ELSEVIER

Nuclear Medicine and Biology 28 (2001) 809–814

NUCLEAR MEDICINE — AND — BIOLOGY

Biodistribution, binding specificity and metabolism of [^{18}F]fluoroethylflumazenil in rodents

Philippe Levêque[a], Daniel Labar[b], Bernard Gallez[a,c,*]

[a]*Unité de chimie pharmaceutique et de radiopharmacie, Université catholique de Louvain, Avenue Mounier 73.40 B-1200 Brussels, Belgium*
[b]*Unité de tomographie par positrons, Université catholique de Louvain, Chemin du Cyclotron 2, B-1348 Louvain-la-Neuve, Belgium*
[c]*Unité de résonance magnétique biomédicale, Université catholique de Louvain, Avenue Hippocrate 10, B-1200 Brussels, Belgium*

Received 15 April 2001; received in revised form 23 May 2001; accepted 30 May 2001

Abstract

Pre-clinical studies were carried out in order to characterize in rodents the biodistribution, the binding specificity and the metabolism of [^{18}F]Fluoroethylflumazenil ([^{18}F]FEF), a potential candidate for in vivo imaging of the benzodiazepine receptors. In vivo competition with flumazenil indicates that [^{18}F]FEF binds specifically to the benzodiazepine receptor in the brain. The accumulation of [^{18}F]FEF was significantly lower than using [^{3}H]Flumazenil. The rather low accumulation in the brain is due to a rapid metabolism of [^{18}F]FEF in hydrophylic metabolites which cannot cross the blood brain barrier, and are rapidly eliminated in the urine. Inhibition of the metabolism by acetaminophen (chemically induced hepatitis) led to a significant increase of the radioactivity found in the circulating blood and in the brain, while these results were not observed using classical inhibitors of the cytochrome CYP450, cimetidine and ketoconazole.

Keywords: [^{18}F]Fluoroethylflumazenil; Metabolites, biodistribution

1. Introduction

Flumazenil (Ro-1722, Anexate®) is a well known inverse agonist of the benzodiazepine receptor, and can be labeled with ^{11}C, a positron emitting radionucleide. It has been widely used for mapping the central benzodiazepine receptor, in vivo, using Positron emission tomography (PET) [7,8,10,14,17–19]. Nevertheless, due to its binding kinetics, mathematical modeling is required to obtain relevant quantitative parameters such as Bmax or affinity constant. These models usually require arterial blood sampling and determination of the unmetabolised fraction of the radiotracer, which is hardly convenient in routine experiment [3,4,9].

The synthesis of [^{18}F]Fluoroethylflumazenil (or 5-[2′-^{18}F] fluoroethylflumazenil; (ethyl-8-fluoro-5,6-dihydro-5-[2′-(^{18}F)fluoroethyl]-6-oxo-4H-imidazo[1,5a] benzodiazepine-3-carboxylate)), a Flumazenil analogue labeled with ^{18}F, was proposed earlier by Moerlein. It has the advantages of a longer half life, of a good affinity for the benzodiazepine receptor (Ki = 11.6 nM), and was a potential candidate for in vivo imaging [15,16].

However, little was known about its biodistribution in vivo, its rate of biotransformation, and the fate of the metabolites [2,12,13]. These parameters must be known in order to be able to develop adequate modeling tools, and to validate the [^{18}F]FEF as a radiotracer that allows accurate quantification of the benzodiazepine receptors. We recently investigated the metabolism of [^{18}F]FEF in vitro, and isolated the main metabolites [11].

The aim of the present study is to investigate the biodistribution of [^{18}F]Fluoroethylflumazenil in rodents, to check the specificity of the radiotracer binding in the brain, and to evaluate its metabolism in various compartments (blood, brain, urine). We first studied the kinetics profile for the organ distribution in rat and in mice, and compared it to the biodistribution of [^{3}H] Flumazenil as standard. The specificity of binding in the brain was evaluated by a displacement experiment using cold Flumazenil. Various metabolism inhibitors were also used to assess the influence of the metabolism on the uptake of the radiotracer in the brain.

