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Review

^{68}Ga-DOTA and analogs: Current status and future perspectives

*Krzysztof Kilian**

Heavy Ion Laboratory, University of Warsaw, Pasteur 5a, 02093 Warsaw, Poland

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ABSTRACT

The construction of the ^{68}Ge/^{68}Ga generator has increased application of radiopharmaceuticals labeled with this isotope in medicine. ^{68}Ga-PET is widely employed in the management of neuroendocrine tumors but favorable chemistry with tri- and tetraaza-ring molecules has opened wide range of ^{68}Ga application in other fields of PET imaging. This review covers the radiopharmaceuticals labeled with gallium in molecular imaging and shows perspectives on the use of gallium-68 as a substitute for technetium-99, fluorine-18 and carbon-11 in some applications.

1. Background

The positron emission tomography (PET) technique is applied in the study of functioning and functional changes in the human body, enabling, among others, precise oncology diagnostics. It is as well an appreciated diagnostic method in neurology and cardiology, allowing to evaluate the condition of the respective systems in a non-invasive way.

The dominant isotope in these types of diagnostics is fluorine-18, widely used in the research and the clinical applications. The source of the isotope is a proton medical cyclotron with particle energies from 10 to 20 MeV range which limits the availability of the isotope to the diagnostic centers, where the operation of such a machine is economically justified and qualified personnel to operate and maintain such an equipment is available. Looking at the history of another isotope, technetium-99m, widely used in the related field of nuclear medicine – scintigraphy, it can be seen that it is critical to the development of the clinical applications to increase the availability of the isotope.

Three isotopes of gallium can be used in nuclear medicine: gallium-66, potentially useful as a PET tracer, gallium-67, for the scintigraphy of the inflammation sites as citrate or with labeled leukocytes, still considered as a routine tool for infection imaging and gallium-68, a fast growing PET radionuclide, especially in cancer diagnostics.

In opposition to fluorine-18 or carbon-11-based PET diagnostics, gallium-68 does not require the use of cyclotrons, thereby reducing costs and increasing flexibility in the practice of nuclear medicine diagnostic imaging. In recent years, gallium-68 has gained importance in molecular imaging by

* Tel.: +48 22 55 46 214.
E-mail address: kilian@slcj.uw.edu.pl
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Table 1 – Chemical properties of Fe, Ga, Tc and In.

	Iron	Gallium		Technetium	Indium
Atomic number	26	31		43	49
Ionic radius [pm]	64	62		136	80
Oxidation state	III	III		I, III, IV, V, VII	III
Coordination number	6	4, 5, 6		5, 6, 7	6, 7, 8
Complex geometry	Octahedral	Octahedral		Octahedral, trigonal bipyramid, square pyramidal	Octahedral, square antiprismatic
Radionuclide	•	^{67}Ga	^{68}Ga	^{99m}Tc	^{111}In
Diagnostic mode	•	SPECT	PET	SPECT	SPECT
Half-life	•	3.56 d	1.13 h	6.02 h	2.83 d
Source	•	Cyclotron	Generator	Generator	Cyclotron

positron emission tomography, due to the advantages of the easy availability both from the ^{68}Ge/^{68}Ga generator and the cyclotrons (^{nat}Zn(p,n)^{68}Ga) with 7–16 MeV protons,[1] good radiation properties and rich Ga^{3+} coordination chemistry. The quality of imaging is in some applications comparable to the quality of imaging with fluorine-18 isotope. In the nearest future these factors may cause a serious increase in the interest in gallium-68 labeled compounds in biology and medicine.[2–4]

This review indicates the growing importance of the radiopharmaceuticals labeled with gallium in molecular imaging and shows the perspectives on the use of gallium-68 as a substitute for technetium-99 in some applications.

2. Gallium chemistry

Gallium is a metal in group 13 of the periodic table. In aqueous acidic solutions, it occurs as a free, hydrated Ga^{3+} ion, in slightly acidic and neutral pH hydrolyses to insoluble $Ga(OH)_3$ but nanomolar concentrations, it is used for the formulation of radiopharmaceuticals, can be obtained without precipitation. In contrast to Tc(V), where improper reaction conditions lead to insoluble and unreactive TcO_2, amphoteric properties of gallium allow the redissolution of the formed hydroxide. If the concentration exceeds the nanomolar level, addition of the carboxylic acids (citrate, oxalate, acetate) prevents precipitation. At higher pH, above 7, gallium hydroxide redissolves as $[Ga(OH)_4]^-$.

