Radiopharmaceuticals in Positron Emission Tomography: Radioisotope Production and Radiolabelling Procedures at the Austin & Repatriation Medical Centre

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SUMMARY. Positron Emission Tomography (PET) is an imaging technique to study physiological processes *in vivo* using compounds labelled with short-lived positron-emitting radioisotopes. The Austin & Repatriation Medical Centre (A&RMC) Centre for PET is equipped with a facility consisting of radioisotope production (cyclotron), radiolabelling production (automated synthesis) and quality control laboratory as an integrated unit. The Centre for PET has been in operation for six year and produces a range of radiotracers labelled with ^{15}O, ^{13}N, ^{11}C and ^{18}F for clinical and research studies in the fields of oncology, cardiology, neurology, psychiatry and brain activation.

1. INTRODUCTION

Positron emission tomography (PET) is a relatively new technique for imaging the distribution of radiolabelled pharmaceuticals and biochemical tracers within the body. PET offers the unique possibility of studying metabolic and physiological processes in living human subjects without disturbing the system under investigation (1). The technique uses short-lived radioisotopes to label substances which occur naturally in the body.

One of the attractive aspects of PET is that the radioactive tracer can be labelled with short-lived radioisotopes of the natural elements of the biochemical constituents of the body. For example, natural atoms of carbon, nitrogen and oxygen are replaced with the short-lived positron-emitting radioisotopes carbon-11 ($t_{½}$=20.4 min), nitrogen-13 ($t_{½}$=10 min) and oxygen-15 ($t_{½}$=2 min). In addition, fluorine-18 ($t_{½}$=109.6 min) can be exchanged for hydrogen in the molecule.

This article describes the basic principles of the PET technique and reviews the cyclotron-produced radioisotopes and radiolabelling procedures of the ARMC PET Centre.

2. BASIC PRINCIPLES OF PET

In a typical PET experiment, a very small amount of labelled compound (called radiopharmaceutical or radiotracer) is introduced into the patient usually by intravenous injection. During its decay process, the radionuclide emits a positron which, after travelling a short distance (3-5 mm), encounters an electron from the surrounding environment. The two particles combine and "annihilate" each other, resulting in the emission in opposite directions of two gamma rays of 511 KeV each. The image acquisition is based on the external detection, in coincidence, of these γ-rays. Therefore the localisation of the positron-emitting radionuclides inside the patient and the concentration of the tracer in the tissue can be measured with the PET scanner.

The commercial PET scanner (Siemens/CTI 951/3R) installed at the Austin & Repatriation Medical Centre in Melbourne comprises of 16 rings of bismuth germanate (BGO) detectors, covering an axial length of 10.8 cm with a ring aperture of 56.7 cm in diameter. The cyclotron in operation at the PET Centre (2) is a negative ion design (Cyclone 10/5 from ION BEAM APPLICATIONS, Belgium) which accelerates protons (H^- ion) to 10 MeV and deuterons (D^- ion) to 5 MeV.

3. CYCLOTRON PRODUCED RADIONUCLIDES

The energy of the particle and the beam current, as well as the cross section of the nuclear reaction itself, determine the quantity of radionuclide that can be produced in any time period. Appropriate amounts of the four positron emitters commonly used in PET can be obtained with 10 MeV protons

and 5 MeV deuterons. Table 1 lists the physical half-life and the typical production yield of these commonly used short-lived positron emitters.

radionuclides	half-life	production yield
Oxygen-15	2.0 min	300mCi (11GBq)
Nitrogen-13	10.0 min	100mCi (4GBq)
Carbon-11	20.4 min	800mCi (30GBq)
Fluorine-18	109.6 min	800mCi (30GBq)

Table 1. Typical production yields for the currently used positron emitters at the A&RMC PET Centre

Oxygen-15 is produced by deuteron bombardment of natural nitrogen gas, as the target material, through the $^{14}N(d,n)^{15}O$ nuclear reaction. Oxygen-15 can be produced as molecular oxygen ($^{15}O_2$), or directly as carbon dioxide ($C^{15}O_2$) by mixing the target gas with 5% of natural carbon dioxide as a carrier. Carbon monoxide ($C^{15}O$) can also be easily produced by reduction of $C^{15}O_2$ on activated charcoal at 900°C.

