[18F]Fluoroethylflumazenil: a novel tracer for PET imaging of human benzodiazepine receptors

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Abstract. 5-(2′-[18F]Fluoroethyl)flumazenil ([18F]FEF) is a fluorine-18 labelled positron emission tomography (PET) tracer for central benzodiazepine receptors. Compared with the established [11C]flumazenil, it has the advantage of the longer half-life of the fluorine-18 label. After optimisation of its synthesis and determination of its in vitro receptor affinities, we performed first PET studies in humans. PET studies in seven healthy human volunteers were performed on a Siemens ECAT EXACT whole-body scanner after injection of 100–280 MBq [18F]FEF. In two subjects, a second PET scan was conducted after pretreatment with unlabelled flumazenil (1 mg or 2.5 mg i.v., 3 min before tracer injection). A third subject was studied both with [18F]FEF and with [11C]flumazenil. Brain radioactivity was measured for 60–90 min p.i. and analysed with a region of interest-oriented approach and on a voxelwise basis with spectral analysis. Plasma radioactivity was determined from arterial blood samples and metabolites were determined by high-performance liquid chromatography. In human brain, maximum radioactivity accumulation was observed 4±2 min p.i., with a fast clearance kinetics resulting in 50% and 20% of maximal activities at about 10 and 30 min, respectively. [18F]FEF uptake followed the known central benzodiazepine receptor distribution in the human brain (occipital cortex >temporal cortex >cerebellum >thalamus >pons). Pretreatment with unlabelled flumazenil resulted in reduced tracer uptake in all brain areas except for receptor-free reference regions like the pons. Parametric images of distribution volume and binding potential generated on a voxelwise basis revealed two- to three-fold lower in vivo receptor binding of [18F]FEF compared with [11C]flumazenil, while relative uptake of [18F]FEF was higher in the cerebellum, most likely owing to its relatively higher affinity for benzodiazepine receptors containing the α6 subunit. Metabolism of [18F]FEF was very rapid. Polar metabolites represented about 50%–60% of total plasma radioactivity at 5 min and 80%–90% at 20 min p.i. Although [11C]flumazenil has some advantages over [18F]FEF (higher affinity, slower metabolism, slower kinetics), our results indicate that [18F]FEF is a suitable PET ligand for quantitative assessment of central benzodiazepine receptors, which can be used independently of an on-site cyclotron.

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

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Introduction

Benzodiazepines are used for their anxiolytic, anticonvulsant, muscle-relaxant and sedative-hypnotic properties. The existence of a specific benzodiazepine receptor linked to the γ-aminobutyric acid (GABA) receptor/chloride ionophore has been demonstrated by means of tritiated diazepam [1]. The in vivo potency of benzodiazepines correlates with their affinity for central benzodiazepine receptors [2]. Alterations of benzodiazepine receptor densities have been described in post-mortem studies of several neuropsychiatric disorders including Alzheimer's disease [3], Huntington's disease [4] and schizophrenia [5, 6]. Positron emission tomography (PET) or single-photon emission tomography (SPET) methodolo-

gy has been used to demonstrate alterations in benzodiazepine receptor binding in various forms of epilepsy [7], Huntington's disease [8] and anxiety disorders [9].

After the first description of the potent benzodiazepine antagonist flumazenil (Ro 15-1788), the compound was initially labelled with carbon-11 for use in PET [10]. The successful labelling of Ro 16-0154 with iodine-123 for SPET ([^{123}I]iomazenil) was described shortly thereafter [11]. When binding affinity to benzodiazepine receptors is determined from human and monkey brain homogenates, iomazenil has a tenfold higher affinity for the benzodiazepine receptor than flumazenil (K_D 0.5 n*M* vs 5.8 n*M* [12]). Both compounds display high specific to non-specific binding ratios, with their regional brain uptake representing the known distribution of benzodiazepine receptors in the brain [2, 13]. Other ligands used for PET imaging of benzodiazepine receptors include the partial inverse benzodiazepine agonist [^{11}C]Ro 15-4513 [14] and the cyclopyrrolone derivative [^{11}C]suriclone [15]. 2′-[^{123}I]iododiazepam has been proposed for use in SPET [16].

The synthesis of a fluorinated analogue of flumazenil, 5-(2′-[^{18}F]fluoroethyl)flumazenil ([^{18}F]FEF) [17], has been previously reported. Our efforts to improve the synthesis and to determine the binding affinities to benzodiazepine receptor subtypes are described elsewhere [18; Lüddens et al., unpublished work]. Here we report on the first PET studies in healthy human volunteers and describe an approach for in vivo quantification of benzodiazepine receptor binding on a voxelwise basis suitable for this compound.

