

In Vitro and In Vivo Evaluation of Iodine-123-Ro 16-0154: A New Imaging Agent for SPECT Investigations of Benzodiazepine Receptors

Hans-Frieder Beer, Peter A. Bläuenstein, Peter H. Hasler, Bernard Delaloye, Georg Riccabona, Isolde Bangerl, Walter Hunkeler, Erico P. Bonetti, Lorenzo Pieri, J. Grayson Richards, and P. August Schubiger

Radiopharmacy Division, Paul Scherrer Institute, Villigen, Switzerland; Division Autonome, CHUV, Lausanne, Switzerland; Universitätsklinik, Innsbruck, Austria; and F. Hoffman-La Roche AG, Basel, Switzerland

The flumazenil analogue, Ro 16-0154, a benzodiazepine partial inverse agonist, has been labeled by halogen exchange to enable SPECT investigations of central benzodiazepine receptors in the human brain. The purified ^{123}I -Ro 16-0154 was found to be stable in rat brain preparations and to be metabolized in rat liver preparations. Its pharmacologic properties were comparable to those of flumazenil. The biodistribution in rats (1 hr postinjection) resulted in a high brain-to-blood ratio of 16. Clinical studies revealed images of the benzodiazepine receptor density in the brain. Since the receptor labeling was markedly reduced by injection of flumazenil, it was considered to be specific. Storage defects due to pathologic cerebral blood flow and changed receptor density were detected; this shows the potential usefulness of the substance for diagnostic purposes, e.g., the differential diagnosis of various forms of epilepsy.

J Nucl Med 1990; 31:1007-1014

Since the first description of the potent benzodiazepine antagonist flumazenil (Ro 15-1788) by Hunkeler et al. (1), numerous clinical investigations with this compound have been undertaken. Several studies with the hydrogen-3 (^3H) and carbon-11-labeled (^{11}C) compounds in mice, rats, baboons, and human volunteers (2-6) have demonstrated the selective binding of flumazenil to benzodiazepine receptors. Positron emission tomography (PET) studies (4-7) have allowed the imaging of the benzodiazepine receptor density in the living brain. Alterations in the density of these receptors have been reported for experimental seizures (8), Huntington's disease (9, 10), Alzheimer's disease (11), anxiety disorders, ethanol dependence (12), and hepatic encephalopathy (13). Recently, the reduced benzodi-

azepine receptor binding in human epileptic foci was demonstrated (14). The successful imaging of the benzodiazepine receptors by PET, the continuing importance of benzodiazepines as tranquilizers in clinical practice, and the intensity of basic research on the role of benzodiazepines in alleviating anxiety, insomnia, muscle tension, and epilepsy, prompted us to label an analogue of flumazenil (Ro 16-0154, Fig. 1) with iodine-123 (^{123}I) for single-photon emission computer tomography (SPECT) imaging of the GABA/benzodiazepine receptors in the living human brain. The advantage of flumazenil as an imaging agent (benzodiazepine antagonist with high affinity for the receptor and lacking major intrinsic pharmacologic effects) was combined with the ideal radiation properties of ^{123}I to obtain a new brain imaging agent. In contrast to the usual short-lived positron-emitting nuclides for PET, the suitable half-life of ^{123}I enables investigations in nuclear medicine departments which lack a nearby cyclotron facility.

MATERIALS AND METHODS

Labeling and Purification

Sodium (^{123}I) iodide in 0.1 N NaOH with a specific activity between 0.2 and 1.5 TBq/mg (0.04-3.7 GBq/ml) was produced at PSI. The bromoprecursor (Ro 19-3797) was synthesized at F. Hoffmann-La Roche in Basle/CH. All chemicals used were either of p.a. or high-performance liquid chromatography (HPLC) quality. Thin-layer chromatography (TLC) was performed on silica (Kieselgel 60, layer 0.25 mm, 5 × 20 cm glass plates, Merck).

Two different solvent systems were used:

Mixture A—ethylacetate/ NH_4OH 200/1 (v/v).

Mixture B— $\text{CHCl}_3/\text{CH}_3\text{COOH}/\text{H}_2\text{O}$ 65/35/5 (v/v/v).

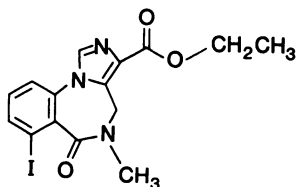
The radioactivity on the developed plates was detected on a positron-sensitive detector (Chromelec, Numelec SA, Geneva).

A new and very simple labeling procedure for halogen exchange was developed and performed in a conical reaction vial tightly closed by a teflon laminated silicon septum. The solution containing the ^{123}I activity (up to 11.1 GBq) in 0.1 N

Received Jun. 2, 1989; revision accepted Jan. 9, 1990.
For reprints contact: Dr. P. A. Schubiger, Paul Scherrer Institute, Radiopharmacy, CH-5232 Villigen PSI, Switzerland.

FIGURE 1

Ro 16-0154: ethyl-5,6-dihydro-7-iodo-5-methyl-6-oxo-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxylate.



NaOH was evaporated to dryness by means of a gentle stream of nitrogen at 90°C. Afterwards, 1.5 mg of the bromoprecursor (Ro 19-3797) dissolved in 100 μ l glacial acetic acid was added and the reaction mixture heated for 1 hr at 155°C. After cooling, the mixture was dissolved in 5 ml water and purified by HPLC, using the following conditions: RP-18 column (8 \times 250) Knauer Lichrosorb 10 μ m, MeOH/H₂O 45/55, 2 ml/min, iodide: $k^1 = 0.0$, Ro 19-3797: $k^1 = 2.75$, Ro 16-0154: $k^1 = 4.00$. The purification was performed on a device consisting of a valco 6-port valve with 20 ml loop, a Waters 510 pump, a Kontron 740 LC detector, and a NaI scintillation detector. Labeling and purification was done in a lead box equipped for remote handling. The labeling was virtually quantitative (monitored by HPLC). A substance with the same retention value ($k^1 = 1.63$) as the free acid of Ro 16-0154 was detected by HPLC as a byproduct. During the HPLC separation, the ¹²³I-Ro 16-0154 peak was collected and afterwards evaporated to dryness with a Rota-Vapor. The residue was dissolved in a solution containing 5% glucose and passed through a silver powder column to adsorb iodine liberated during the Rota-Vapor treatment. After sterile filtration and adjustment of the activity concentration to 37 MBq/ml, the new radiopharmaceutical was ready for use.

