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Quantification of human brain benzodiazepine receptors using [18F]fluoroethylflumazenil: a first report in volunteers and epileptic patients

Philippe Levêque[1, 2], Sandra Sanabria-Bohorquez[3], Anne Bol[1], Anne De Volder[1], Daniel Labar[1], K. Van Rijckevorsel[4], Bernard Gallez[2, 5]

[1] Unité de tomographie par positrons, Université catholique de Louvain, Louvain-la-Neuve, Belgium
[2] Unité de chimie pharmaceutique et de radiopharmacie, CMFA/REMA, Université catholique de Louvain, Bruxelles, Belgium
[3] Imaging Research, Merck Research Laboratories, West Point, Philadelphia, USA
[4] Service de neurologie, Cliniques Universitaires Saint-Luc, Bruxelles, Belgium
[5] Unité de résonance magnétique biomédicale, Université catholique de Louvain, Bruxelles, Belgium

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Abstract. Fluorine-18 fluoroethylflumazenil ([18F]FEF) is a tracer for central benzodiazepine (BZ) receptors which is proposed as an alternative to carbon-11 flumazenil for in vivo imaging using positron emission tomography (PET) in humans. In this study, [18F]FEF kinetic data were acquired using a 60-min two-injection protocol on three normal subjects and two patients suffering from mesiotemporal epilepsy as demonstrated by abnormal magnetic resonance imaging and [18F]fluorodeoxyglucose positron emission tomography. First, a tracer bolus injection was performed and [18F]FEF rapidly distributed in the brain according to the known BZ receptor distribution. Thirty minutes later a displacement injection of 0.01 mg/kg of unlabelled flumazenil was performed. Activity was rapidly displaced from all BZ receptor regions demonstrating the specific binding of [18F]FEF. No displacement was observed in the pons. Plasma input function was obtained from arterial blood sampling, and metabolite analysis was performed by high-performance liquid chromatography. Metabolite quantification revealed a fast decrease in tracer plasma concentration, such that at 5 min post injection about 70% of the total radioactivity in plasma corresponded to [18F]FEF, reaching 24% at 30 min post injection. The interactions between [18F]FEF and BZ receptors were described using linear compartmental models with plasma input and reference tissue approaches. Binding potential values were in agreement with the known distribution of BZ receptors in human brain. Finally, in two patients with mesiotemporal sclerosis, reduced uptake of [18F]FEF was clearly observed in the implicated left hippocampus.

Keywords: Fluoroethylflumazenil – Flumazenil – Benzodiazepine receptor – Positron emission tomography – Epilepsy

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Bernard Gallez (✉)
Unité de chimie pharmaceutique et de radiopharmacie,
CMFA/REMA, Université catholique de Louvain,
73-40 Avenue Mounier, 1200 Bruxelles, Belgium
e-mail: Gallez@cmfa.ucl.ac.be
Fax: +32-2-7642790

Introduction

The GABA-A receptor has been reported to be a potential marker of various pathological states, such as Parkinson's disease, Huntington's chorea, Alzheimer's disease and epilepsy. Its distribution can be evaluated in nuclear medicine either with the single-photon emission tomography tracer iodine-123 iomazenil, or with the positron emission tomography (PET) tracer carbon-11 flumazenil ([11C]FMZ). As an alternative to [11C]FMZ, the fluorinated analogue of flumazenil, fluorine-18 fluoroethylflumazenil ([18F]FEF), was first proposed by Moerlein and Perlmutter [1]. [18F]FEF has a good affinity for the benzodiazepine (BZ) receptor, and was used for in vivo imaging of the BZ receptor in the baboon [2]. In addition, due to its longer half-life, [18F]FEF can be synthesised outside the hospital, thus avoiding the need for an on-site cyclotron facility. As initial steps in the preclinical validation process, we previously investigated the in vitro metabolism of [18F]FEF [3]. We also reported its biodistribution and binding specificity in rodents, and observed a rapid biotransformation in vivo [4]. The use of [18F]FEF in human brain has recently been reported by

Gründer et al. [5]. These authors demonstrated the specificity of [^{18}F]FEF binding in vitro and in vivo, and applied spectral analysis using plasma input function to assess the BZ receptor distribution in the human brain for the first time.

In the present study, we describe the blood and brain kinetics of [^{18}F]FEF in healthy human volunteers and in two patients suffering from mesiotemporal lobe epilepsy. The specificity of [^{18}F]FEF binding to BZ receptors was investigated, as was the pattern of metabolisation and its influence on the modelling processes.