Materials and methods

Chemistry. 5-(2′-[^{18}F]fluoroethyl)flumazenil was synthesised as described in references [17, 19] following N-alkylation of the desmethyl-flumazenil precursor using [^{18}F]fluoroethyltosylate in a one-pot synthesis. In the present work, [^{18}F]fluoroethyltosylate was first separated by means of high-performance liquid chromatography (HPLC), followed by ^{18}F-N-fluoroalkylation using *N*-desmethyl-flumazenil, the latter being deprotonated using NaH. DMSO was used as the solvent and a temperature of 90°C with a reaction time of 10 min was applied. The solvent was isolated adsorbing the product on a C18 cartridge, then both [^{18}F]FEF and the precursor were eluted using ethanol (2 ml) [18; Lüddens et al., unpublished work].

[^{18}F]FEF was finally purified using HPLC (250×10 RP18, CH_3CN: 0.05 *M* Na_2HPO_4 40:60, 5 ml/min). The fraction of pure [^{18}F]FEF was isolated and adsorbed on a second C18 cartridge in order to remove the HPLC solvent system. The column was washed with 2 ml water and eluted using 10 ml of an ethanol:water mixture (1:9). The elute was sterile filtrated. Before injection, quality control was performed including chemical, radiochemical and radionuclidic purity, specific activity, pH and absence of pyrogens. For determination of chemical and radiochemical purity, HPLC was applied (250×4 RP18, CH_3CN:H_2O 55:45, 1 ml/min). [^{18}F]FEF was analysed as >99% pure with respect to any other radiochemical contamination.

The specific activity was analysed by means of the same HPLC technique using the calibrated 5-(2′-[^{19}F]fluoroethyl)flumazenil UV intensity. Specific activities were in the range between 6 and 10 GBq/μmol. Accordingly, the injected tracer mass for all studies was less than 10 μg. The volume of [^{18}F]FEF injected as ethanol:water mixture (1:9) was 5±1 ml.

[^{11}C]Flumazenil was labelled with carbon-11 according to the original method reported by Maziere et al. [10] with slight modifications described by Watkins et al. [20]. Briefly, precursor in DMSO was fixed on a self-made Teflon reacting column (ID 1.6 mm, length 30 mm) filled with Al_2O_3/KOH and dried with a stream of nitrogen. ^{11}C methylation was carried out by passing [^{11}C]methyliodide in a stream of nitrogen through the column. Subsequently, the column was eluted by a mixture of CH_2Cl_2/CH_3Cl (20/80, v/v). For purification purposes, the elute was passed through an alumina N-cartridge (Waters). After evaporation of the solvent, the residue was dissolved with 0.5 ml ethanol followed by 9.5 ml isotonic saline. The specific activity of the radiopharmaceutical used for this study was 19 GBq/μmol.

In vitro studies. Rat $GABA_A$ receptors of the configurations $\alpha1\beta1\gamma2$ and $\alpha6\beta1\gamma2$ were transiently expressed in human embryonic kidney cells (HEK 293) following standard procedures [21]. Steady-state binding of [^{3}H]Ro 15-4513, a benzodiazepine receptor ligand which binds non-selectively to all benzodiazepine receptor subtypes, was assessed in the presence of increasing concentrations of iomazenil and FEF, and compared with values for flumazenil obtained previously in our group with the same methodology [22]. Details are reported in a paper by Lüddens H et al., "Improved synthesis and in-vitro determination of benzodiazepine receptor affinities of 5-(2'-[^{18}F]fluoroethyl)flumazenil", that has been submitted for publication.

PET studies in humans. The study was approved by the local ethics committee and the radiation protection authorities.

Subjects. PET studies were performed in seven healthy male volunteers. All subjects gave written informed consent to participate in the study. Four subjects were scanned only once. In two other volunteers, a second PET scan was performed 4 weeks after the baseline scan. Three minutes before the second tracer injection, the subjects received 1 or 2.5 mg unlabelled flumazenil intravenously as a bolus. Two PET scans were also performed in the seventh subject. For comparison of the two radiotracers, he was scanned both with [^{11}C]flumazenil and, approximately 6 weeks later, with [^{18}F]FEF. While all PET studies with [^{18}F]FEF were performed at the Department of Nuclear Medicine at the University of Mainz, the [^{11}C]flumazenil scan was done at the Department of Nuclear Medicine at the Klinikum rechts der Isar, Technical University of Munich. The same PET camera and an identical acquisition protocol were used at the two locations.

