Technetium-99m-MRP20, A Potential Brain Perfusion Agent: In Vivo Biodistribution and SPECT Studies in Normal Male Volunteers

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The lipophilic neutral complex [^{99m}Tc][TcO(MRP20)] [MRP20 is (N-(2(1H pyrolylmethyl))N'-(4-pentene-3-one-2) ethane-1,2-diamine)] is known to cross the blood-brain barrier in non-primate animals. We report here its in vivo biodistribution, radiation dosimetry, and single-photon emission computed tomography (SPECT) characteristics in man. Following i.v. administration of 15–25 mCi of the tracer, the maximum uptake of activity in the brain peaked at 1 min p.i. Fifteen minutes p.i., the percentage retained in the brain was 5.2 ± 1.6, which remained fairly constant over 24 hr. Blood clearance was relatively slow with an apparent affinity of the compound for the cellular fraction of the blood, however, soft-tissue and facial activity cleared at a rate four times faster than that of the brain. SPECT images obtained at 15 min, 1 hr, 3 hr, 7 hr, and 24 hr p.i. showed no redistribution of the tracer within the brain. The dosimetry is favorable for administration of 25–30 mCi of MRP20. Our results indicate that this compound is rapidly extracted and retained by the brain and may be used for SPECT imaging of regional blood flow.

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The search for suitable single-photon emission computed tomography (SPECT) imaging agents for regional cerebral blood flow (rCBF) studies has yielded in the past years [^{123}I]IMP amphetamine (*1*), [^{123}I-]HIPDM (*2*), ^{99m}Tc-HMPAO (*3,4*) and ^{99m}Tc-ECD, which is currently under development (*5,6*). The limited availability of ^{123}I and the relative cheap cost of generator-produced ^{99m}Tc as compared to cyclotron-produced ^{123}I favors the technetium-labeled agents. [^{99m}Tc][TcO(MRP20)], N-(2(1H pyrolylmethyl))N'-(4-pentene-3-one-2) ethane 1,2 diamine, belongs to a new class of neutral, lipophilic technetium complexes that are under investigation as potential brain perfusion agents (*7*). Preliminary animal studies in rats and in the dog have shown that MRP20 readily crosses the blood-brain barrier and is taken up and retained by the brain tissues long enough for SPECT studies to be obtained in a dog up to 4 hr after injection (*8*). Since these studies suggested favorable characteristics of MRP20 as a potential cerebral blood flow marker, and after acute animal toxicity studies had proven the safety of the compound, we determined the biodistribution, dosimetry, safety, and distribution pattern of MRP20 in reconstructed SPECT images of normal brain in eight human adult volunteers.

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MATERIALS AND METHODS

Subjects

Studies were performed on eight healthy male volunteers, ranging in age between 23 and 37 yr. The subjects were asked to abstain from any medication for 72 hr prior to the study. Written informed consent was obtained from each volunteer, and the protocol was reviewed and accepted by the institutional review board of the university. Blood samples were taken for biochemical analysis 10 min prior to administering the material and again 24 hr postinjection (p.i.). Blood pressure, pulse, respiration, and temperature were recorded 15 min prior to injection and again 15–40 min p.i.

Study Protocol

The study was divided in two parts. Four subjects were studied to determine biodistribution and metabolism by planar whole-body imaging. In this group, a SPECT study of the brain was performed at 1 hr and 7 hr p.i. Another four subjects were studied to determine the optimum imaging time and the pattern of distribution in normal brain. In this group, successive SPECT studies were performed at 15 min, 1 hr, 3 hr, 7 hr and 24 hr after injection.

Preparation of the Radiopharmaceutical

MRP20 was supplied in freeze-dried vials for reconstitution with sodium pertechnetate obtained in isotonic saline from a commercial ^{99}Mo/^{99m}Tc generator. Each vial contained 2 mg MRP20, 10 μg stannous chloride dihydrate, and 0.3 mg Na HCO_3. The radiopharmaceutical was prepared according to our formulation protocol, which is currently under review.

However, experimentally it was found that the radiochemical purity of the reaction was always greater than 90% at the time of injection as determined by a rapid octanol/saline extraction technique.