The interactions between [^{18}F]FEF and BZ receptors were described using linear compartment models with both plasma input function [6] and reference tissue approaches [7, 8] for modelling of the kinetic PET data. Finally, we report the usefulness of [^{18}F]FEF in delineating the abnormal brain area implicated in mesiotemporal lobe epilepsy, as compared with [^{18}F]fluorodeoxyglucose (FDG) PET and magnetic resonance imaging (MRI).

Materials and methods

Synthesis of [18F]FEF. [^{18}F]FEF was synthesised according to the method proposed by Moerlein and Perlmutter [1].

Subjects. Three healthy male volunteers aged 21, 22 and 23 years gave their informed written consent to undergo this study. The protocol was approved by the local ethics committee and complied with the Declaration of Helsinki. None of the volunteers was under any medication at the time of the study or during the previous five days. Two female patients (aged 26 and 21 years) also participated in this study. They were suffering from temporal lobe epilepsy due to mesiotemporal sclerosis ascertained by high-resolution MRI (atrophy of the left hippocampal region). A [^{18}F]FDG PET scan showed the classical pattern of temporal hypometabolism on the left side.

Experimental protocol. The experimental protocol lasted 60 min and consisted of two injections. An injection of 314±17 MBq of [^{18}F]FEF was performed, followed 30 min later by a displacement injection of cold flumazenil (Anexate, 0.01 mg/kg).

Typical specific activity of [^{18}F]FEF was 37±17 GBq/μmol. [^{18}F]FEF and flumazenil were injected, each as a 30-s bolus, through a 22-gauge catheter (Abbocath) inserted in a forearm vein. Arterial blood samples (~2 ml) were withdrawn manually from the opposite arm. After centrifugation of the samples, plasma was collected and counted in an NaI well counter (Wallac-Oy, Turku, Finland), cross-calibrated with the PET camera. Counts were corrected for ^{18}F decay, and expressed in μCi/ml.

Metabolite analysis. Metabolite analysis was performed on plasma samples at selected time points (1, 2, 5, 7, 10, 20 and 30 min) in order to assess the amount of unchanged [^{18}F]FEF in arterial blood. We used the high-performance liquid chromatography (HPLC) method we had developed for the analysis of metabolites in mice [3].

PET and MRI. PET studies were performed using the ECAT EXACT HR tomograph (Siemens/CTI, Knoxville, TN, USA) in three-dimensional (3D) mode, with a total axial field of view of 15 cm. Prior to tracer injection, a 15-min transmission scan was performed using germanium sources. After each injection, dynamic emission scans were obtained in a sequence of 16 frames (8×15 s, 3×60 s and 5×300 s), for a total acquisition time of 60 min. Forty-seven contiguous transaxial slices were reconstructed with a voxel size of 2 mm in the tomographic direction, and 3.125 mm in the axial direction.

[^{18}F]FDG scans were performed on a separate day with an injection ranging from 240 to 270 MBq.

T1-weighted MR images were obtained on a 1.5-T GE Signa unit using a fast spin echo technique. They were obtained in the bicommissural (AC-PC) orientation.

For each subject, MR images were co-registered to a summed PET image obtained from the first 16 frames, and then resampled to the PET voxel size, using an interactive home-made image display software (iv_align) [9, 10]. Regions of interest (ROIs) were then drawn on the PET images based on anatomical landmarks from MR images, and taking into account the activity distribution. This process was performed with the image analysis program "Mediman" [11]. Volumes of interest were calculated by assembling 2D ROIs drawn on consecutive planes on frontal, parietal, lateral temporal, mesiotemporal and occipital cortices, as well as on the pons, cerebellum and thalamus.

Data analysis: kinetic modeling. Interactions between [^{18}F]FEF and BZ receptors were described using linear models with both the plasma input function model [6] and the generalised reference tissue model (GRTM) [7, 12]. In addition, Logan graphical analysis using a reference region was also applied to estimate [^{18}F]FEF distribution volume ratios [8]. The system was described by a two-compartment model with three parameters, the two rate constants K_1 and k_2 and the vascular contribution [6]. The information on receptor concentration was obtained from the apparent distribution volume, K_1/k_2.