* Corresponding author. Laboratory of medicinal chemistry and radiopharmacy, Université catholique de Louvain, Avenue Mounier 73.40, B-1200 Brussels. Tel.: +32-2-764-7348; fax: +32-2-764-7363.
E-mail address: gallez@cmfa.ucl.ac.be (B. Gallez).

0969-8051/01/$ – see front matter
PII: S0969-8051(01)00251-7

Finally, the fate of the radiotracer in the blood, brain and urine was monitored using HPLC.

2. Materials and methods

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

[^{18}F]FEF was synthesized according to a previously published scheme [15]. Briefly, desmethylflumazenil (Hoffman LaRoche, Basel, Switzerland), and [^{18}F]fluorotosylethane were reacted in anhydrous acetonitrile, using Kryptofix 2.2.2/CO_3^{2-} to give [^{18}F]FEF. [^{18}F]fluorotosylethane was synthetized from ditosylethane and $^{18}F^-$, obtained from the IBA Cyclone 30 cyclotron (Center de Recherche du Cyclotron, Université catholique de Louvain, Louvain-la-Neuve, Belgium). [^{18}F]FEF was purified by semi preparative HPLC on a Silica column, using dichloromethane-methanol (98.5: 1.5 v/v) as eluent. The radiochemical and chemical purity were higher than 99%, and determined by reverse-phase HPLC on a C18 Alltima column (Alltech, Laarne, Belgium) with a UV and radioactive detection. The specific activity was higher than 37 GBq per micromole.

Solution for injection was prepared as follows: [^{18}F]FEF was dissolved in ethanol and briefly sonicated, diluted with sterile and non pyrogenic 4% human serum albumin (Croix-Rouge, Brussels, Belgium), and filtered aseptically on a 0.22 μm filter (Millex-GV, Millipore, Molsheim, France).

2.2. Biodistribution studies

All animal experiments were performed in compliance with the local ethics committee.

Male wistar rats (Animalerie facultaire, Université catholique de Louvain, Brussels, Belgium) weighing 250–300 g were anesthetized with ketamine/xylazine (80 mg/kg + 8 mg/kg IP) and injected with 3700–5550 kBq of [^{18}F]FEF in the femoral vein. Animals were sacrificed by decapitation at various time points. Blood and various organs (kidneys, spleen, liver, heart, lungs, muscle, brain) were rapidly removed, and weighted samples of each organ were counted on a Wallac NaI well counter (Wallac 1480 Wizard 3, Wallac Oy, Turku, Finland). Standard solutions of [^{18}F]FEF were counted in the same conditions. Data were corrected for dead time (less than 10%) and for physical decay. Results are expressed as percent of the injected dose per gram of wet tissue (%ID/g), and are the mean of five animals per time point.

A biodistribution study in male NMRI mice (Animalerie facultaire, Université catholique de Louvain, Brussels, Belgium) weighing 25–35 g was similarly carried out except that [^{18}F]FEF was injected in the tail vein, without anesthesia.

A control study was conducted in male NMRI mice, using ^{3}H-Flumazenil (N-methyl-^{3}H Ro15–1788, specific activity 2619 GBq/mol, Dupont NEN, Boston, MA, USA) as standard. A dose of [^{3}H]FMZ ranging from 110 kBq to 260 kBq was injected in the tail vein without anesthesia, and the animals were killed by cervical dislocation at various time points. Blood and other organs were quickly removed and treated as follows. For beta counting: three samples of each organ were weighted and dissolved in 1 ML Soluene (Soluene 350, Packard Instruments, Meriden, CT, USA) at 50°C for 4 h. When necessary, the solution were discolored with water peroxide (Merck). Ten ML of liquid scintillation cocktail were added (HionicFluor, Packard, Meriden, CT, USA) and the samples were allowed to stand for 12h before counting in a Wallac beta counter (Wallac 1180, Wallac Oy, Turku, Finland). Counting efficiency for ^{3}H was at least higher than 20%. The result for each organ is the mean of the three measurements, and expressed as %ID/g.

