FLUORINE-18: CURRENT APPROACH IN RADIOLABELLING AND RADIATION SAFETY ASPECTS

(Fluorin-18: Pendekatan Semasa dalam Pengradiopenglabelan dan Aspek Keselamatan Sinaran)

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Abstract

Positron Emission Tomography (PET) imaging has currently become an important technique to study physiological, biochemical and pharmacological functions in humans. The radiopharmaceuticals or tracers for the PET scan incorporating the positron emitting radioisotopes such as Fluorine-18, Carbon-11, Nitrogen-13 and Oxygen-15. A Fluorine -18 (^{18}F) is oftenly used in development of radiopharmaceuticals due to its favourable physical and nuclear characteristics. By far, the most common radiopharmaceutical used in PET imaging is 2-[^{18}F]-fluoro-2-deoxy-D-glucose, or [^{18}F]FDG. There are several approaches in radiolabelling using ^{18}F and the disadvantage is the time consuming multi-step reactions. Therefore, there is a need to make the radiolabelling prosess more speedy. Once working with radionuclide, the radiation safety is concerned and must be addressed. This paper will discuss on the current approach in the ^{18}F radiolabelling using "click reaction" based on paper review and a practical aspects of radiation safety. The advantages of this system are cheap, does not require an inert atmosphere, can be perfomed in the presence of water and eliminates the need for a base. As a result, the radiolabelling prosess can be performed in shorter time and a good yield.

Keywords: Fluorine-18, click reaction and radiation safety

Abstrak

Pengimejan Tomografi Pancaran Positron (PET) kini telah menjadi satu teknik penting untuk mengkaji fungsi fisiologi, biokimia dan farmakologi pada manusia. Radiofarmaseutikal atau pengesan untuk imbasan PET menggabungkan radioisotop pemancar positron seperti Fluorin-18, Karbon-11, Nitrogen-13 dan Oksigen-15. Fluorine -18 (^{18}F) kerap digunakan dalam pembangunan radiofarmaseutikal ini disebabkan oleh ciri-ciri fizikal dan nuklearnya yang menggalakkan. Setakat ini, radiofarmaseutikal yang paling biasa digunakan dalam pengimejan PET ialah 2 - [18F]-fluoro-2-deoxy D-glukosa, atau [^{18}F] FDG. Terdapat beberapa pendekatan dalam radiopenglabelan menggunakan ^{18}F dan kelemahannya ialah melibatkan pelbagai langkah tindakbalas yang memakan masa. Oleh itu, terdapat keperluan untuk membuat proses radiopenglabelan yang lebih cepat. Apabila bekerja dengan radionuklid, keselamatan sinaran dititikberatkan dan mesti ditangani. Kertas kerja ini akan membincangkan pendekatan semasa dalam radiopenglabelan ^{18}F menggunakan "tindak balas klik" berdasarkan kajian semula kertas kerja dan aspek-aspek praktikal keselamatan sinaran. Kelebihan sistem ini adalah ia murah, tidak memerlukan atmosfera yang lengai, boleh dibuat dengan kehadiran air dan tanpa keperluan asas. Keputusannnya, proses radiopenglabelan boleh dilakukan dalam masa yang lebih singkat dan hasil yang baik.

Kata kunci: Fluorin-18, tindak balas klik dan keselamatan sinaran

Introduction

Positron Emission Tomography (PET) imaging has become an important technique to study physiological, biochemical and pharmacological functions in humans. It is a non-invasive imaging technique that can measure the concentration of the tracer in tissues accurately due to its high sensitivity and high spatial resolution.[1,2] The radiopharmaceuticals or tracers for the PET scan incorporating the positron emitting radioisotopes such as Fluorine-

18, Carbon-11, Nitrogen-13 and Oxygen-15. But, a Fluorine -18 (^{18}F) is oftenly used for radiolabelling due to its favourable physical and nuclear characteristics [3] .