Gallium is classified as hard Lewis acid and can form octahedral complexes with hard Lewis bases, such as oxygen and nitrogen atoms. Carboxylic, phosphonate, thiol and amino groups form strong six-coordinate complexes, thermodynamically and kinetically stable at physiological conditions. Existing five and four-coordinate gallium complexes are more sensitive to hydrolysis but are enough inert on the time-scale of the gallium applications.

The coordination chemistry and biological properties of Ga are similar to Fe ion (Table 1). Both are 3+ ions with comparable ionic radius and dominating octahedral complex geometry, thus the biomolecules with high Fe affinity could be easily labeled with Ga–ligand exchange between intravenously applied gallium citrate and transferrin, abundant plasma protein, forms a complex used for imaging of inflammatory sites.

Another element whose coordination chemistry is of great interest for the development of gallium compounds, is indium. As a hard Lewis acid, In^{3+} prefers hard bases and forms stable complexes with nitrogen and oxygen atoms. Only higher ionic radius (80 pm vs. 60 pm for Ga) differentiates the ligands against a cavity size, where In fits perfectly the tetraaza-macrocyclic ligands (DOTA, Fig. 1a), while Ga prefers smaller triaza rings (NOTA, Fig. 1b).

3. Generators

Gallium-68 is a positron emitter with a half-life of 67.6 min and dominantly decays via 1.92 MeV positron emission (89%) and electron capture (11%). The relatively short half-life and hydrophilic nature are beneficial for the rapid renal clearance and reduce effective dose for the patient. Gallium-68 can be conveniently and economically obtained from the ^{68}Ge/^{68}Ga generator and the long half-life of the parent nuclide ^{68}Ge ($t_{1/2}$ = 271 days) allows long-term PET imaging on-site. Thereby, the introduction of gallium-68 generator has opened access to the PET radioisotopes for diagnostic sites, located outside production centers equipped with cyclotrons. The availability of the ^{68}Ge/^{68}Ga generator system also provides imaging centers with a PET nuclide that is routinely available in a manner similar to the generator-produced single-photon emission computed tomography nuclide technetium-99m, with added value of better resolution and sensitivity. Thus, the development of gallium radioisotopes and diagnostics procedures combined with on-demand production and operation in the absence of cyclotron facilities provides a reasonable development pathway for gallium-68 labeled compounds in the molecular imaging.

Although the first gallium-68 generators have been used already in the 60s, the chemical forms of the isotope as the inert complexes, prevented the practical application and the development of gallium radiochemistry. The second problem was the presence of significant amounts of parent nuclide and other metallic impurities in eluate. Both of these factors resulted in the temporary loss of interest in applications of gallium-68, but as a complex with EDTA has been used in clinical practice that time and was the β+ source in the development of the PET scanners. Practical applications of the first gallium generators have been described recently.[5]

Beginning of the 21st century saw new developments in the construction of generators. Various resins, using inorganic oxides (TiO_2, IGG100 ^{68}Ga generator; Eckert&ZieglerEurope),[6] SnO_2[7] and nanoparticles[8] or organic polymers, containing

