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Biological evaluation of 2′-[^{18}F]fluoroflumazenil ([^{18}F]FFMZ), a potential GABA receptor ligand for PET

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Abstract

[^{11}C]Flumazenil, a highly selective benzodiazepine antagonist is the most extensively used $GABA_A$ ligand for PET so far. To overcome half life disadvantages of ^{11}C a [^{18}F]-labeled flumazenil derivative, 2′-[^{18}F]fluoroflumazenil (FFMZ) was developed and biologically evaluated with respect to the $GABA_A$ receptor. Organ with the highest uptake was the pituitary gland. Brain uptake was high and followed the order cortex>thalamus>cerebellum>rest brain. Fluoroflumazenil displaced [^{3}H]flumazenil binding from membrane $GABA_A$ receptors with an IC_{50}value (3.5 nM) comparable to that of Flumazenil (2.8 nM). The presented data confirm the potential of [^{18}F]FFMZ for PET imaging of the GABA-ergic system. © 2004 Elsevier Inc. All rights reserved.

Keywords: FFMZ; Flumazenil; Fluoroflumazenil; GABA; PET

1. Introduction

Benzodiazepines are used as sedative, anxiolytic, hypnotic, anticonvulsant and muscle-relaxant drugs. These drugs evolve their action via a special binding pocket on the central gaba receptor (CBR). [1] Various diseases, such as epilepsy, Huntington's disease, Alzheimer's disease or schizophrenia can be caused by alterations of the CBR. Thus imaging and quantification of CBRs with positron emission tomography (PET) can be helpful for the diagnosis of neurological and psychiartric diseases [2,3]. [^{11}C]Flumazenil ([^{11}C]FMZ, Ro15 1788-C11), a highly selective benzodiazepine antagonist has been introduced for PET in the early eighties [4], and is the most extensively used CBR ligand for PET so far. However, there are some disadvantages due to its short half life (20.3 min). To overcome this disadvantage a [^{18}F]-labeled FMZ derivative (5-(2′-[^{18}F]fluoroethyl)flumazenil, [^{18}F]FEFMZ) has been presented showing high binding affinity but rapid metabolism [5-7]. The [^{18}F]fluoroethyl label was attached to the nitrogen function of the molecule possibly causing the rapid metabolism. We have presented recently, that [^{18}F]fluoroethylation at a carboxylic group could yield in a stabilization of the resulting ester [8,9]. With this rationale we synthesized 3-(2′-[^{18}F]fluoro-FMZ) ([^{18}F]FFMZ) [10] (Fig. 1). During our evaluation of the synthesis another study was presented using a different synthetic approach for the first preparation of [^{18}F]FFMZ. This publication showed displaceable uptake of this tracer on autoradiographic images [11]. Therefore the present work aimed to further evaluate the binding affinity of FFMZ to the CBR and to investigate the biodistribution of FFMZ in rats.

2. Materials and methods

2.1. Chemicals

Inactive Precursor (8-fluoro-5-methyl-6-oxo-5,6-dihydro-4H-benzo-[f]imidazo[1,5-a] [1,4]diazepine-3-carboxy-

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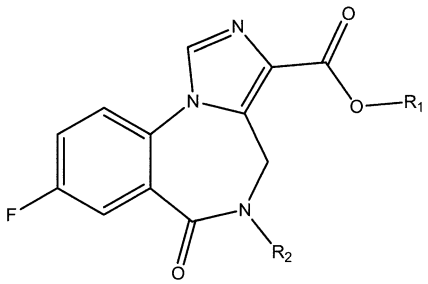

	R1	R2	reference
FMZ	Ethyl-	Methyl-	4
FEFMZ	Ethyl-	2-Fluoroethyl	5-7
FFMZ	2-Fluoroethyl-	Methyl-	10,11
N-desmethyl FFMZ	2-Fluoroethyl-	H-	10,11
Precursor	H-	Methyl-	10

Fig. 1. Structures of various analogues of FMZ.

lic acid (3-desethylflumazenil)), FFMZ standard (2′-fluoroethyl 8-fluoro-5-methyl-6-oxo-5,6-dihydro-4H-benzo-[f] imidazo[1,5-a]-[1,4]diazepine-3-carboxylate; 3-(2′-fluoro)-flumazenil; 3-(2′-fluoroethyl)-3-desethylflumazenil) such as FMZ were purchased from ABX–Advanced Biochemical Compounds (Dresden, Germany).

2.2. Radiochemistry

[^{18}F]FFMZ was synthesized by reacting 3-desethyl-FMZ with 2-Bromo-1-[^{18}F]fluoroethane in less than 80 minutes [10]. Radiochemical purity of [^{18}F]FFMZ exceeded 98%. So far, starting from 52±8 GBq [^{18}F]fluoride, 5.2.3 GBq of [^{18}F]FFMZ were obtained (n=5, EOB). Specific radioactivity exceeded 89.4 GBq/μmol (2418.9 Ci/mmol).