Scanning procedure. All PET studies were performed on an ECAT EXACT (Siemens/CTI, Knoxville, Tenn.) tomograph in 2D mode with a total axial field of view of 16.2 cm. Attenuation for the studies was corrected using a transmission scan of 10 min with an external ^{68}Ge/^{68}Ga ring source obtained prior to injection.

The positioning of the head in the scanner was parallel to the canthomeatal line. The subjects underwent a dynamic study following the administration of 183±63 MBq [^{18}F]FEF (mean±SD, range 100–280 MBq) as an intravenous bolus injection. Dynamic emission data were collected in time frames ranging from 30 s to 10 min [23] and reconstructed into 31 contiguous horizontal image planes (filtered back-projection, Hamming, cut-off 0.4). In five subjects, the total scanning time was 60 min; in the other two the scanning time was

extended to 90 min. Prior to scanning, a 22-gauge cannula was inserted into the radial artery after subcutaneous infiltration with 0.5% lidocaine. In the two subjects who were scanned at baseline and after injection of unlabelled flumazenil, the plasma input function was generated from arterialised venous blood ("heated hand", temperature 50°C, pO_2 60 mmHg, pCO_2 40 mmHg). Blood samples were immediately centrifuged and plasma radioactivity counted in a gamma scintillation spectrometer. The arterial input function was calculated from approximately 60 arterial samples, corrected for metabolites derived from a fitted curve of 12 time point values.

For the scan with [^{11}C]flumazenil, 701 MBq of the tracer was injected. The scanning procedure and the determination of the metabolite-corrected arterial input curve was identical to the [^{18}F]FEF studies.

Data analysis. Polygonal regions of interest (ROIs) were drawn on planes, where the respective regions (various cortical regions, striatum, thalamus, cerebellum, white matter, pons) have maximal areas. ROIs were drawn bilaterally and right and left values were averaged for subsequent analysis. Radioactivity values in specific ROIs were then plotted over time and subsequently fitted by nonlinear least squares analyses.

In a second analytical step, parametric images of the regional transport rate of the tracer from blood to brain, K_1, and its volume of distribution, VD, were obtained using a version of the spectral analysis described by Cunningham and Jones [24] applied for analyses on a voxel by voxel level [25]. This technique uses the time courses of the label in tissue and in arterial blood to produce a spectrum of kinetic components which relates to the tissue's response to the blood activity curve. From the summary of the kinetic components the tissue's unit impulse response function (IRF) is derived for each voxel. This can be viewed as smoothed deconvolution allowing the extraction of the IRF from the tissue data. The integral of the curve of best fit for IRF, extrapolated to time point infinity, provides the VD of the tracer and the image derived at the 1-min time point of the curve reflects K_1 [25].

This analysis was chosen because the IRF is independent of the particular plasma input function and therefore enables direct subsequent comparisons of different data sets [26]. Another advantage of this method is that it makes no assumptions as to the number of components or compartments required to describe the time course of the label in tissue.

From the volumes of distribution, binding potentials for specific brain regions were calculated according to the following equation [27]:

$$\text{Binding potential} = (VD_{Region} - VD_{Pons})/VD_{Pons}.$$

The pons was chosen as a reference region, because it is generally considered to be benzodiazepine receptor free [28, 29].

Since the blocking studies with unlabelled flumazenil were not obtained with arterial, but arterialised venous blood sampling, a spectral analysis was not performed on these subjects' data. Thus, Table 2 presents only VDs and binding potentials (BPs) acquired from the remaining five subjects.

Plasma metabolite studies. Blood samples of 2.5 ml per time point were used for plasma metabolite studies. These samples were centrifuged for 10 min at 5.5 *g*. Aliquots of 0.5 ml plasma were taken for determination of labelled metabolites. A volume of 0.5 ml of acetonitrile was added for precipitation. These mixtures were centrifuged again for 10 min at 5.5 *g*. Aliquots of 10–30 µl of the supernatant were transferred to thin-layer chromatography (acetonitrile/acetic acid 97:3) on Kieselgel 60 plates (Merck, Darmstadt). Activity distribution was detected using an Instant Imager with r_f values of 0.8–0.9 for [^{18}F]FEF and 0–0.1 for a polar metabolite.

Results

In vitro studies

The K_i values given in Table 1 were determined after conversion of IC_{50} values to K_i values following the Cheng-Prusoff equation. They indicate that FEF has a $GABA_A$ receptor subunit selectivity somewhat different from flumazenil. Specifically, the ratio between inhibition constants for α6 and α1 subunit-containing benzodiazepine receptors is lower for FEF than for flumazenil (Table 1). The absolute affinity of FEF for benzodiazepine receptors is about one order lower of magnitude compared with the affinity of flumazenil.

