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Evolution of PET Radiochemistry: Synthesis and Clinical Application of Radiopharmaceuticals

Continuing Education for Nuclear Pharmacists
And
Nuclear Medicine Professionals

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The Evolution of PET Radiochemistry: Synthesis and Clincial Application of Radiopharmaceuticals

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EVOLUTION OF PET RADIOCHEMISTRY: SYNTHESIS AND CLINICAL APPLICATION OF RADIOPHARMACEUTICALS

STATEMENT OF LEARNING OBJECTIVES:

1. Describe the principle of molecular imaging and the characteristics of PET radiopharmaceuticals
2. Describe the basic concepts involved in the synthesis of
   ^{18}F and ^{11}C labeled radiotracers
   ^{68}Ga and ^{64}Cu labeled peptides and Antibodies
3. Describe the application of automated synthesis modules in the routine production of PET radiopharmaceuticals
4. Describe the potential clinical applications of new PET radiopharmaceuticals

COURSE OUTLINE

Presented at 2009 Annual APhA Meeting

EVOLUTION OF PET RADIOCHEMISTRY: SYNTHESIS AND CLINICAL APPLICATION OF RADIOPHARMACEUTICALS

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INTRODUCTION

Georg de Hevesy in 1920s coined the term *radioindicator* or *radiotracer* and introduced the *tracer principle* in biomedical sciences. A radiotracer can be defined as a specific radiolabeled molecule (or probe) that resembles or traces the in vivo behavior of a natural molecule and can be used to provide information about a specific biological process. The degree of similarity between radiotracer and the natural substance, however, may vary depending on the particular radiotracer. For example, [^{11}C]glucose and [^{14}C]glucose are true tracers of glucose since they are chemically identical to natural glucose, while [^{18}F]fluorodeoxyglucose (FDG), an analog of glucose, also traces glucose, but does not behave identically to glucose since it is chemically different. One of the most important characteristics of a true radiotracer, however, is the ability to study the components of a homeostatic system without disturbing their function. Occasionally, the term *radioligand* is also used in the context of imaging studies. All radiolabeled compounds or substances used for the purpose of diagnosis or therapy have been defined as *radioactive drugs* or *radiopharmaceuticals* (RP) by the U.S. food & Drug Association (FDA).

In the early 1970's Michael Phelps and his colleagues at the Washington University in St. Louis built the first PET camera for clinical studies and demonstrated the potential clinical utility of positron tracers such as [^{11}C]glucose, [^{13}N]ammonia and [^{15}O]water. Following the development of FDG synthesis by Dr. Walter Wolf and his colleagues at Brookhaven National Laboratory, the first FDG-PET imaging studies were performed at UCLA in 1977. In the last 3 decades, several hundred positron emitting radiotracers have been developed for PET.

RADIONUCLIDES FOR PET

The most important radionuclides useful for the development of PET radiopharmaceuticals are listed in Table 1. The physical half-life ($T_{½}$) and the theoretical specific activity (SA) are important for radiotracer development and synthesis. The β^+ abundance, energy and the associated gammas determine the imaging characteristics such as resolution, sensitivity, and radiation dosimetry of the PET radiopharmaceutical.

Among the radionuclides listed in Table-1, we have three groups: organic nuclides (^{11}C, ^{13}N), halogens (^{18}F, ^{124}I) and metals (^{68}Ga, ^{64}Cu, ^{62}Cu, ^{89}Zr and ^{82}Rb). Developing a specific RP involves careful design of the structural requirements in a molecule in order to optimize target specificity and at the same time optimize the pharmacokinetic and pharmacodynamic behavior of the probe to meet the demand of the imaging technique. The physicochemical properties of the RP such as size, charge, solubility, lipophilicity, and specific activity are very important criteria and must be addressed in designing the structural features of the molecule. Factors such as rapid metabolism and plasma protein binding and non-specific binding in non-target tissues are not desirable for optimal in vivo behavior. It is important to identify the most appropriate structural analog that meets most of the criteria for an ideal RMIP.

Table 1.

RADIONUCLIDES FOR PET: HALF-LIFE, β^+ EMISSION AND SPECIFIC ACTIVITY*

Nuclide	$T_{½}$ (min)	SA* (Ci/μmol)	Mean β^+ Energy (MeV)	Mean Range (mm)
^{82}Rb	1.20	150,400	1.5	4.0
^{13}N	10.0	18,900	0.492	2.0
^{11}C	20.4	9,220	0.386	1.7
^{68}Ga	68.3	2,766	0.829	2.9
^{18}F	110	1,710	0.250	1.4
^{64}Cu	768	245	0.230	1.4
^{89}Zr	884	213		
^{124}I	6048	31	0.8	3.3

* Maximum Theoretical SA

CHEMISTRY OF ^{18}F

To develop PET RPs, ^{18}F appears to be an ideal radionuclide for the following reasons:

- Low positron energy (0.64 MeV) with a short range in tissue (Max. 2.4 mm) and provides high resolution images
- Can be produced in high specific activity
- Can be produced in large amounts (>10 Ci) in a cyclotron
- Fluorine is the most electronegative of all the elements and can react with many organic and inorganic chemicals.
- It can react as an electrophile or a neutrophile chemical species.
- Relatively high labeling yields (20-40%) in the synthesis of ^{18}F-PET tracers
- Acceptable radiation dosimetry for multiple studies in a patient
- The physical $T_{½}$ (110 min) allows for transport from the production site to the PET centers.