2.3. Animal experiment

All biodistribution studies followed a protocol of the NIH Animal Care and Use Committee also approved by the Austrian law on animal experiments. Male Sprague-Dawley rats/Him:OFA (n=20, 239-270 g) were injected with 1.58-2.72 MBq [^{18}F]FFMZ in 180-225 μl physiological phosphate buffer through the tail vein. Subsequently the rats were sacrificed by exsanguination from the abdominal aorta in ether anesthesia after 5 (n=5), 15 (n=5), 30 (n=5) and 60 minutes (n=5). The organs were removed, weighed and counted (Cobra II auto-gamma counter, Canberra Packard, Canada). Radioactivity is expressed as percentage injected dose per gram tissue (% I.D./g).

2.4. Radioligand binding studies to membranes from rat forebrains

Adult female rats (Sprague-Dawley/Him:OFA) were sacrificed and the forebrains were rapidly dissected. A membrane suspension was prepared [8] and stored at −80°C. For binding studies, frozen membranes were thawed and resuspended in 50mM Tris-citrate buffer (TC 50) at pH 7.4 by ultra-turraxing, at a protein concentration of about 0.1-0.3 mg/ml as measured by the BCA-protein assay kit of Pierce Chemical with bovine serum albumin as standard. Membranes (0.8ml) were then incubated for 90 min at 4°C in a total of 1 ml of a solution containing TC-50 buffer, 150 mM NaCl, 2 nM of [^{3}H]FMZ (78.6 Ci/mmol, PerkinElmer Life Sciences) in the absence or presence of 100 μM diazepam (Hoffmann La Roche, Basle, Switzerland) or various concentrations (0.1, 0.3, 1.0, 3.0, 10, 30, 100 nM) of FFMZ or FMZ. Membranes were filtered (Whatman International Ltd, Maidstone, England), filters were rinsed twice with 5 ml ice-cold TC-50 buffer, transferred to scintillation vials and after addition of 3.5 ml scintillation fluid (Ultima Gold^{TM} MV, Packard) subjected to scintillation counting (2100 TR Tri-Carb® Liquid Scintillation Analyzer, Packard). Non-specific binding determined in the presence of 100 μM diazepam was substracted from total [^{3}H]FMZ binding to calculate specific binding.

3. Results

3.1. Biodistribution

The organ with the highest uptake was the pituitary gland showing 0.95±0.35 %I.D./g (15 min) followed by cortex (0.73±0.07I.D./g, 5 min) and liver (0.70±0.11%I.D./g, 5 min). The organs with the lowest uptake was fat (0.11±0.02%I.D./g, 5 min) and femur (0.36±0.02%I.D./g, 5 min). Blood activity was 0.48-0.51%I.D./g throughout the whole experiment. The only organ with increasing uptake over time was the colon (0.27±0.07, 5 min. to 0.47±0.1%I.D./g, 60 min.). All other organs were in a range from 0.31±0.04%I.D./g for the testes (5min) to 0.67±0.06%I.D./g for the thalamus (5 min). Remaining activity in the carcass was 0.53±0.04 %I.D./g. All values are shown in Table 1.

3.2. Radioligand binding

FFMZ displaced [^{3}H]FMZ binding from membrane $GABA_A$ receptors with an IC_{50} value (3.5 nM) comparable to the IC_{50} value of FMZ (2.8 nM). Values are shown in Fig. 2.

4. Discussion

4.1. Biodistribution

Organ with the highest uptake was the pituitary gland. Since there is affinity of FMZ to the GABA receptors expressed on the pituitary gland, these findings can be

Table 1
Biodistribution study of [18F]FFMZ in rats expressed as % I.D./g (mean ± SD, n=5)