PET studies in humans

Injection of [^{18}F]FEF did not lead to significant changes in vital signs (blood pressure, heart rate).

Plasma metabolites. [^{18}F]FEF was rapidly metabolised. After 5 min less than 50% of the injected radiotracer was unchanged in arterial plasma. Twenty minutes after tracer injection, the metabolite fraction had increased to about 80%–90% (Fig. 1). When the metabolism of [^{18}F]FEF was compared with that of [^{11}C]flumazenil in the same subject, its rapid breakdown was even more apparent (Fig. 1).

By means of thin-layer chromatography, the metabolites were determined to be polar in nature, which is in agreement with findings reported by other investigators [17, 30]. Because it is well justified to assume that the polar metabolites are not able to cross the blood-brain barrier, their number and chemical identity were not determined.

With regard to total plasma radioactivity, after a first sharp peak immediately after tracer injection, a second increase occurred after 10–15 min, which most likely correlates with the development of the metabolites.

Table 1. Inhibition constants for flumazenil, FEF and iomazenil, at two different benzodiazepine receptor configurations. Data are means ± standard deviations. Data for Ro 15-4513 and for flumazenil are from ref. [22]

	α1β1γ2	α6β1γ2	Ratio α6/α1
K_D [^{3}H]Ro15-4513 (n*M*)	15±4	5.4±0.4	0.36
K_i flumazenil (n*M*)	0.5±0.2	90±20	180
K_i FEF (n*M*)	5.2±1.5	428±55	82
K_i iomazenil (n*M*)	0.59±0.23	26±13	44

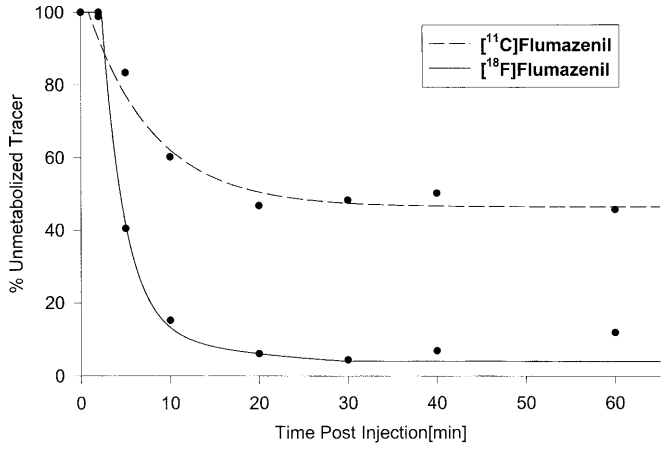

Fig. 1. Fraction of unchanged concentrations (individual data points and fitted curves) of [18F]FEF and [11C]flumazenil in percent of injected total radiotracer concentration as measured in the same human volunteer (*n*=1)

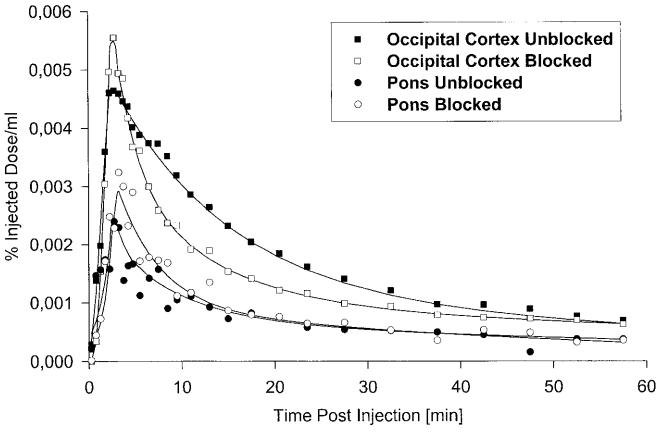

Fig. 3. Time-activity curves (original data and fitted curves) for the occipital cortex and the pons in the same human healthy volunteer, who was scanned twice with [18F]FEF. The second PET scan was performed after injection of 2.5 mg unlabelled flumazenil (Anexate®) 3 min prior to tracer injection

