Original Article

간질 PET영상을 위한 플루마제닐(벤조디아제핀 수용체)유도체의 신속하고 간단한 합성방법 소개

서울대학교병원 핵의학과
조용현 · 김형우 · 황기영 · 임진균 · 이홍재 · 우재룡 · 김현주

A Fast and Simple Synthesizing Method of ^{18}F-Flumazenil as Derivative Benzodiazepine Receptor for Epilepsy PET Imaging

Yong Hyun Cho, Hyung Woo Kim, Ki Young Hwang, Jin Koon Lim,
Hong Jae Lee, Jae Ryong Woo, Hyun Ju Kim
PET center, Nuclear Medicine, Seoul National University Hospital, Seoul, Korea

Department of Nuclear Medicine in Seoul National University Hospital (SNUH) had developed ^{18}F-Flumazenil as Benzodiazepine receptor imaging agent for PET diagnosis of Epilepsy. But production Activity of ^{18}F-Flumazenil is decreased owing to this method has difficult synthesis procedures and pretty long synthesis time. In this study, we can modify synthesizing method to have more simple procedure and less spend time and help to increase production Activity. **Old method:** Radioactivity was produced by cyclotron was captured by QMA cartridge that was activated. Captured radioactivity was eluted into the reaction vial by using kryptofix solution and delivered. After evaporation of eluent, the azeotrophic drying step repeated two times. tosylflumazenil in anhydrous Acetonitrile was added to a reaction vial while bubbling. The reaction mixture was evaporated until the mixture volume was 0.5 mL. Reaction vial washed with 20 % Acetonitrile and that solution went into the reaction vial. The reaction mixture was loaded to the HPLC loop by hand and purified ^{18}F-Flumazenil by HPLC column. **New method:** We used $TBAHCO_3$ solution as a eluent. After the eluent was evaporated, tosylflumazenil in anhydrous acetonitrile was added to a reaction vial and the reaction mixture was bubbled for 15 minutes. It was evaporated until the mixture volume became 0.5 mL. It was loaded to the HPLC loop. In old method, ^{18}F-Flumazenil was synthesized via 6 steps synthesis procedures in 105 minutes with 30~35% synthesizing yield (non-decay correction) and specific activity was about $0.5\sim2\times10^5$ Ci/mole. In new method, It had 3 steps synthesis procedures in 53 minutes with 40~45% synthesizing yield and specific activity was about $3\sim8\times10^5$ Ci/mole. This method leads to improve of minimizing synthesis time, increasing synthesis yield and specific activity. While we can load reaction mixture to the HPLC loop, we can expose high radiation field thanks to used by hand. **(Korean J Nucl Med Technol 2008;12(3):176-180)**

Key Words : ^{18}F-Flumazenil, Benzodiazepine receptor, Tosylflumazenil, Synthesis method

INTRODUCTION

Epilepsy is a chronic neurological disorder, and also one of the oldest disease in human history. In the early days, people believed that a person with epilepsy was seized by a supernatural force or power. The word "Epilepsy" being derived from the Greek word "epilambanein" means "to seize or attack". Epilepsy is characterized by recurrent unprovoked seizures. Seizures are similar to muscle jerks or convulsions. Especially, those are classified according to where in the brain they

· Received: July 15, 2008. Accepted: September 1, 2008.
· Corresponding author: Yong Hyun Cho
Department of Nuclear Medicine, Seoul National University Hospital
28 Yeongon-dong, Chongno-gu, Seoul, 110-744, Korea
Tel: +82-2-2072-2537, Fax: +82-2-745-7690
E-mail: hiuni486@hanmail.net