Patient Preparation and Blood and Urine Sampling

Ten minutes prior to the study an 18-gauge intracathether line was installed in an antecubital vein for bolus injection of the tracer and a similar venous access was installed in a contralateral vein for blood sample collection. Fifteen to 25 mCi of MRP20 were injected in a bolus followed by 10 ml of saline. Blood samples were collected in the contralateral arm at 2, 5, 10, 20, 30, 60 min and 2, 4, and 24 hr p.i. Urine collection was conducted in 0–4 hr, 4–8 hr, and 8–24 hr samples. Feces were collected up to 24 hr.

Whole blood and plasma samples as well as aliquots of the urinary samples were counted against appropriate standards of known dilution in an automatic gamma well counter and expressed as a percentage of the injected dose. The blood volume of each volunteer was estimated according to weight and height in order to determine the individual volunteer blood dilution factor for blood clearance analysis. Feces specimens were counted in duplicate on the gamma camera for 2 min each with a standard.

Initial Brain Uptake

In order to study initial kinetics of brain uptake, serial dynamic images of the head in an anterior position were obtained for the first 10 min after injection. The images were recorded in a 64 × 64 matrix with a frame rate of 1/sec for the first 2 min followed by a frame rate of 1/10 sec for the next 8 min. To generate time-activity curves a region of interest (ROI) including the whole brain, was selected. The activity retained in the brain at the end of the dynamic study was expressed as a percentage of the injected dose by means of counting appropriate standards under the gamma camera.

Biodistribution Studies

A number of anterior and posterior views covering the whole body were taken with a large field of view camera (Siemens ZLC 37) and collected on a Sopha S2000 data processing system in a 64 × 64 matrix. These whole-body measurements were made at 30 min, 2–4 hr, 6–8 hr, and 22–24 hr p.i. Using ROIs, the total number of counts over the whole body was measured for each image series in the anterior and posterior views. In order to correct for attenuation, the geometric mean of the anterior and posterior counts was calculated as a measure of total injected activity remaining in the body. The fraction of activity remaining in a particular organ was then measured by comparing the geometric mean of the total counts in the ROI drawn around the anterior and posterior views of the organ with the above estimate of total-body counts. Specific ROIs were drawn for brain, thyroid, lungs, heart, liver, spleen, kidneys, urinary bladder, gonads and legs (muscle). For the estimation of intestinal activity, a ROI including the whole abdomen was drawn and the total number of counts in this region minus the number of counts in liver, spleen and kidneys was considered as intestinal activity. Radiation dose estimates were calculated from the biodistribution data using the MIRD scheme (*9*). The corresponding effective dose equivalent (EDE) was determined according to the ICRP 26 (*10*) and ICRP 53 (*11*) recommendations.

SPECT Imaging

Tomographic images were obtained using a rotating gamma camera equipped with a low-energy high-resolution collimator. Data were collected in 64 × 64 matrices, using 64 angular increments over 360°, with an acquisition time of 30 sec/view. Before reconstruction, a 50% scatter correction was performed by subtracting 50% of the image acquired in the scatter window (100 keV, 20%) from the image acquired in the technetium peak window (140 keV, 15%). Transaxial, sagittal, and frontal slices were generated by filtered backprojection, using a Hamming-Hann filter. Representative ROIs were determined in both hemispheres (frontal, temporal, parietal, occipital regions, the basal ganglia and the cerebellum), the mean counts per pixel were calculated and, after correction for physical decay, were normalized to 100% of maximum activity from the first SPECT acquisition.

RESULTS

Safety Data

The injection of [^{99m}Tc][TcO(MRP20)] was well tolerated by all subjects and no adverse reactions attributed to the injection of the drug were observed in any of the volunteers. Pre- and postinjection vital signs were stable and remained unchanged throughout the study. Blood cell counts, blood, and urine chemistry values were within normal limits and remained unaltered.

Initial Dynamic Planar Brain Imaging

Figure 1 shows the planar brain time-activity curve for the first 10 min. Each point represents the mean activity in %ID for the eight subjects within a brain ROI drawn over anterior view acquisitions. Activity in the brain reached a maximum within 1 min after injection. This was followed by a 10% decrease in activity, which plateaued by about 5 min. At the end of the dynamic study, 10 min p.i., the absolute amount of injected activity was 5.14 ± 1.25 %ID.

