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研究課題名（和文）Development of F-18 radiopharmaceutical for PET imaging of infections

研究課題名（英文）Development of F-18 radiopharmaceutical for PET imaging of infections

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研究成果の概要（和文）：我々は動物モデルにおいては細菌性感染症と非感染性炎症の識別することができた2-デオキシ-2- [18 F]フルオロアセトアミド-D-グルコピラノースの標識合成法に掲載された。 さらに、同様の標識合成条件下で[18 F]フッ化物の取り込みと同じ効率で新しい前駆体を開発し、最終的な[18 F] FAGの品質管理のためのHPLC法を開発しました。Ts前駆体を用いる最終比活性は、Br前駆体を用いるよりも高かった。しかも、最終比活性及び放射化学的収率は以前の新規標識合成法よりも低く、したがって臨床試験には適してない。最後に、新規UDP-N-アセチルグルコサミン前駆体の合成法が設計され、現在進行中である。

研究成果の概要（英文）：We had reported the radiosynthesis of 2-deoxy-2-[18F] fluoroacetamido-D-glucopyranose, which was able to distinguish infection from inflammation in animal models. Furthermore, we developed new precursors with the same efficiency of [18F]fluoride incorporation under similar radiosynthesis conditions and also developed a high-performance liquid chromatography (HPLC) method for quality control of final [18F]FAG. Final specific activity using tosylate precursor was higher than using bromo precursor. Nevertheless, final specific activity and radiosynthesis yield were lower than previous radiosynthesis procedure therefore not suitable for clinical trials. Finally, the synthesis a new UDP-N-acetylglucosamine precursor was designed and is currently ongoing.

研究分野：　Nuclear medicine

キーワード：　PET　Infection　Inflammation　Glucosamine　Bacterial imaging agent

１．研究開始当初の背景

Japan has been rated number one on the World Health Organization's life expectancy list. One consequence of this accomplishment is the high cost of treating and caring for the country's booming population of senior citizens. As life expectancy increases, orthopedic affections and associated surgical procedures become more frequents. Complicated infections like osteomyelitis and hip replacements are among the most common orthopedic procedures.

The diagnosis of infection and the ability to distinguish infection from inflammation by nuclear medicine remain as a challenge. A wide variety of radiotracers are under pre-clinical development, however the clinical imaging agents most currently employed, 2-[^{18}F]fluoro-2-deoxy-D-glucose and radiolabelled leucocytes, are non-specific and may accumulate at sites of sterile inflammation or other lesions, resulting in high rate of false positive results. We had reported the radiosynthesis of 2-deoxy-2-[^{18}F]fluoroacetamido-D-glucopyranose ([^{18}F]FAG), which was able to distinguish infection from inflammation in animal models despite the final radiochemical yield and specific activity were low. The mechanisms of uptake for [^{18}F]FAG in infected tissue still require to be studied in-depth, but the results suggested that [^{18}F]FAG could be incorporated into bacterial cells. (Figure 1)

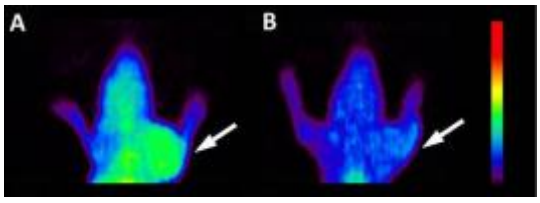

Fig. 1. Representative static maximun intensity projection (MIP) PET images on rats with an induced bacterial infection (A) or nonbacterial inflammation (B) in the right front leg muscle. Images were acquired 60 min after intravenous injection of 16.7-22.2 MBq of [^{18}F]FAG.

２．研究の目的

The results of this research are aimed to improve the use of PET as non-invasive diagnostic imaging modality for screening of infections and non-bacterial inflammations. Our strategy will be focus in the development of imaging tracers specific for bacteria capable of detecting infection in its earliest stages and distinguishing between infection and sterile forms of inflammation.

The present project propose to evaluate new *N*-acetylglucosamine (NAG) derivatives as precursors for the radiosynthesis of [^{18}F]FAG. Besides, evaluate [^{18}F]FAG as a tool for non-invasive diagnosis in animal experiments. Moreover, since NAG is incorporated into bacterial cell wall polysaccharides by glycosyltransferases (GTs) using UDP-NAG the incorporation of a positron emitter into UDP-NAG structure could be another approach for a more specific bacterial imaging agent for PET.

３．研究の方法

Unless otherwise noted, all reagents and solvents were obtained from commercial suppliers and were used without further purification. Non-radioactive FAG, as well as bromo, mesylate and tosylate precursors were synthesized in our laboratory. ^{1}H-NMR measurements were determined on a JEOL LA-600 spectrometer (600 MHz and Me_4Si was used as the internal reference). Radiosynthesis of [^{18}F]FAG was performed using microwave heating followed by alkaline hydrolysis. Final [^{18}F]FAG was purified by HPLC using: 1) amino-bonded silica phase column, Wakopak® Wakosil 5NH2 (5 μm, 250 mm × 10 mm i.d.) or 2) amino-bonded silica phase column plus a C18 bonded silica phase column, Cosmosil 5C_{18}-AR-II (5 μm, 250 mm × 10 mm i.d.). Evaluation of hydrolysate was performed by LC-MS/MS with electrospray source, operating in positive or negative ionization MRM mode of a triple quadrupole mass spectrometer using a different amino-bonded silica phase column

４．研究成果

We successfully synthesized 2-methanesulfonyl and 2-(*p*-toluenesulfonyl) derivatives of NAG. New derivatives were suitable for radiofluorination using microwave irradiation or conventional heating conditions (Figure 2).