The most common radiopharmaceutical used in PET imaging is 2-[^{18}F]-fluoro-2-deoxy-D-glucose, or [^{18}F]FDG. However, its specificity in cellular dynamics in regards to energy requirements has limited its use in molecular imaging. [4] Therefore, there is a need for new PET radiopharmaceuticals other than [^{18}F]FDG as there are still many biological aspects of cancer that cannot be measured by [^{18}F]FDG alone.[5] To date, several approaches in radiolabelling using ^{18}F were developed. But the disadvantage is the time consuming multi-step reactions which is need for improvement to make the radiolabelling proses more speedy.Table 1 shows some of the different approaches for labelling biomolecules with ^{18}F.

Recently, a click chemistry which uses fewer chemical reactions and milder conditions to generate labelled substrates compared to other current methods is discovered.[6] The click reaction is high yielding and easy to perform using readily available reagents and starting materials. It is also tolerant to water, and the subsequent work-up and product isolation are straight-forward.[7,8] A variety of Cu(I) sources have been used in the Huisgen cycloaddition reaction including CuI salts such as copper iodide [9] and copper bromide [10] however, this type of reaction needs a large of excess of copper and ligand to work efficiently.[11] The use of metallic copper has also been employed, however, the reaction with copper turnings took a long time to form the desired triazole in good yield [12].

The most common click reactions is using an in situ reduction of a Cu(II) salt system to produce Cu(I),such as Cu(II) sulfate with sodium ascorbate as reducing agent. The click reaction forms a 1,2,3-triazole via the Cu(I) catalyzed 1,3-cycloaddition of azides and terminal alkynes (Fig.1). The advantages of this system is it is cheap, does not require an inert atmosphere, can be perfomed in the presence of water and eliminates the need for a base.[12,13] Marik and Sutcliff (2006) were demonstrated that peptides could be efficiently labelled with [^{18}F]alkynes in high yield, under mild conditions, and with rapid preparation times of 30 min.[14] The first sugar analog sucessfully labelled via click chemistry was also demonstrated by Korean.[15] This paper described how 4-[^{18}F]fluoro-1-butyne was succesfully synthesized for labelling of biomolecules.

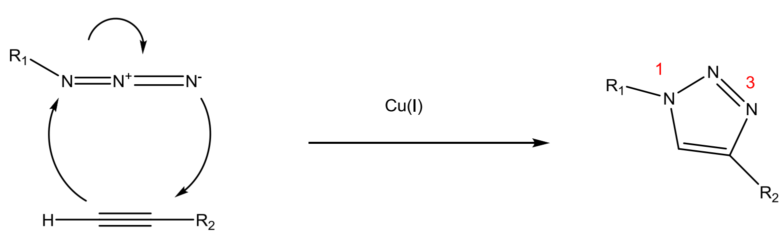


Fig. 1: The 'click reaction' of an azide and terminal alkyne to give a 1,2,3-triazole.(Adapted from ref.16)

Table 1: Different approaches for labelling biomolecules with ^{18}F. (Adapted from ref.17, 18)

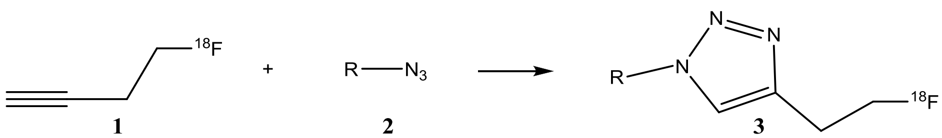
Method	^{18}F labelling agent	Preparation time (min)	Radiochemical yield (%)
Acylation	4-Nitrophenyl-2-[^{18}F]fluoropropionate [NPFP] N-Succinimidyl-4 [^{18}F]fluorobenzoate [SFB]	90 35-100	60 25-60
Imidation	3- [^{18}F]Fluoro-5-nitrobenzimidate	45	20-23
Alkylation	4- [^{18}F]Fluorophenacyl bromide	75	28-40
Click reaction	[^{18}F]fluoroalkynes	10-15	36-81

Once working with radionuclide, the radiation safety is concerned and must be addressed. Fluorine-18 has physical half-life of 109.8 min. The principle radiations of ^{18}F are 511-keV annihilation photons (1.94 per decay) and 640-keV (Emax) positron (0.97 per decay). It can be exposed to human by ingestion, inhalation, puncture, wound and skin contamination absorption.Thus, As Low As Reasonable Achievable (ALARA) guideline should be followed.