It is very important to appreciate the fact that fluorine atom is only slightly larger than a hydrogen atom (Table 9.1) and the introduction of a fluorine atom to substitute a hydrogen atom or a hydroxyl group (F for H^+ or OH-) in a drug or metabolic substrates, does not cause any sterical changes, but may induce changes in the in vivo behavior of fluorinated molecule. For example, as a substituent, fluorine can alter the metabolic properties of the parent molecule and prolong drug action by preventing hydroxylation. Also, the higher electronegativity of fluorine atom may alter the lipophilicity of the compound. A large number of pharmaceuticals contain fluorine atom and in the last 3-4 decades, hundreds of ^{18}F labeled radiotracers have been developed.

Basically there are 2 kinds of targets used to produce two different chemical forms of fluorine. A water target for the production of ^{18}F as nucleophilic fluoride ion ($^{18}F^-$) is based on the nuclear reaction $^{18}O(p, n)^{18}F$. Several curies of ^{18}F can easily be made in 1-2 hours using 10-19 MeV protons 20-35 μA. While the theoretical SA of ^{18}F is 1700Ci/μmoles, the NCA ^{18}F produced is generally <10Ci/μmoles. The most common nuclear reaction to produce ^{18}F as electrophilic fluorine gas ($[^{18}F]F_2$) based on the reaction, $^{20}Ne(d, \alpha)$. Following bombardment for 1-2 hours with 8-9 MeV deuterons, <1.0 Ci of $[^{18}F]F_2$ is generated with very low SA (10-20 mCi/μmoles).

In addition, to the two primary ^{18}F precursors (fluoride and fluorine gas), several secondary precursors have been developed to radiolabel a number of organic molecules. Fluoride ion needs to be activated prior to fluorination reactions. Metal fluorides (such as $K^{18}F$, $Cs^{18}F$), and tetra-n-

butyl ammonium fluoride ($nBu_4N^{18}F$) are the most widely used ^{18}F precursors in nucleophilic fluorination reactions reactions. Among them, [^{18}F]CH_3COOF (acetyl hypofluorite) is the important precursor.

Fluorination Reactions

The most successful approach for preparing high SA ^{18}F radiotracers is based on **nucleophilic fluorination** reactions since [^{18}F]fluoride can be produced in high SA (>2 Ci/μmoles). Synthesis of ^{18}F RPs using fluoride ion utilize 2 general categories or types of chemical reactions as shown in the Figure-1; 1) aliphatic nucleophilic substitution, also known as substitution nucleophilic bimolecular (S_N2) and 2) aromatic nucleophilic substitution (S_NAr).

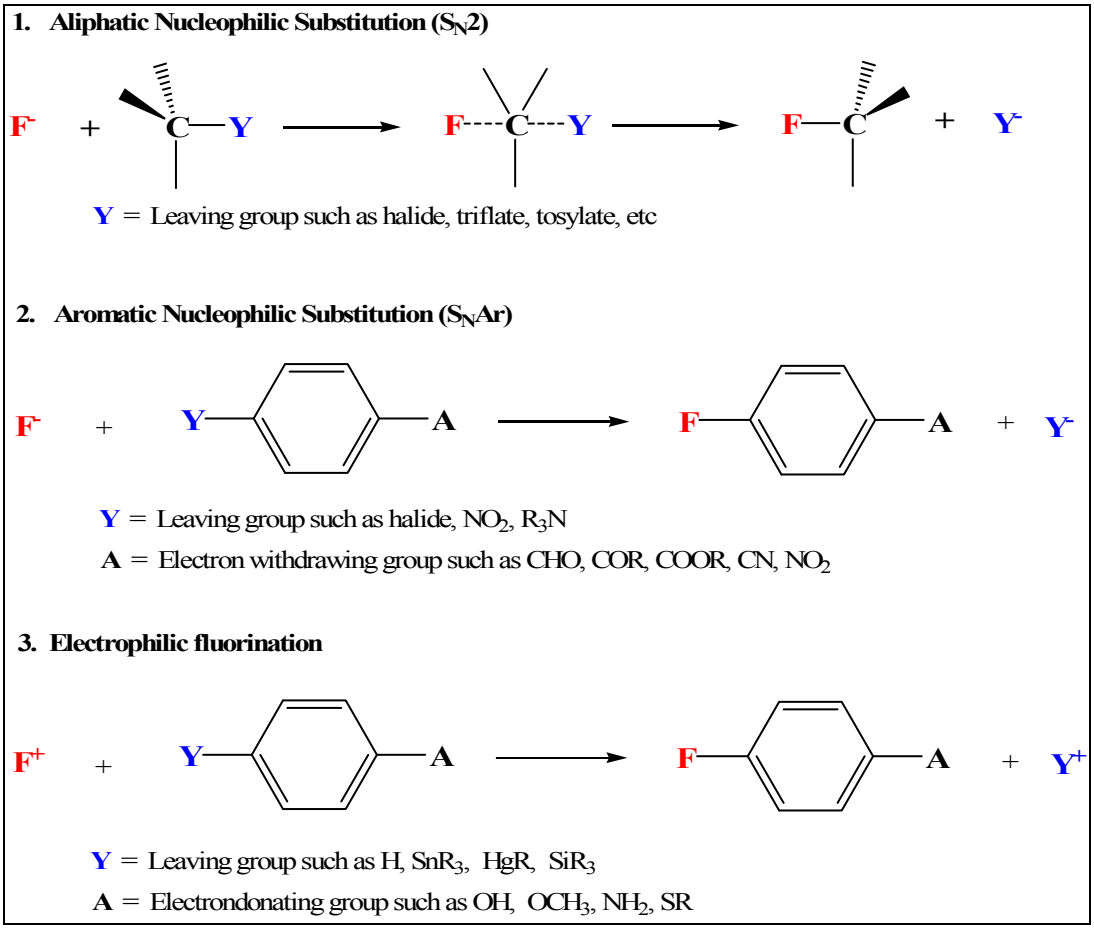

Figure 1. Nucleophilic Fluorination Reactions

The fluoride ion in aqueous solution is very unreactive and has to be activated to increase its solubility and lipophilicity. [^{18}F]fluoride ion is generally activated by complexing with a metal or