Stability and Metabolism

The metabolism and the stability of ¹²³I-Ro 16-0154 were investigated by means of TLC, using extracts from rat liver and brain and homogenates of these tissues. Five to 6 g of the organs, stored in liquid nitrogen, were thawed and rinsed with phosphate-buffered saline (100 mM NaCl, 45 mM phosphate buffer pH 7.3). After addition of 50 ml TRIS buffer solution (50 mM pH 7.4), the tissues were homogenized in a glass homogenizer, halved, and stored at 4°C. The halves were centrifuged at 4°C and 1,000 rpm (280 g) for 5 min (Beckman J-6). The supernatants were further used as extracts.

For comparison, the behavior of ¹²³I-Ro 16-0154 was tested in three different solutions, i.e., TRIS buffer, blood serum, and artificial cerebrospinal fluid (15), a solution of 126 mM NaCl, 6 mM KCl, 1 mM NaH₂PO₄, 0.88 mM MgSO₄, 1.45 mM CaCl₂, and 25 mM Hepes (pH 7.2).

Five-tenth milliliters of the final product were added to 5 ml of the respective solution or suspension and incubated at 37°C. The homogenates of the tissues were gently shaken during the whole incubation period. After 0.5 and 20 hr samples were withdrawn and analyzed by TLC using the mixtures A and B (see Labeling section). In the case of the homogenates, the solid contents were eliminated by means of a centrifuge (Eppendorf 5417; 10 min, 1,400 rpm, ca. 1,500 g) prior to the analysis.

Pharmacologic Investigations

Binding Studies in Rat Brains. The antagonistic properties of flumazenil and Ro 16-0154 with respect to the diazepam

binding in vitro and the flunitrazepam binding in vivo were investigated.

Hydrogen-3-diazepam (NEN, North Billerica, MA) binding assays were performed with membranes from whole rat brain in vitro as described in Reference 16. The concentrations necessary for half-maximal inhibition (IC₅₀ values) were determined.

To assess the interaction of Ro 16-0154 and flumazenil with benzodiazepine receptors in vivo, the inhibition of ³H-flunitrazepam binding to rat brain was studied using the method of Williamson et al. (17). Various doses of Ro 16-0154 or flumazenil (1.0, 3.0, 10, 30, 100 mg/kg, suspension in 0.3% Tween 80; two animals per dose) were administered orally; 13 min later, ³H-flunitrazepam (8.108 kBq/kg dissolved in 1.0 ml 0.9% NaCl, specific activity 2.96 TBq/mole, Amersham) was intravenously injected; decapitation followed 2 min thereafter. Aliquots of whole brain homogenates (excluding brain stem) in TRIS-buffer pH 7.4 were applied to Whatman GF/B filters and washed twice with 5 ml TRIS-buffer, before counting the radioactivity bound to the tissue. Nonspecific binding of ³H-flunitrazepam was determined after oral administration of 100 mg/kg diazepam.

Radioautography. Hydrogen-3-Ro 16-0154 (311.91 GBq/mole, F. Hoffmann-La Roche, Basle) and ¹²³I-Ro 16-0154 (> 5.476 GBq/mole, PSI, Villigen-PSI) were used. In vitro binding assays were performed with cryostat sections of lightly fixed rat brain as previously described (18). Brain regions labeled with the radioligands (total binding with 2 nMole) were revealed by radioautography using LKB Ultrafilm.

Proconvulsant Activity. In order to test the proconvulsant effect, the mice received 60 mg/kg i.p. pentylenetetrazol (PTZ) which by itself induced tonic convulsions in 0%–20% of animals. Flumazenil and Ro 16-0154 were administered p.o. 15 min before PTZ, and the number of mice exhibiting tonic convulsions was noted. A statistical difference between mice receiving Ro 16-0154 plus PTZ and those receiving only PTZ was assessed by the chi-squared test.

Antagonism of Diazepam: Pentylenetetrazol Test. The antagonism was studied in mice given 5 mg/kg diazepam i.p. 1 hr before 120 mg/kg PTZ i.p. The antagonists Ro 16-0154 and flumazenil were given by oral gavage 15 min (0.68–6.8 mg/kg p.o. and 0.32–2.1 mg/kg p.o., respectively) before PTZ (19). The ED₅₀ values were calculated by use of a probit analysis program.

Horizontal Wire Test. In this test (19,20), the mice were lifted by the tail and allowed to grasp a horizontally strung wire with the forepaws and released. The number of animals from a total of ten per treatment group was determined that did not actively grasp the wire with at least one hind paw within 3 sec or did not grasp the wire with the forepaws. In untreated control animals, this number was consistently zero. After two trials, performed at 5-min intervals, diazepam (3 mg/kg i.p.) was injected. This dose usually prevents grasping in nine or ten animals. At 15 min after the injection of diazepam, the vehicle or the test compound were administered by oral gavage. Antagonism was quantified by comparing maximum effects within 45 min after administration of either the vehicle or the test compound in two groups of animals. A dose-dependent antagonism (0.03–3.0 mg/kg p.o. and 0.1–3.0 mg/kg p.o. for flumazenil and Ro 16-0154, respectively) of the depressant effect of diazepam in the horizontal wire test was found.

Biodistribution

The biodistribution of the ^{123}I -Ro 16-0154 was determined 2, 10, 20, 40 min, 1, 6, and 15 hr after i.v. injection. At each of those times, three rats (female, Wistar, SPF, alimentum ad libitum) were killed after narcotization in an ether atmosphere, and the radioactivity of their organs was measured in an ionisation chamber. The weights of the animals ranged between 113 and 144 g. The injected doses varied between 6.8 and 13.1 MBq in a volume of 0.2 ml.