Carbon-11 is produced by proton bombardment of natural nitrogen-14 through the $^{14}N(p,\alpha)^{11}C$ nuclear reaction. A target gas mixture of a few percent oxygen in natural nitrogen will produce radioactive carbon dioxide ($^{11}CO_2$) and a few percent hydrogen in natural nitrogen will produce methane ($^{11}CH_4$). To date, one of the most commonly used methods for ^{11}C-radiolabelling of PET radiotracers is through methylation using ^{11}C-methyl iodide ($^{11}CH_3I$). The current method of production of $^{11}CH_3I$ is through the reduction of $^{11}CO_2$ using $LiAlH_4$, followed by aqueous HI reaction. This method suffers from the major disadvantage of natural carbon dioxide ($^{12}CO_2$) contamination, resulting in a much lower specific activity of $^{11}CH_3I$ than the original $^{11}CO_2$. The theoretical specific activity of $^{11}CO_2$ produced could be as high as 10 Ci/pmol but could drop below 5 Ci/μmol after production of the $^{11}CH_3I$ (3). To overcome this problem, an alternative gas phase production of $^{11}CH_3I$ from $^{11}CH_4$ has been recently investigated (4).

Nitrogen-13 is produced by proton bombardment of distilled water (1.5 mL) through the $^{16}O(p,\alpha)^{13}N$ nuclear reaction. Even with the relatively low energy proton beam delivered by our cyclotron (10 MeV) a useful yield of 100 mCi can be achieved with 20 minutes irradiation. Until recently, Nitrogen-13 was recovered from the target mainly as nitrogen oxides ($^{13}NO_x$) in aqueous solution, and a Devarda alloy was necessary to reduce the nitrite and nitrate into the more useful chemical form ammonia ($^{13}NH_3$). Today, the use of a scavenger for oxidising radicals, such as ethanol (5 mM), has been successfully used as to prevent in-target oxidation and to produce ammonia directly in the target (5).

Fluorine-18 is produced by proton bombardment of oxygen-18 enriched water (1 mL) through the $^{18}O(p,n)^{18}F$ nuclear reaction. Fluorine-18 is recovered as an aqueous solution of fluoride-18 ($H_2O/^{18}F^-$), and can be easily extracted by ion exchange chromatography. Ionic fluoride-18 can be transfered into an organic solvent and used for stereospecific nucleophilic substitutions. Routinely 800 mCi of fluorine-18 can be produced in one hour of irradiation. It is important to mention that fluorine-18 can also be produced as a radioactive gas through the $^{20}Ne(d,\alpha)^{18}F$ nuclear reaction. This method of production, which is useful for electrophilic substitution, requires the addition into the target of fluorine-19 gas as carrier, and is currently seen as a less attractive method.

4. RADIOLABELLING PROCEDURE

Several hundred relevant molecules have been labelled world-wide with positron emitters during the past two decades, and about 30 are presently considered to be of major interest in clinical PET (6).

The number of tracers produced and used on a regular basis at the A&RMC PET Centre has steadily increased since 1992, and currently nine different molecules are routinely labelled for clinical use. Table 2 gives the list of these tracers/radiopharmaceuticals and an example of their clinical applications.

Radiotracers & radiopharmaceuticals	Examples of biomedical applications
^{15}O-oxygen	oxygen metabolism
^{15}O-carbon monoxide	blood volume
^{15}O-carbon dioxide	blood flow
^{15}O-water	blood flow
^{13}N-ammonia	blood flow
^{18}F-FDG	glucose metabolism
^{18}F-FMISO	hypoxic cell tracer
^{11}C-SCH23390	dopamine D1 marker
^{11}C-Flumazenil	benzodiazepine marker

Table 2. PET Radiotracers and radiopharmaceuticals produced at the A&RMC PET Centre and example of biomedical applications

^{15}O-oxygen, ^{15}O-carbon monoxide, ^{15}O-carbon dioxide and ^{13}N-ammonia

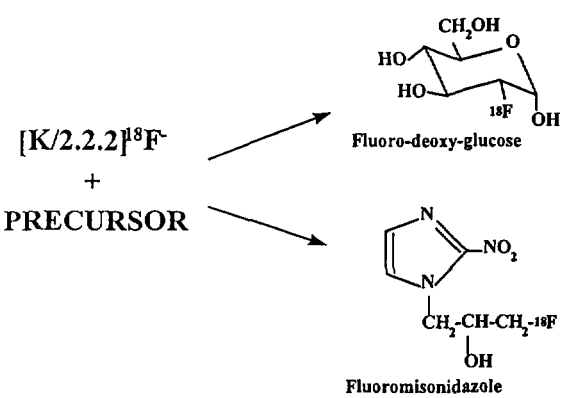
As previously mentioned, some PET radiotracers can be directly produced out of the target without further chemistry. This is the case for ^{15}O-labelled oxygen or carbon dioxide and for ^{13}N-labelled ammonia. ^{15}O-labelled carbon monoxide can also be easily produced by reduction of $^{11}CO_2$ on activated charcoal at 900°C.