positively charged ion. When alkali metal halides ($K^{18}F$, $Cs^{18}F$, $Rb^{18}F$) are used, it is essential to have the metal coordinated by cryptands and polyaminoethers (such as Kryptofix 2.2.2) so that the relatively free fluoride can show very good reactivity. Instead of alkali metal halides, a variety of tetraalkylammonium [^{18}F]fluorides have also been used. These reactions generally take place in basic or neutral conditions in the presence of an appropriate dipolar aprotic solvent (such as acetonitrile, dimethylsulfoxide (DMSO), and dimethylformamide (DMF)) in which the reactants show good solubility. Sometimes, [^{18}F]fluoride ion is first converted to another reactive species such as alkyl halides ([^{18}F]fluorobromoethane, [^{18}F]fluorobromopropane) and benzylic halides.

The [^{18}F]F_2 precursor has only one of the atoms as ^{18}F while the other is stable ^{19}F atom. Therefore the labeling yield are always <50%. Since fluorine is a nonselecctive electrophile and acts as an oxidizing agent, it is frequently converted into [^{18}F]acetyl hypofluorite, a milder fluorinating, and much less reactive with greater solubility than elemental fluorine. With these precursors, direct electrophilic fluorinations are not necessarily regioselective and the ^{18}F atom can attack any of the C-C double bond in the molecule. Therefore, these precursors are used only in rare situations where nucleophilic reactions are not appropriate.

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Synthesis of 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG)

The original synthesis developed by Brookhaven group in 1976 was based on electrophilic reaction. Most of the current synthesis procedures use a modification of a nucleophilic procedure developed in 1986. In the last 2 decades, the synthesis of FDG is the most established procedure and hundreds of PET centers all over the world use automated synthesis modules (GE, Nuclear

Interface, CTI, IBA) for the production and distribution of FDG. Almost all these modules involve the following basic steps:

[^{18}F]fluoride ion is trapped on a small column of anion exchange resin and the target water is collected in a vial for future use. The[^{18}F]fluoride ion is then eluted into a reaction vial using a solution of aqueous base, potassium carbonate (K_2CO_3), Kryptofix 222 in acetonitrile. The residual water is removed by repeated azeotropic distillations using anhydrous acetonitrile and stream of nitrogen. The organic precursor (Fig. 2), mannose triflate (10-25 mg) in acetonitrile is added to the dried fluoride ion and the mixture is heated at 80-90^{o}C for 5 min. In order to generate FDG from [^{18}F]acetyl protected FDG (intermediate complex) hydrolysis of acetyl groups (deprotection) is done using an acidic (HCl) solution by heating the mixture at 130^{o}C for 10-15 min. In recent techniques, the acetylated FDG intermediate is loaded on a solid support (C_{18} Sep-Pak® cartridge) and hydrolysis is performed under basic conditions at room temperature using KOH or NaOH solution. The purification procedures involve passing the intermediate mixtures or the final FDG solution through C_{18} Sep-Pak and alumina cartridges and washing with water to eliminate Kryptofix and organic solvent contamination. The purified FDG is finally obtained from the cartridge by eluting with physiological saline and sterilized by passing through 0.2µ membrane filter and collecting it in a sterile vial. Almost all the automated FDG synthesis modules provide FDG with a radiochemical yield of 40-70% and radiochemical purity of >90% in 30-45 min of total synthesis time.

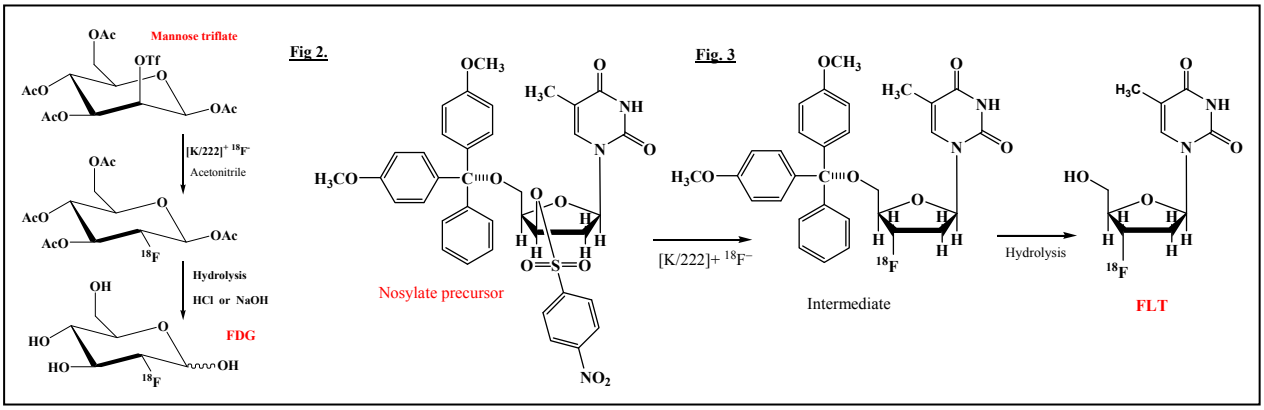

Figure 2 and 3.

