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Nuclear Medicine and Biology 32 (2005) 263–268

NUCLEAR MEDICINE — AND — BIOLOGY

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Biological properties of 2′-[^{18}F]fluoroflumazenil for central benzodiazepine receptor imaging

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Received 2 September 2004; received in revised form 23 December 2004; accepted 23 December 2004

Abstract

A novel positron emitting agent, 2′-[^{18}F]fluoroflumazenil (fluoroethyl 8-fluoro-5-methyl-6-oxo-5,6-dihydro-4*H*-benzo-[*f*]imidazo [1,5-*a*][1,4]diazepine-3-carboxylate, FFMZ), has been reported for benzodiazepine imaging. In the present study, biological properties of [^{18}F]FFMZ were investigated. Stability tests of [^{18}F]FFMZ in human and rat sera were performed. Biodistribution was investigated in mice and phosphorimages of brains were obtained from rats. A receptor binding assay was performed using rat brain (mixture of cortex and cerebellum) homogenate. A static positron emission tomography (PET) image was obtained from a normal human volunteer. Although [^{18}F]FFMZ was stable in human serum, it was rapidly hydrolyzed in rat serum. The hydrolysis was 39%, 63% and 92% at 10, 30 and 60 min, respectively. According to the biodistribution study in mice, somewhat even distribution (between 2~3% ID/g) was observed in most organs. Intestinal uptake increased up to 6% ID/g at 1 h due to biliary excretion. Bone uptake slowly increased from 1.5% to 3.5% ID/g at 1 h. High uptakes in the cortex, thalamus and cerebellum, which could be completely blocked by coinjection of cold FMZ, were observed by phosphorimaging study using rats. Determination of K_d value and B_{max} using rat brain tissue was performed by Scatchard plotting and found 1.45±0.26 nM and 1.08±0.03 pmol/mg protein, respectively. The PET image of the normal human volunteer showed high uptake in the following decreasing order: frontal cortex, temporal cortex, occipital cortex, cerebellum, parietal cortex and thalamus. In conclusion, the new FMZ derivative, [^{18}F]FFMZ appears to be a promising PET agent for central benzodiazepine receptor imaging with a convenient labeling procedure and a specific binding property.

Keywords: Benzodiazepine receptor; FFMZ; PET; Flumazenil; Fluoroflumazenil

1. Introduction

Central benzodiazepine receptors have been visualized and quantified in vivo by positron emission tomography (PET) or single photon emission computed tomography (SPECT). The potent benzodiazepine antagonist flumazenil (Ro 15-1788, ethyl 8-fluoro-5-methyl-6-oxo-5,6-dihydro-4*H*-benzo-[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate) has been labeled with ^{11}C and ^{18}F for PET [1–4], and ^{123}I labeled Ro 16-0154 [^{123}I]iomazenil was described shortly thereafter for SPECT [5–8]. The above compounds all show high regional specific binding that corresponds to the known distribution of cerebral benzodiazepine receptors.

[^{11}C]Flumazenil is the most commonly used agent for imaging central benzodiazepine receptor using PET [9–11]. However, the short half-life of ^{11}C (20 min) limits its use. To overcome this problem, 5-(2′-[^{18}F]-fluoroethyl)flumazenil ([^{18}F]FEFMZ), a flumazenil analogue labeled with ^{18}F, has been developed using a two-step synthesis procedure [12]. However, its preparation was time-consuming and inefficient because of the two-step reaction. A further improvement in the synthetic procedure was achieved by synthesizing a new derivative 2′-[^{18}F]fluoroflumazenil(FFMZ) (scheme 1) that employs a tosylated precursor for one-step labeling reaction [13]. A two-step reaction procedure has also been applied to label [^{18}F]FFMZ later [14].

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doi:10.1016/j.nucmedbio.2004.12.004

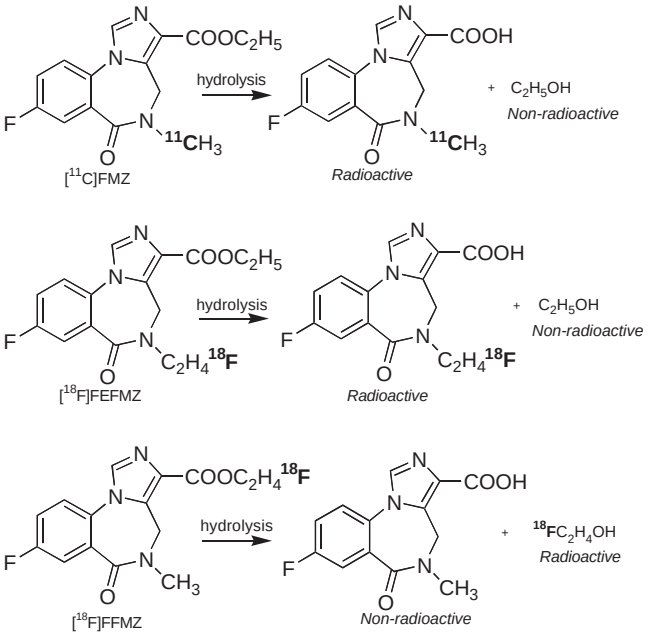

Fig. 1. Hydrolysis of [^{11}C]FMZ, [^{18}F]FEFMZ and [^{18}F]FFMZ. In cases of [^{11}C]FMZ and [^{18}F]FEFMZ, radioactivity remains in hydrophilic metabolites, that is, [^{11}C]FMZ acid and [^{18}F]FEFMZ acid, respectively. However, radioactivity remains to [^{18}F]fluoroethanol in the case of [^{18}F]FFMZ.

