Aspects of the Preparation of ^{18}F -2-deoxy-2-fluoro-D-glucose (^{18}F FDG) for Medical Use

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Fluorine-18-2-deoxy-2-fluoro-D-glucose (^{18}F FDG) for medical use had been prepared with the attention regarding radiochemical yield and purity, specific activity and quality control.

More than four ^{18}F -by-products in ^{18}F -adducts from the reaction of triacetyl glucal with $^{18}\text{F}_2$ were detected by the autoradiography of thin layer plates in which two by-products could not be perfectly removed by a column chromatography, and selective collection of the eluate gave ^{18}F FDG with the purity of 96.7% as an average in 12 runs. At end of synthesis (EOS) 259~925 MBq (7~25 mCi) of a sterilized, isotonic solution of ^{18}F FDG was obtained with the specific activity of 629~851 MBq (17~23 mCi)/mg at end of bombardment (EOB) after preparation time of 3~3.5 hr, in which bacteria and pyrogen were not detected.

Key Words: fluorine-18, fluorine-18-2-deoxy-2-fluoro-D-glucose, radiopharmaceuticals, labeled compounds

1. Introduction

2-Deoxy-2-fluoro-D-glucose (FDG) was first labeled with ^{18}F using the reaction of labeled $^{18}\text{F}_2$ with 3,4,6-tri-O-acetyl glucal (TAG) by Ido, et al.^(1),2) (Fig. 1) at Brookhaven National Laboratories. The first tomographic studies of the brain in man with ^{18}F FDG^(3),4) was performed by Reivich, Kuhl, Wolf and coworkers. And there is increasing interest in using ^{18}F FDG for the measurement of glucose utilization rates of the brain and heart in conjunction with positron computed tomography (PCT)^(5)~7). Clinical application is now performed in a limited number

of institutes. There are a few reports^(2),8),9) in detail for the preparation of ^{18}F FDG for medical use.

We currently prepare ^{18}F FDG for clinical use. In this paper we report the production of $^{18}\text{F}_2$, the reaction of $^{18}\text{F}_2$ with TAG, column chromatographic isolation of the ^{18}F -adducts, purification after hydrolysis, preparation of a sterilized, isotonic solution of ^{18}F FDG and quality control for dosing to man.

2. Materials and Methods

2.1 Targetry

The body of the target was constructed of

Fig. 1 Synthesis of ^{18}F FDG.

the purest nickel with a volume of 80 ml. The front window consisted of a sandwich of 20 μ m nickel foil (facing to the target gas) backed with 600 μ m aluminum foil. It was the usual target condition that F_2^* and pure neon^{*2} were loaded at a pressure of 20~25 atmosphere, containing 0.1~0.15% (85~120 μ M). F_2 in the target chamber after passivation with 1 atm F_2 overnight. These target design and target gas handling were similar to Brookhaven group's methods¹⁰.

2.2 $^{18}F_2$ production and recovery from target

Fluorine-18 (half-life=109.8 min)-labeled F_2 was produced by the deuteron bombardment (E_d = 22.5 MeV, incident 16.03 MeV) by our cyclotron^{*3}. The beam was shaken during bombardment to minimize danger to foil breakage. The bombardment was performed under the condition of beam current 10~15 μ A, for about 2 hr. After bombardment, the target gas was purged from the target chamber through an automatic flow controller into the reaction system.

2.3 Preparation of ^{18}F FDG

2.3.1 Reaction of $^{18}F_2$ with TAG and column chromatography of the ^{18}F -adducts

Remote preparation system(except hydrolysis and purification procedures)was assembled before run. The target gas was purged into the reaction vessel containing a solution of 30~40 mg (110~150 μ M) of 3,4,6-tri-*O*-acetylglucal^{*4} (recrystallized from EtOH/*n*-hexane) in 10 ml of Freon 11^{*5} ($CFCl_3$), which was cooled with an acetone/dry-ice bath at $-78^\circ C$. After purging, the Freon reaction solution was transferred onto a silica gel column (1.5 cm $\times$ 8 cm) and the reaction vessel was rinsed with *n*-hexane. After adsorption of the Freon and hexane solution on a silica gel column, the ^{18}F -adducts were eluted with *n*-hexane/ether (3/2). The eluate was fractionally collected by a fraction collector.