Synthesis of [^{18}F]-3'-Deoxy-3'-Fluorothymidine (FLT)

The synthesis of FLT was first reported in 1991, but the low radiochemical yield prevented routine clinical use of the compound. Subsequent improvements in synthesis and radiochemical yields help start clinical evaluation of FLT. Since then, a number of precursors and different labeling protocols have been evaluated to improve labeling yields and the radiochemical purity. The most reliable [^{18}F]FLT radiosynthesis methods involve a simple three step procedure based on a protected nosylate precursor known as 3-N-Boc-5'-O-dimethoxytrityl-3'-O-nosyl-thymidine (Fig. 3). Fluorination reactions involve displacement of 3-O-nosyl group with [^{18}F]fluoride and yield an ^{18}F labeled protected intermediate, which following hydrolysis generates [^{18}F]FLT. The final radiochemical yields of FLT vary (10-40%) and depend on the mass amount of precursor and kryptofix used. Increased amounts of precursor may yield higher labeling yields, but may also increase chemical impurities in the final product. We found that the kryptofix plays a very important role and that by carefully adjusting the precursor/kryptofix molar ratio, higher labeling yields with minimal chemical impurities can be obtained. HPLC purification is essential to reduce chemical impurities. A fully automated method for the synthesis of FLT with a 50% radiochemical yield, by modifying a commercial FDG synthesizer and its disposable fluid pathway was also reported.

CHEMISTRY OF ^{11}C

All natural organic molecules or biochemicals in the human body and many drug molecules are made up of carbon and hydrogen. Among the three organic radionuclides, ^{11}C offers the greatest potential to develop RMIPs for routine clinical applications because ^{11}C, as a label, can be easily substituted for stable carbon in an organic compound without changing the biochemical and pharmacological properties of the molecule. Also the short $T_{½}$ of ^{11}C provides favorable radiation dosimetry to perform multiple studies in the same subject under different conditions. The short $T_{½}$ of ^{11}C, however, may be disadvantageous for commercial production of radiotracers, but has significant potential for developing radiotracers with high SA to study drug interactions associated with very small concentrations of neuroreceptors.

^{11}C was first produced in 1934 and the first biological application was based on the use of [^{11}C]CO_2 to investigate the photosynthesis in plants. [^{11}C]CO was the first radiotracer used in

human subjects to investigate the fixation of CO by red blood cells. The most commonly used method of ^{11}C production is based on the nuclear reaction, $^{14}N(p,\alpha)^{11}C$, in which the natural nitrogen gas is used as the target. With trace amounts of oxygen in the target (<1%), the [^{11}C]CO_2 and [^{11}C]CO are formed. With relatively higher proton energies (>13 MeV), longer irradiation times (>30 min) and higher beam currents (>30 μA), the most predominant ^{11}C precursor generated is [^{11}C]CO_2 gas. In the presence of hydrogen (5%) in the target, [^{11}C]methane (CH_4) and [^{11}C]hydrogen cyanide (HCN) can be produced in the target. Subsequently, [^{11}C]CO_2 and [^{11}C]CH_4can be converted into several secondary precursors such as methyl iodide, and methyl triflate, the most common precursors for ^{11}C radiolabeling. Automated commercial modules (from BioScan, GE, Siemens) are available for the synthesis of these two precursors.

Historically several different approaches have been developed for the production of ^{11}C labeled radiotracers, but the most practical approaches have been based on either a) organic synthetic methods, or b) enzyme catalysis. The methods based on organic synthesis typically involve alkylations of C, N, O, and S neucleophiles with [^{11}C]methyl iodide or methyl triflate. The alkylation reactions require an organic precursor, also known as *nor* compound (a molecule of interest without a methyl group on a specific C, N, O or S atom). If a molecule of interest has several reactive groups, the organic precursors must have protective groups that can be easily de-protected by hydrolysis following methylation to generate the final drug product. This is the most common synthetic approach used in the routine production of ^{11}C labeled RPs.

Synthesis of [*O*-methyl-^{11}C]Raclopride

Raclopride is a dopamine D_2 receptor antagonist and is one of the most extensively used neuroreceptor imaging probe. Raclopride is labeled with ^{11}C by *O*-methylation using [^{11}C]methyl iodide (Fig. 4). The enantiomerically pure *S*-precursor (*O*-desmthylraclopride) in DMSO is reacted with [^{11}C]CH_3I in the presence of sodim hydroxide. The purified drug product, [^{11}C]raclopride is obtained following reverse phase HPLC of the reaction mixture using a C-18 column and 10 mM phosphoric acid and acetonitrile (70:30 v/v) as an eluant. The fraction containing [^{11}C]raclopride is subsequently evaporated to remove acetonitrile and reformulated in physiological saline and sterilized by membrane filtration.