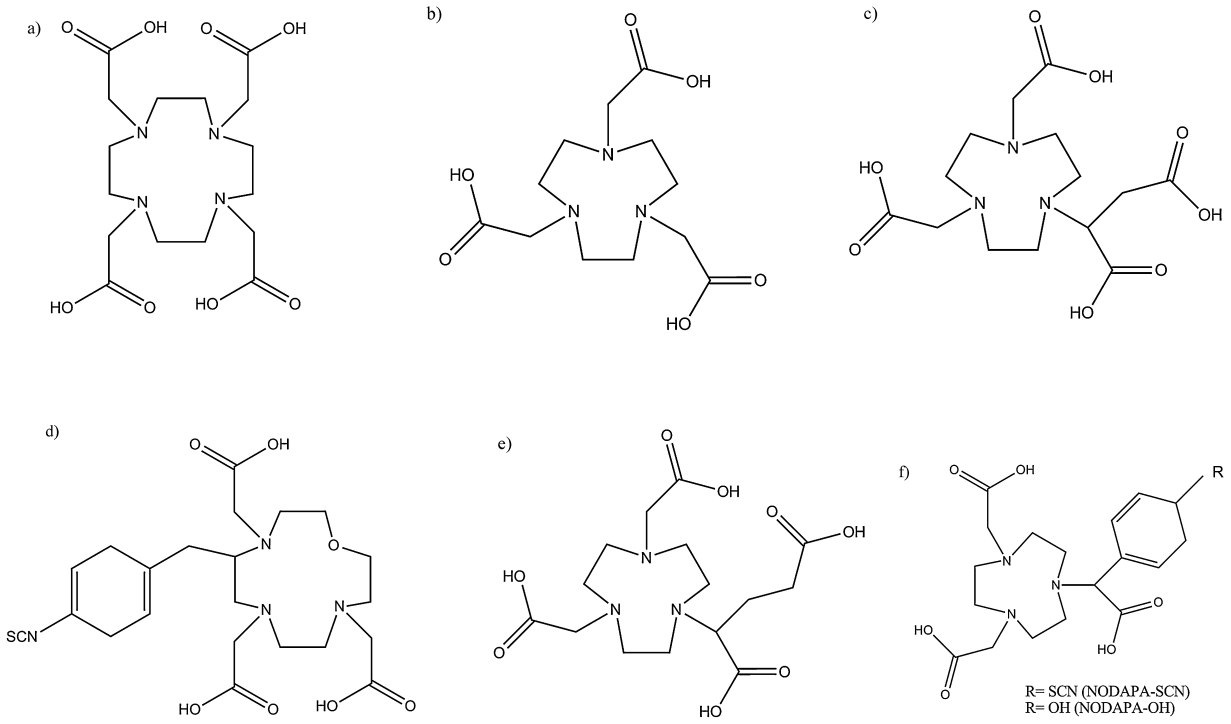

Fig. 1 – Examples of the ligands DOTA (a), NOTA (b), and derivatives: NODAGA (c), OXO-DO3A (d), NODASA (e) and NODAPA (f).

N-methylglucamine groups[9] or lauryl gallate (ITG GmBH, Germany) were constructed and successfully applied. Contemporary ^{68}Ge/^{68}Ga generator can be used for 1–2 years and the gallium-68 build-up is rapid enough to allow multiple radiotracer preparations daily.

The gallium-68 eluted from the inorganic generator often suffered from the contamination by other metals, which influenced negatively radiolabelling.[10] Commonly observed impurities are: Fe(III), Al(III) and Zn(II) at the mg/L levels, Ni(II), Co(II), Cd(II) and Cu(II) at 0.01–0.1 mg/L and traces of Mn, Pb, Cr, V.[7,11] Average gallium-68 breakthrough varies from 10^{-4} to 10^{-5}%. The most critical impurities are ^{68}Ge, Fe(III), Zn(II), Mn(II), thus additional purification and removal of the metal contaminants has to be applied. The most popular procedures differentiate gallium species (Ga^{3+} cations and anionic $[GaCl_4]^-$) depending on pH and chloride concentration and then separate on the respective ion exchangers. Typically, $^{68}Ga^{3+}$ is trapped on the strong cation exchange cartridge (SCX), then converted with HCl, HCl/acetone or NaCl into $[^{68}GaCl_4]^-$ and transferred with a small volume of concentrated HCl to the strong anion exchange cartridge (SAX) and finally eluted with water or slightly acidic solution.[12–15] The process could be easily automated for the reproducible preparation of ready-to-use $^{68}Ga^{3+}$ in optimal activity and chemical form for labeling.[16]

The generators based on zirconium nanoparticles and organic matrix could be eluted under milder conditions and gallium-68 ions are easier to form suitable complexes for clinical use. Furthermore, they suffer less from the metallic contaminants and the purification processes could be omitted or simplified.[17]

At the time of this publication, at least four types of the generators with different construction and scalable activity are available commercially and more than 100 centers across the world use this type of generator, both for the basic research on gallium-68 radiochemistry and the clinical trials.[18]

4. Radiopharmaceuticals

The rapid development of radiopharmaceuticals labeled with gallium is due to the synergy between a growing importance of the PET diagnostics in the molecular imaging and the use of long-term experience with the technetium generators. The trivalent cation $^{68}Ga^{3+}$ is eluted from the generator and its chemical form allows simple and universal application in radiopharmaceutical preparations with an appropriate chelator. Ga^{3+} ion has complexing properties comparable to those of Fe(III) and In(III) but can easily adapt to various Tc targeting concepts (Table 2), resulting in a large group of molecules (vectors), with well-established and proved practical applications in the molecular imaging.

4.1. *Gallium-68 bifunctional chelators (BFC) in the neuroendocrine tumor imaging*

The bifunctional chelators are compounds acting as a conjugate between the radioisotope and a vector. The BFC's key features are the ability to quickly and steadily incorporate the isotope and create covalent bonds with biomolecules, responsible for targeting. Over the past decade, the interest in it is inseparably connected with the synthesis and use of

Table 2 – ^{99m}Tc tracers with their ^{68}Ga analogs.