The reference tissue approach constitutes a good alternative that avoids the need for blood sampling by taking advantage of the negligible BZ receptor concentration in the pons. When data were analysed using GRTM, reversible [^{18}F]FEF kinetics were assumed in both reference and target regions. The general equation for the reference tissue input compartmental model is given by [12]:

$$C_T(t) = \mathrm{R}_I C_R(t) + \sum_{i=1}^{m+n-1} \phi_i e^{\theta_i t} \otimes C_R(t) \quad (1)$$

where m and n are the total number of tissue compartments in the reference and target tissues, respectively, and ϕ_i and θ_i are the parameters of the system impulse response function. C_T and C_R are the tracer concentration courses in the target and reference tissues, respectively, and R_I is the tracer delivery between the target and reference regions. The [^{18}F]FEF volume of distribution ratio (DVR) can be obtained from the integral of the impulse response function [7, 12]:

$$\mathrm{DVR} = \mathrm{R}_I + \sum_{i=1}^{m+n-1} \frac{\phi_i}{\theta_i} \quad (2)$$

In Logan graphical analysis [8], DVR was calculated with a linear regression from 10 to 30 min according to the following equation:

$$\frac{\int_0^t C_T(\tau)d\tau}{C_T(t)} \approx \mathrm{DVR}\frac{\int_0^t C_R(\tau)d\tau}{C_T(t)} + c \quad (3)$$

The vascular contribution was neglected when using both reference tissue models.

Results

Blood kinetics

After intravenous bolus injection of [^{18}F]FEF, the total plasma activity rapidly increased and reached its maximum level after less than 1 min (52±6 s) (Fig. 1, *top*). The distribution phase was very rapid, with a half-life of less than 30±7 s. The blood activity level stabilised after 5 min, then slowly increased again to reach a second plateau 10 min after injection. This second mild rise in total blood activity did not seem to have any influence on the brain time-activity curves. The same kind of pattern was observed in all subjects. In contrast, the blood curve corrected for metabolites did not show any further increase around t=10 min, and a regular exponential decrease was observed (Fig. 1, *top*).

Metabolite analysis

Metabolite analysis showed that [^{18}F]FEF was rapidly transformed, following a mono-exponential decay, with a half-life of 8.2±1.2 min. Unmetabolised [^{18}F]FEF accounted for 50% of the plasmatic circulating dose after less than 10 min. After 20–25 min, more than 70% of plasmatic activity was due to metabolites (Fig. 1, *centre*). The HPLC analysis showed the development of only one radiolabelled metabolite (Fig. 1, *bottom*). In the present study, intact [^{18}F]FEF was absent in urine, where only one metabolite was found (data not shown).

Brain kinetics and specificity of binding

Tracer uptake in the brain reached its maximum level around 2.5 min and then cleared rapidly following a mono-exponential elimination (Fig. 2). Relative uptake and elimination half-life were consistent with the known distribution of BZ receptors. High uptake and a long elimination half-life (8.4±2.6 min, ranging from 5.8 to 11.9 min) were observed in receptor-rich occipital cortex, whereas reduced uptake and a short half-life (5.5±0.3 min, ranging from 5.2 to 5.9 min) were observed in the pons.

Injection of a high dose of cold flumazenil (0.01 mg/kg) as a competitor for BZ receptor occupancy triggered a fast wash-out of the radiotracer from its binding sites (Fig. 2). This displacement was particularly evident in receptor-rich regions (neocortex such as occipital, frontal or temporal areas), where the specific signal was relatively bigger than in the thalamus and cerebellum (Fig. 3). The negligible specific binding in the pons [13] was confirmed by the lack of displacement observed at 30 min in the subjects who received the second injection of cold flumazenil (Fig. 2). After displacement, activity levels in the different brain regions were of the same magnitude, and reached the low binding level observed in the pons.