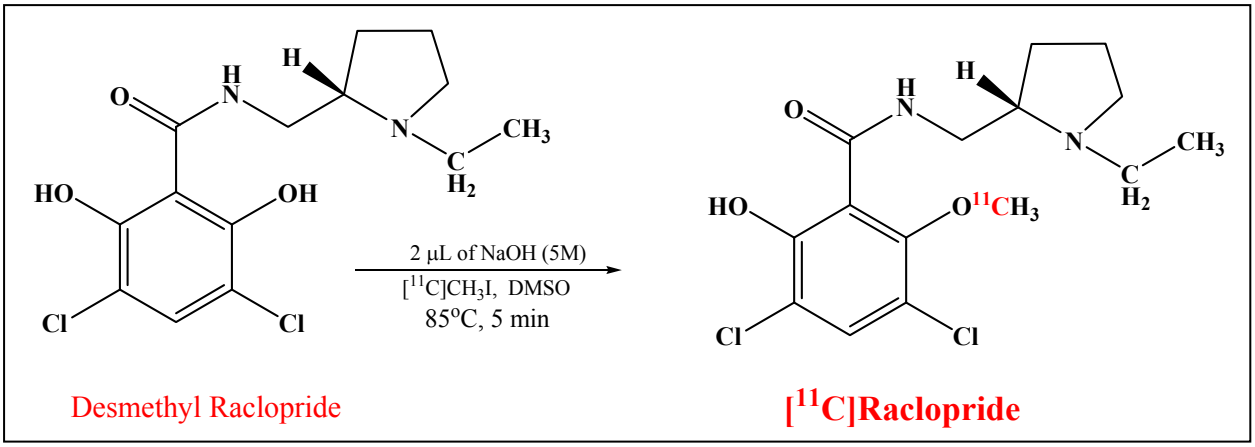

Figure 4.

CHEMISTRY OF RADIOMETALS

In nuclear medicine, a number of RPs have been developed in the last four decades based on ^{99m}Tc, ^{67}Ga and ^{111}In. In addition, a number of therapeutic radiopharmaceuticals have also been developed based on β^- emitting radiometals (^{90}Y, ^{177}Lu and ^{67}Cu). The extensive knowledge, experience and understanding of the metal chemistry at the tracer level, would enable the development of new molecular imaging radiotracers based on β^+ emitting radiometals. The advantages of metal labeled molecular imaging radiotracers can be summarized as follows:

- Easy availability: ^{62}Cu and ^{68}Ga generators are available for easy in house preparation based on kit production. Cyclotron production of metallic nuclides has been optimized using medical cyclotrons using primarily (p,n) nuclear reactions
- Ability to label target specific biomolecules (peptides and proteins)
- Availability of radionuclide pairs for imaging and therapy (^{68}Ga/^{67}Ga ^{62}Cu/^{64}Cu /^{67}Cu, ^{110}In /^{111}In, ^{86}Y /^{90}Y)
- High specific activity (SA) of radiometal and metal-labeled peptide or protein
- High in vivo stability of metal-labeled tracers
- Favorable radiation Dosimetry

Some of the important physical properties and the electron configuration of various metals useful in developing molecular imaging probes are summarized in the Table 2. Among these metals, gallium, and indium belong to group IIIB while yttrium belongs to group IIIA of the periodic table. All other metals useful for developing radiopharmaceuticals are transition metals with complex coordination chemistries. Among the β^+ emitting metallic nuclides, ^{64}Cu ($T_{½}$=12.6 hr), and ^{89}Zr ($T_{½}$=3.27 d) are more appropriate to develop commercial PET RPs that can be transported across the country. For most of these metallic radionuclides, cyclotron production

methods have been optimized using medical cyclotrons using primarily (p,n) nuclear reactions. $^{82}Sr(T_{½}=25\ d) \rightarrow {}^{82}Rb\ (T_{½}=1.25\ m)$ generator (CardioGen-82®) was FDA approved for myocardial perfusion studies. The two nuclides with short half-lives, $^{68}Ga\ (T_{½}=68.3\ min)$ and $^{62}Cu\ (T_{½}=9.76\ min)$ can also be produced on demand from generator systems without the need for an on-site cyclotron.

Table 2.

PHYSICAL PROPERTIES OF RADIOMETALS				
Physical property	**Element**			
	Cu	Ga	Zr	In
Atomic number	29	31	40	49
Atomic radius (pm)	128	122	160	163
Ionic radius (pm)	2^+, 72	3^+, 62	2^+, 109	3^+, 92
Electron structure	[Ar] $3d^{10}$ $4s^1$	[Ar] $3d^{10}$ $4s^2$ $4p^1$	[Kr] $4d^1$ $5s^2$	[Kr] $4d^{10}$ $5s^2$ $5p^1$
Electronegativity	1.90	1.81	1.65	1.78
Oxidation state	+1, +2	+3	+4, +2	+3

Chemistry of ^{68}Ga

The aqueous chemistry of Ga and In is dominated by their ability to form strong complexes (both soluble and insoluble) with the hydroxyl ion. The fully hydrated (hexaaquo) M^{3+} ions are only stable under acidic conditions. As the pH is raised above 3, these 3 metals form insoluble hydroxides ($M(OH)_3$). A variety of OH intermediates are formed as a function of pH and the mass of the metal. Among these 3 metals, gallium is more amphoteric than indium and yttrium. As a result, at physiological pH, gallium exists predominantly as a soluble species, $[Ga(OH)_4]^-$ (gallate).

^{68}Ga generator was first developed in 1960s for brain imaging studies. Subsequent generators utilized ^{68}Ge germanate adsorbed on tin dioxide and ^{68}Ga was eluted with HCl. Use of relatively high concentrations of HCl (1.0 N) presents a problem due to the volatility of $GeCl_4$ and subsequent spread of airborne, long-lived ^{68}Ge contamination. In addition, the ^{68}Ga is eluted in a large volume of acid (>5 mL), containing metal impurities that are known to bind with high affinity to DOTA. A commercial generator is available (Obninsk, Russia) based on the use of

TiO_2 as an inorganic matrix to immobilize ^{68}Ge in the oxidation state IV+ and ^{68}Ga (III) can be easily separated by eluting with dilute HCl. It has been reported that the SA of generator eluted ^{68}Ga can be as high as 27 Ci/μmol. These generators, however, are not necessarily optimized for the synthesis of ^{68}Ga-labeled radiopharmaceuticals. In order to avoid metal impurities, additional concentration and purification can be performed using a miniaturized column with organic cation-exchanger resin and hydrochloric acid/acetone eluant. The processed ^{68}Ga fraction can be directly transferred to solutions containing labeling precursors such as DOTATOC (Fig. 5). Labeling yields of >95% and specific activities of (50-500 MBq/nmol) can be obtained under optimized conditions. Fully automated synthesis modules have been developed to prepare ^{68}Ga radiopharmaceuticals for clinical use.