2.3.2 Hydrolysis of 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucopyranosyl fluoride (2)

Combined fractions of glucopyranosyl fluoride (2) was evaporated and the residue was hydrolyzed with 2.5 ml of 1N HCl at $130^\circ C$ for 30

min. The hydrolyzate was colored with brown because the fraction contained the unreacted TAG.

2.3.3 Purification of ^{18}F FDG

In order to remove the colored by-products, the hydrolyzate was added with a small amount of active charcoal, filtered through a Millipore filter, and washed out with sterilized water. The filtrate was passed through a column of AG 11A8 resin* (0.8 cm $\times$ 7 cm) and a column of alumina(0.8 cm $\times$ 7 cm) respectively to neutralize and remove fluoride ion. The eluate was prepared to be isotonic by addition of NaCl and sterilized by a Millipore filtration.

2.4 ^{18}F recovery and product analysis

2.4.1 ^{18}F recovery and distribution

Fluorine-18 recovery was given as the sum of the activities, corrected to end of bombardment (EOB), in the components of the reaction system; the reaction vessel, a soda lime trap, a gas trap, a silica gel column and the eluate.

2.4.2 Analysis of ^{18}F -adducts

The fraction of (2) and the other ^{18}F -adducts were detected by thin-layer chromatography(TLC-C) or TLC-ARG (autoradiography) technique using a silica gel plate^{*2} and *n*-hexane/ether (1/1) (R_f of (2) and (3)=3.5 and 1.3).

2.4.3 Analysis of ^{18}F FDG

The radiochemical purity of ^{18}F FDG was determined by TLC using a cellulose plate^{*2} and isobutyric acid/ NH_3/H_2O (66/1/33), or by high performance liquid chromatography (HPLC) using Bodapak C18 Carbohydrate column^{*3} and CH_3CN/H_2O (85/15), 1 ml/min of flow rate. The R_f values of impurity and FDG were respectively about 0.7 and 0.45. The retention time of impurity and FDG were respectively about 3.8 min and 6.8 min.

2.5 Sterilization and quality control for bacteria and pyrogen

All glass equipments, alumina and charcoal were sterilized before use by heating at $250^\circ C$ for 3 hr. Plastic equipments were sterilized by steam at $121^\circ C$ for 20 min. A part (0.2 ml) of final sample of ^{18}F FDG solution was checked on

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* Bio-Rad Laboratories, ^{*2} Merk, ^{*3} Nihon Waters Ltd. 4 mm $\times$ 30 cm

bacteria and pyrogen (endotoxin) using Bactec R301* and Pyrogen*² respectively.

3. Results and Discussion

The target chamber was treated before loading target gas usually by admitting 1 atm of F_2 and standing overnight, in the case of the first use of a new target or a new window foil, by heating the target chamber loaded with 1 atm of F_2 . Theoretical yield of ^{18}F were ranging from 23.68~33.3 GBq (640~900 mCi) at EOB for doses of 90~130 mC.

Table 1 Distribution of ^{18}F recovered from target^{a)}

Percent recovery of ^{18}F from target ^{b)}	$63.6 \pm 6.2\%$
Distribution in the reaction system	
Reaction solution	$86.6 \pm 2.3\%$
Reaction vessel walls	$9.8 \pm 1.2\%$
Soda lime trap	$2.4 \pm 1.3\%$
Gas trap	$1.3 \pm 0.4\%$

- a) The yield data represent an average $\pm$ S.D. in 12 runs
 b) % Recovery = (reaction solution + reaction vessel walls + soda lime + unreacted gas) / (calculated yield^{c)} of ^{18}F $\times 100$
 c) Based on $^{20}\text{Ne}(d, \alpha)^{18}\text{F}$ excitation function (100.5 mCi/ μA sat.)