Clinical Trials: Displacement Kinetics and SPECT

With healthy volunteers, studies ($n = 10$) were performed at three nuclear medicine departments, with protocols adapted to the individual camera systems, to prove the receptor binding properties of ^{123}I -Ro 16-0154 (Phase I). Details of the methods are published elsewhere (21–23).

The displacement kinetics in two normal volunteers were investigated using a single head rotating gamma-camera (Siemens-Gammasonc Orbiter ZLC 37 with a Dec-PDP-11/34 computer, Des Plaines, IL). After injection of 122 MBq ^{123}I -Ro 16-0154 (0.6–4.3 μg), the uptake of the radioactivity into the brain was monitored by a dynamic study with 1 frame/30 sec until 15 min postinjection. The first SPECT investigation started ~40 min postinjection. The second dynamic study was recorded with the injection of cold flumazenil at the indicated time points, followed at ~115 min postinjection by the second SPECT imaging. The third and final dynamic study was done ~150 min postinjection.

Another study (24) was performed, comparing the CBF pattern, using ^{123}I -isopropyl-p-iodoamphetamine (IMP) and $^{99\text{m}}\text{Tc}$ -HM-PAO, with the ^{123}I -Ro 16-0154 distribution in patients (Phase II) with partial epilepsy ($n = 9$), and Lennox-Gastaut-Syndrome ($n = 15$). The Lennox-Gastaut-Syndrome is a form of epilepsy starting between 1 and 6 yr of age. Frequently the syndrome is accompanied by myoclonal symptoms and atypical absences. These patients suffer from mental retardation and show an insufficient response to conventional antiepileptic therapy (25,26). The patients taking part in this Phase II study were investigated 30 min after injection of 185 MBq ^{123}I -Ro 16-0154 (0.9–6.5 μg) with SPECT. For SPECT, 64 angles were measured with an average acquisition time of 30 sec/angle until 50,000 counts were collected. All patients were additionally investigated 1 day later with 185 MBq ^{123}I -IMP or 555 MBq $^{99\text{m}}\text{Tc}$ -HM-PAO. In the case of the IMP investigation, the rotation started 30 min after the injection and for $^{99\text{m}}\text{Tc}$ -HM-PAO 10 min after the injection.

RESULTS

Stability and Metabolism

The R_f values for the different TLC-systems are as follows: with mixture A the ^{123}I -Ro 16-0154 has an $R_f = 0.3$. Iodide, the free acid of the ^{123}I -Ro 16-0154 and an unknown third compound are retained at the origin ($R_f = 0$). Chromatograms with mixture A prove the absence of labeled impurities with an R_f higher than 0.3 (less polar than ^{123}I -Ro 16-0154). With mixture B, different metabolites can be determined. With this TLC system, ^{123}I -Ro 16-0154 migrates to the front ($R_f = 1.0$). Iodide has $R_f = 0.04$, the free acid $R_f = 0.38$, and the third compound $R_f = 0.55$. In the control experiment

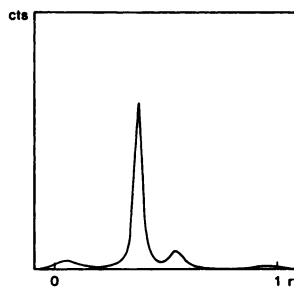


FIGURE 2
Radiochromatogram developed with solvent mixture B, 20 hr incubation of 0.5 ml ready for use radiopharmaceutical in 5 ml rat liver homogenate.

with TRIS buffer, the product was virtually stable within 20 hr. The sum of the decomposition products did not exceed 4%.

The investigation with the liver tissue showed no significant differences between the homogenate and the extract. After 30 min incubation time, 5%–6% iodide and 5%–11% of the free acid were found. After 20 hr, a greater augmentation of the free acid concentration (27%–72%, Fig. 2) was seen as compared to the iodide (7%–11%). In addition, 8%–17% of the third unidentified compound was detected. After 20 hr incubation in rat brain preparations, the ^{123}I -Ro 16-0154 remained nearly undecomposed as shown in Figure 3. About 1% iodide and 2%–3% free acid were found, values comparable to those obtained with the TRIS buffer. Centrifugation of the brain homogenate after the incubation resulted in a diminution of the product peak to ~4% in the supernatant.

No detectable decomposition products were found in artificial cerebrospinal fluid. In human blood serum, 9% of decomposition products were detected.

Chemical and Pharmacologic Properties of Ro 16-0154

The chemical and pharmacologic properties of Ro-0154 and flumazenil are given in Tables 1 and 2. Ro 16-0154, but not flumazenil, showed slightly proconvulsive properties in animal experiments. With 0.1 mg/kg, 11 out of 20 mice had tonic convulsions, whereas up to 100 mg flumazenil/kg failed to potentiate the convulsant effect of PTZ. In acute toxicity experiments, the maximum tolerated dose was estimated to be >5,000 mg/kg p.o.

Radioautography

A comparable distribution in Ro 16-0154 binding with both isotopes was observed (Fig. 4 A-B). This is consistent with results previously reported for ^3H -flu-

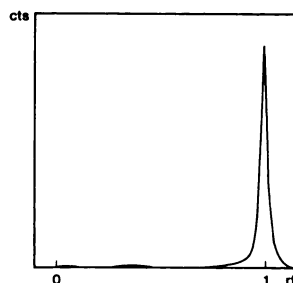


FIGURE 3
Radiochromatogram of the brain homogenate, conditions, as in Figure 2.

TABLE 1
The Chemical Properties of Ro 16-0154 and Ro 15-1788 (Flumazenil)

	Ro 16-1054	Ro 15-1788
Melting point	246°C	200°C
Solubility in water	1.2×10^{-4} mole/l	14×10^{-4} mole/l
pK	1.6	1.7
Partition coefficient between n-octanol/0.02 M phosphate buffer (pH 7.4):	30	15

mazenil in vitro and in vivo (3,18). Hydrogen-3 and ^{125}I -Ro 16-0154 bindings were competitively inhibited by 1 μmole flumazenil.