Tissue	5 min	15 min	30 min	60 min
Adrenals	0.56 ± 0.04	0.44 ± 0.03	0.40 ± 0.05	0.41 ± 0.08
Blood	0.48 ± 0.04	0.52 ± 0.05	0.53 ± 0.02	0.51 ± 0.08
Carcass	0.53 ± 0.04	0.53 ± 0.04	0.49 ± 0.05	0.49 ± 0.08
Cerebellum	0.63 ± 0.06	0.48 ± 0.02	0.46 ± 0.07	0.41 ± 0.06
Colon	0.27 ± 0.07	0.34 ± 0.04	0.34 ± 0.04	0.47 ± 0.10
Cortex	0.73 ± 0.07	0.53 ± 0.02	0.43 ± 0.03	0.38 ± 0.06
Duodenum, Ileum, Jejunum	0.68 ± 0.05	0.55 ± 0.04	0.49 ± 0.04	0.46 ± 0.06
Fat	0.11 ± 0.03	0.11 ± 0.01	0.10 ± 0.02	0.10 ± 0.02
Femur	0.35 ± 0.02	0.33 ± 0.04	0.32 ± 0.02	0.36 ± 0.05
Heart	0.52 ± 0.05	0.49 ± 0.03	0.45 ± 0.02	0.40 ± 0.05
Kidneys	0.58 ± 0.05	0.52 ± 0.02	0.46 ± 0.03	0.41 ± 0.05
Liver	0.70 ± 0.09	0.51 ± 0.03	0.39 ± 0.02	0.33 ± 0.05
Lung	0.50 ± 0.05	0.49 ± 0.03	0.43 ± 0.03	0.38 ± 0.05
Muscle	0.52 ± 0.04	0.45 ± 0.03	0.37 ± 0.02	0.29 ± 0.04
Pancreas	0.48 ± 0.04	0.38 ± 0.03	0.33 ± 0.02	0.30 ± 0.03
Pituitary gland	0.78 ± 0.17	0.95 ± 0.35	0.68 ± 0.09	0.90 ± 0.19
Rest brain	0.61 ± 0.09	0.49 ± 0.03	0.40 ± 0.10	0.44 ± 0.06
Spleen	0.50 ± 0.07	0.46 ± 0.03	0.42 ± 0.02	0.35 ± 0.05
Testes	0.31 ± 0.04	0.48 ± 0.03	0.41 ± 0.02	0.34 ± 0.04
Thalamus	0.67 ± 0.06	0.52 ± 0.03	0.47 ± 0.03	0.48 ± 0.09
Thyroid gland	0.43 ± 0.19	0.35 ± 0.04	0.34 ± 0.07	0.37 ± 0.08
Whole brain (all regions)	0.69 ± 0.07	0.52 ± 0.02	0.43 ± 0.02	0.41 ± 0.06

explained by the close structural relation between FMZ and FFMZ (Fig. 1) [12,13]. Maximum was reached at 15 minutes, slightly decreasing towards 60 minutes. Enhanced uptake in the liver could be due to the hepatical microsomal cytochrome P450 enzyme system being responsible for metabolism and hepatobilary excretion. However, [^{18}F]FFMZ appeared metabolically stable, as low levels of fluoride accumulation was shown in the femur (maximum value 0.36±0.05 %I.D./g after 60 minutes). The major elimination route of [^{18}F]fluoroethanol and [^{18}F]fluoroacetic acid—metabolic products formed after cleavage of the fluoroethylester—would be renal [8]. Since uptake in the kidneys was moderate (max. 0.57±0.04 %I.D./g, 5min) and decreasing with time, no significant cleavage of the [^{18}F]fluoroethylester is supposed. The increase in uptake of the colon over time without a concomitant increase in uptake in the kidneys is in line with the hepatobiliary excretion of [^{18}F]FFMZ. Calculating tissue to blood ratios of different brain sections at 5 minutes, uptake of [^{18}F]FFMZ in the cortex shows a factor 1.53, cerebellum 1.32 and thalamus 1.41 (Fig. 3). The ratio for the whole brain is 1.46 for [^{18}F]FFMZ, compared to 0.73 for [^{18}F]FEFMZ [5]. Since the lipophilicity of [^{18}F]FFMZ is lower than [^{18}F]FEFMZ, this increased ratio could be explained by the higher stability of [^{18}F]FFMZ. (see chapter logD) Rest body activity was 0.49±0.05 to 0.53±0.04%I.D./g, comparable to blood (0.48-0.51% I.D./g). Fig. 4 shows the kinetics of [^{18}F]FFMZ with peak uptake for all sampled brain regions at about 5 minutes after application. These kinetics are considerably faster than those reported for [^{11}C]FMZ, and comparable to [^{18}F]FEFMZ [6]. Combining the autoradiographic evaluation [11] with the present biodistribution and pharmacodynamic findings [^{18}F]FFMZ appears to be a promising ligand for human in vivo studies.

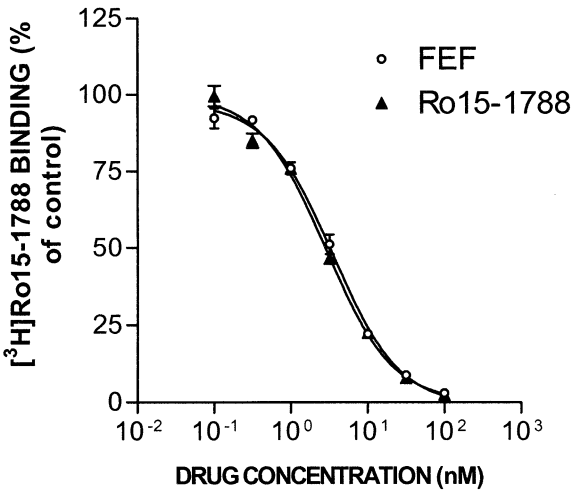

Fig. 2. Competitive binding curves: displacement of [^{3}H]FMZ binding by FFMZ and FMZ. Membranes of rat forebrains were incubated with 2 nM [^{3}H]FMZ in the absence or presence of 100 μM diazepam (to calculate the 100% control value) or various concentrations of drugs as indicated. Data were analyzed by non-linear regression with a curve-fitting computer software package (Graph-Pad Prism 3.0, San Diego; mean values ±S.D. from three different experiments each performed in triplicates).