Diagnostics	^{99m}Tc	^{68}Ga
Peptide receptors	^{99m}Tc-HYNIC-peptide	^{68}Ga-DOTA-peptide
Bone metastases	^{99m}Tc-MDP	^{68}Ga-phosphonates
Renal function	^{99m}Tc-DTPA/MAG3/DMSA	^{68}Ga-EDTA
Cardiac function	^{99m}Tc-RBC/MIBI	^{68}Ga-BAPEN
Lung function	^{99m}Tc-MAA	^{68}Ga-MAA
Hepatobiliary	^{99m}Tc-IDA	^{68}Ga-IDA
Infection	^{99m}Tc-WBC	^{68}Ga-citrate
Brain imaging (perfusion)	^{99m}Tc-ECD	^{68}Ga-ECD

Abbreviations: HYNIC, hydrazinonicotinamide; MDP, methylenediphosphonic acid; DTPA, diethylenetriaminepentaacetic acid; MAG3, mercaptoacetyltriglycine; DMSA, dimercaptosuccinic acid; RBC, red blood cell; MIBI, methoxyisobutylisonitrile; MAA, macroaggregated albumin; IDA, iminodiacetic acid; WBC, white blood cell; ECD, ethyl cysteinate dimer; EDTA, ethylenediaminetetraacetic acid; BAPEN, Tris(4,6-dimethoxysalicylaldimine)-N,N′-bis(3-aminopropyl)-N,N′-ethylenediamine.

radiolabeled peptides for positron emission tomography (PET) in the clinical and preclinical diagnostics, as well as with the radiation-based therapeutics.

The macrocyclic chelators with three or four nitrogen atoms in a ring have been established as frequently considered routes for the introduction of gallium-68. Between them, the molecules with tri- and tetraaza-ring have become the most prevalent since irreversible complexation of the gallium atom, thermodynamic stability and superior kinetic even at room temperature. External substituents, usually in the form of carboxyl groups allow convenient conjugation to various targeting molecules.

DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) remains the most frequently used chelator because of its availability and well-recognized coordination chemistry.[2] Its six coordinate triaza-ring analog NOTA ((1,4,7-triazanonane-1,4,7-triyl)triacetic acid) forms slightly deformed octahedral complexes with gallium-68 which display higher stabilities and faster incorporation of Ga(III) at lower temperatures.[18] A significant number of compounds, similar to DOTA and NOTA have been used for specific applications (Fig. 2): bifunctional derivatives of NOTA – NODAGA (1,4,7-triazacyclononane,1-glutaric acid-4,7-acetic acid) and NODASA (1,4,7-triazacyclononane-1-succinic acid-4,7-diacetic acid) are limited to coupling peptides through an amide bond, NODAPA (1,4,7-triazacyclononane-1,4-diacetic acid-7-p-phenyl-acetic acid) with hydroxyl group (NODAPA-OH) or thiocyanate groups (NODAPA-$(NCS)_n$) have been successfully applied as versatile bifunctional chelators.[19]

Many receptors are overexpressed in cancer cells, thus the targeted peptides with aminoacids sequences specific for activity centers, labeled with appropriate radioisotope via BFC, have been used for the development of highly specific imaging probes for PET and/or therapeutic agents.

^{68}Ga-DOTA-peptides are a group of PET tracers that specifically bind to somatostatin receptors (SST) that are over-expressed on the neuroendocrine tumor (NET) cells.[20] Targeting agents are coupled via the BFC leveraging standard intermediates that enable covalent attachment of peptides, including active octapeptides (octreotride, OC) and related peptide analogs, TOC (Tyr^3-OC), TATE (Tyr^3-Thr^8-OC) and NOC (NaI^3-OC) (Fig. 2). Six different SST receptors have been identified (SST1, 2A, 2B, 3, 4, 5) in humans. Of the analogs mentioned above, DOTA-TOC and DOTA-TATE show a high affinity for the SSTR2, although a 10-fold higher affinity for the SSTR2 has been demonstrated for DOTA-TATE as compared to DOTATOC in vitro in the transfected cell cultures. Their binding affinity to SSTR5 is quite low and that to SSTR3 is almost negligible and there is no considerable affinity to SSTR1 and SSTR4. For DOTA-NOC, high affinities for SSTRs' 2,3 and 5 were reported,[21] which improves the binding profile and extends the spectrum of targeted tumors.