The binding specificity of the radiotracer in the brain was evaluated by displacement of the bound [^{18}F]FEF with a saturating dose of a cold ligand, Flumazenil, in mice. After injection of [^{18}F]FEF or [^{3}H]FMZ for control, cold flumazenil was injected at an other point in the tail vein (0.1 mg/kg). Animals were killed after 5 minutes, and the organs treated as described above.

2.3. Metabolism studies

The metabolism of [^{18}F]FEF was investigated in NMRI mice, using reverse phase HPLC or TLC, to separate and quantify intact [^{18}F]FEF from its metabolites, as previously described [11]. Briefly, after IV injection of the radiotracer, mice were killed and blood was collected, centrifugated and the plasma deproteinised with acetonitrile. After centrifugation, the supernatant was evaporated, reconstituted with acetonitrile-water and analyzed by HPLC. The brain was homogenized in a phosphate buffer (150 mM, pH = 7.4) using a Potter-Eveljhem. This homogenate was extracted twice with a mixture of dichloromethane-ethylacetate (90:10 v/v), and the organic layer evaporated to dryness. The recovery of the radioactivity in the organic layer was higher than 97%. The residue was then reconstituted with a mixture of water and acetonitrile, and analyzed by HPLC as described earlier.

Table 1
Biodistribution of [^{18}F] FEF in rats

Organ	Time (min)			
	5	13	20	30
Blood	1.15 ± 0.14	0.69 ± 0.04	0.17 ± 0.03	0.09 ± 0.01
Kidneys	9.08 ± 2.73	2.64 ± 0.14	2.29 ± 0.22	1.67 ± 0.18
Spleen	0.57 ± 0.07	0.53 ± 0.05	0.23 ± 0.03	0.09 ± 0.01
Liver	7.85 ± 0.66	4.22 ± 1.68	1.83 ± 0.54	1.43 ± 0.41
Heart	0.77 ± 0.12	0.57 ± 0.03	0.3 ± 0.04	0.12 ± 0.01
Lungs	1.03 ± 0.25	0.67 ± 0.05	0.17 ± 0.03	0.08 ± 0.01
Brain	0.84 ± 0.05	0.49 ± 0.03	0.20 ± 0.05	0.04 ± 0.01
Muscle	0.70 ± 0.17	0.58 ± 0.05	0.15 ± 0.02	0.06 ± 0.01

Results are mean of 5 animals ± S.E. unit is % injected dose/g of tissue.

Table 2
Biodistribution of [^{18}F] FEF in mice

Organ	Time (min)			
	5	13	20	30
Blood	0.95 ± 0.12	0.32 ± 0.10	0.44 ± 0.25	0.13 ± 0.06
Kidneys	4.34 ± 0.54	1.77 ± 0.63	0.82 ± 0.17	0.57 ± 0.22
Spleen	0.48 ± 0.06	0.47 ± 0.25	0.11 ± 0.03	0.07 ± 0.04
Liver	9.11 ± 0.93	4.29 ± 0.53	1.85 ± 0.32	0.72 ± 0.24
Heart	0.57 ± 0.07	0.28 ± 0.05	0.10 ± 0.01	0.08 ± 0.03
Lungs	0.94 ± 0.10	0.24 ± 0.03	0.08 ± 0.01	0.05 ± 0.01
Brain	0.60 ± 0.10	0.24 ± 0.03	0.08 ± 0.01	0.05 ± 0.01
Muscle	0.55 ± 0.11	0.38 ± 0.15	0.10 ± 0.01	0.11 ± 0.07

Results are mean of 5 animals ± S.E. unit is % injected dose/g of tissue.

