed within the ISOLPHARM collaboration.

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