Biodistribution

In Figure 5, the biodistribution in percentage of injected radioactivity per gram of tissue for some selected organs is shown. The highest accumulation occurs in liver and kidneys 10 min postinjection. Within 1 hr, a very rapid decline takes place. The intestine activity increases correspondingly to a declining liver value. At the first time point, the other organs contain a low and decreasing radioactivity. The spleen, muscles, and bones shown a similar timecourse of accumulation.

The blood and the brain radioactivities are plotted in Figure 6 and the brain/blood ratio is shown. The highest value in the brain is reached within 10 min after injection. The decrease of radioactivity in the blood is faster than in the brain, and is comparable to the other organs. After 1 hr, nearly all activity is eliminated from the blood. The elimination from brain is considerably slower, with a residual brain content of 0.05% ID/g at 6 hr. Correspondingly, the brain/blood ratio increases from 3 at 2 min to 16 after 1 hr postinjection and is still >1 after 6 hr. These results are comparable with those described by d'Argy and Persson (2) for ^3H -flumazenil accumulation in mice. The higher concentration of ^3H -flumazenil in the brain than in the rest of the body was described as a "dominating finding." In their experiment, no other tissue showed such remarkable accumulation.

In Figure 7, the results of ^{123}I -Ro 16-0154 are compared with those of $^{99\text{m}}\text{Tc}$ -HM-PAO (27) and [^{123}I]IMP (28). The most important difference is the higher clearance rate of ^{123}I -Ro 16-0154 observed in all organs. Thus, all organs except the brain retain a very low amount of the new product, while the other two sub-

stances show similar values at a higher level 10 min and 1 hr after injection. A striking difference between ^{123}I -IMP and the ^{123}I -Ro 16-0154 is found in the lung uptake of both substances, where the value for IMP is very high and stable.

The regional distribution pattern of the benzodiazepine receptors in the rat brain has been published (29,30). By comparison of the images in Figure 4 with those in References 29 and 30, it is seen that the regional distributions of flumazenil and Ro 16-0154 in the brain are similar and closely resemble the known distribution of the benzodiazepine receptors.

Based on the reported animal data, we estimated the radiation burden per organ in man. The activity for each organ in man was calculated according to Roedler (31) and combined with MIRD values (32). The results of this estimation are shown in Table 3. The main radiation burden is given to the intestine. The MIRD value for the brain is not given. The similar uptake of Ro 16-0154 and IMP in the brain (Fig. 7) indicates that

TABLE 2
Pharmacologic Properties of Ro 16-0154 and Ro 15-1788 (Flumazenil)

	Ro 16-0154	Ro 15-1788
Diazepam binding: IC50 [nmole/l]	2.2	2.5
Flunitrazepam binding: ED50 [mg/kg]	4.6	3.9
Antagonism of diazepam versus PTZ: ED50 [mg/kg]	0.7	2.8
Horizontal wire test: ED50 [mg/kg]	0.3	0.2

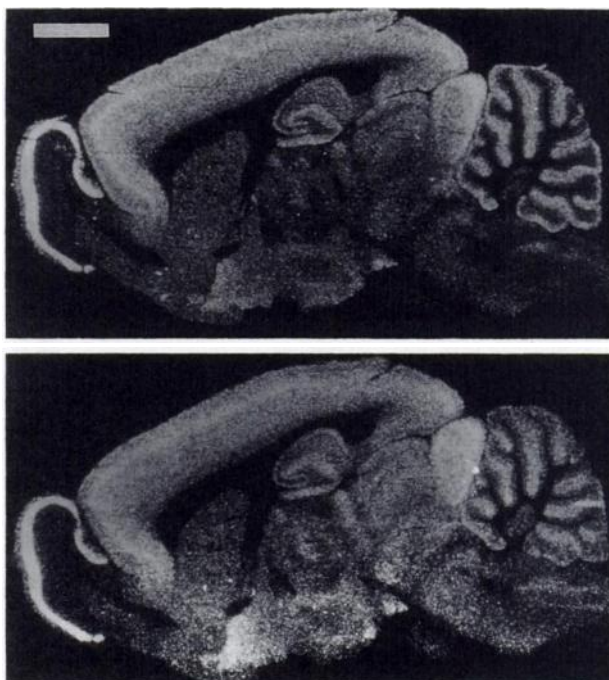


FIGURE 4
Radioautographic distribution of binding sites of ^3H -Ro 16-0154 (top) and ^{125}I -Ro 16-0154 (bottom) in semi-adjacent rat brain sections. White areas represent highest intensities of radiolabeling (calibration bar = 3 mm)

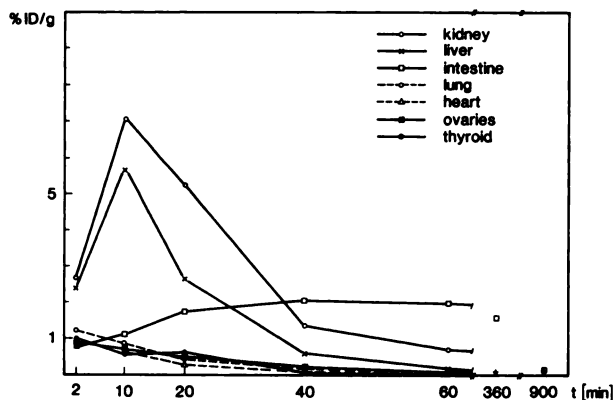


FIGURE 5
Percent of injected dose of the ^{123}I -Ro 16-0154 per gram of tissue, mean value of three rats, the s.d. is typically ranging between 5% and 25%.

a similar radiation burden is caused by both substances (i.e., $29 \mu\text{Gy}/\text{MBq}$) (33).