2.4. Biodistribution—metabolism inhibition studies

The influence of the metabolism level, and the role of the liver on the biodistribution was investigated through inhibition studies using various inhibitors. In the first two series, male NMRI mice were pretreated by IP administration of cimetidine 100 mg/kg (Sigma, Bornem, Belgium) [5,20], or ketoconazole 100 mg/kg (Sigma, Bornem, Belgium) [6,21] one hour before injection of [^{18}F]FEF. Mice were killed by cervical dislocation five minutes after injection of the radiotracer, the organs removed and counted as described above.

In the third series, a hepatitis was induced with acetaminophen. Acetaminophen was dissolved in PEG 300, diluted with saline (PEG final concentration 40%) and injected IP (300 mg/kg) 24 hours before the biodistribution experiment [1,20]. Five minutes after administration of the radiotracer, animals were killed, the organs removed and counted as described above. The liver damages were noted visually.

3. Results

3.1. Biodistribution in rat and mice

The biodistribution of [^{18}F]FEF in rat shows that [^{18}F]FEF is rapidly distributed in the body, and also rapidly eliminated. As there is a continuous wash-out of the tracer from the tissues, no equilibrium state is reached. The accumulation of [^{18}F]FEF in the brain is rather low if compared to non specific binding organs such as the muscle or the spleen (Table 1).

The general profile of the biodistribution in mice shows the same characteristics than in rat: a rapid distribution with a rapid wash-out from the tissues (Table 2). As in rats, accumulation of [^{18}F]FEF in the brain is low in comparison with other organs (muscles, lung etc.).

It can be assumed that the anesthetics (ketamine/xylazine) do not interfere in the biodistribution process, since the [^{18}F]FEF uptake in the brain of non anesthetized mice was comparable to the brain accumulation of the tracer in anesthetized rats.

3.2. Specificity of the binding

Although the accumulation of [^{18}F]FEF in the rodents brain was surprisingly low, the competition studies of the tracer uptake with cold Flumazenil indicate that the binding is specific to the benzodiazepine receptor (Fig. 1).

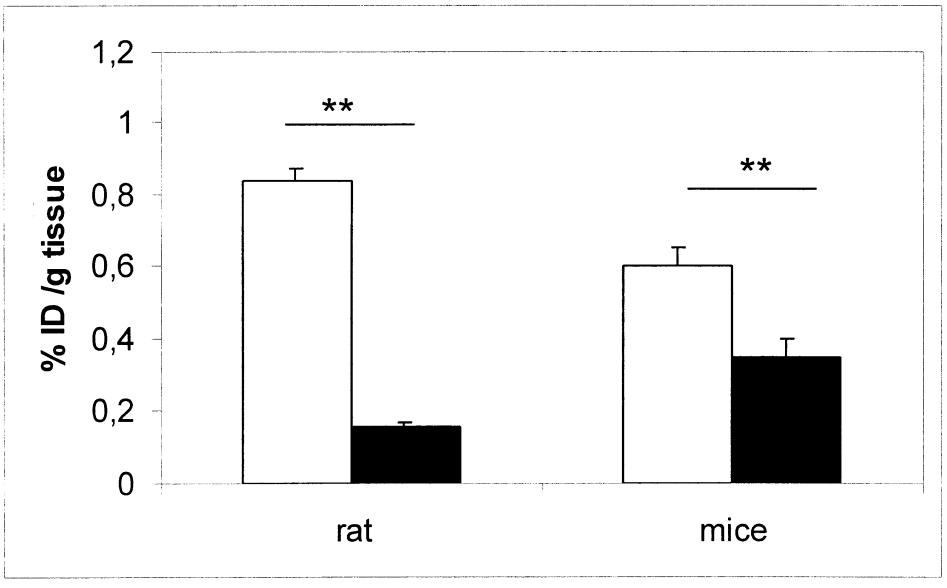

Fig. 1. Accumulation of [^{18}F] FEF in the brain in controls animals (white box) or in animals which received a second injection of cold Flumazenil (black box). The results are expressed as mean ± S.E. On the left: experiment carried out in rats; on the right: experiment carried out in the mice. **$p < 0.01$ (Student t-test).