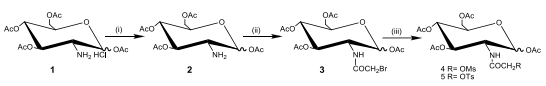

Fig 2. Synthesis scheme. Reagents and conditions (i) water, AcONa; (ii) $(BrCH_2CO)_2O$, CH_2Cl_2/Py; (iii) AgR/MeCN reflux.

Radiosynthesis of [^{18}F]FAG using bromo or tosylate as a precursors showed similar

radiochemical yields, radiochemical purities and synthesis times. A new HPLC method was validated for specificity, linearity, solution stability, accuracy, precision, limit of detection, and limit of quantitation using FAG and NAG as standard (Figure 3). The new method exhibited better sensitivity and detection than our previous reported HPLC method. The LC-MS/MS method was validated for monosaccharides originating from NAG analogues after basic hydrolysis. The method effectively quantified FAG and NAG analogues produced after hydrolysis. Purification using only amino-bonded silica phase column gave specific activity values of $1.4\pm0.3\times10^{-3}$ and $2.1\pm0.2\times10^{-3}$ GBq/μmol for bromo and tosylate precursors respectively. Whereas, when amino column was followed by a C18 column the specific activity values obtained were $7.8\pm4.4\times10^{-2}$ and $8.0\pm1.9\times10^{-2}$ GBq/μmol for bromo and tosylate precursors respectively.

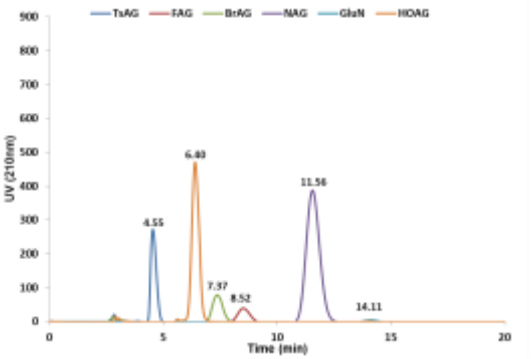

Fig 3. HPLC chromatogram of compounds of interest (see above) using Wakopak ® Wakosil 5NH2 (5 μm, 250 mm × 4.6 mm i.d.), CH_3CN/H_2O=80/20 (v/v), 1.0 mL/min at 30 °C.

Further analysis by ESI-MS found that purification using amino column was affected by a common by-product contaminant with similar elution time that final [^{18}F]FAG. The addition of a C18 bonded silica phase column help to separate this by-product and can explain the observed increment by ten-fold in the final specific activity. Nevertheless, the inclusion of a second HPLC purification procedure dropped the radiochemical yield to 2.3% (bromo) and 0.9% (tosylate). Considering that animal or clinical experiments will be difficult to perform, following the original plan, the synthesis of a new UDP-glucosamine precursor was started.

Figure 4 shows the synthesis scheme of UDP-NAG analogue modifying the 2-acetamido group in the acetylglucosamine moiety by a *N*-bromoacetyl group. The scheme utilizes Khorana's morpholidate coupling method and the introduction of bromoacetyl group followed previous FAG precursor methodology. Intermediaries from 2 to 7 were synthesized and structure were confirmed by ^{1}H-NMR.

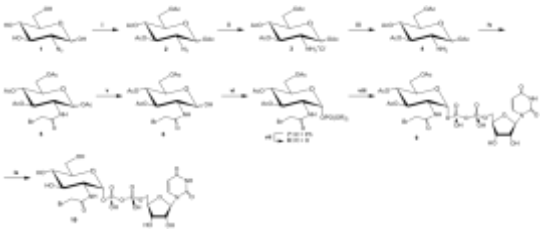

Fig. 4. Synthesis scheme. i) C_7H_8, $(CH_3CO)_2O$, C_5H_5N, 81%; ii) Lindlar's catalyst, H_2, HCl, 23%; iii) CH_3COONa, H_2O, quant.; iv) $(BrCH_2CO)_2O$, DCM/Py, 87%; v) $NH_2NH_2\cdot AcOH$, DMF; vi) $(PhO)_2POCl$, DMAP; vii) H_2, PtO_2, $EtOAc/CH_3CH_2OH$ (1:1); viii) 4-Morpholine-N,N'-dicyclohexylcarboxamidinium uridine 5'-monophosphormorpholidate, tetrazole, dry pyridine. ix) TEAB, CH_3OH, H_2O, -20 ° C.

There were not significant differences regarding the use of bromo or tosylate precursor for radiosynthesis of [^{18}F]FAG. The addition of a C18 bonded silica phase column after the amino-bonded silica phase column help to increase by 10-fold the specific activity of final [^{18}F]FAG. The new UDP-NAG analogue synthesis scheme includes nine reaction steps and six of them were done successfully, but whole synthesis scheme and radiolabeling evaluation was not accomplished. Since all required reagents had already been purchased, completion of synthesis scheme as well as evaluation could be performed in the near future.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　　件）

〔学会発表〕（計　　４件）

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Medicine Association Congress. 2016, October 15-19. Barcelona, Spain.
② MARTINEZ Miguel, MAKINO Akira, MORI Tetsuya, TOKUNAGA Yuji, OKAZAWA Hidehiko, KIYONO Yasushi. Comparison of bromo and tosyl precursor for radiosynthesis of 2-deoxy-2-[^{18}F]fluoroacetamido-D-glucopyranose. 11th Annual Meeting of Japanese Society for Molecular Imaging. 2016, May 28-29, Kobe Portopia Hotel (Kobe, Hyogo).
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〔図書〕(計　　件)

〔産業財産権〕

○出願状況（計　　件）

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権利者：
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国内外の別：

○取得状況（計　　件）

名称：
発明者：
権利者：
種類：
番号：
取得年月日：
国内外の別：

〔その他〕
ホームページ等

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