CLINICAL RESULTS

Organ Distribution in Man

The organ distribution of ^{123}I -Ro 16-0154 was determined in five healthy volunteers 2 and 90 min after applications of the radiopharmaceutical (21). The results are shown in Table 4 and indicate the high and stable uptake of the tracer in the human brain, the small burden in other tissues, and the excretion through liver and bladder.

Displacement Kinetics and SPECT

The results of the ^{123}I -Ro 16-0154 uptake and displacement study are shown in Figure 8. Immediately after administration of the radiopharmaceutical, a steep increase of the count rate in the brain was observed. A plateau of $\sim 160\text{--}170 \text{ imp}/(\text{pixel}\cdot\text{min})$ is reached within 20 min postinjection. In one of the two volunteers, the count rate stayed constant up to 100 min postinjection. In the other, a decrease of the count rate was observed.

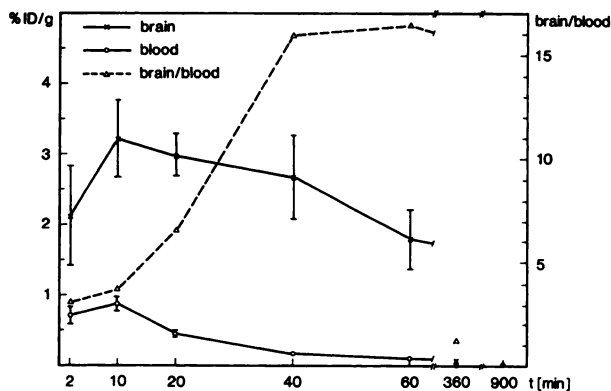


FIGURE 6
Percent of injected dose of the ^{123}I -Ro 16-0154 per gram of tissue, mean value of three rats \pm s.d. for the brain, the blood, and the brain/blood ratio.

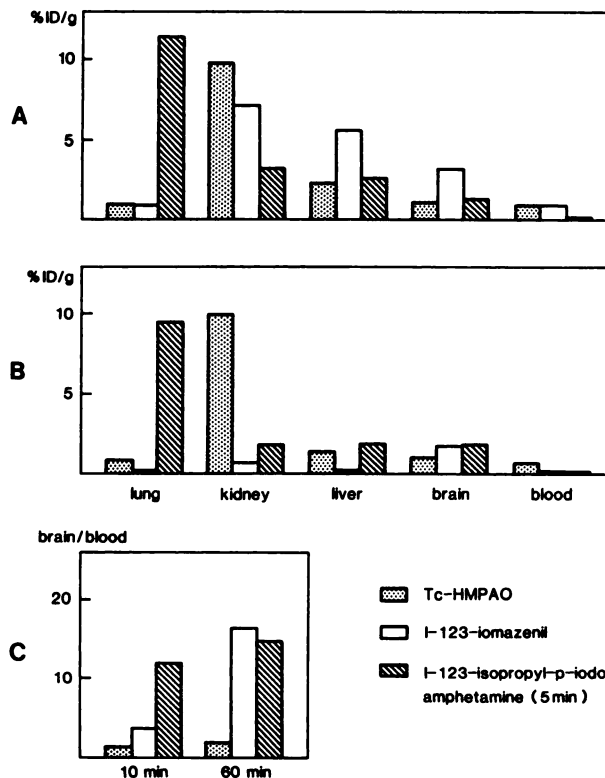


FIGURE 7
Comparison of the distribution in selected organs of $^{99\text{m}}\text{Tc}$ -HM-PAO (27), ^{123}I -Ro 16-0154, and isopropyl-p-iodo-123-aminphetamine (28) 10 min (A)*, 1 hr (B) postinjection, and the brain/blood ratio (C). (iodazenil = flumazenil analogue Ro 16-0154).* In the case of isopropyl-p-iodoamphetamine, no values determined 10 min after injection are reported in (28), therefore the values taken 5 min after injection were included here.

Injection of $0.05 \text{ mg}/\text{kg}$ Anexate[®], containing flumazenil as active compound, caused in both cases a sudden loss of radioactivity in the brain. No reaction of the two volunteers after injection of Anexate[®] was observed.

The initial uptake of the ^{123}I -Ro 16-0154 in the 10 normal volunteers was similar to the rCBF seen with [^{123}I]IMP and $^{99\text{m}}\text{Tc}$ -HM-PAO, with the exception of the

TABLE 3
Estimation of the Radiation Burden in Man Caused by the ^{123}I -Ro 16-0154 Based on Animal Data

Organ	Dose ($\mu\text{Gy}/\text{MBq}$)
Thyroid	4.7
Liver	4.1
Spleen	3.3
Kidneys	5.6
Stomach	17.2
Intestine	48.1
Lungs	1.9
Ovaries	11.0
Red bone marrow	5.2
Whole body	3.7

TABLE 4
Organ Distribution in Man Expressed as Percent of
Injected Dose per Organ (mean value of $5 \pm \text{s.d.}$)

	2 min	90 min
Head	8.28 ± 1.02	7.07 ± 0.74
Heart	3.60 ± 0.40	0.96 ± 0.19
Lungs	9.04 ± 1.89	2.78 ± 1.03
Liver	16.26 ± 1.47	4.89 ± 2.44
Bladder	—	26.30 ± 2.18

basal ganglia showing a lower activity as demonstrated in Figure 9. The unbound Ro 16-0154 was virtually completely cleared from the brain within 30 min post-injection and the distribution followed the known distribution of the benzodiazepine receptors as measured with ^{11}C -flumazenil (5). In the patient group, the investigations 30 min postinjection showed the distribution pattern of these receptors, but defects in the uptake were also seen. The investigations of the patients with Lennox-Gastaut-Syndrome resulted in a considerable difference between the perfusion pattern seen with $^{99\text{m}}\text{Tc}$ -HM-PAO and the receptor distribution seen with ^{123}I -Ro 16-0154. Two examples of transverse slices with $^{99\text{m}}\text{Tc}$ -HM-PAO and ^{123}I -Ro 16-0154 are shown in Figure 10.