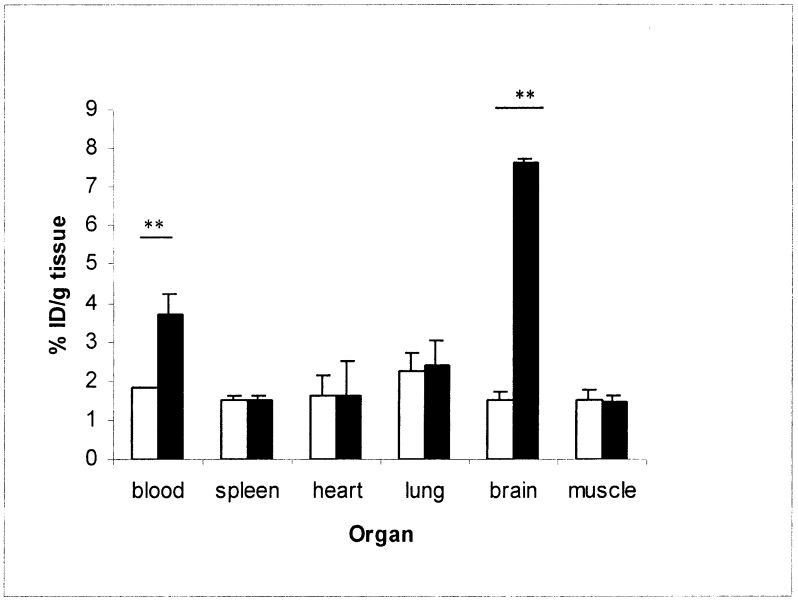

Fig. 2. Biodistribution of [^{3}H] FMZ (black box) and [^{18}F] FEF (white box) in mice, radioactivity found in the organ five minutes after injection.

3.3. Comparison in the biodistribution of [^{18}F]FEF and [^{3}H]FMZ

The biodistribution of [^{18}F]FEF contrasts markedly with the behavior of [^{3}H]FMZ which was chosen as reference compound. The distribution of [^{3}H]FMZ and [^{18}F]FEF was comparable in various organs (Fig. 2), which are nonspecific binding organ, but we observed a significant difference in the brain. In that organ, the accumulation of [^{3}H]FMZ was more than three times higher than for [^{18}F]FEF. It must be mentioned that the blood level for [^{3}H]FMZ is higher than for [^{18}F]FEF.

3.4. Metabolism pattern in mice

Analyses of blood and urine samples in mice showed a rapid and important in vivo biotransformation. A typical radiochromatogram of serum analysis (Fig. 3) shows two, sometimes three radiolabeled metabolites. These accounts for up to 60% of the total radioactivity recovered by HPLC. These hydrophilic metabolites are rapidly eliminated through urine, and no intact [^{18}F]FEF was found in urine (data not shown). According to our previous study using HPLC-MS-MS, these metabolites correspond to compounds for which the ester lateral chain is hydroxylated, and the diazo ring N-alkyl chain is also modified. Analyses of brain homogenates showed that only the intact [^{18}F]FEF was present in the CNS (Fig. 3). Metabolites clearly do not cross the blood brain barrier.

3.5. Influence of metabolism inhibition on the biodistribution

In order to assess the influence of the metabolism on the biodistribution pattern, we used different treatments aimed at inhibiting CYP450 dependent metabolism, using cimetidine and ketoconazole, or at decreasing the number of hepatocytes (chemical hepatitis induced by acetaminophen).