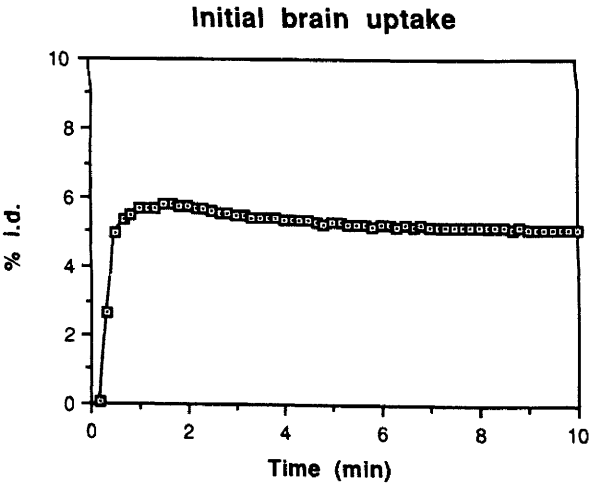

FIGURE 1
Dynamic planar brain time-activity curve showing the initial uptake kinetics of MRP20. Each point represents the mean of eight normal subjects.

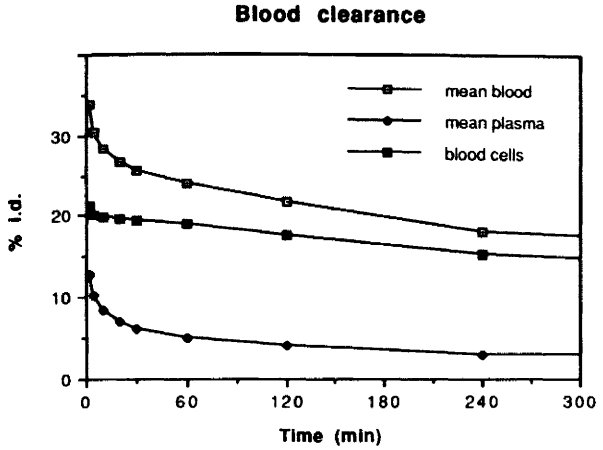

FIGURE 2
Venous blood clearance of MRP20 in eight normal subjects.

Blood Clearance

As depicted in Figure 2, clearance of MRP20 from plasma was much faster than that from the whole blood. The uptake in the cellular fraction of blood was calculated by subtracting the plasma values from the whole-blood values. There was a rapid uptake of nearly 20% injected dose and a long retention of the TcO(MRP20) by the blood cells. After 1 hr, 80% of the blood activity was in the cellular fraction.

Biodistribution

The quantitative data describing the kinetics of organ uptake and clearance are summarized in Table 1.

Brain uptake at 15 min was 5.2% ± 1.6%. In all cases, the level of uptake remained approximately constant over 24 hr at which time it was still 4.4% ± 0.9%. At 7 hr p.i., only 5% of the initial activity had cleared out of the brain.

MRP20 was excreted both in the urine and through the hepatobiliary system. Total urinary excretion over 24 hr was 28.5% ± 3.3%. Gallbladder activity showed a high degree of variation from one subject to another. No significant amount of activity was observed in the stomach, so that it may be assumed most of the intestinal activity entered the small intestines from the biliary tract and was subsequently excreted. Cumulated fecal and colon activity at 24 hr was 25.4% ± 4%. Soft-tissue distribution of the tracer was predominantly found in the skeletal muscle. About 2% of the activity was retained in the myocardium. Initial lung uptake was 15%, decreasing to about 10% at 24 hr. No significant accumulation, other than blood-pool activity, was observed in thyroid and gonads. Variable slight uptake was observed in nasal and oral mucosa and in the parotid glands. Some trapping of the tracer occurred in the vein into which the tracer was injected, which is a common finding with other perfusion agents as well.

The results of the radiation dose estimates are shown in Table 2. For the calculation of the EDE, intestines, kidneys, liver, spleen, and brain were considered to be the five remaining organs receiving the highest radiation dose. The corresponding EDE was 36 mrem/mCi.

SPECT Studies

High quality brain SPECT images of $>4 \times 10^6$ counts could be obtained until 7 hr p.i. Qualitatively, these brain images showed an uptake similar to the pattern seen with other perfusion agents and there was good differentiation between gray and white matter. Figure 3 displays equivalent transverse slices of the successive SPECT studies performed in one of the subjects. Regional tracer distribution remained constant with no redistribution between gray and white matter. Quantitatively, no significant variations in clearance were observed between left and right hemispheres nor between different brain regions (Fig. 4) (Table 3). Brain clearance was only 5%–10% of initial activity until 6 hr after injection. Soft-tissue activity in the facial region decreased by nearly 40% during the same period.