Materials and Methods

Chemicals and solvents were obtained from Sigma – Aldrich Chemical Company and used without further purification. All reactions were performed in standard glassware. Fluorine-18 was produced on a Cyclotron via the $^{18}O(p, n)^{18}F$ nuclear reaction. A fluorination reactions were carried out in the presence of potassium carbonate and the amino polyether $Kryptofix_{2.2.2}$ in acetonitrile under nitrogen.

Firstly, the non-radiolabelled for standard on High Purification Liquid Chromatography (HPLC) was obtained. Next, radiolabelled the material which radiofluorination was carried out prior to the click chemistry. The scheme of the radiolabelling of targetted material using click reaction was shown in (Fig.2).



1 **2** **3**

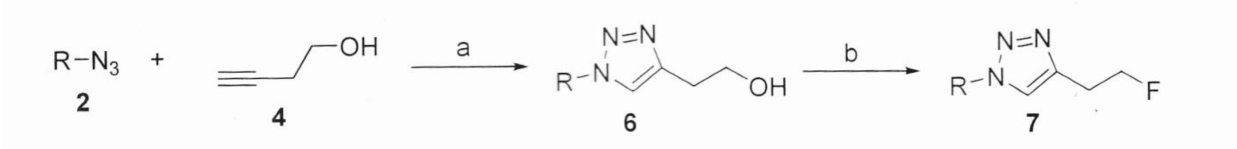
Fig.2: Radiolabelling of biomolecule using click reaction.

Butynyl tosylate (**5**) (refer scheme 1 in Results and Discussion) was synthesized as in literature.[15] The product was purified using flash column chromatography (4:1 hexane-ethyl acetate). The terminal fluoroalkyne (**1**) was prepared by nucleophilic substitution of the corresponding tosylate, (**5**) with [^{18}F]fluoride. The [^{18}F]fluoride anion was produced by the $^{18}O(p,n)^{18}F$ nuclear reaction in the cyclotron machine. A solution of Kryptofix (K_{222}) and potassium carbonate, K_2CO_3 were added to the [^{18}F]fluoride vial. The solvent was evaporated under a stream of nitrogen at 100°C with a reducing vacuum. This azeotropic drying was repeated twice by further addition of anhydrous acetonitrile. The precursor, butynyl tosylate (**5**) was dissolved in CH_3CN and added to the dried $K_{2.2.2}.K_2CO_3.K^{18}F$ complex. The reaction was heated for 10-20 min and the volatile product, 4-[^{18}F]fluoro-1-butyne (bp 45 ^oC) distilled and transfered into another vial for use in the click reaction.

In term of radiation safety, dosimetry should always be monitored. This can be performed by wearing radiation dosimetry monitoring badges [body & ring] whenever handling ^{18}F.[21] The labeling work should be done behind a shielding and the activity of ^{18}F also should always monitored. General precautions include the personal protection equipment such as glove, safety glass and lab coat.

Results and Discussion

1. A route to obtain the cold standard for HPLC

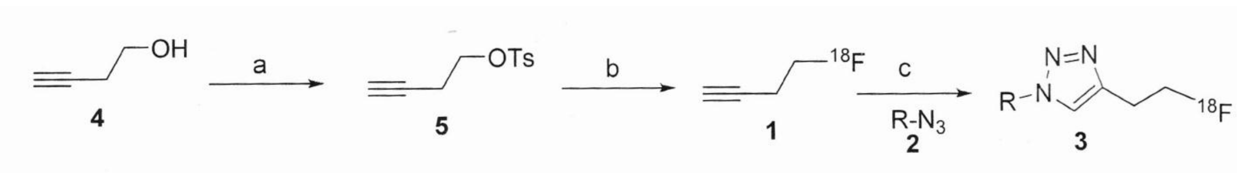


Scheme 1: A route to obtain the cold standard for HPLC

Table 2: The result of reaction in scheme 1(Adapted from ref.15)

No.	Reagents	Conditions	Yield (%)
a	Cu(II) sulfate, sodium ascorbate, tert- butanol, water	room temperature, 2 hours	84
b	Diethylaminosulfur trifluoride, Dichloromethane	0 ^{0}C, 30 minute	68

2. A route to label the target material



Scheme 2: A route to label the target material.