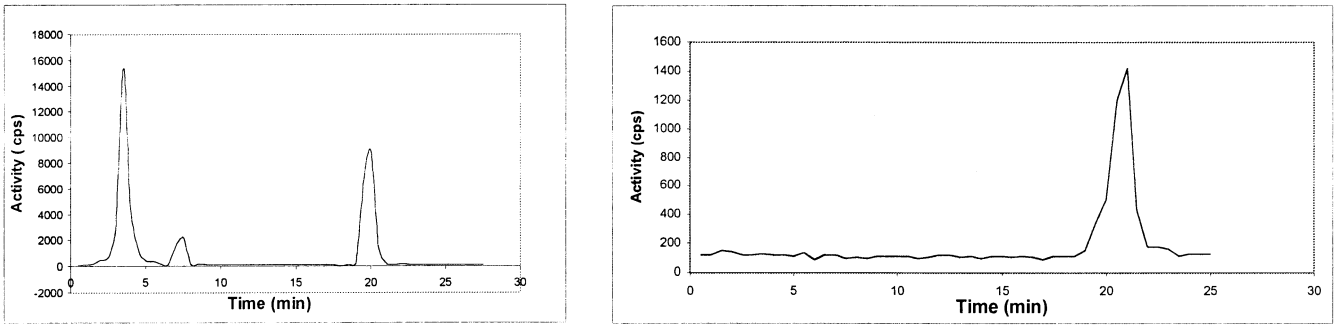

Fig. 3. Left: typical radiochromatogram of plasma in mice 3 minutes post injection. Right: typical radiochromatogram of brain homogenate in mice 3 minutes post injection. The peaks with Rt of approximately 20 minutes correspond to intact [^{18}F]FEF.

Table 3
% change of [^{18}F] FEF in various organs after pre-treatment of mice with inhibitors of metabolism (compared to control mice)

Organ	Treatment		
	Cimetidine	Ketoconazole	Acetaminophen
Blood	+14% (N.S.)	−23% (N.S.)	+74% (**)
Liver	−15% (N.S.)	−43% (*)	−45% (**)
Kidneys	−33% (N.S.)	−13% (N.S.)	+25% (N.S.)
Brain	−8% (N.S.)	+15% (N.S.)	+51% (**)

N.S.: non significant.
* $p < 0.0083$ (ANOVA/Bonferroni corrected t-test).
** $p < 0.01$ (ANOVA).

The modification of the accumulation of [^{18}F]FEF in critical organs is shown in Table 3.

Pre-treatment of mice by ketoconazole led to a significant decrease of the radioactivity found in the liver (ANOVA one way analysis/Bonferroni corrected T-test for multiple comparison, $p < 0.0083$). However, no significant change was found in the blood and in the brain. Cimetidine had no statistically significant effect. Interestingly, hepatitis due to acetaminophen led to more dramatic changes in the distribution of the radioactivity among the different organs: a significant increase of the [^{18}F]FEF was found in the blood as well as in the brain (ANOVA analysis, $p < 0.01$).

4. Discussion

Overall, the present study carried out in rodents clearly demonstrates that the [^{18}F]FEF binds specifically to the benzodiazepine receptors of the brain, as it is selectively inhibited by cold Flumazenil (Fig. 1). We also found that [^{18}F]FEF is rapidly metabolized in more hydrophilic labeled compounds, which are eliminated in the urine (Fig. 3). These metabolites do not cross the blood brain barrier as they were not found in the brain (Fig. 3). Therefore, the radioactivity found in the brain is only due to the intact [^{18}F]FEF. This intense metabolism (less than 40% of [^{18}F]FEF remaining intact three minutes after injection) is responsible for a lower accumulation in the brain compared to Flumazenil (Fig. 2). Interestingly, the metabolism can be significantly inhibited by an hepatitis model, with a consequent increase of the intact compound in the circulating blood as well as in the brain (Table 3).