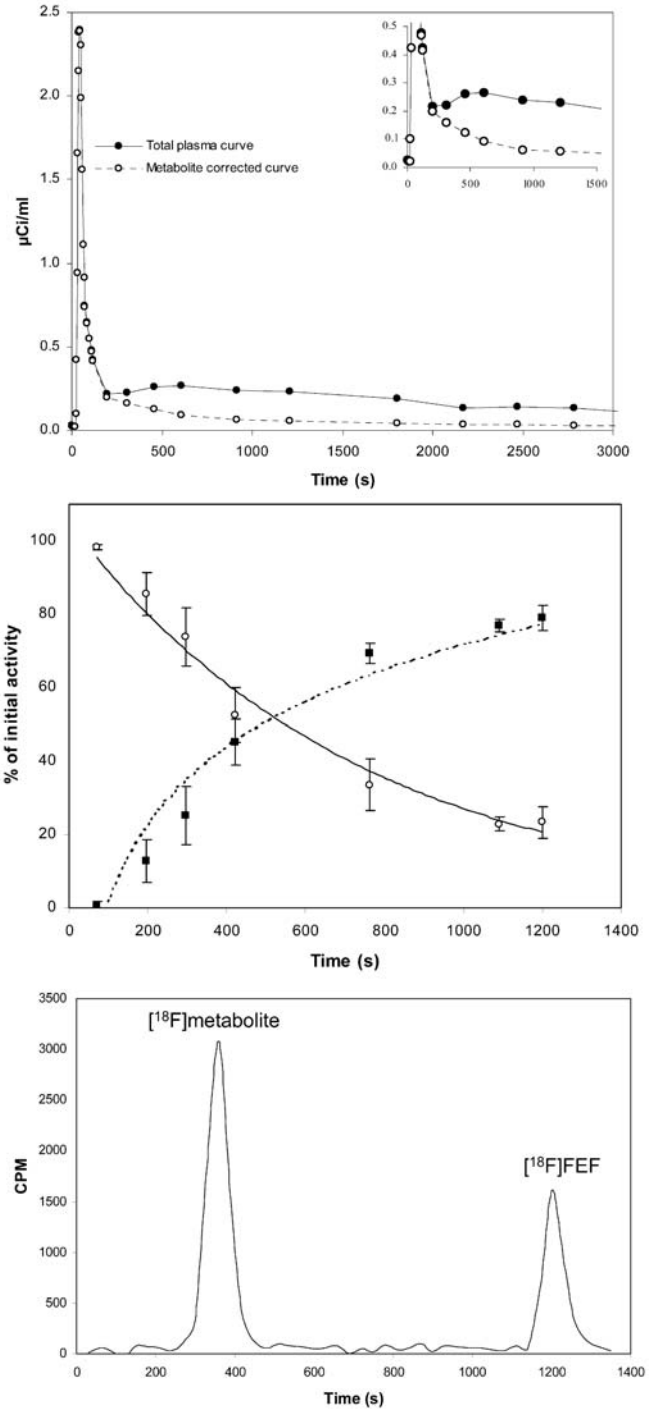

Fig. 1. *Top:* Typical total plasma curve (*solid line*) and curve corrected for metabolites (*dashed line*). Both curves are corrected for decay. *Centre:* Kinetics of metabolite appearance (and [^{18}F]FEF disappearance). *Points* are mean±SD, and *lines* are fitted curves (the *solid line* represents [^{18}F]FEF and the *dashed line*, metabolites). *Bottom*: Metabolite analysis using radio-HPLC. Typical radiochromatogram from plasma sample obtained 10 min after tracer injection

Imaging

A typical set of images is presented in Fig. 4, obtained in one of the patients suffering from mesiotemporal lobe epilepsy. In this patient, the anatomical T1-weighted MR images showed clear atrophy of the left hippocampus. The corresponding slices from interictal co-registered [^{18}F]FDG PET showed a marked hypometabolism in the left mesiotemporal area, as well as in the whole left temporal lobe. This extensive hypometabolism in structurally intact regions is usually attributed to remote functional de-afferentation (diaschisis) in the ipsilateral temporal neocortex. In the same patient, the regional distribution of [^{18}F]FEF showed a more precise pattern, with a decreased binding that was restricted to the mesiotemporal area (hippocampus) without a remote effect in the ipsilateral temporal neocortex. The same observations applied to the second patient.