DISCUSSION

Most tests demonstrated the high stability of ^{123}I -Ro 16-0154. Degradation was observed only in liver preparations, indicating an enzymatic process. The assumption that the necessary enzymes are contained in the cytosol is supported by the observation that no signif-

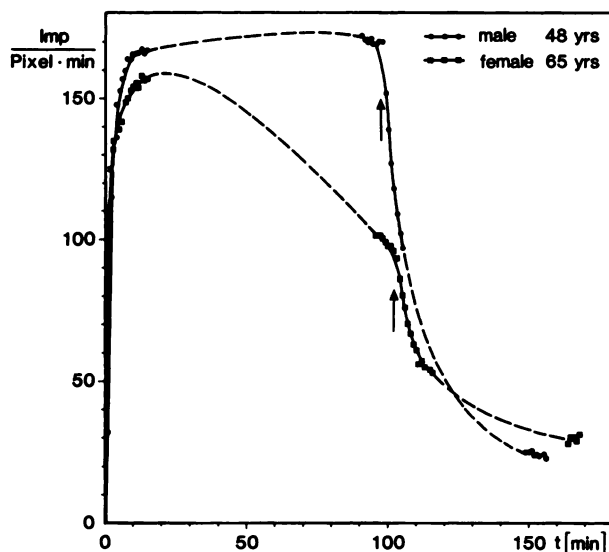


FIGURE 8
Uptake and displacement study in two volunteers. Initial phase: rapid uptake in the brain after injection of 122 MBq ^{123}I -Ro 16-0154. Arrows indicate the injection of Anexate® (0.05 mg/kg), the dotted lines indicate SPECT investigations.

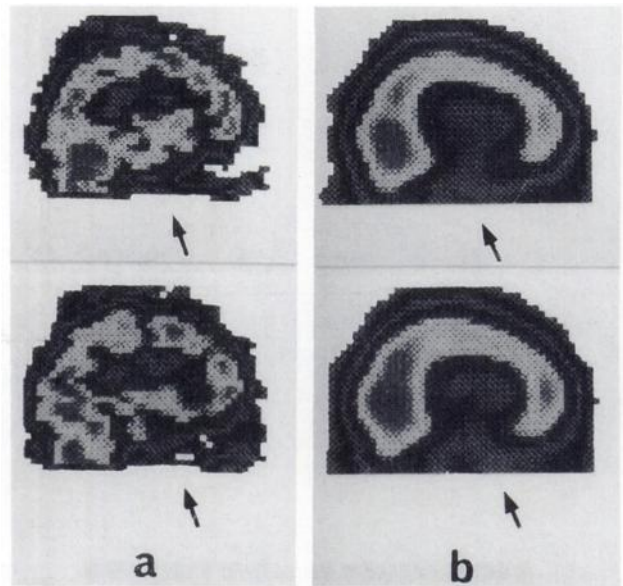


FIGURE 9
Comparison of SPECT images (two adjacent sagittal slices) with $^{99\text{m}}\text{Tc}$ -HM-PAO and ^{123}I -Ro 16-0154 in a normal volunteer. (A) 555 MBq $^{99\text{m}}\text{Tc}$ -HM-PAO and (B) 185 MBq ^{123}I -Ro 16-0154.

icant difference between the degradation in the liver homogenate and centrifugate was found. In the brain homogenate and centrifugate, no degradation was seen, indicating that no metabolism takes place. The dimi-

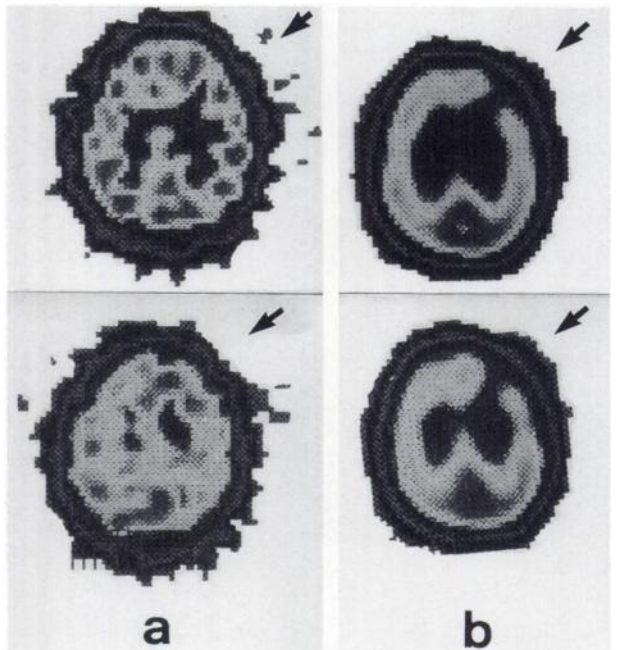


FIGURE 10
Comparison of CBF and benzodiazepine receptor distribution in two adjacent transverse brain slices found in a patient with Lennox-Gastaut-Syndrome. (A) 555 MBq $^{99\text{m}}\text{Tc}$ -HM-PAO and (B) 185 MBq ^{123}I -Ro 16-0154. The slices are shown from the lower side. The storage defect in example B is found in the left hemisphere. (Images courtesy G. Riccabona, Innsbruck)

nution of the unchanged product after centrifugation of the rat brain homogenate mixture can be explained by the binding of the product to parts of the cells.

Because of the close chemical relationship between Ro 16-0154 and flumazenil, a comparison of their pharmacologic properties is given. With the exception of the antagonism of diazepam versus PTZ (where Ro 16-0154 has partial inverse-agonist properties), the two substances have similar properties. The order of magnitude of the IC₅₀ and EC₅₀ values is the same for both.

The radioautographic images indicated that Ro 16-0154 binding sites have the same distribution in the brain as those for ³H-flumazenil and, with the exception of the thalamus, the same as known for GABA receptors (3,18,29,30).