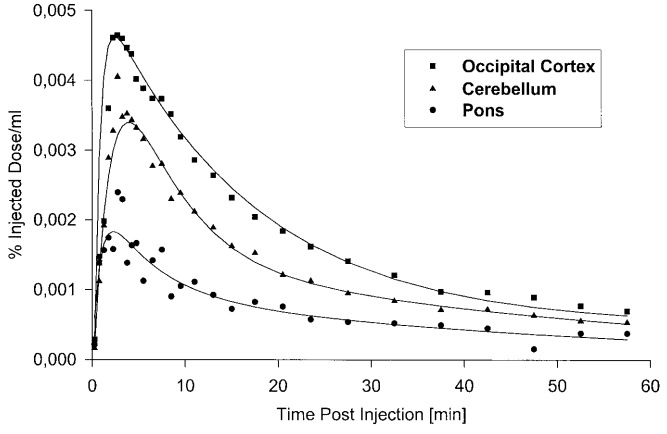

Fig. 2. Typical set of time-activity curves (original data and fitted curves) in three representative regions of interest for [18F]FEF in a healthy human volunteer

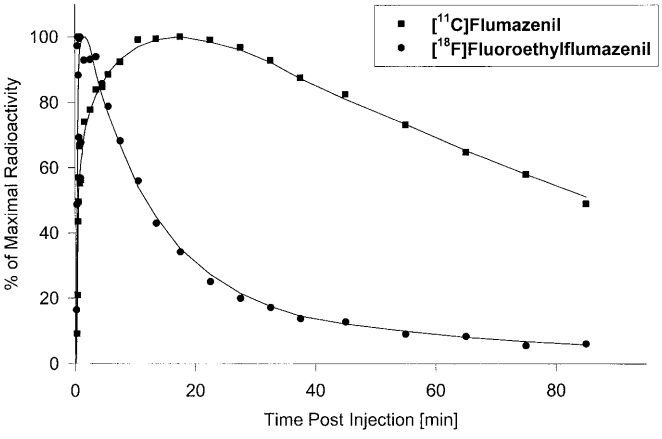

Fig. 4. Time-activity curves (original data and fitted curves) after injection of [18F]FEF or [11C]flumazenil in a temporal cortical ROI in the same healthy human volunteer, expressed as percent of maximal measured radioactivity. Activity is corrected for different radioactive decay of the two isotopes

Blocking studies with unlabelled flumazenil. In human brain, maximum accumulation of [18F]FEF was observed 4±2 min p.i. with fast clearance kinetics resulting in approximately 50% and 20% of maximum activities at about 10–15 and 30 min, respectively. [18F]FEF uptake followed the known central benzodiazepine receptor distribution in the human brain (occipital cortex >temporal cortex >cerebellum >thalamus >pons; Fig. 2). Pretreatment with unlabelled flumazenil resulted in reduced tracer uptake in all brain areas with benzodiazepine receptors (Fig. 3). However, initial tracer uptake was increased in all brain regions by the pharmacological manipulation, most likely owing to an increase in blood flow.

Comparison with [11C]flumazenil. When brain uptake of [18F]FEF was compared with uptake of [11C]flumazenil in the same healthy human volunteer based on an ROI-oriented approach, its kinetics was more rapid in all brain regions. This was even more apparent when the time-activity curves for [11C]flumazenil were corrected for its faster radioactive decay compared with the fluorine-18 isotope (Fig. 4).

Secondly, when the distribution of [18F]FEF and [11C]flumazenil, in the same human brain, was analysed with spectral analysis on a voxelwise basis [24], the distribution volumes (VD) for [11C]flumazenil were between two- and threefold higher than for [18F]FEF (Tables 2 and 3; Fig. 5). VD values obtained from the whole 60-min time-activity curves were not significantly different from VD values acquired from time-activity curves as short as only 20 min (e.g. occipital cortex, 60 min: 1.80±0.56 ml blood/ml tissue, 20 min: 1.75±0.41; cerebellum, 60 min: 1.43±0.35, 20 min: 1.37±0.21; pons,

60 min: 0.85±0.20, 20 min: 0.84±0.11). Table 2 presents VDs generated from 20-min scans.

Interestingly, specific binding of [^{18}F]FEF, expressed as binding potential (BP), in the cerebellum, the only brain region with benzodiazepine receptors with α6 subunit composition, was relatively higher than that of [^{11}C]flumazenil when cerebellar binding was compared with occipital binding. While the BP ratio occipital cortex/cerebellum was 1.71 for [^{18}F]FEF, it was 2.32 for [^{11}C]flumazenil in our single subject analysed with spectral analysis and 2.33 for data from a two-compartment model taken from the literature [31] (Tables 2 and 3).