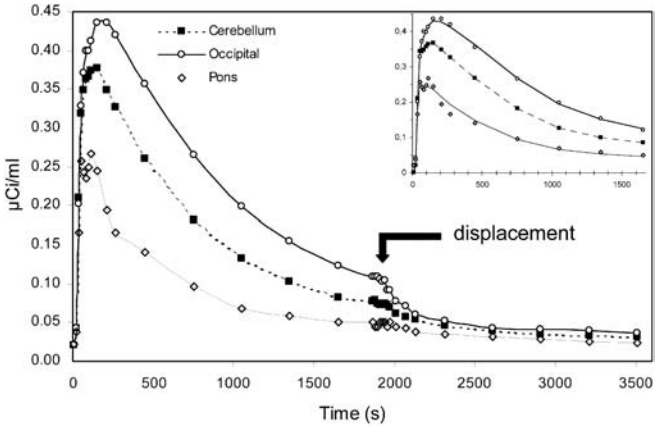

Fig. 2. Typical total activity curves in three representative brain regions, after bolus injection of [^{18}F]FEF. Displacement with cold flumazenil was performed after 30 min (*arrow*). The pons shows a lack of displacement. The enlargement shows the corresponding fitted curves (two-compartment model)

Kinetic modelling results

The parameter estimates obtained using the proposed models are shown in Table 1. Using the plasma input function approach with the two-compartment model, the

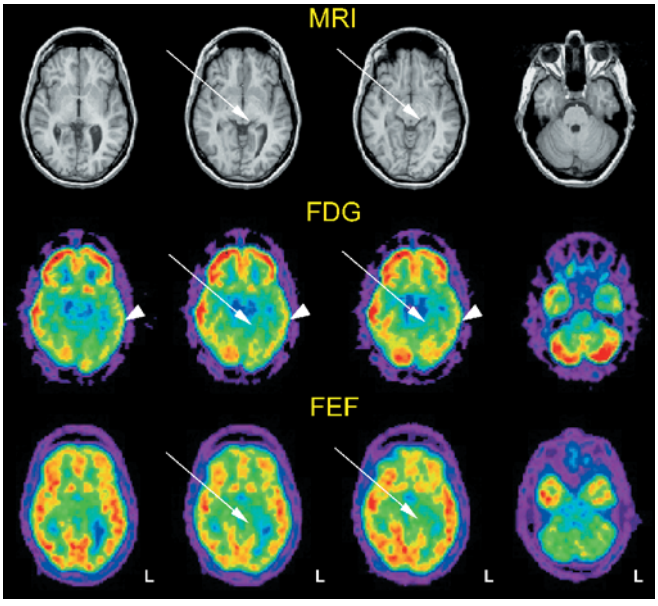

Fig. 4. Typical set of MR images and the corresponding interictal [^{18}F]FDG and [^{18}F]FEF scans from a patient with temporal epilepsy due to mesiotemporal sclerosis. Three contiguous axial slices at the level of the hippocampus and a remote one at the level of the cerebellar are shown. The left side of the brain is on the *right*, as indicated. *Top row:* MR images (T1-weighted) show hippocampal atrophy on the left side (*arrows*) without any other lesion. *Middle row:* the co-registered interictal [^{18}F]FDG PET demonstrates hypometabolism (*arrows*) as well as remote dysfunction in the interconnected, ipsilateral, neocortical areas (*arrowheads*). *Bottom row:* a clear asymmetry of [^{18}F]FEF binding is demonstrated in the left hippocampal area (*arrows*), corresponding to the structural lesion

Fig. 3. Binding of [^{18}F]FEF in several brain regions, before (*black boxes*) and after (*white boxes*) injection of flumazenil competitor. Data are mean±SD for all subjects, and normalised to 100%

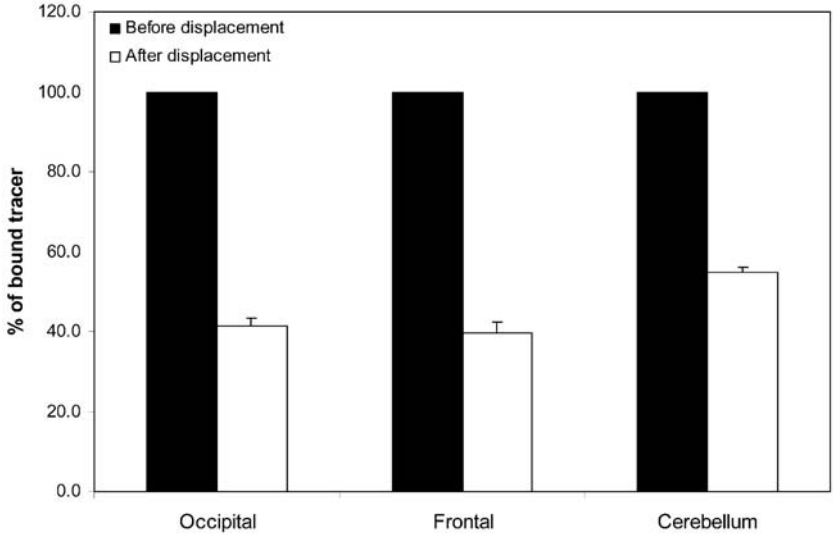

Table 1. Parameter estimates in different brain regions, obtained using a two-compartment model with plasma input function (2-comp plasma, left three columns), a generalised reference tissue model (GRTM) or graphical analysis (Logan, last column)