Despite the variations in somatostatine receptors affinity, all described compounds (^{68}Ga-DOTA-TOC, -NOC, -TATE) have been reported to be accurate for the localization of well differentiated NET lesions, performing better than CT.[22]

Comparing to other modalities, studies have shown the superiority of PET over ^{111}In-pentetreotide-SPECT in the detection of metastases and unknown primary neuroendocrine tumors. PET imaging using ^{68}Ga-SSTR has also been found to be more accurate than ^{18}F-FDOPA-PET in non-carcinoid tumors. [^{68}Ga]-DOTA-TATE detected more tumor sites than [^{123}I]-MIBG in patients with the neural crest tumors (including paragangliomas). Only one bone lesion in one patient with adrenal paraganglioma was detected on [^{123}I]-MIBG scan but not in [^{68}Ga]-DOTA-TATE.[23–29]

Radionuclide-labeled DOTA-SSTR analogs are used for diagnostics and therapy of the neuroendocrine tumors. Typically, ^{68}Ga-DOTA-TOC is used for diagnosis, while the same compound labeled with high- or medium-energy β-emitters such as yttrium-90 or lutetium-177 is used for therapy. The use of coupled diagnostic and therapeutic isotopes with ligand for treatment of NET allows better planning of therapy and to evaluate the therapeutic outcome as in personalized medicine where the diagnosis is tailored to the subsequent therapy. It influenced radiopharmaceutical sciences with the "theranostics" concept, which combines gallium-68 diagnostics with trivalent therapeutic radiometals (yttrium-90, lutetium-177, bismuth-213)[30] with improved personal dosimetry.[31]

4.2. *Behind the NET – applications of ^{68}Ga-BFC-core*

Two main trends could be observed in the development of methods and applications of the gallium-68 radiopharmaceuticals, other than NET-focused. First, using the technetium "shake'n'shoot" concept, has focused attention to the use of gallium-68 as technetium-99m substitute in the part of applications. Recently published papers have presented efficient