In addition, a 1-min tissue impulse response image (IRF1) was generated. IRF1 parametric images of other tracers (e.g. [^{11}C]diprenorphine) have been shown to strongly correlate with K_1 [24, 25]. The absolute values for the above-mentioned regions derived by this approach were, as expected, somewhat lower than those reported for K_1 in the literature derived by a two-compartment model [31]. However, IRF1 values for [^{18}F]FEF and K_1 values for [^{11}C]flumazenil derived by a two-compartment model correlated significantly (Spearman's rank order correla-

Table 2. Volumes of distribution (VD; ml blood/ml tissue) for [^{18}F]FEF as calculated by spectral analysis (*left column*) as well as binding potentials (BP) calculated from VDs (*right column*) in five healthy male volunteers

	VD [^{18}F]FEF	BP [^{18}F]FEF
Occipital cortex	1.75±0.41	1.07±0.29
Temporal cortex	1.43±0.31	0.70±0.17
Frontal cortex	1.46±0.28	0.74±0.15
Thalamus	1.21±0.28	0.43±0.17
Cerebellum	1.37±0.21	0.63±0.08
Pons	0.84±0.11	–

Table 3. Volumes of distribution (VD; ml blood/ml tissue) and binding potentials (BP) as calculated by spectral analysis for [^{11}C]flumazenil in a single healthy subject (*left two columns*), as compared to VD and BP values for [^{11}C]flumazenil from a two-compartment model reported in the literature (Lit.*, *right two columns*, ref. [31])

	VD [^{11}C]Flu	BP [^{11}C]Flu	VD [^{11}C]Flu Lit.*	BP [^{11}C]Flu Lit.*
	Spectral analysis (n=1)		Two-compartment model (n=6)	
Occipital cortex	6.20	5.74	6.04	4.59
Temporal cortex	5.33	4.79	–	–
Frontal cortex	5.02	4.46	5.13	3.75
Thalamus	3.02	2.28	3.32	2.07
Cerebellum	3.17	2.47	3.21	1.97
Pons	0.92	–	1.08	1.08

[^{11}C]Flu: [^{11}C]Flumazenil

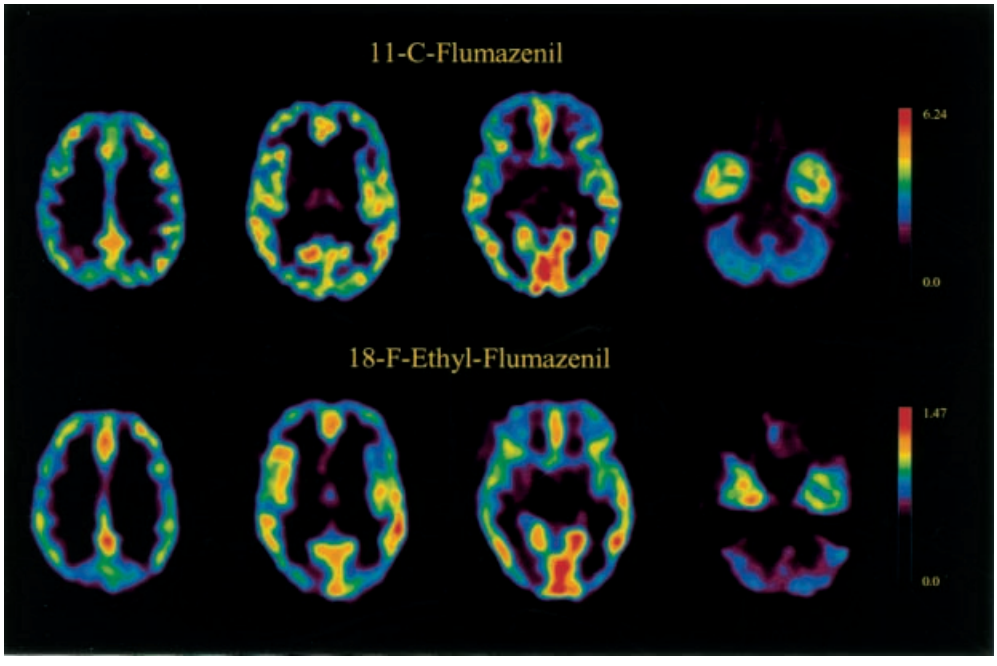

Fig. 5. Voxelwise maps of binding potentials (BP) for [^{11}C]flumazenil (*top*) and [^{18}F]FEF (*bottom*), generated by mathematical transformation of the VD images derived by spectral analysis with the pons as the reference region, according to the equation: VD(region) – VD(pons)/VD(pons). BP maps for [^{11}C]flumazenil are displayed with the colour scale rainbow and a range from 0 to a maximum of 6.24 representing the voxel with the highest BP. BP maps for [^{18}F]FEF were similarly adapted to its maximum BP of 1.47

tion, r_S=0.90, P=0.037; IRF1 [^{18}F]FEF vs K_1 [^{11}C]flumazenil: occipital cortex 0.25 vs 0.34 ml blood/ml tissue × 1/min, frontal cortex 0.20 vs 0.30, thalamus 0.23 vs 0.37, cerebellum 0.22 vs 0.31, pons 0.16 vs 0.29).