Somatostatin receptor binding peptide octreotide its analogs, DOTATOC, DOTATATE and DOTANOC have all been labeled with ^{68}Ga and were evaluated to determine the diagnostic potential in patients with neuroendocrine tumors. Preclinical studies have demonstrated that $^{67/68}$Ga-DOTA-octapeptides show distinctly better preclinical pharmacological performances than the ^{111}In-labeled peptides, especially on SST2-expressing cells and the corresponding animal models. ^{68}Ga- octreotide analogs may be excellent candidates for further development for clinical studies.

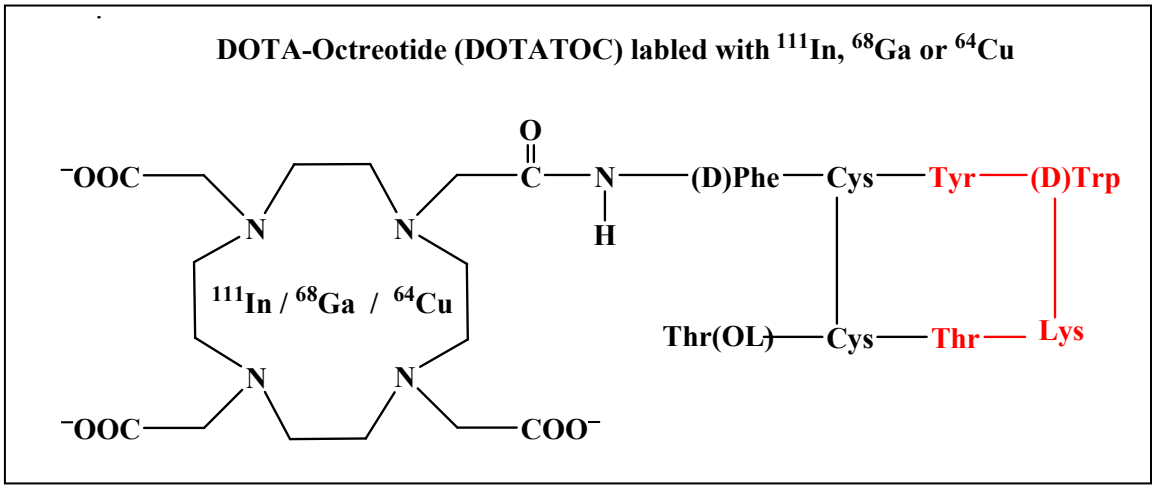

Figure 5.

Chemistry of ^{64}Cu

The chemistry of copper is dominated by 2 oxidation states, I and II. Copper salts form the aqua ion $[Cu(OH)_6]^{2+}$ Compounds of Cu (I) oxidation state are unstable in aqueous solution and readily oxidize to Cu(II) which can form 4, 5, or 6 coordination bonds with ligands. In Cu (II) oxidation state, the metal binds strongly with N and S containing molecules forming co-ordination complexes. Complex formation with chelating agents occurs at pH <7 since formation of insoluble $Cu(OH)_2$ is not a major concern.

In order to bind radionuclides of copper to peptides and antibody molecules, macrocyclic chelators such as TETA have been developed. But Cu (II)-TETA complexes were not optimal as imaging agents since they are not stable in vivo. Recently, a new class of bicyclic tetraazamacrocycles (Fig. 6), the ethylene "crossbridged" cyclam derivatives (CB-2ETA) were developed which form highly kinetically stable complexes with Cu(II) and less susceptible to transchelation in vivo. Similarly, another series of TETA analogs known as hexa-aza-cryptand ligands, SarAr and SarArNCS were also reported to form strong and stable Cu (II) complexes by wrapping the Cu atom more tightly. Based on these new chelating agents, a number of ^{64}Cu labeled RPs based on biomolecules such as peptides and proteins are being developed.