That pattern was not observed after pre-treatment of mice by two CYP-450 inhibitors, cimetidine and ketoconazole. According to these results, we can assume that the liver plays an important role in the metabolism of [^{18}F] FEF, but is not significantly dependent on the CYP450 isoforms inhibited by cimetidine and ketoconazole. In our previous study describing the metabolites of FEF, we found that one major metabolite was a hydroxylated form of FEF with the hydroxyl group on the lateral chain. As this type of metabolite is not classically found in the class of benzodiazepine, it is possible that the metabolism leading to this compound is not depending on the cytochrom CYP450 isoforms inhibited by cimetidine and ketoconazole. Further studies need to be carried out to determine the specific enzymes responsible of the metabolism of [^{18}F] FEF.

In a previous work, we showed that Fluoroethylflumazenil metabolism is more extensive in rat microsomes than in human microsomes [11]. As the metabolism is depending on the species, it is possible that the degradation of [^{18}F] FEF could be less rapid and less extensive in humans. First studies on human volunteers with competition studies will definitively assess the usefulness of this tracer to quantify benzodiazepine receptors in humans.

Acknowledgments

Desmethylflumazenil was a kind gift of Hoffmann-La Roche, Basel, Switzerland.

References

[1] D.C. Davis, W.Z. Potter, D.J. Jollow, J.R. Mitchell, Species differences in hepatic glutathione depletion, covalent binding and hepatic necrosis after acetaminophen, Life Sci. 4 (11) (1974) 2099–2109.
[2] D. Debruyne, P. Abadie, L. Barre, F. Albessard, M. Moulin, E. Zarifian, J.C. Baron, Plasma pharmacokinetics and metabolism of the benzodiazepine antagonist [11C] Ro 15–1788 (flumazenil) in baboon and human during positron emission tomography studies, Eur. J. Drug Metab. Pharmacokinet. 16 (2) (1991) 141–152.
[3] J. Delforge, A. Syrota, M. Bottlaender, M. Varastet, C. Loc'h, B. Bendriem, C. Crouzel, E. Brouillet, M. Maziere, Modeling analysis of [11C]flumazenil kinetics studied by PET: Application to a critical study of the equilibrium approaches, J. Cereb. Blood Flow Metab. 13 (3) (1993) 454–468.
[4] J. Delforge, L. Spelle, B. Bendriem, Y. Samson, M. Bottlaender, S. Papageorgiou, A. Syrota, Quantitation of benzodiazepine receptors in human brain using the partial saturation method, J. Nucl. Med. 37 (1) (1996) 5–11.
[5] P.V. Desmond, R. Patwardhan, R. Parker, S. Schenker, K.V. Speeg Jr., Effect of cimetidine and other antihistaminics on the elimination of aminopyrine, phenacetin and caffeine, Life Sci. 26 (15) (1980) 1261–1268.
[6] J.M. Fahey, G.A. Pritchard, L.L. Moltke, J.S. Pratt, J.M. Grassi, R.I. Shader, D.J. Greenblatt, Effects of ketoconazole on triazolam pharmacokinetics, pharmacodynamics and benzodiazepine receptor binding in mice, J. Pharmacol. Exp. Ther. 285 (1) (1998) 271–276.
[7] P. Hantraye, M. Kaijima, C. Prenant, B. Guibert, J. Sastre, M. Crouzel, R. Naquet, D. Comar, M. Maziere, Central type benzodiazepine binding sites: A positron emission tomography study in the baboon's brain, Neurosci. Lett. 27, 48 (2) (1984) 115–120.
[8] M. Iyo, T. Itoh, T. Yamasaki, H. Fukuda, O. Inoue, H. Shinotoh, K. Suzuki, S. Fukui, Y. Tateno, Quantitative in vivo analysis of benzodiazepine binding sites in the human brain using positron emission tomography, Neuropharmacology 30 (3) (1991) 207–215.
[9] R.A. Koeppe, V.A. Holthoff, K.A. Frey, M.R. Kilbourn, D.E. Kuhl, Compartmental analysis of [11C]flumazenil kinetics for the estimation of ligand transport rate and receptor distribution using positron emission tomography, J. Cereb. Blood Flow Metab. 11 (5) (1991) 735–744.