The recovery of ^{18}F from target was taken as the sum of activities in the components in the reaction system, and is shown in Table 1. Under the employed condition the recovery yield seemed not to be affected by a target pressure (20~25 atm) and F_2 concentration (0.1~0.15%), but to be affected by a trace of water in the nickel tube line from the target to the reaction system. The recovery yield was 63.6% as an average in 12 runs. Contamination by oily substances increased activity in the unreacted gas fraction, which was identified to be mainly CF_4 -from. The recovery obtained by our experiments was comparable to the data reported by Fowler, et al.⁹⁾.

A small excess amount of TAG to F_2 was used in the reaction with $^{18}\text{F}_2$ to minimize the undesirable reaction. The reaction mixture was

separated by a silica gel column using *n*-hexane/ether (3/2) and 3 ml/min flow rate, and was fractionated in about 2 ml volume. Its elution curve of ^{18}F -adducts is shown in Fig. 2, in which mannopyranosyl fluoride (3) is not eluted and three radioactive peaks are seen. Peak 1 was eluted in a void volume. TLC analysis using ARG technique (Fig. 3) indicated that peak-3 was mainly a fraction of glucopyranosyl fluoride (2), but there were at least two unidentified ^{18}F -adducts in peak-3, and their R_f values were very close to that of (2). In typical preparation of ^{18}F FDG, in order to keep high radiochemical purity of ^{18}F FDG about 5 fractions of the top of peak-3 were collected and combined to be used in hydrolysis. Unreacted TAG was also eluted in peak-3. The yield of the combined

→ Indicates the fraction used for TLC-ARG analysis (see Fig. 3).

Fig. 2 Elution curve in a silica gel column chromatography. (1Bq=27.0pCi)

I: peak-1, II: peak-2, III: A fraction of lower retention time in peak-3, IV: A top in peak-3, V: A fraction of higher retention time in peak-3.

Fig. 3 TLC-ARG of ^{18}F -adducts.

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fractions was $21.2 \pm 2.7\%$ of the recovered ^{18}F as an average in 12 runs.

Table 2 Radiochemical yield in the steps of preparation and purification of $^{18}\text{F}\text{DG}^{\text{a)}$

	Yield (%)
Isolation of glucopyranosyl fluoride ^{b)}	$21.2 \pm 2.7^{\text{c)}$
Decolorization by charcoal	$88.2 \pm 5.2^{\text{d)}$
Neutralization by resin	$88.7 \pm 1.8^{\text{d)}$
Removement of F ion by alumina	$53.1 \pm 2.3^{\text{d)}$

a) The yield data represent an average $\pm$ S.D. in 12 runs

b) Yield of fraction of glucopyranosyl fluoride

c) Percentage based on the total ^{18}F -activities recovered from target

d) Percentage based on the ^{18}F -activity obtained in the preceding operation

Purification after hydrolysis was performed by three steps and the radiochemical yield on the each step is given in Table 2. The solution hydrolyzed with HCl was colored due to decomposition of TAG. Decolorization was successfully performed by an active charcoal treatment, but not by the subsequent purification steps. Neutralization of HCl and removal of ^{18}F ion were perfectly carried out by passage through AG 11A8 resin and alumina columns. Radiochemical purity was determined by TLC or HPLC (Fig. 4) using fractional counting of TLC or radiodetecting on line. Both analysis gave a similar radiochemical purity. HPLC analysis data, shown in Table 3, of samples in the each purification steps suggests that three purification procedures are not sufficient to remove radiochemical impurity. Therefore, in preparation of $^{18}\text{F}\text{DG}$ with high radiochemical purity, it seems important to isolate (2) from the mixture of ^{18}F -adducts as purely as possible. Radiochemical purity were $96.7 \pm 1.0\%$ as an average in 12 runs.

Specific activities of the final $^{18}\text{F}\text{DG}$ product were ranging from 629~851 MBq (17~23 mCi)/mg to EOB, which was calculated by a known molar of F_2 in the target chamber and theoretical yield of ^{18}F activity. Amount of $^{18}\text{F}\text{DG}$ at EOS (end of synthesis) was from 629~925 MBq (7~25 mCi) after about 3~3.5 hr from EOB.