DISCUSSION

MRP20 is readily available from a freeze-dried vial and may be prepared with a radiochemical purity (RCP) of >90% by following a simple formulation protocol. For each subject in the clinical trial, the technetium complex was prepared using fresh generator eluate (less

TABLE 1
Biodistribution in Normal Human Subjects

Organ	% ID/organ			
	15 min	3 hr	7 hr	24 hr
Brain	5.2 ± 1.6	4.8 ± 1.0	4.8 ± 1.2	4.5 ± 1.0
Lungs	15.0 ± 1.6	12.0 ± 2.4	11.5 ± 1.2	9.9 ± 2.2
Heart	2.4 ± 0.5	1.9 ± 0.3	1.8 ± 0.3	1.5 ± 0.2
Liver	15.2 ± 3.3	11.7 ± 3.1	10.2 ± 2.7	7.4 ± 3.1
Kidneys	8.2 ± 0.6	6.7 ± 0.8	6.1 ± 0.9	4.5 ± 1.4
Thyroid	0.4 ± 0.1	0.3 ± 0.05	0.3 ± 0.07	0.3 ± 0.06
Skeletal muscle	19.9 ± 4.2	17.8 ± 3.2	14.7 ± 2.8	13.8 ± 2.5
Gall bladder and intestines	8.9 ± 1.7	11.4 ± 3.4	13.2 ± 4.1	17.7 ± 3.2
Urine and bladder	3.7 ± 0.3	16.7 ± 2.3	22.6 ± 2.6	28.9 ± 3.3
Total body	100.0	86.2 ± 1.7	80.8 ± 2.8	70.7 ± 3.4

TABLE 2
Radiation Dose Estimates

Organ	mRad/mCi	mGy/GBq
Brain*	22	6
Bladder	8	2
Intestines*	170	46
Kidney*	115	31
Liver*	51	14
Lungs*	59	16
Ovaries*	14	4
Red bone marrow*	10	3
Testes*	2	1
Spleen*	68	18
Muscles	8	2
Thyroid*	3	1

* Effective dose equivalent: 36 mrem/mCi (9.84 mSv/GBq).

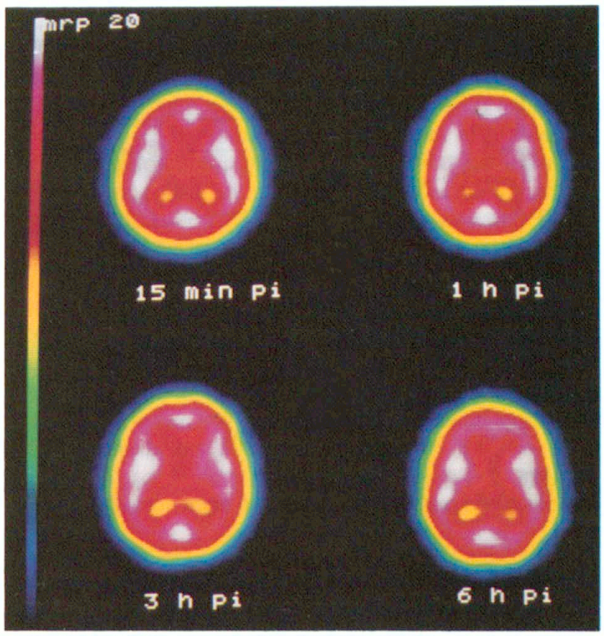

FIGURE 3
Equivalent transverse cross sections obtained in successive SPECT studies in Subject 8. No substantial differences in regional tracer distribution were observed between 15 min and 6 hr p.i.

than 2-hr-old) from a generator that had always been eluted less than 24 hr earlier. Although the complex was prepared immediately prior to its use, the in vitro stability does allow a minimum delay of 2 hr before the RCP falls below 80%. The RCP was determined by octanol/saline extraction, using a procedure previously described and confirmed retrospectively by instant thin-layer chromatography. At no time were the impurities of TcO_2 and TcO_4^- found to be >5%.