All these findings suggest that ¹²³I-Ro 16-0154 is a benzodiazepine receptor ligand which in vivo is enriched rapidly in the brain. This uptake is in accordance with the high n-octanol-to-phosphate buffer partition coefficient. The high percentage of radioactivity measured in the liver, intestine, and kidneys, prove that the substance is excreted by these two excretion paths. No other tissue showed much enrichment.

The clinical displacement study indicates the fast and efficient uptake of ¹²³I-Ro 16-0154 in the human brain, but shows also large individual differences between both investigated persons. The displacement of ¹²³I-Ro 16-0154 by unlabeled flumazenil proved that Ro 16-0154 binds to the same central binding sites as does flumazenil. The specific binding of ¹¹C-labeled flumazenil to the benzodiazepine receptors in the human brain has been demonstrated by Mazière et al. (4). The fact that 15%–19% of the radioactivity in the brain is not displaced in these experiments is obviously not caused by metabolism of Ro 16-0154, as was seen in the studies with the rat brain. Three possible reasons can be put forward to explain this remaining radioactivity. The first and simplest is the assumption that 0.05 mg/kg cold flumazenil is not able to displace all the specifically bound radioactive tracer. The second may be some type of nonspecific binding of the radiotracer. Finally, the third could be the difference in the excretion velocity of Ro 16-0154 and flumazenil expressed as the half-life of the plasma or (respective) serum clearance of both substances. Flumazenil has a very short plasma clearance with $t_{1/2} = 53$ min (34), whereas the half-life of Ro 16-0154 has been reported (22) to be 2 hr and 45 min. After the elimination of cold flumazenil, a “back occupation” of the benzodiazepine receptors with ¹²³I-Ro 16-0154 can be expected. This would lead to a remaining part of radioactivity in the brain because it is not possible to distinguish between radioactivity bound to the receptors or displaced but not crossed the blood brain barrier. Due to the higher polarity of flumazenil, it is washed out faster from the brain than Ro

16-0154. After the complete clearance of flumazenil, a certain amount of Ro 16-0154 is still present in the brain tissue and can occupy the receptors again. A similar “back occupation” was observed during the antagonism of midazolam or flunitrazepam in patients with Anexate® (flumazenil) as reported in (34), where a resedation phenomenon is described. The reason for the slower elimination of the iodocompound compared to the fluorocompound is probably the higher octanol/phosphate buffer ratio of the iodocompound, rather than the higher molecular weight. In vivo studies with additional amounts of either cold flumazenil or Ro 16-0154 (i.e., ¹²⁷I-Ro 16-0154) might allow us to elucidate the real reason. However, the remaining radioactivity in the brain did not exceed 20% of the maximal uptake, which is considered to be so low that the value of the results would not justify experiments with patients or volunteers.

SPECT investigations (21–23) with ¹²³I-Ro 16-0154 resulted, immediately after the injection (10–15 min postinjection) in images of the cerebral blood flow, indicating the transport of the compound through the blood brain barrier, and the distribution of the radioactivity according to the cerebral blood flow. Delayed images (30 min postinjection) after the washout of the free product with the blood flow showed a distribution of the radioactivity consistent with the known distribution of the benzodiazepine receptors in the human brain. The comparison of images gained with a blood flow tracer and ¹²³I-Ro 16-0154 enables the physician to distinguish between uptake defects caused by a pathologic cerebral blood flow, and storage defects caused by a change in the receptor density, as is clearly demonstrated in all cases (n = 15) of the Lennox-Gastaut-Syndrome.

Different forms of epilepsy were chosen for investigation because of their well-defined clinical status. The nuclear medicine departments involved in these Phase II clinical studies will present their results elsewhere. Studies concerning other diseases mentioned in the introduction are in progress.

CONCLUSIONS

Iodine-123-Ro 16-0154 is the first SPECT tracer for the clinical investigation of central benzodiazepine receptors. It is hoped that it will enable neurologists, psychiatrists, and nuclear physicians to investigate the involvement of these receptors in neurologic disorders using an accessible method.

ACKNOWLEDGMENTS

The authors wish to express their thanks to Dr. H. Carman, F. Hoffmann-La Roche & Co., AG, Basle (currently with Roche Vienna), for encouraging the contact between PSI and F. Hoffmann-La Roche & Co., AG.

The displacement study shown in Figure 8 was kindly left

at our disposal by Dr. H. Fill, University of Innsbruck, shortly before his tragic death.

Special thanks are given to Th. Mäder, Ph. Scheurer, and J. Huser for their valuable technical assistance.