Fig. 4 HPLC radiochromatogram of $^{18}\text{F}\text{DG}$.

Table 3 Radiochemical purity in the purification steps

	Purity(%)*
After hydrolysis	95.9
Treatment by charcoal	96.2
Passage through resin column	96.3
Passage through alumina column	97.0

* Based on organic- ^{18}F

In increasing radiochemical yield of $^{18}\text{F}\text{DG}$, the two improvement would be useful; (1) collection of peak-3 in wider range in a column chromatography of ^{18}F -adducts, (2) reduction in preparation time by fully automatic preparating system. Only 40~60% (21% of recovered ^{18}F) of activity of peak-3 are collected to remove ^{18}F -by product near (2) (see Fig. 2), but if the activity of peak-3 could be collected in wider range, the yield would be increased about 1.5 times, although radiochemical purity would be decreased. At present there are no detailed data of the proportion of ^{18}F -by products and physiological studies of ^{18}F -impurity, which are under study. Increase of about 30% of radiochemical

yield would be achieved by about one hour shortening in preparation time by a fully remote preparation system, which is in progress. Sterilization was performed by the methods mentioned in materials and methods. Resin column was washed with sterilized water(500 ml) overnight. The final ^{18}F FDG solution after passage through a Millipore filter and determination of radiochemical purity was examined for bacteria and pyrogen contamination using Bactec R301 and Pyrogen respectively. All final products in 12 runs resulted in safety for bacteria and pyrogen.

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References

- 1) Ido, T., Wan, C.-N., Fowler, J., et al.: *J. Org. Chem.*, **42**, 2341 (1977)
- 2) Ido, T., Wan, C.-N., Casella, V., et al.: *J. Label. Compd. Radiopharm.*, **XIV**, 175 (1978)
- 3) Reivich, M., Kuhl, D., Wolf, A., et al.: *Cir. Res.*, **44**, 127 (1970)
- 4) Kuhl, D., Hoffman, E., Phelps, M., et al.: In Medical Radionuclide Imaging Vol. 1, IAEA Symposium, Los Angeles, Calif. 1976, p. 309, Vienna IAEA (1977)
- 5) Phelps, M., Hoffman, E., Selin, C., et al.: *J. Nucl. Med.*, **19**, 1311 (1978)
- 6) Kuhl, D., Phelps, M., Kowell, A., et al.: *Ann. Neurol.*, **8**, 47 (1980)
- 7) Reivich, M., Kuhl, D., Wolf, A., et al.: *Acta Neurol. Scand.*, **56**, 192 (1977)
- 8) Fowler, J., Macgregor, R., Wolf, A., et al.: *J. Nucl. Med.*, **22**, 376 (1981)
- 9) Barrio, J., Macdonald, N., Robinson, G., et al.: *ibid.*, **22**, 372 (1981)
- 10) Casella, V., Ido, T., Wolf, A., et al.: *ibid.*, **21**, 750 (1980)

要 旨

医学利用のための ^{18}F -2-デオキシ-2-フルオロ-D-グルコース(^{18}F FDG)の合成

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放射化学的収率および純度, 比放射能, 品質を十分考慮して ^{18}F -標識 2-デオキシ-2-フルオロ-D-グルコース(^{18}F FDG)の標識合成を行った。

トリアセチルグルカール(TAG)と $^{18}\text{F}_2$ の反応物中に, 4つ以上の ^{18}F -副生成物が存在することが, 薄層クロマト・プレートのオートラジオグラフィによって確認された。この中の2つの ^{18}F -副生成物は, カラムクロマトグラフィによっても完全に分離することは不可能で, 溶出液を選択的に捕集することによって, 平均96.7%の放射化学的純度を有する最終 ^{18}F FDGが与えられた。

^{18}F FDGの滅菌, 等張化水溶液は, 合成終了時点で259~925 MBq(7~25 mCi), 比放射能は照射終了時換算で629~851 MBq(17~23 mCi)/mg, 合成時間は3~3.5hrであった。この ^{18}F FDG水溶液はバクテリア, パイロジェン(発熱性物質)はフリーであった。