MRP20 appears to be safe and useful for brain perfusion SPECT studies in healthy human volunteers. The rapid initial brain uptake and long retention of the tracer in the brain are very favorable characteristics for SPECT imaging with conventional rotating gamma cameras. These SPECT studies show a regional cerebral distribution, unchanged over time, that is similar to the distribution pattern observed with other brain perfusion agents, especially to that of ^{99m}Tc-HMPAO. This high degree of similarity in brain pharmacokinetics and in regional brain distribution of both agents is very much in agreement with the earlier studies in dogs. While it has not yet been proved experimentally that the cerebral distribution of MRP20 is proportional to regional blood flow, we can nevertheless conclude that in man, MRP20 possesses the two basic requirements for a brain perfusion agent: rapid passage across the blood-brain barrier and an intrinsic mechanism by which the tracer after having crossed the blood-brain barrier does not freely diffuse out again. This mechanism has not been defined but it is presumed to be related to the in vitro tendency of the neutral complex to hydrolyse, generating a cationic species unable to back-diffuse out of the brain.

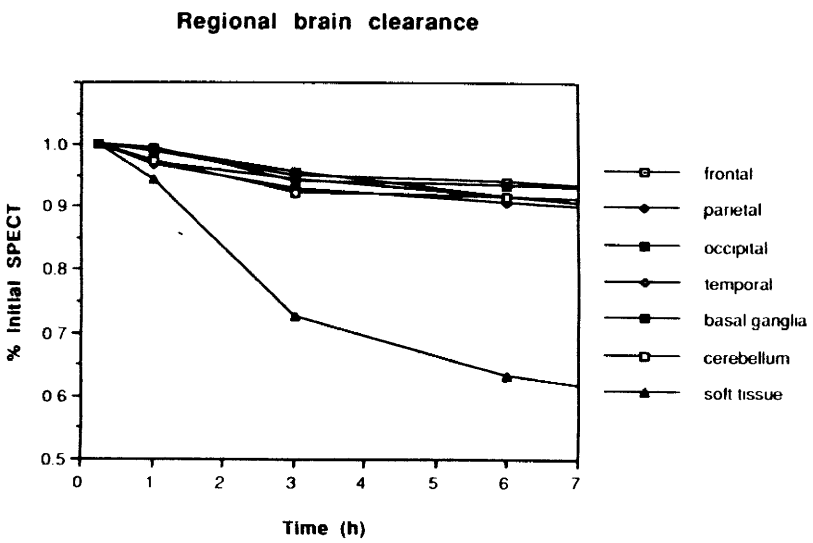

FIGURE 4
Regional brain clearance of MRP20 observed in the successive SPECT studies performed in Subject 8. All values are expressed in percentage of the regional count rate in the SPECT study started at 15 min p.i.

TABLE 3
Regional Uptake Values in Successive SPECT Studies*

Regions	mean ± s.d. 15 min p.i.	mean ± s.d. 1 hr	mean ± s.d. 3 hr	mean ± s.d. 7 hr
Frontal	1.013 ± 0.012	1.013 ± 0.016	1.012 ± 0.018	1.021 ± 0.013
Parietal	1.002 ± 0.008	1.000 ± 0.009	0.995 ± 0.004	0.990 ± 0.007
Temporal	1.071 ± 0.019	1.069 ± 0.003	1.087 ± 0.011	1.081 ± 0.024
Occipital	1.004 ± 0.009	0.995 ± 0.010	1.005 ± 0.014	1.998 ± 0.015
Basal ganglia	0.954 ± 0.005	0.960 ± 0.015	0.950 ± 0.025	0.961 ± 0.019
White matter	0.568 ± 0.026	0.572 ± 0.024	0.559 ± 0.025	0.561 ± 0.021
Cerebellum	1.148 ± 0.067	1.146 ± 0.064	1.131 ± 0.067	1.127 ± 0.145

* Data are expressed as (mean cts/voxel of a ROI)/(mean cts/voxel of whole brain).