REFERENCES

1. Hunkeler W, Möhler H, Pieri L, et al. Selective antagonists of benzodiazepines. *Nature* 1981; 290:514–516.
2. d'Argy R, Persson A. Autoradiographic findings in mice with Ro 15-1788: the distribution of H-3 labelled Ro 15-1788 studied by whole-body autoradiography. *Psychopharmacology* 1987; 92:8–13.
3. Richards JG, Glinz R, Schoch P, Moehler H. New trends in mapping benzodiazepine receptors. In: Biggio G, Costa E, eds. *Chloride channels and their modulation by neurotransmitters and drugs. Volume 45: advances in biochemical psychopharmacology*. New York: Raven Press; 1988:27–46.
4. Mazière M, Hantraye P, Prenant C, Sastre J, Comar D. Synthesis of ethyl 8-fluoro-5,6-dihydro-5-[C-11]methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (Ro 15-1788-C-11): a specific radioligand for the in vivo study of central benzodiazepine receptors by positron emission tomography. *Int J Appl Radiat Isot* 1984; 35:973–976.
5. Shinotoh H, Yamasaki T, Inoue O, et al. Visualization of specific binding sites of benzodiazepine in human brain. *J Nucl Med* 1986; 27:1593–1599.
6. Pappata S, Samson Y, Chavoix C, Prenant C, Mazière M, Baron JC. Regional specific binding of [C-11] Ro 15-1788 to central type benzodiazepine receptors in human brain: quantitative evaluation by PET. *J Cereb Blood Flow Metab* 1988; 8:304–313.
7. Samson Y, Hantraye P, Baron JC, Soussaline F, Comar D, Mazière M. Kinetics and displacement of [C-11] Ro 15-1788, a benzodiazepine antagonist, studies in human brain in vivo by positron tomography. *Eur J Pharmacol* 1985; 110:247–251.
8. Paul SM, Skolnick P. Rapid changes in brain benzodiazepine receptors after experimental seizures. *Science* 1978; 202:892–893.
9. Trifiletti RR, Snowman AM, Whitehouse PJ, Marcus KA, Snyder SH. Huntington's disease: increased number and altered regulation of benzodiazepine receptor complexes in frontal cerebral cortex. *Neurology* 1987; 37:916–922.
10. Whitehouse PJ, Trifiletti RR, Jones BE, et al. Neurotransmitter receptor alterations in Huntington's disease: autoradiographic and homogenate studies with special reference to benzodiazepine receptor complexes. *Ann Neurol* 1985; 18:202–210.
11. Owen F, Poulter M, Waddington JL, Mashal RD, Crow TJ. [H-3]Ro 05-4864 and [H-3]flunitrazepam binding in kanate-lesioned rat striatum and in temporal cortex of brains from patients with senile dementia of Alzheimer type. *Brain Res* 1983; 278:373–375.
12. Chugani HT, Olsen RW. Benzodiazepine/gaba receptor binding in vitro and in vivo analysis of clinical disorders. In: Chugani HT, Olsen RW, eds. *Benzodiazepine/Gaba receptors and chloride channels: structural and functional properties*. New York: Alan R. Liss; 1986:315–335.
13. Samson Y, Bernuau J, Pappata S, Chavoix C, Baron JC, Mazière MA. Cerebral uptake of benzodiazepine measured by positron emission tomography in hepatic encephalopathy. *N Engl J Med* 1988; 316:414–415.
14. Savic I, Roland P, Sedvall G, Persson A, Pauli S, Widen L. In-vivo demonstration of reduced benzodiazepine receptor binding in human epileptic foci. *Lancet* 1988; 863–866.
15. Rivier JE, Lazarus HL, Perrin MH, Brown MR. Neurotensin analogues. Structure-activity relationships. *J Med Chem* 1977; 20:1409–1412.
16. Möhler H, Okada T. Benzodiazepine receptor: demonstration in the central nervous system. *Science* 1977; 198:849–851.
17. Williamson MJ, Paul SM, Skolnick P. Labeling of benzodiazepine receptors in vivo. *Nature* 1978; 275:551–553.
18. Richards JG, Möhler H, Haefely W. Mapping benzodiazepine receptors in the CNS by radiohistochemistry and immunohistochemistry. In: Panula P, Päiväranta H, Soinila S, eds. *Neurology and neurobiology, Volume 16: neurochemistry, modern methods and applications*. New York: Alan R. Liss; 1986:629–677.
19. Bonetti EP, Pieri L, Cumin R, et al. Benzodiazepine antagonist Ro 15-1788: neurological and behavioral effects. *Psychopharmacology* 1982; 78:8–18.
20. Courvoisier S. Pharmacodynamic labels for the use of chlorpromazine in psychiatry. *J Clin Exp Psychopathol* 1956; 17:25–37.
21. Bischof-Delaloye A, Oloachea-Toro M, Bogousslavsky J, et al. First experience in normal volunteers with a I-123 labeled benzodiazepine (BZD) antagonist. *Eur J Nucl Med* 1988; 14:251.
22. Riccabona G, Fill H, Bangerl I, Hinterhuber H, Vogl G. Preliminary report on I-123-flumazenil as radiotracer for brain SPECT. In: Hoefler R, Bergmann H, eds. *18th international symposium*. Badgastein, January 11–14. New York, Stuttgart: Schattauer; 1988:289–295.
23. Hoell K, Deisenhammer E, Dauth J, Loeffler W, Carmann H, Schubiger PA. SPECT mapping of human brain benzodiazepine receptors. *J Nucl Med* 1988; 29:759.
24. Bangerl I, Riccabona G, Bauer G, Bohr K, Hasler PH, Schubiger PA. ¹²³I-lomazenil brain SPECT in various forms of epilepsy (a preliminary report). *Eur J Nucl Med* 1989; 15:408.
25. Wolf P, Wagner G, Amelung F. *Anfallskrankheit*. Heidelberg: Springer Verlag; 1987:45.
26. Schmidt D. *Behandlung der epilepsien*, 2nd edition. Stuttgart: Georg Thieme Verlag; 1984:6.
27. Nowotnik DP, Canning LR, Cumming SA, et al. Development of a Tc-99m-labelled radiopharmaceutical for cerebral blood flow imaging. *Nucl Med Comm* 1985; 6:499–506.
28. Winchell HS, Baldwin RM, Lin Th. Development of I-123-labeled amines for brain studies: localization of I-123 iodo-phenylalkyl amines in rat brain. *J Nucl Med* 1980; 21:940–946.
29. Young WS, Kuhar MH. Radiohistochemical localization of benzodiazepine receptors in rat brain. *J Pharmacol Exp Ther* 1980; 212:337–346.
30. Richards JG, Möhler H. Benzodiazepine receptors. *Neuropharmacology* 1984; 23:233–242.
31. Roedler HD. Strahlenbelastung durch Radiopharmaka—Entwicklung eines mathematischen Dosiskonzeptes und Ergebnisse von Neuberechnungen der Energiedosis. PhD Thesis, 1974:137.
32. Snyder WS, Ford MR, Warner GG, Watson SB. "S" absorbed dose per unit cumulated activity for selected radionuclides and organs. *MIRD Pamphlet No. 11*. New York: Society of Nuclear Medicine; 1975.
33. Radiation Protection. *ICRP publication 53. Radiation dose to patients from radiopharmaceuticals*. Oxford: Pergamon Press; 1988:280.
34. Geller E, Thomson D. Proceedings of the International Symposium on Flumazenil—the first benzodiazepine antagonist. Geneva, Switzerland, February 1988. *Eur J Anaesthesiology* 1988; (suppl.) 2: 332.