Discussion

In agreement with the observations from baboon studies published by Moerlein and Perlmutter [17], our results demonstrate specific and displaceable binding of [^{18}F]FEF to human central benzodiazepine receptors. As shown in Fig. 2, tissue uptake follows the well-known distribution of benzodiazepine receptors in human brain. Highest B_{max} values have been reported to be present in the occipital cortex, followed by temporal and frontal cortices, the cerebellum and the thalamus [32]. Pre-injection of unlabelled flumazenil blocked tracer uptake in regions with notable benzodiazepine receptor densities (Fig. 3). A mild initial increase in tracer uptake in the blocking experiments, which was observed in all brain regions (Fig. 3), is most likely due to a change in blood flow induced by the pharmacological manipulation. Blood flow changes are commonly observed with application of pharmacological stimuli, pain or anticipatory anxiety [33, 34]. It has been demonstrated that in subjects with low-level anxiety, cerebral blood flow increases with anxiety [34]. Since all studies except for the blocking experiments, which were simply designed to demonstrate specific tracer binding, were performed under the same experimental – "baseline" – conditions, we believe that this probable change in blood flow does not confound the results presented here.

While maximal uptake of the tracer to cortical areas was reported to be up to 0.014% of the injected dose/ml in the baboon [17], it did not exceed 0.006% injected dose/ml in our human studies. Also, the kinetics of the tracer in the human brain seems to be more rapid than in the baboon brain (for comparison see Fig. 1 and ref. [17]). Although the reason for this difference is not quite clear, the observation of a more rapid metabolism in humans compared with baboons was also reported for [^{11}C]flumazenil [35].

While, because of the rapid kinetics of the tracer, the fluorine-18 label of [^{18}F]FEF cannot fully exert the benefit of its longer half-life, an advantage of [^{18}F]FEF could be the convenient laboratory analysis of blood samples for the input function and especially the metabolite correction. It has been argued that another benefit of [^{18}F]FEF is that only polar metabolites are generated from this ligand, which do not pass the blood-brain barrier, whereas the acidic main metabolite Ro 15-3890 formed from [^{11}C]flumazenil would penetrate into the brain, thereby complicating kinetic modelling [17]. However, it is now generally accepted that the brain penetration of Ro 15-3890 is negligible [36]. Although it cannot be totally excluded that very small amounts of the metabolite are formed within the brain, it is now widely assumed that all or almost all brain radioactivity measured following [^{11}C]flumazenil injection originates from the parent compound [37].

Our in vitro results extend findings of Moerlein and Perlmutter, who were the first to report on the substitution of the methyl group of flumazenil by a fluorine-18 containing fluoroethyl group, which leads to [^{18}F]FEF [17, 20]. They determined a K_i of 11.6±1.0 n*M* for benzodiazepine receptors in primate frontal cortex membranes [38]. When the fluoroethyl group of [^{18}F]FEF is replaced by the larger fluoropropyl moiety, the affinity of the ligand for benzodiazepine receptors further decreases, making the resulting tracer unattractive for PET scanning [39]. Moerlein and Perlmutter determined a K_i of 24.5 n*M* for the resulting compound [^{18}F]fluoropropylflumazenil [17]. We have been able to show that FEF has a somewhat different benzodiazepine receptor subtype selectivity compared with flumazenil. When we transiently expressed specific rat $GABA_A$ receptors with the subunit configurations $\alpha1\beta1\gamma2$ and $\alpha6\beta1\gamma2$ in human embryonic kidney cells (HEK 293), K_i values for [^{18}F]FEF were determined to be 5.2±1.5 and 28±55 n*M*, respectively. Although especially the K_i of 5.2±1.5 n*M* is in the range known from other PET tracers, the affinities are about one order of magnitude lower than those determined for flumazenil or iomazenil with the same methodology [21] (Table 1). These in vitro binding data indicate that the ratio between K_i values of FEF for $GABA_A$ receptors of the composition $\alpha6\beta1\gamma2$ and those of the composition $\alpha1\beta1\gamma2$ is lower than the respective ratio for flumazenil.