4.2. GABA-ergic system

$GABA_A$ receptors (γ-aminobutyric receptors, type A) are the major inhibitory neurotransmitter receptors in the

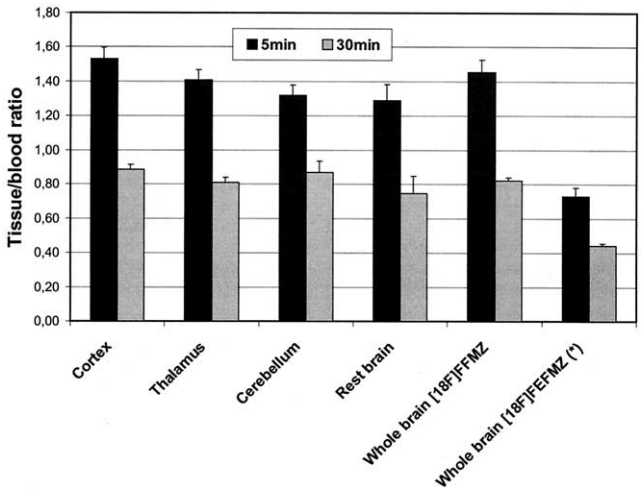

Fig. 3. Shows tissue to blood ratios of brain regions: Cerebellum, Cortex, Thalamus Whole brain and Rest brain. (*) Values taken from reference [5].

central nervous system and the site of action of many pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, neurosteroids and anesthetics [1]. Evidence has accumulated that the most abundant isoform of these pentameric ion channels is composed of two α, two β and one γ subunit [14]. The benzodiazepine binding site is an allosteric modulatory site located at the α/γ-interface of $GABA_A$ receptors [15]. Binding of benzodiazepines to this site causes a conformational change in the receptor that usually enhances GABA-induced chloride flux into the cell. This effect can be inhibited by FMZ, that binds with high affinity to the benzodiazepine binding site of CBR but has only weak intrinsic efficacy for changing GABAergic transmission. To investigate whether FFMZ can bind to the same site as FMZ, functional competition experiments were carried out with membrane preparations from rat forebrains. The similar IC_{50}values of FMZ and FFMZ indicate that the structural modification of FMZ resulting in FFMZ did not cause a significant change in the affinity of this compound.

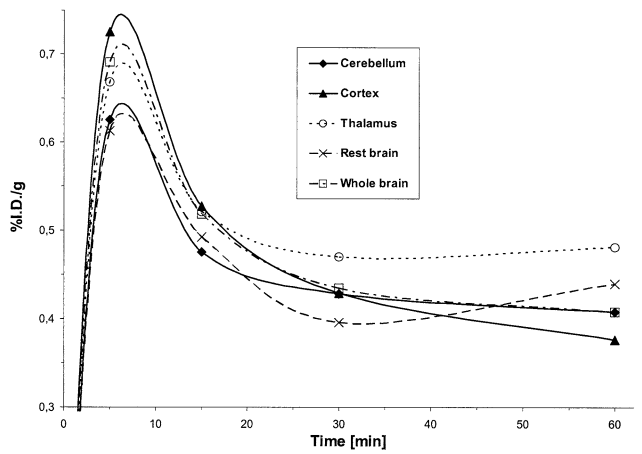

Fig. 4. Shows time-activity curves of FFMZ for various brain regions.

LogD values (calculated logP for neutral pH) were determined with ACD/logD Suite Version 5.0 for Microsoft Windows. We calculated 0.68 for FFMZ and interestingly 0.84 for N-desmethyl-FFMZ, a major potential metabolite. This higher value could be explained by the formation of intramolecular hydrogen bonding between the unsubstituted nitrogen atom in position 5 and the carboxylic function - calling for further investigation. The FFMZ precursor, also a potential metabolite shows a logD of 0.04. For comparison: [^{18}F]FEFMZ shows a logD of 1.25.

5. Conclusion

[^{18}F]FFMZ has a similar affinity to the GABA receptor as FMZ. The biodistribution of [^{18}F]FFMZ in rats shows uptake in the brain and together with recent autoradiographic findings [11] the presented data confirm the potential of [^{18}F]FFMZ for PET imaging of the GABA-ergic system.

Acknowledgments

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