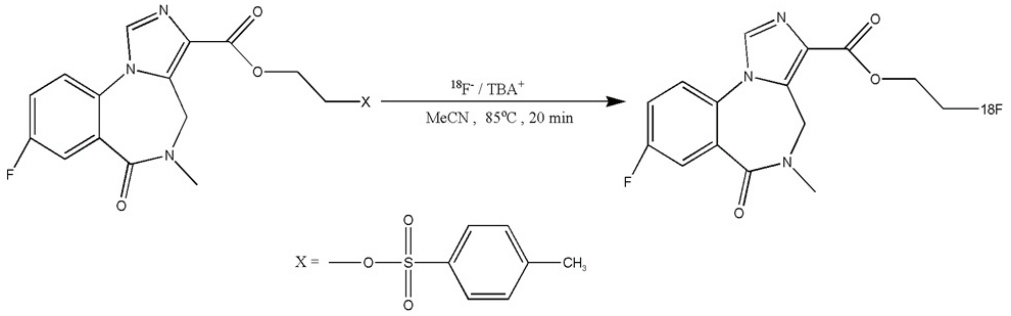

Fig. 1. Synthesis of ^{18}F-Flumazenil.

arise. To find out the epilepsy focal regions, we have been using several diagnostic equipments such as MRI, CT, EEG, MEG, MRS, SPECT, PET. PET image could be better than MRI or CT image because PET image can find out a changing metabolism of disorder regions before MRI or CT image can find out a changing morphology of disorder regions. ^{18}F-FDG is usually used for epilepsy PET image. Many studies have been reported that ^{18}F-FDG metabolism is decreased in epilepsy region during interictal phase. But ^{18}F-FDG has important disadvantages, it shows widely real epilepsy region and it become the important problem according to which phase can be scanned. ^{18}F-FDG image for Epilepsy can get only interictal phase. Ictal phase, other drug and physical condition can affect to the getting ^{18}F-FDG PET image. Using a benzodiazepin compounds with radioisotope can get the specific Epilepsy PET image. Benzodiazepin receptors are distributed with a high density in cerebral cortex, cerebellum, thalamus and it appears that density is decreased in Epilepsy trigger region. There are many derivative benzodiazepine compounds such as flumazenil, iomazenil with ^{11}C or ^{18}F.

EXPERIMENTAL

All of the buffers were used with further purification and Distilled Water, Acetonitrile, $TBAHCO_3$, TBAOH, K222, K_2CO_3 were used without further purification. Before we inject this radiopharmaceutical to patients ^{18}F-flumazenil passed through the 0.22 μm filter to removed microorganisms through the 0.22 μm filter.

1. Produce of radioisotope

No-carrier-added ^{18}F was produced through the ^{18}O(p,n) ^{18}F reaction by the proton bombardment using a EBCO 13 MeV cyclotron. After the irradiation, ^{18}F was transferred in synthesis module under gas stream, and radioisotope was trapped by QMA Sep-Pak in synthesis module.

2. Preparation of Sep-Pak cartridge

QMA was activated by 5 mL of 0.5 M K_2CO_3 and 10 mL of Distilled Water for new method and 5 mL of 0.5 M K_2CO_3 and 10 mL of Distilled Water for old method. Alumina was activated by only 10 mL of Distilled Water. All of the Sep-Pak cartridges must remove solvents by nitrogen gas.

3. Preparation of reagents

There are two eluents in our methods. One is kryptofix solution for old method and another is $TBAHCO_3$ (Tetrabutylammoniumbicarbonate) solution for new

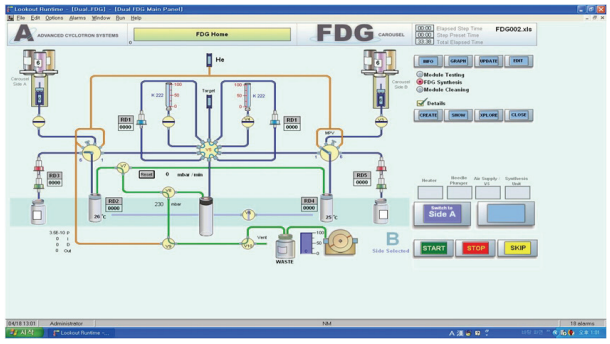

Fig. 2. Automatic synthesis module.