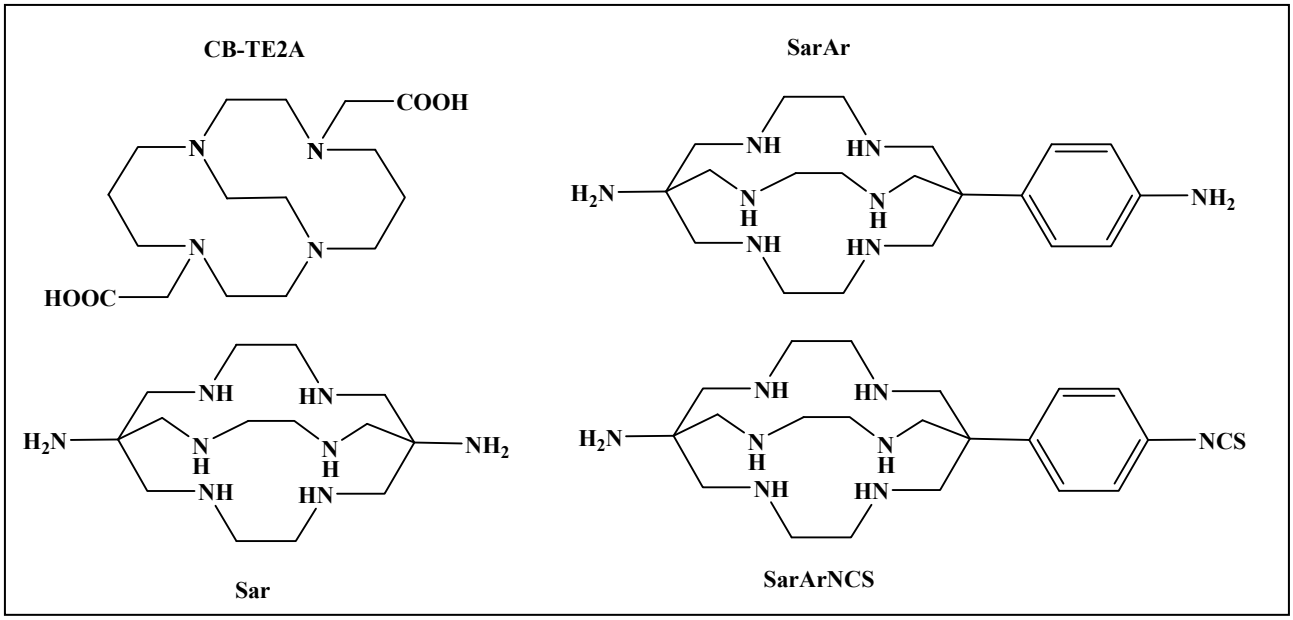

Figure 6.

NEW PET RADIOPHARMACEUTICALS

Most diseases are characterized by specific alteration in chemical homeostasis resulting in specific biochemical abnormalities. The PET technique has the advantage of developing radiopharmaceuticals based on a wide variety of positron-emitting radionuclides. Since the chemistry of positron emitting radionuclides is favorable to label biochemicals and drug molecules, a number of radiopharmaceuticals have been developed as diagnostic radiotracers or molecular imaging probes to detect and quantitate the function of an organ or a unique biochemical process in a specific tissue. However, at present, there are only four PET radiopharmaceuticals ^{82}Rb, [^{13}N]NH_3, [^{18}F]FDG, [^{18}F]Fluoride) that were approved by FDA for routine clinical imaging studies.

A number of molecular imaging radiotracers are being developed for tumor imaging. Also, in neuro-psychiatric diseases, several PET radiopharmaceuticals have shown significant potential as diagnostic tracers. One of major areas of molecular imaging is the detection of coronary atherosclerosis/thrombosis. The success of future PET RPs (Table 3) depends on the potential for commercialization and documentation of clinical utility based on multi-center clinical trials (Phase I, II, and III). At present most of the RPs listed here are in the early stages of clinical evaluation and it would take 3-5 years before any any of these tracers can be commercially available for routine clinical use.

Table 3.

NEW PET RADIOPHARMACEUTICALS IN ONCOLOGY		
Radiopharmaceutical	**Mechanism/Target**	**Application**
3'-deoxy-3-[^{18}F]Fluorothymidine (FLT)	DNA synthesis. Substrate for *thymidine kinase*	Tumor cell proliferation
[^{18}F]Fluoromethylcholine (FCH)	Synthesis of phaosphatidylcholine. Substrate for *Choline kinase*	Tumor cell proliferation
[^{18}F]Fluoromisonidazole (FMISO), (5-[^{18}F]fluoro-5-deoxy-α-D-arabinofuranosyl)-2-nitroimidazole (FAZA) ^{62}Cu-ATSM	Tumor hypoxia. Uptake is inversely proportional to tissue oxygenation	Hypoxia imaging to optimize radiation therapy
[^{18}F]Fluoro-17β-estradiol (FES)	Binds specifically to estrogen receptors	Predicting response to breast cancer hormonal therapy
^{88}Ga-DOTA-tyrosine-Octrotide	Binds to somatosatin receptors	Imaging neuroendocrine tumors

(DOTATOC)		
L-3-[^{18}F]fluoro-α-methyltyrosine (FMT) 1-amino-3-[^{18}F]fluorocyclobutane-1-carboxylic acid (FCCA)	Amino acid transport, Protein synthesis	Cell proliferation, protein synthesis
9-(4-[^{18}F]-fluoro-3-hydroxy-methylbutyl) guanine (FHBG)	Substrate for HSV1-thymidine kinase	gene expression and to asses response to gene therapy
[^{18}F]FP-CIT, [^{18}F]FE-CIT	Binds to dopamine transporters in presynaptic nigrostriatal nerve terminals	Parkinson's disease
[^{18}F]FDDNP and [^{11}C]PIB	Binds to β-amyloid plaques in brain	Alzheimer's disease
[^{11}C]5-Hydroxytryptophan (5-HTP) [^{18}F]Fluorodopa (FDOPA)	Precursor for serotonin synthesis Dopamine metabolism	Neuroendocrine tumors
^{64}Cu-Annexin, ^{124}I-Annexin V	Binds to plasma membrane-bound phosphatidylserine (PS)	Apoptosis in tumor and heart
N-4-[^{18}F]fluorobenzoyl labeled cyclic RGD peptide [^{18}F]FB-RGD	cell adhesion integrin $\alpha_v\beta_3$ receptors	Tumor angiogenesis

SUMMARY

Molecular imaging is the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in a living system. A number of PET radiopharmaceuticals based on ^{18}F, ^{11}C, ^{68}Ga, ^{62}Cu, ^{64}Cu, ^{68}Ga, ^{89}Zr, and ^{124}I are under development and clinical evaluation in oncology, neurology, and. Cardiology. Among the positron emitting radionuclides, ^{18}F is ideal for the development of radiotracers for small organic molecules. In order to develop molecular imaging probes based on peptides and proteins, metallic radionuclides are more appropriate.