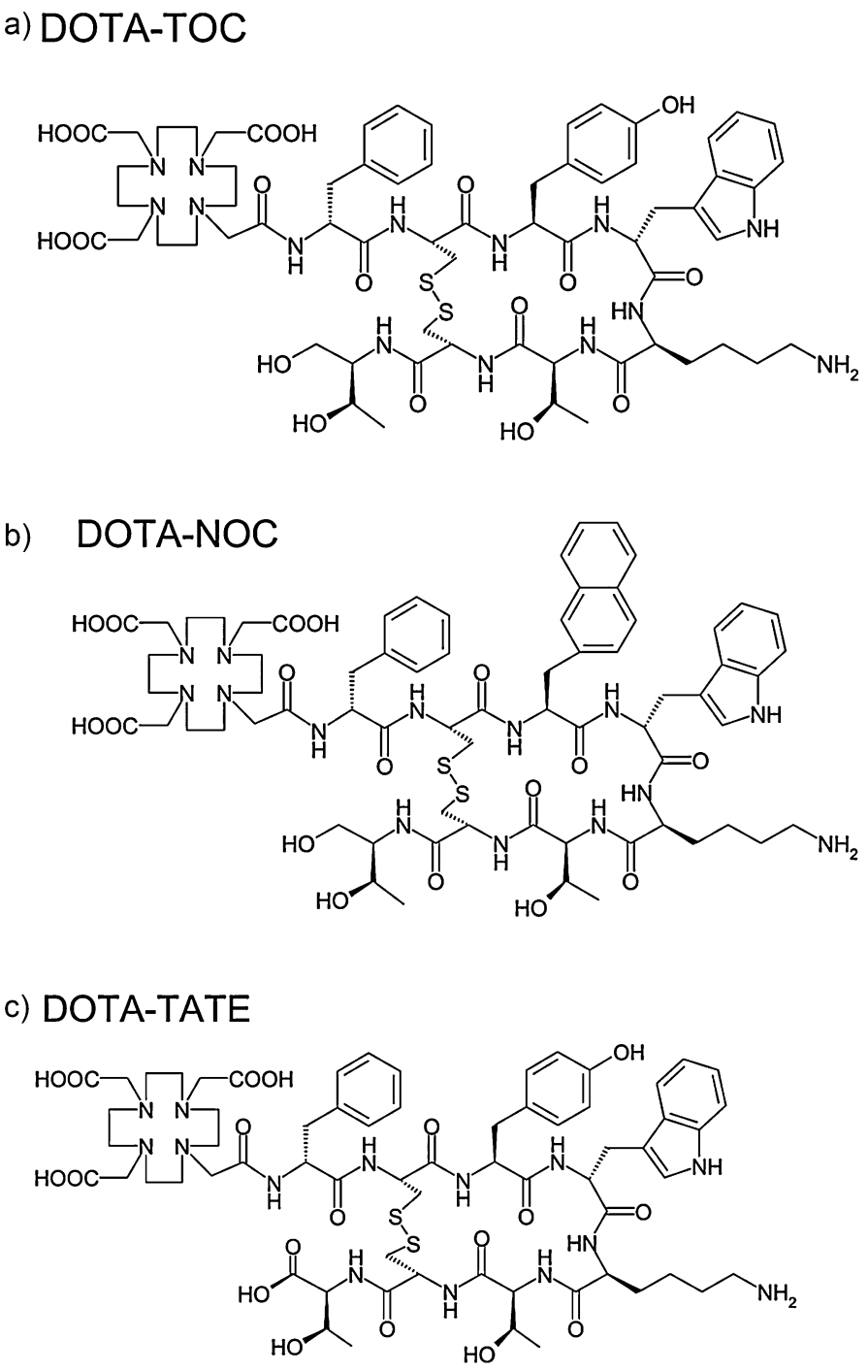

Fig. 2 – Structures of DOTA-TOC (a), DOTA-NOC (b), and DOTA-TATE (c). The most clinically applied ^{68}Ga-labeled pharmaceuticals in nuclear medicine.

gallium-68 labeling of commercially available phosphonate kits for scintigraphy of bone lesions,[32,33] MAA (macroaggregated human serum albumin) particulate for perfusion studies,[34,35] ^{68}Ga-NOTA-MSA (mannosylated human serum albumin) for immune system imaging,[36] and ^{68}Ga-BAPEN (Tris(4,6-dimethoxysalicylaldimine)-N,N′-bis(3-aminopropyl)-N,N′-ethylenediamine) myocardial uptake as substitute for MIBI.[37] All these compounds profit from similar affinity of gallium and technetium to some vectors and only slightly differentiate biodistribution and pharmacokinetics (Table 2).

The second trend, taking the generator advantages of independence from the cyclotron, much cheaper operation and production, resulted in the synthesis of ^{68}Ga labeled radiopharmaceuticals as an alternative or support to fluorine-18 and carbon-11 compounds. Routine fluorine-18 production runs are bulk and it is hard to scale them for single patient. Therefore, flexibility of gallium-68 generators opens the

Table 3 – ^{68}Ga analogs to commonly used ^{18}F and ^{11}C PET tracers.

Diagnostics	^{18}F/^{11}C	^{68}Ga
Angiogenesis	^{18}F-galacto-RGD	[^{68}Ga]DOTA-RGD, ^{68}Ga-VEGF
General cancer imaging	^{18}FDG	^{68}Ga-CXCR4 biomarker, ^{68}Ga-uPAR biomarker, ^{68}Ga-SCN-NOTA-BZA
Hypoxia	^{18}F-nitroimidazloes (FAZA, FMISO, FETNIM)	^{68}Ga-DOTA-imidazoles
Proliferation	^{18}FLT	^{68}Ga-DO3A-thymidine
Glioma	^{18}FET, ^{11}C-methionine	^{68}Ga-glutamine, ^{68}Ga-DO3A-alanine, ^{68}Ga-DO2A-tyrosine,
Prostate cancer	^{18}FDG, ^{11}C-acetate, ^{18}F-choline, ^{11}C-choline	^{68}Ga-DOTA-PSMA

Abbreviations: RGD, arginylglycylaspartic acid; FAZA, [^{18}F]-1-α-D-(2-deoxy-2-fluoroarabinofuranosyl)-2-nitroimidazole; FMISO, [^{18}F]-fluoromisonidazole; FETNIM, [^{18}F]-fluoroerythronitroimidazole; FLT, [^{18}F]-fluorothymidine; FET, [^{18}F]-fluoroethyl-L-tyrosine; VEGF, vascular endothelial growth factor; CXCR4, chemokine receptor; BZA, benzamide; PSMA, prostate specific membrane antigen; UPAR, urokinase-type plasminogen activator.

possibility to produce short series or even single doses. While it is difficult today to imagine the replacement of ^{18}FDG or leading carbon-11 radiopharmaceuticals in routine clinical diagnostics, the use of gallium-68 equivalents in targeted diagnostics seems to be rapidly developing (Table 3).

4.2.1. *Angiogenesis*

Receptors playing a key role in angiogenesis are important targets for several experimental drugs. The expression of the αvβ3 integrin has been detected on blood vessels with the intensive angiogenesis and indicates tumors with high metastatic potential. One of the most intensely evaluated compounds so far is [^{18}F]Galacto-RGD[38] which specifically binds to the αvβ3 integrin and shows very good pharmacokinetics. Comparable results were received for [^{68}Ga]DOTA-RGD[39] or other RGD peptides linked with modified bifunctional ligands: [^{68}Ga]NS3-RGD, [^{68}Ga] Oxo-DO3A-RGD,[40] or four nitrogen atoms open chain H2dedpa.[41] Effective diagnosis of angiogenesis was reported as well with the VEGF (vascular endothelial growth factor) receptors. The tracer contained single chain VEGF (scVEGF), linked with the polyethylene glycol (PEG) bridge to NOTA, which was suitable for labeling with gallium-68 at ambient temperature. Gallium-68 labeled scVEGFPEG-NOTA was injected intravenously into HT-29 (human colon adenocarcinoma) tumor-bearing mice, and clearly visualized tumor structure.[42] Labeling procedure was proposed as a kit-formulated radiopharmaceutical for the targeted imaging of tumor angiogenesis.