Although some biological evaluations of [^{18}F]FFMZ, such as the biodistribution in rats and the IC_{50} value of displacing [^{3}H]FMZ, have been reported [15], many other biological properties have not been reported. In the present study, we investigated the binding affinity of [^{18}F]FFMZ to mouse brain tissue, stability in serum, biodistribution in mice, phosphorimages of rat brain and static PET image of a normal human volunteer.

In addition, we tested the possibility that hydrolysis of the ester bond of [^{18}F]FFMZ would produce [^{18}F]fluoroethanol. On the other hand, [^{11}C]FMZ and [^{18}]FEFMZ would produce hydrophilic nondiffusible [^{11}C]FMZ acid and [^{18}F]FEFMZ acid, respectively (Fig. 1).

2. Materials and methods

2.1. Preparation of [^{18}F]FFMZ

[^{18}F]Fluoride was produced using a TR13 cyclotron (Ebco Technologies) by bombarding ^{18}O-enriched water (97%) with an accelerated proton beam. The synthesis of 2′-tosyloxyflumazenil and its labeling with ^{18}F were performed as previously published method [13]. The [^{18}F]fluoride produced was captured by an AG1-X8 microcolumn and eluted with a 1-ml solution of Kryptofix 2.2.2. (19.26 mg, 0.05 mmol)/K_2CO_3 (6.43 mg, 0.0465 mmol) in MeCN/H_2O (86/14) (v/v). In a reaction vessel located in an oil bath at 105°C, the eluted [^{18}F]fluoride was dried by purging with helium gas (flow rate, ~20 ml/min). After drying the azeotropic mixture, 1 ml of MeCN was added and the mixture was dried two more times to remove water thoroughly. To the dried [^{18}F]fluoride, 10 mg of TFMZ or MFMZ in 2.5 ml in MeCN was added, and the mixture was heated at 85°C for 12 min under continuous purging with helium for mixing. The reaction mixture was purified by a preparative HPLC (column: C_{18}, 300 mm×7.8 mm, 10 mm, Waters), and [^{18}F]FFMZ was eluted at 24.4 min by gradient elution (100% water, 7 min; 0% to 40% MeCN in water, 10 min; and continuous 40% MeCN in water) at a flow rate of 5 ml/min. The radiochemical purity of the [^{18}F]FFMZ was higher than 98% when checked by TLC (ethyl acetate/ethanol/hexane=1/1/2, AR2000, Bioscan, Washington, DC, USA) and an analytical HPLC (column: Bio-Rad RSiL C_{18}HL, 250×4.6 mm, 10 μm, MeCN/water=3/7, retention time=12.4 min). The specific activity of [^{18}F]FFMZ was $4.5\times10^9\pm1.6\times10^9$ MBq/mol as determined by an analytical HPLC. All other reagents used were of analytical grade and were obtained commercially.

2.2. Preparation of the rat brain membranes

Cerebral membranes for binding assay were prepared as previously described with some modifications [16]. Male Sprague–Dawley rats, weighing 270–290 g, were killed by decapitation and brains were rapidly removed. The cerebral cortex and the cerebellum were separated from the brain stem and immediately frozen in liquid nitrogen, and then stored in a deep freezer (−70°C) until use. The frozen cerebral cortex and the cerebellum (about 1 g) were placed in 1.4 ml of ice-cold 50 mM Tris–citrate buffer (pH 7.5) containing protease inhibitor cocktail (Sigma, St. Louis, USA), homogenized for 30 s using a polytron homogenizer (Kinematica, Westbury, Canada) on setting 7 and centrifuged at 48,000×g for 30 min at 4°C. The supernatant was then decanted and the pellet obtained was suspended in 1.4 ml of the same buffer, rehomogenized and recentrifuged at 48,000×g for 30 min at 4°C. The crude membranes were then suspended in 1 ml of the same buffer and frozen in liquid nitrogen. After freezing, the membranes were thawed and centrifuged at 48,000×g for 15 min at 4°C. These freezing and thawing steps were then repeated twice. The resulting pellet was suspended in 1 ml of 50 mM Tris–citrate buffer (pH 7.5) without protease inhibitor cocktail and stored at −70°C until required for binding studies. Protein concentrations were determined using a BCA protein assay kit (Pierce, Rockford, USA).

2.3. Binding assay

[^{18}F]FFMZ binding assays were carried out by incubating aliquots of membranes suspension (0.1~1.6 mg) for 60 min at 0°C in 0.4 ml of 50 mM Tris–citrate buffer (pH 7.5) containing 0.76 nM [^{18}F]FFMZ. Nonspecific binding was determined in the presence of 10 μM cold FMZ. Binding reactions were stopped by rapid filtration through Whatman GF/B glass fiber filters presoaked with buffer under reduced pressure. Filters were washed twice with 5 ml of ice-cold 50 mM Tris–citrate buffer and