^{15}O-water

^{15}O-labelled water can be produced on-line from the cyclotron-produced ^{15}O-oxygen itself (7). ^{15}O-oxygen is mixed with hydrogen, in a stoichiometric proportion, and passed over a palladium catalyst in an oven at 150°C. The radioactive water vapour diffuses across a semi-permeable membrane (cellulose acetate) into a sterile saline solution (0.9% NaCl). The saline solution is pumped continuously through the system with a medical infusion pump to generate a solution containing ^{15}O-labelled water, which can be infused directly into the patient.

^{18}F-FDG and ^{18}F-FMISO

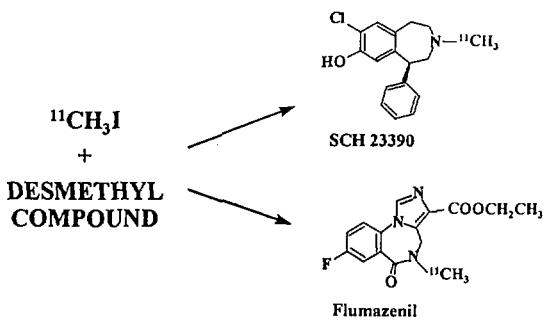
Radiotracers such as 2-Fluoro-2-Deoxy-D-Glucose (FDG) and Fluoromisonidazole (FMISO), radiolabelled with ^{18}F, require more sophisticated radiochemistry procedures (8,9). Radiofluorination of both compounds is performed using the nucleophilic substitution reaction of aminopolyether potassium complex $[K/2.2.2]^{18}F^-$ with the corresponding protected precursor.



The trifluoromethansulfonyl analogue of mannopyranose and the tosyl analogue of misonidazole are used as the precursors for the preparation of ^{18}FDG and ^{18}FMISO respectively. This method allows for a radiochemical yield close to 50% in a synthesis time of about 60 min from the end of bombardment.

^{11}C-SCH23390 and ^{11}C-Flumazenil

Both ^{11}C-labelled drugs are prepared by the methylation of suitable precursors (desmethyl compound) using ^{11}C-iodomethane.



^{11}C-iodomethane can be routinely prepared from ^{11}C-carbon dioxide by reaction with lithium aluminium hydride and subsequent addition of hydriodic acid (10). Due to the rapid radioactive decay of carbon-11 ($t_{½}$=20 min), time is an important constraint and the multi-step radiosynthesis is usually performed using an automated chemistry module (11) including High Pressure Liquid Chromatography purification. In a typical experiment, approximately 50 mCi (2 GBq) of purified ^{11}C-radiopharmaceutical is prepared (decay corrected yield 26%) with a specific activity close to 0.5 Ci/μmoles at the end of synthesis (12).

5. QUALITY CONTROL

Each radiolabelling procedure was validated by testing batches of production for sterility, presence of endotoxins, heavy metal contamination, pH, chemical purity, radiochemical purity, radionuclide identification and radionuclidic purity prior to human use. Initial validation and continual quality assurance is vital, as the main body of the labelling system is not disposable and cannot be dismantled easily for routine sterilisation of components (14).
In addition, prior to human studies quality control of each batch of PET radiopharmaceuticals produced is performed. This consists of daily testing for pH, radiochemical purity using thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC) and radionuclide identification. The present system has been in operation for over six years and has proved to be a safe and efficacious method for delivery of PET radiopharmaceuticals.

6. CONCLUSIONS

The main applications of PET to date, have been for studies of the human brain (15-19) and heart (20,21). More recently applications in oncology have shown very promising results (22-24).
The interest in PET is now well established in medical research. The strength of PET lies in its ability to provide quantitative functional information

about physiology *in vivo*. The proven ability of PET to image and quantify physiological and chemical processes provides clinicians with a unique means of guiding diagnosis and treatment. The role for PET will continue to be expanded as more chemically specific tracers are developed for probing normal and abnormal biological function.

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