method. Kryptofix solution was made by 12.6 mg K222 in 0.602 mL of Acetonitrile and 2.03 mg K_2CO_3 in 0.098 mL of Distilled Water. $TBAHCO_3$ solution was made by 0.029 mL of TBAOH (Tetrabutylammoniumhydroxide), 0.586 mL of Acetonitrile and 0.085 mL of Distilled Water. Tosylflumazenil (FUTURECHEM.Co.LTD in Korea) was used as a precursor in methods. We prepared 6.25 mg of precursor in 3 mL acetonitrile.

4. Preparation of equipments

^{18}F-Flumazenil was synthesized automatically in module

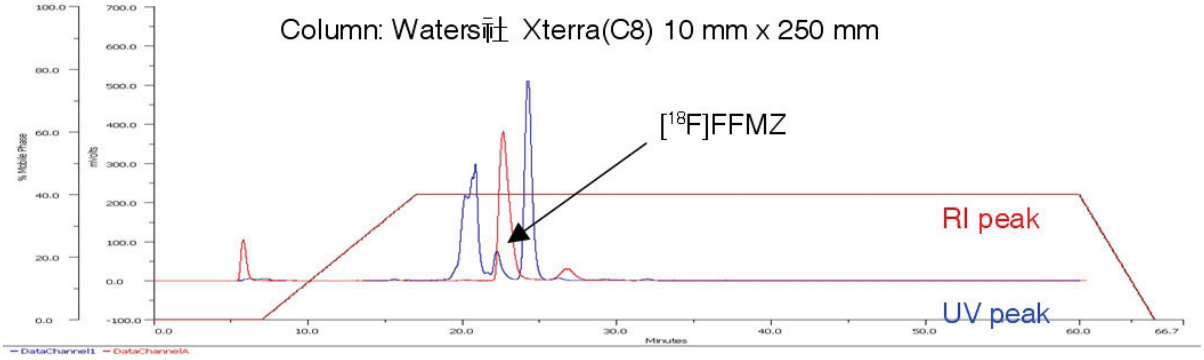

Fig. 3. Prep-HPLC chromatogram, Ethanol : D.W (0:100→40:60), flow rate 4 mL/min.