4.2.2. *General oncology imaging*

Mapping of the chemokine receptor CXCR4 in tumors is used for rating tumor aggressiveness and estimation of the metastatic seeding probability in breast, prostate and lung cancer. Specific tracer for the imaging of CXCR4, gallium-68 labeled DOTA-4-FBn-TN14003 (fluorinated 14-aminoacids peptide) was synthesized as a potential PET tracer for this purpose.[43]

Similar studies were performed with the urokinase-type plasminogen activator receptor (uPAR) which is a well-established biomarker for tumor aggressiveness and metastatic potential. The synthetic peptide (Asp-cyclohexylalanine-Phe-D-Ser-D-Arg-Tyr-Leu-Trp-Ser (AE105)) has been identified to have a high affinity for human uPAR. The use of ^{68}Ga-DOTA-AE105-NH2 and ^{68}Ga-NODAGA-AE105-NH2 as the first gallium-68 labeled uPAR radiotracers for PET imaging were reported, where NODAGA is a favored tracer providing highest tumor-to-background ratio.[44] These new tracers constitute an interesting alternative to the 64Cu-labeled version (64Cu-DOTA-AE105 and 64Cu-DOTA-AE105-NH2) for detecting uPAR expression in tumor tissue.[45]

Radiolabeled benzamides (BZA) have been reported to be the attractive agents for targeting malignant melanoma as they bind melanin and display high accumulation in the melanoma cells. The ^{68}Ga-labeled benzamide via NOTA was presented as a potential PET agent for malignant melanoma. Biodistribution and micro-PET studies of ^{68}Ga-SCN-NOTA-BZA in B16F10-bearing mice showed selective uptake into the tumor. The radiotracer was effectively cleared via renal excretion without further metabolism.[46]

4.2.3. *Hypoxia imaging*

Wide range of fluorine-18 nitroimidazloes have been developed such as FETNIM ([^{18}F]-fluoroerythronitroimidazole), FAZA ([^{18}F]-1-α-D-(2-deoxy-2-fluoroarabinofuranosyl)-2-nitroimidazole), FETA ([^{18}F]-fluoroetanidazole) and especially [^{18}F]FMISO, still considered as a gold standard for hypoxia imaging.[47] Copper-64 ATSM (diacetyl-bis(N4-methylthiosemicarbazone) was indicated as an alternative, but suffers from less favorable dosimetry, associated with emission of β^- (38.5%), and solid target cyclotrone production (^{64}Ni(p,n)^{64}Cu) which limits availability. Several ^{68}Ga-complexes with nitroimidazoles were proposed for hypoxic tumor imaging: DOTA-derivative with one or two metronidazole moieties,[48] imidazole coupled to DOTA by conjugating via an amide or thiourea bond.[49]

The results obtained were comparable with reference images obtained with [^{18}F]FMISO and [^{18}F]FAZA, but the usage of methylated 5-nitroimidazole improved performance of ^{68}Ga-DOTA-nitroimidazoles in hypoxia imaging.[50]

4.2.4. *Proliferation*

Radiolabeled thymidine analogs specifically addressing DNA synthesis have significant potential for imaging of tissue proliferation in vivo. [^{18}F]-fluoro-3′-deoxy-3′-L-fluorothymidine ([^{18}F]FLT) has been developed and well-established as a proliferation tracer.

Thus, thymidine analogs substituted by BFC with gallium-68, were proposed as alternative to [^{18}F]FLT. DO3A macrocycle

in N-3 position was attached to thymidine and then labeled with gallium-68.[51]

Amino acids labeled with carbon-11 or fluorine-18 play an important role in clinical diagnostics and understanding the basic processes in biochemistry and physiology.[52–54] They are involved in the synthesis of peptides and proteins, thus tumor cells concentrate labeled amino acids at a high rate, while the uptake in normal cerebral tissue is relatively low. ^{11}C-methionine (MET) and ^{18}F-fluoroethyl-L-tyrosine (FET) are routinely applied for gross tumor volume delineation in brain gliomas and for the differentiation between treatment-related changes and residual/recurrent tumor.