The basic chemistry of ^{18}F, ^{11}C, ^{68}Ga and ^{64}Cu are briefly discussed here with specific examples giving details of synthetic procedures. An overview of design and development of ^{18}F labeled PET radiopharmaceuticals, radiochemistry, and mechanism(s) of tumor cell uptake and localization of radiotracers is presented here. The potential routine clinical utility of ^{18}F labeled PET radiopharmaceuticals depends on regulatory compliance in addition to documentation of potential safety and efficacy in carefully designed clinical studies.

REFERENCES

1. AL-Nahhas A, Win Z, Szyszko T, et al (2007) What can gallium-68 PET add to receptor and molecular imaging? Eur J Nucl Med Mol Imaging 34:1897–1901

2. Anderson CJ, Dehdashti F, Cutler et al (2001). Copper-64-TETA-octreotide as a PET imaging agent for patients with neuroendocrine tumors. J Nucl Med 42:213-221.

3. Anderson CJ, Green MA, Fujibayashi Y (2003) Chemistry of copper radionuclides and radiopharmaceutical products. In: Welch MJ, Redvanly CS (eds) Handbook of radiopharmaceuticals, Wiley, West Sussex, England

4. Antoni G, Kihlberg T, Langstrom B (2003) Aspects on the synthesis of ^{11}C-labeled compounds. In: Welch MJ and Redvanley CS (eds) Handbook of Radiopharmaceuticals, Wiley, West Sussex, UK

5. Börjesson PKE, Jauw YWS, Boellaard R et al (2006) Performance of immuno-positron emission tomography with zirconium-89-labeled chimeric monoclonal antibody U36 in the detection of lymph node metastases in head and neck cancer patients. Clin Cancer Res 12: 2133-2140

6. Breeman WAP, Verbruggen AM (2007) The ^{68}Ge/^{68}Ga generator has high potential, but when can we use ^{68}Ga-labelled tracers in clinical routine? Eur J Nucl Med Mol Imaging 34:978-981

7. Fowler JS, Ding Y-S (2002) Chemistry. In: Wahl RL, Buchanan JW (eds) Principles and practice of positron emission tomography, Lippincott Williams & Wilkins, Philadelphia

8. Hamacher K. et al (1986). Efficient stereospecific synthesis of no-carrieradded 2-[^{18}F]fluoro-2-deoxy-D-glucose using amino-polyether supported nucleophilic substitution. J Nucl Med 27:235-238

9. Kilbourn MR (1990). Fluorine-I8 labeling of radiopharmaceuticals. National Academy of Sciences, National Academy Press, Nuclear Science Series no. NAS-NS-3203.

10. Långström B, Kihlberg T, et al (1999) Compunds labeled with short-lived β+ emitting radionuclides and some applications in life sciences. The importance of time as a parameter. Acta Chem Scand 53:651-669

11. Maecke HR, Andre JP (2007) ^{68}Ga-PET radiopharmacy: A generator-based alternative to ^{18}Fradiopharmacy. Ernst Schering Res Found Workshop 62:215-242

12. Maecke HR, Hofmann M, Haberkorn U (2005). ^{68}Ga-labeled peptides in tumor imaging. J Nucl Med 46:172S-178S.

13. Shiue CY, Welch MJ (2004) Update on PET radiopharmaceuticals: life beyond fluorodeoxyglucose. Radiol Clin N Am 42:1033-1053

14. Stocklin GL (1998) Is there a future for clinical fluorine-18 radiopharmaceuticals (excluding FDG)? Eur J Nucl Med 25:1612–1616

15. Vallabhajosula S (2007). Radiopharmaceuticals for PET, In Biersack HJ, Freeman L (eds.) Practical Nuclear Medicine, Springer-Verlag, Berlin

16. Vallabhajosula S (2007) ^{18}F-Labeled PET Radiopharmaceuticals in Oncology: An Overview of Radiochemistry and Mechanisms of Tumor Localization. Semin Nucl Med 37:400-419

17. Zhernosekov KP, Filosofov DV, Baum RP, et al (2007) Processing of generator-produced ^{68}Ga for medical application. J Nucl Med 48:1741-1748

ASSESSMENT QUESTIONS

1. An ideal PET radiotracer must be designed to have

    a) High specific activity
    b) Minimal or no in vivo metabolism
    c) Target specificity
    d) All of the above

2. In general ^{18}F radiopharmaceuticals are prepared based on

    a) Electrophilic fluorination reactions only
    b) Nucleophilic fluorination reactions based on ^{18}F as fluoride
    c) The use of chelating agents to complex the radionuclide
    d) Enzymatic reactions

3. Preparation of ^{11}C radiopharmaceuticals involves using

    a) $[^{11}C]CH_3I$ (methyl iodide) for alkylation reactions
    b) $[^{11}C]CO$ gas
    c) $[^{11}C]CO2$ gas directly
    d) None of the above

4. As a trivalent metal, ^{68}Ga forms strong coordination complexes with

    a) Small inorganic molecules
    b) Chelating agents such as DTPA and DOTA
    c) Peptides directly
    d) Proteins at acidic pH only

5. ^{64}Cu labeled radiopharmaceuticals are also based on

    a) Chelation chemistry
    b) Alkylation reactions
    c) Iodination reactions only
    d) Antibody molecules only

6. Synthesis of PET radiopharmaceuticals requires the use of automated synthesis modules

    a) To reduce radiation exposure for the chemists
    b) To ensure routine synthesis with consistent radiolabeling
    c) To produce large amounts of activity for commercialization
    d) All of the above