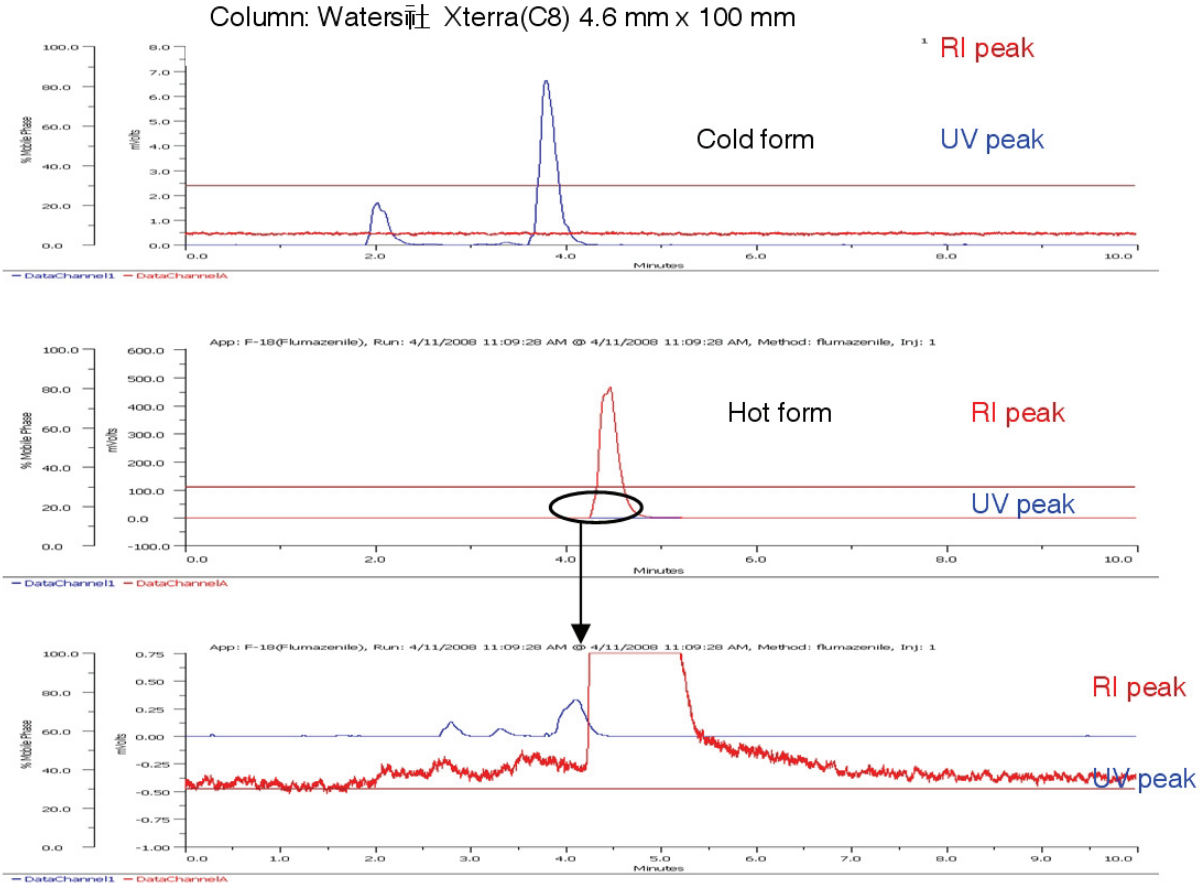

Fig. 4. Anlytical HPLC chromatogram, Acetonitrile : D.W (30:70), flow rate 1 mL/min (0.0046 umole cold form in DMF /^{18}F-Flumazenil/enlarge the circle area to confirm the UV peak retention time).

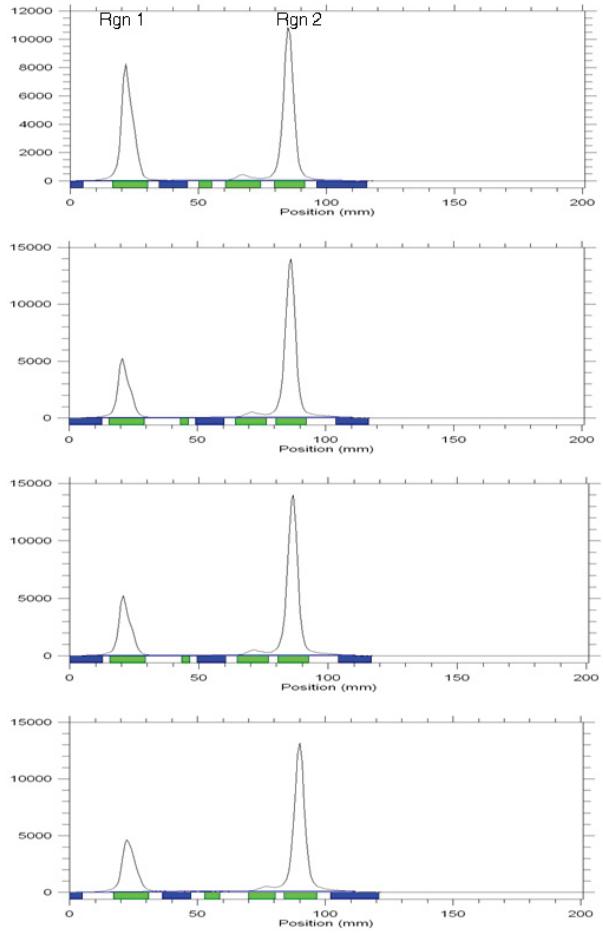

Fig. 5. TLC Chromatogram (fluorination time 5 min/10 min /15 min/20 min).