Following i.v. administration, blood clearance of [^{99m}Tc][TcO(MRP20)] is slow ($T_{1/2}$ > 4 hr). This is mainly due to a rapid and persistent uptake in the cellular fraction of the blood of about 20% injected dose. For a brain perfusion agent, the high intravascular activity (20% injected dose after 1 hr) is a drawback: assuming that the brain contains 112 ml of blood, the activity contribution would be about 0.5%. This is not dissimilar from ^{99m}Tc-HMPAO (*12*). Technetium-99m-ECD, however, has a much more favorable blood clearance (<5 %ID after 1 hr). The total-body retention of MRP20 after 24 hr was about 70%, the tracer being excreted both by urinary and hepatobiliary excretion. Following initial uptake, no accumulation was observed in other organs that could be an indication of in vivo reoxidation to pertechnetate. Taking into account the ICRP weighting factors for the calculation of EDE, the organs having the greatest contribution to radiation dosimetry are the intestines, the lungs and the kidneys. The EDE corresponding to an administered dose of 25 mCi would be 0.9 rem, which is of the same order of commonly performed radionuclide studies.

No redistribution of activity within the brain was observed between the successive SPECT studies. During the relatively long acquisition period necessary with rotating cameras, activity changes within the field of view are more likely to be related to physical decay than to variations in tracer distribution. This study, an initial Phase I clinical evaluation of [^{99m}Tc][TcO(MRP20)] in normal volunteers, suggests that the radiopharmaceutical is safe to administer intravenously with favorable dosimetry for an injection of 25–30 mCi. As far as can be determined from the reconstructed SPECT slices, the tracer distributes according to regional blood flow and remains within the cerebral matter for several hours. Because of the apparent lack of redistribution between gray and white matter, it is possible and perhaps preferable to scan two hours after injection or thereafter, thus allowing the background activity in the facial area and the blood-pool activity to diminish. We conclude therefore that [^{99m}Tc][TcO(MRP20)] warrants further evaluation as a compound suitable for SPECT imaging in clinical assessment of rCBF.

REFERENCES

1. Winchell HS, Horst WA, Braum L, Oldendorf WH, Hattern R, Parker H. N-isopropyl (I123) p-iodoamphetamine: single-pass brain uptake and washout: binding to brain synaptosomes and localization in dog and monkey brain. *J Nucl Med* 1980;21:947–952.
2. Holman BL, Lee RGL, Hill TC, et al. A comparison of two cerebral perfusion tracers N-isopropyl I-123-p-iodoamphetamine and I-123-HIPDM in the human. *J Nucl Med* 1984;25:25–30.
3. Neirinckx RD, Canning LR, Nowotnik DP, et al. Technetium-99m d,l-HM-PAO: a new radiopharmaceutical for SPECT imaging of regional cerebral blood perfusion. *J Nucl Med* 1987;28:191–202.
4. Sharp PF, Smith FW, Gemmell HG, et al. Technetium-99m-HM-PAO stereoisomers as potential agents for imaging regional cerebral blood flow: human volunteer studies. *J Nucl Med* 1986;27:171–177.
5. Holman BL, Hellman RS, Goldsmith SJ, et al. Biodistribution, dosimetry, and clinical evaluation of technetium-99m-ethyl cysteinate dimer in normal subjects and in patients with chronic cerebral infarction. *J Nucl Med* 1989;30:1018–1024.
6. Léveillé J, Demonceau G, De Roo M, et al. Characterization of technetium-99m-L,L-ECD for brain perfusion imaging, part 2: biodistribution and brain imaging in humans. *J Nucl Med* 1989;30:1902–1910.
7. Morgan GF, Deblaton M, Clemens P, et al. Tc-99m-MRP20—a new class of neutral complex with implications for brain scintigraphy [Abstract]. *J Nucl Med and Allied Sci* 1989;33:305–306.
8. Morgan GF, Deblaton M, Clemens P, et al. Technetium-99m-MRP20: a potential new brain perfusion agent. Part 1: The in vivo biodistribution and SPECT studies in non-primate animals. *J Nucl Med* 1991;32:500–505.
9. Snyder WS, Ford MP, Warner GG, et al. "S" absorbed dose per unit accumulated activity for selected radionuclides and organs. *MIRD pamphlet no. 11.* New York: The Society of Nuclear Medicine, Inc: 1975.
10. Sowby FD. Recommendations of the International Commission on Radiological Protection. *Annals of the ICRP. ICRP publication 26.* England, 1977.
11. Sowby FD. Radiation dose to patients from radiopharmaceuticals. *ICRP publication 53.* England, 1987.
12. Podreka E, Suess E, Goldenberg G, et al. Initial experience with technetium-99m-HM-PAO brain SPECT. *J Nucl Med* 1987;28:1657–1666.