Table 2: The result of reaction in scheme 1(Adapted from ref.14,15)

No.	Reagents	Conditions	Yield (%)
a	p-toluenesulfonyl chloride, Triethylamine, Dichloromethane	0 ^{0}C, 3 hours	72
b	K[^{18}F]fluoride/Kryptofix$_{222}$ complex , Acetonitrile,	100 ^{0}C, 10-20 minutes	Not determined as through distillation process
c	Cu(I) Iodide, sodium ascorbate, 2,6-lutidine/DIEA	90 ^{0}C, 10 minutes	-

The click reaction is recent method in radiolabelling field. First of all, the non-radiolabelled standard was synthesized (Scheme 1). To obtain this standard, the fluorination was carried out using diethylaminosulfur trifluoride (DAST), a common fluorinating agent for the conversion of aliphatic alcohol into alkyl fluorides.[19] The cold standard product (**7**) (non radiolabelled) is necessary to be used in authenticating the ^{18}F radiolabelled derivative and in developing HPLC conditions for radiolabelling and purification.

The labeling of (**2**) began with radiofluorination of the tosylated precursor (**5**) to give 4-[^{18}F]-fluoro-1-butyne (**1)** via nucleophilic substitution (Scheme 2). To a dried K[^{18}F]fluoride/Kryptofix$_{222}$ complex, the tosylated alkyne (**5**) in acetonitrile was added and the reaction facilitated by heating, after which distillation was used to obtain the desired compound. The next step was click reaction of (**1)** with the molecule (**2**) (scheme 2). Marik et. al and Kim et. al used acetonitrile for the click reaction during the radiofluorination instead of tert butanol (m.p. 23-26^{o}C) , as the latter would freeze during the distillation at -50^{o}C.

It was mention earlier that the most common click reactions is use an in situ reduction of a Cu(II) salt system to produce Cu(I), such as Cu(II) sulfate with sodium ascorbate as reducing agent (Scheme 1). But, in the click reaction to incorporate the [^{18}F]fluoroalkyne (**1**) with molecule (**2**), the optimisation of catalytic system using Cu(I) with the presence of nitrogen base (2,6-lutidine, DIEA) showed a better result (Scheme 2). Sodium ascorbate was required to prevent oxidation of Cu(I) to Cu(II) by atmospheric oxygen (Scheme 2). The reagents were combined and the reaction stirred for 10 min after which, the reaction mixture containing the desired radiolabelled product (**3**) was filtered to remove any precipitate. The reaction mixture was then directly injected onto a HPLC column and the product (**3**) was collected. An aliquot from this fraction was co-injected with the cold fluorine standard (**7**) which was confirming the presence of the radiolabelled target (**3**)[14, 15] The biomolecule was succesfully labelled with ^{18}F via click chemistry.

Above all, occupational radiation exposure is concerned. The dose limit for radiation workers is 20 mSv/year. By reinforcing the ALARA concepts; time, distance and shielding, it will minimize radiation exposure to the occupational worker. Radiation dose is directly related to the time exposure. Accordingly, the time of handling the ^{18}F must be done as fast as posible. These study, 10 min of labelling time seems as the quick method and complies with the ALARA concepts. In the literature, 1.61 in. of lead are required to shield the 511-keV photons of ^{18}F effectively.[20] The rate of exposure will be considerably reduced according to the inverse square law. Therefore, if possible, include the use of long-handled tongs while handling the ^{18}F. An operational survey meter present in the work area and turned on whenever ^{18}F is handled, the activity of the radiation source also always measured, so that any external exposure issues will be immediately apparent and hence quickly addressed.[21]

Conclusion

PET imaging of tumours is important because it is a non-invasive functional imaging modality which can provide information not only about the location of the disease but also about how the target area (organ) is functioning. These group, Marik et.al (2006) and Kim et.al (2008) have described the development of a new method for radiolabelling various biomolecule (sugar and peptide) for use in PET imaging through the utilisation of click chemistry. Their work resulted in the labelling of biomolecules via click chemistry is more easy and fast (10 min) compared to other methods as described in Table 1. The radiochemical yield was reported as very good which is 98%.[14] This method is promising to be used in the development of PET radiopharmaceuticals. For the safety purposes, ALARA concepts must be applied to reduce an occupational radiation exposure.

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