(Carousel, ACS company, Canada). We used Prep HPLC (321 pump, Gilson, USA) to separate ^{18}F-Flumazenil from others and used analytical HPLC (Gilson, USA) to confirm whether it is ^{18}F-Flumazenil or not. UV detector system (UV/VIS-155, Gilson, USA) and RI detector system (Gabi, Raytest, Germany) were installed in HPLC System.

5. Synthesis procedure

^{18}F-Flumazenil was produced by cyclotron and tapped on QMA cartridge. Eluent was passed through the QMA cartridge and transfered into the reaction vial at 105℃. The solvent was evaporated using a helium gas. After evaporation, precursor was added and fluorination step kept going for 20 minutes. After fluorination, the solvent passed through the alumina Sep-Pak and delivered into the product vial. 2 mL product mixture injected to HPLC loop and it was separated by HPLC column (Waters, Xterra (C8) 10 mm×250 mm).

6. Old method and New method

Old method : Trapped ^{18}F-Flumazenil was eluted by 0.8 mL of kryptofix solution and the azeotropic drying step repeated two times. 10 mg tosylflumazenil in 4 mL anhydrous Acetonitrile was added to a reaction vial and was bubbling for 20 minutes. It was evaporated until the mixture volume was 0.5 mL and then it delivered into the product vial. 20% Acetonitrile was washed reaction vial then, delivered into the product vial.

New method : We used 0.7 mL $TBAHCO_3$ solution as a eluent. After eluent was evaporated, 6.25 mg tosylflumazenil in 3 mL anhydrous acetonitrile was added to a reaction vial and the reaction mixture was bubbled for 10 minutes at 85℃. It was evaporated until the mixture volume became 0.5 mL. It went into the product vial with washed solution.

RESULT AND DISCUSSION

^{18}F-Flumazenil solution was separated through the prep-HPLC column. It's RI peak appeared 23 minutes and we took it in product vial by three way valve. ^{18}F-Flumazenil was determined by analytical HPLC. ^{18}F-FFMZ's UV peak appeared 4 minutes and we compared standard solution to ^{18}F-Flumazenil solution.

Compare to standard UV peak areas and ^{18}F-Flumazenil UV peak areas, we calculated a specific activity. Compare to old method, synthesis time should be taken at least 50 minutes less than old one and product activity should be at least 50% more than old method. Product yield and specific activity were improved more than old method. What we change eluent from kryptofix to $TBAHCO_3$ affect to improve a product activity and synthesis yield. But, we connected the alumina sep-pak to end of the synthesis module line to remove a $TBAHCO_3$. This led to a loss of product activity. We checked TLC chroma-

togram to confirm how to change the labelling yield during the fluorination step was continuing. Region 1 is free form, fluoride 18 and region 2 is bound form, Flumazenil. We take it 4 times every 5 min. Labelling yield sharply increase from start point, 0 min to 5min. Between 5 and 10 min, labelling yield increase mildly and after 15min, labelling yield didn't increase any more.

CONCLUSION

For years, we can get some difficulties when we synthesis flumazenil such as complex step, complex procedures, low product activity and so on. But, we can never experience those when we used to new synthesis method. We could save a lot of synthesis time using new method due to removing of the azeotropic drying step and reducing of fluorination time from 20 minutes to 10 minutes, even 5 minutes. In case of using krpytofix, we could not remove the azeotropic drying step. When we have done without azeotropic step, we have known that product activity and specific activity is decreased. The advantages of the using new method were decrease synthesis step and time, increase product yields and specific activity and decrease fluorination time. Nevertheless, important thing is how we can find a solution of loading to the HPLC loop. When we loaded product mixture to HPLC loop, we should only expose the radiation field.

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