[10] M.J. Koepp, A. Hammers, C. Labbe, F.G. Woermann, D.J. Brooks, J.S. Duncan, 11C-flumazenil PET in patients with refractory temporal lobe epilepsy and normal MRI, Neurology 25, 54 (2) (2000) 332–339.
[11] Ph. Leveque, E. de Hoffmann, D. Labar, B. Gallez, Assesment of [^{18}F]fluoroethylflumazenil metabolites using high performance liquid chromatography and tandem mass spectrometry, J. Chromatogr. B 754 (2001) 35–44.
[12] C. Loc'h, Ph. Hantraye, M. Khalili Varasteh, B. Maziere, J. Delforge, E. Brouillet, A. Syrota, M. Maziere, C-11 Flumazenil metabolism: Analysis of unchanged ligand in baboon plasma, J. Nucl. Med. 31 (1990) 897.
[13] C. Loc'h, Ph. Hantraye, M. Khalili Varasteh, B. Maziere, J. Delforge, E. Brouillet, A. Syrota, M. Maziere, J. Labelled Cpd. Radiopharm. 30 (1991) 234.
[14] M. Maziere, P. Hantraye, C. Prenant, J. Sastre, D. Comar, Synthesis of ethyl 8-fluoro-5,6-dihydro-5-[11C]methyl-6-oxo-4H-imidazo [1,5-a] [1,4]benzodiazepine-3-carboxylate (RO 15.1788–11C): A specific radioligand for the in vivo study of central benzodiazepine receptors by positron emission tomography, Int. J. Appl. Radiat. Isot. 35 (10) (1984) 973–976.
[15] S.M. Moerlein, N-w-[F-18]fluoroalkylated flumazenil: Potential agent for mapping cerebral benzodiazepines recpetors with PET, J. Nucl. Med. 31 (1990) 902.
[16] S.M. Moerlein, J.S. Perlmutter, Binding of 5-(2′-[18F]fluoroethyl) flumazenil to central benzodiazepine receptors measured in living baboon by positron emission tomography, Eur. J. Pharmacol. 21, 218 (1) (1992) 109–115.
[17] F. Nagy, D.C. Chugani, C. Juhasz, E.A. da Silva, O. Muzik, W. Kupsky, A. Canady, C. Watson, J. Shah, H.T. Chugani, Altered in vitro and in vivo flumazenil binding in human epileptogenic neocortex, J. Cereb. Blood Flow Metab. 19 (9) (1999) 939–947.
[18] I. Savic, L. Widen, J.O. Thorell, G. Blomqvist, K. Ericson, P. Roland, Cortical benzodiazepine receptor binding in patients with generalized and partial epilepsy, Epilepsia 31 (6) (1990) 724–730.
[19] S. Sanabria-Bohorquez, D. Labar, Ph. Leveque, A. Bol, A.G. De Volder, C. Michel, C. Veraart, [11C] Flumazenil metabolite measurement in plasma is not necessary for accurate brain benzodiazepine receptor quantification, Eur. J. Nucl. Med. 27 (2000) 1674–1683.
[20] K.V. Speeg Jr., R.V. Patwardhan, G.R. Avant, M.C. Mitchell, S. Schenker, Inhibition of microsomal drug metabolism by histamine H2-receptor antagonists studied in vivo and in vitro in rodents, Gastroenterology 82 (1) (1982) 89–96.
[21] L.L. von Moltke, D.J. Greenblatt, J. Schmider, S.X. Duan, C.E. Wright, J.S. Harmatz, R.I. Shader, Midazolam hydroxylation by human liver microsomes in vitro: Inhibition by fluoxetine, norfluoxetine, and by azole antifungal agents, J. Clin. Pharmacol. 36 (9) (1996) 783–791.