Knowledge of the tracers' affinities to benzodiazepine receptors with a particular subunit composition offers the opportunity to relate the data obtained with PET in vivo to the molecular characteristics. This is important, because affinity and clinical efficacy of most clinically used benzodiazepines critically depend on the specific subunit composition of the receptor [40]. The research questions arising from these facts have not been addressed in previous PET and SPET studies; this is because all currently used tracers, including [^{11}C]flumazenil and [^{123}I]iomazenil, bind non-selectively to all receptor subtypes with the exception of $\alpha4$ and $\alpha6$ subunit-containing benzodiazepine receptors, for which they exhibit a very low affinity. The brain uptake of these tracers follows the distribution of benzodiazepine receptors in the human brain, which itself was determined with non-selective, tritiated agents like [^{3}H]diazepam or [^{3}H]flumazenil [41, 42], but underestimates receptor density in brain areas like the cerebellar granule cell layer containing high amounts of $\alpha6$ subtype-containing receptor complexes. Because [^{18}F]FEF also has a relatively low but different affinity for $\alpha6$ subunit-containing receptors compared to the above-mentioned ligands, it can be expected that conditions primarily affecting binding to $\alpha6$ subunit-containing benzodiazepine receptors may result in a distinct binding pattern.

The differences in the in vivo tissue binding of [18F]FEF and that of [11C]flumazenil in the same human volunteer reflect the differences between the ligands as far as the overall affinity and the subtype selectivity are concerned. The slower metabolism of [11C]flumazenil compared with that of [18F]FEF may also contribute to the higher brain uptake of [11C]flumazenil (Fig. 1). The lower ratio between inhibition constants of [18F]FEF at cerebellar and α1 subunit-containing benzodiazepine receptors is most likely reflected in disparate VD and BP values acquired with the spectral analytical approach ([24]; Table 2, Fig. 5). Although in general, absolute VD values are two- to threefold lower than those obtained with [11C]flumazenil, they clearly reflect the density distribution of benzodiazepine receptors in different brain regions. However, cerebellar values for VD and BP obtained with [18F]FEF are almost as high as in cortical regions (Table 2). While the cerebellar BP for [18F]FEF reaches on average 59% of the highest BP in the occipital cortex, it is only about 43% of the occipital BP for [11C]flumazenil, when the analysis for [11C]flumazenil is based on data from a two-compartment model taken from the literature [31]. Knowledge of these characteristics is especially important for pharmacological studies using benzodiazepine-like agents with higher specificity for certain subunit compositions [22, 43, 44] and for the investigation of disease states that are probably related to α6 subunit-containing benzodiazepine receptors such as alcoholism or anxiety disorders [22, 45]. Such abnormalities may be related not only to abnormal absolute receptor numbers but also to aberrations of specific distribution ratios of particular receptor compositions.

The usefulness of the spectral analytical approach for determination of benzodiazepine receptor distribution on a voxelwise basis in the human brain is demonstrated by the exact agreement of our VD values for [11C]flumazenil binding and published data obtained with a two-compartment model [31]. BP values for [11C]flumazenil determined with spectral analysis for our single subject are also within the normal range compared with values taken from the literature (Table 3). In particular, the cerebellar BP is exactly 43% in magnitude compared with the occipital BP (Table 3). As VD values for [18F]FEF calculated by spectral analysis from the first 20 min of the time-activity data were not significantly different from VD values generated from data acquired over 60 min, it seems justified to reduce the scanning time.

In summary, our studies in healthy male human volunteers demonstrate that [18F]FEF reversibly binds to central benzodiazepine receptors. Its distribution in the human brain follows the known relative benzodiazepine receptor densities. Uptake of the ligand can be reduced by unlabelled flumazenil in receptor-rich brain areas. Compared with [11C]flumazenil, [18F]FEF is characterised by a lower affinity to benzodiazepine receptors, a relatively low specific to non-displaceable ratio, a rapid kinetics in the brain and faster metabolism. Nevertheless, parametric images reflecting binding of [18F]FEF to benzodiazepine receptors can be generated by approaches like spectral analysis in a manner that leads to reasonable results, making this ligand suitable for quantification of benzodiazepine receptor binding.

In conclusion: Although [11C]flumazenil has some advantages over [18F]FEF (higher affinity, slower metabolism, slower kinetics), our results indicate that [18F]FEF could be a suitable PET ligand for quantitative assessment of central benzodiazepine receptors in the human brain, which has the major advantage that it can be used independently of an on-site cyclotron.

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