	2-comp plasma			GRTM	Logan
	K_1 (min^{-1})	DV	BP	BP	BP
Cerebellum	0.28±0.04	0.98±0.13	0.76±0.16	0.48±0.165	0.49±0.17
Frontal right	0.28±0.03	1.33±0.20	1.32±0.28	0.88±0.23	0.92±0.23
Frontal left	0.28±0.03	1.31±0.20	1.30±0.28	0.85±0.23	0.89±0.23
Insula right	0.29±0.03	1.42±0.20	1.50±0.30	1.04±0.25	1.09±0.25
Insula left	0.27±0.03	1.36±0.22	1.39±0.33	0.94±0.27	0.98±0.27
Occipital right	0.30±0.03	1.44±0.23	1.52±0.30	1.02±0.25	1.06±0.26
Occipital left	0.29±0.04	1.40±0.21	1.45±0.32	1.01±0.24	1.06±0.24
Parietal right	0.28±0.04	1.32±0.20	1.31±0.26	0.86±0.22	0.90±0.23
Parietal left	0.28±0.04	1.31±0.19	1.31±0.27	0.85±0.22	0.89±0.22
Pons	0.18±0.03	0.57±0.06	n/a	–	–
Thalamus	0.31±0.05	1.00±0.12	0.76±0.16	0.49±0.16	0.50±0.16
Temporal right	0.28±0.03	1.38±0.23	1.41±0.33	0.93±0.25	0.97±0.26
Temporal left	0.27±0.03	1.37±0.21	1.41±0.32	0.92±0.24	0.97±0.24

Values are mean±SD with SD obtained from the co-variance matrix. In the two-compartment model, BP values were estimated using the pons as reference: $BP=DV/DV_{ref}^{-1}$
n/a, Not applicable

Table 2. Distribution volume and binding potential for the mesiotemporal area in healthy volunteers and epileptic patients

		Healthy volunteers		Epileptic patients	
		Mesiotemporal right	Mesiotemporal left	Mesiotemporal right	Mesiotemporal left
2-comp PIF	DV	1.33±0.24	1.29±0.24	1.08±0.18	0.92±0.11
	BP	1.30±0.40	1.22±0.41	0.96±0.21	0.67±0.11
GRTM	BP	1.00±0.20	0.96±0.20	0.53±0.11	0.35±0.06
Logan	BP	0.96±0.25	0.89±0.26	0.58±0.12	0.38±0.07

Mean values ± SD are given for the different models used
2-comp PIF, Two-compartment model with plasma input function

highest DV values were obtained for the cerebral cortex regions, followed by the cerebellum, the thalamus and the pons. For all considered ROIs, K_1 and DV were accurately estimated, leading to a unique solution and small parameter estimation uncertainties. SD values obtained from the co-variance matrix were below 20% for DV, and mostly below 15% for K_1.

In this model, binding potential (BP) values were determined using the pons as reference region, with $BP=DV/DV_{ref}^{-1}$.

Using the GRTM and Logan graphical methods, BP values were determined as $BP=DVR^{-1}$. A very good agreement was found between BP estimates using both methods. However, BP(GRTM) and BP(Logan) appeared to be underestimated in comparison with the BP estimates using the plasma input model.

In all subjects, results were very similar for left and right homologous regions whichever model was used. BP values were also in good agreement among subjects, with a standard deviation of less than 20% in the major selected areas (Table 1).

In both epileptic patients, a sharp difference in BP was noted between the right (normal) and the left (atrophic) mesiotemporal area (Table 2). The decrease in BP values between the right and the left mesiotemporal area was only 5% (NS, Student's *t* test) in healthy volunteers, but reached 30% ($P<0.01$, Student's *t* test) in epileptic patients.