Radiolabeled with gallium-68 glutamine was used for tracing glutamine for imaging and for determination of protein synthesis rates in tumors. Glutamine was conjugated to DOTA and labeled with gallium-68 by mixing the Ga^{3+} species with the conjugate in ethanol and reacting at 65 °C.[55,56]

Alanine derivatives of DO2A and DO3A were synthesized and labeled with gallium-68. Cell uptake assays were carried out in Hep3B (human hepatoma) and U87MG (human glioma) cell lines at 37 °C. Positron emission tomography (PET) imaging studies were performed using balb/c mice xenografted with CT-26 (mouse colon cancer).

^{68}Ga-DO3A-homoalanine showed the highest uptake value ratio, followed by ^{68}Ga-DO2A-alanine, ^{68}Ga-DO3A-alanine and ^{68}Ga-DO2A-homoalanine, but all derivatives were found to have high tumor cell uptakes, high tumor/nontumor ratio and low nonspecific uptake in normal organs, except for the kidneys.[57]

The novel tyrosine chelate (^{68}Ga-DO2A-(OBu-L-tyr)2) (^{68}Ga-1,4,7,10-tetraazacyclododecane-1,7-diacetic acid-4,10-di-(O-butyl)-L-tyrosine) was proposed as an approach which uses the biological amino acid transporter targeting properties of L-tyrosine.

In vitro studies utilizing the F98-glioblastoma cell line revealed specific uptake of [^{68}Ga]Ga-DO2A-(OBu-L-tyr)2 that was comparable to that of the reference [^{18}F]fluoroethyl-L-tyrosine (FET). These promising results indicate a high potential of tyrosine analogs labeled with GaDOTA for molecular imaging of tumor-driven amino acid uptake by PET.[58]

4.2.5. *Prostate cancer*

Imaging of prostate cancer was dominated by 3 tracers: ^{18}FFDG, acetate and choline, both labeled with fluorine-18 or carbon-11.[59] The prostate specific membrane antigen (PSMA) is a unique membrane bound glycoprotein, which is overexpressed in prostate cancer and is well-established in the diagnosis as highly specific prostate cancer cell-surface protein. Recently, theranostics concept had been applied to label PSMA-ligands with gallium-68 and lutetium-177.[60] Successful synthesis of ^{68}Ga-DOTA-PSMA[61] and ^{68}Ga(HBED-CC)-PSMA[62] was followed by promising results of clinical trials, where at least one lesion suspicious for cancer was indicated with 100% detection ratio at PSA >2.2 ng/ml. Lesions suspicious for prostate cancer were presented with excellent contrast as early as 1 h post injection, with high detection rates even at low PSA levels.[63]

5. Availability

The effective synthesis of DOTA-TOC and DOTA-TATE were presented[64] and numerous strategies of automated[65,66] or semi-automated[67] purification and synthesis were proposed. One of the recent developments influenced radiopharmaceuticals sciences with a new term “autoclabeling”,[68] describing convenient procedure for gallium-68-labeling by combining the labeling reaction and the steam sterilization into one single step to get the final, sterile ready-to-use product. Increase in clinical and radioprotection demands have boosted for extensive automation of the production process and resulted in a number of commercial synthetic modules, but regulatory restrictions, both requesting GMP-compliant generator and GMP regulations for labeling as for the standard drugs, has retarded the implementation in clinical practice. Both these obstacles have been overcome and at least one pharmaceutical ^{68}Ge/^{68}Ga generator was officially approved for the use in clinical studies and some modules were registered as GMP-compliant.[69] Additional positive impact could be caused by the debate on adopting GMP-regulations for microdoses to PET-radiopharmaceuticals production specificity,[70] intending to maintain the required quality aspects but not to hamper innovation and dynamic development of gallium-68 radiopharmaceuticals in clinical trials.

6. Conclusions

Construction of the ^{68}Ge/^{68}Ga generator increased the applications of radiopharmaceuticals labeled with this isotope in medicine. The availability of the commercial, pharmaceutical grade ^{68}Ga/^{68}Ge generator for routine applications and a favorable chemistry using DOTA, NOTA-derived bifunctional chelators have opened a brilliant future for the application of gallium-68 in all fields of PET imaging. Wide range of the PET radiopharmaceuticals based on gallium-68 labeling can be an alternative or complement to the already well-established radiopharmaceuticals based on fluorine-18 or carbon-11.

Nowadays, a complete replacement of these radioisotopes is hard to imagine, but the extension of diagnostic procedures by on-request, short series synthesis of targeted radiopharmaceuticals labeled with gallium-68, seems to be a very reasonable option in the nearest future.

Conflict of interest

None declared.

Financial disclosure

None declared.

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