Discussion

As expected from previous findings in rodents [4], the distribution kinetics of [^{18}F]FEF are extremely rapid. This is observed not only for the blood kinetics, but also for the biotransformation rate (Fig. 1) and for the regional brain distribution (Fig. 2). It is likely that the fast biotransformation and elimination affect the maximum level of tracer uptake in the brain, as seen from the low DV values. Nevertheless, the images display a good contrast 10 min after injection, with an injected activity in the usual range for PET tracers (~370 MBq). On the other

hand, [18F]FEF brain kinetics are much faster than for [11C]FMZ, most probably reflecting a fast dissociation rate of the former from the BZ receptor.

At first sight, these features seem rather unfavourable. However, some interesting points must be highlighted. Firstly, the level of tracer uptake in the various selected brain areas follows the known density of BZ receptors (Table 1). Secondly, [18F]FEF binding in the brain can be considered as specific to the BZ receptors, since the displacement experiment with unlabelled flumazenil showed rapid elimination of the tracer in receptor-rich areas (Figs. 2, 3). This completes and confirms the findings of Gründer et al. [5], who used a different approach (presaturation), with very high doses of competitor.

The metabolisation rate in man was very rapid. Only one metabolite was found, and it is very likely identical to the one identified in mice (Fig. 1, bottom). We previously demonstrated that this metabolite is unable to cross the blood-brain barrier [4]. This is, of course, of crucial interest for the pertinence of studies using [18F]FEF. Moreover, although there is a clear increase in total blood activity after 10 min (Fig. 1, top), probably due to metabolites, such a rise is not observed in any of the brain regions (Fig. 2). Furthermore, this rise disappears when the blood curve is corrected for metabolites. One can reasonably conclude that metabolites do not interfere with the measured brain activity, which only reflects unmetabolised radiotracer uptake.

We applied plasma input function and reference tissue approaches to quantify the binding potential of [18F]FEF to BZ receptors. The plasma input function model requires the determination of the concentration of ligand in plasma and metabolite analysis during PET acquisition. The rapid clearance of [18F]FEF from tissue allowed robust DV estimates to be obtained using a two-compartment model and 30-min acquisition data. In addition, our DV estimates were very similar to those recently reported in the literature using spectral analysis [5].

The second approach presented here uses a reference tissue region devoid of receptors instead of plasma input function. Given the low receptor concentration in the pons [13] and the lack of displacement in this region after injection of cold flumazenil (Fig. 2), we used this region as reference tissue. Although BP values obtained from GRTM or Logan plot were underestimated as compared with the plasma input BP values (R^2=0.84 and 0.90, respectively), the tight correlation (R^2=0.97) between BP(GRTM) or BP(Logan) and BP(2-comp) indicates that the reference region models offer a less invasive alternative for BZ quantification using [18F]FEF. These BP values can be directly compared to results published previously using spectral analysis and are in the same range [5]. The comparison with BP values obtained with [11C]FMZ is much more difficult because BP is a relative index, not an absolute one. In the occipital cortex, BPs with [11C]FMZ are reported to be between 4.79 and 5.74. Using [18F]FEF, BP is markedly lower (1.52±0.30). This is not surprising considering the different kinetics observed between the two tracers. Moreover, other parameters such as protein binding, non-specific binding and rate of brain uptake, which were not individually investigated here, also affect BP results. Therefore it is difficult to make a direct comparison between the two tracers using BP. Nevertheless, the same phenomenon is observed with both tracers, with the highest BP values in the occipital cortex and the lowest in the thalamus. A method that allows absolute determination of K_d and B_{max} would be of great interest for a direct comparison between the two tracers.

In the study with patients, whichever model was used, BP values allowed identification of the pathological state in the hippocampal region. In the two epileptic patients studied with [18F]FEF, the calculated asymmetry was about 30%; it must be remembered that using FDG, an asymmetry of 15% is considered a significant level, above which the pathological state is clearly demonstrated [14].

Conclusion

Although [18F]FEF biotransformation and distribution kinetics are markedly faster in vivo than for [11C]FMZ, [18F]FEF shows a distribution pattern very similar to that of [11C]FMZ. Because binding of [18F]FEF to the BZ receptor is specific, it is possible to quantify the BZ receptor distribution using various models, and without any influence of the tracer metabolite. Together with the first data obtained in two patients suffering from mesiotemporal epilepsy, these results demonstrate that [18F]FEF may be further investigated as an alternative to [11C]FMZ for study of the distribution of BZ receptors in vivo in man, and more particularly in cases of mesiotemporal epilepsy.

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