Human Biodistribution, Dosimetry and Clinical Use of Technetium(III)-99m-Q12

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Technetium(III)-99m-Q12, trans-(1,2-bis(dihydro-2,2,5,5-tetramethyl - 3(2H)furanone - 4 - methyleneimino)ethane)bis(tris(3 - methoxy-1-propyl)-phosphine)technetium(III)-99m, is a nonreducible complex of Tc(III) which is herein evaluated as a myocardial perfusion imaging agent. **Methods:** The biodistribution and dosimetry of ^{99m}Tc-Q12 were assessed in 10 normal volunteers, while its potential clinical use was evaluated in 70 patients. **Results:** Safety parameters measured up to 24 hr postinjection demonstrate no clinically significant drug-related adverse reactions. Technetium(III)-99m-Q12 exhibits good heart uptake (2.2% injected dose at 1 hr postinjection under resting conditions) and no detectable myocardial washout or redistribution up to 5 hr postinjection. The biodistribution is characterized by very rapid hepatobiliary clearance which allows effective myocardial imaging at times as short as 15 min postinjection. Blood and plasma clearances and myocardial uptake are rapid, while lung uptake is minimal. The heart-to-lung and heart-to-liver ratios are higher at stress than at rest, independent of the time elapsed between injection and image acquisition, and independent of whether the patient is fasted or fed after tracer administration. A preliminary correlation shows that 46/47 patients with angiographically demonstrated CAD also have perfusion defects demonstrated by ^{99m}Tc-Q12. **Conclusions:** On the basis of the studies reported herein, ^{99m}Tc-Q12 appears to be a promising myocardial perfusion imaging agent.

Key Words: myocardium; technetium-99m-Q12; myocardial perfusion

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The presence and extent of myocardial perfusion impairment is currently assessed by scintigraphy using appropriate myocardial perfusion imaging agents (MPIAs) (*1–4*). Thallium-201 is the principle MPIA, and it has been in widespread clinical use for over two decades (*5*). In order to overcome the limitations of ^{201}Tl which result from the relatively low energy of its emitted photon and from its poor dosimetric properties, MPIAs based on ^{99m}Tc have recently been developed and proposed for clinical use (*6,7*). The first such ^{99m}Tc MPIA was ^{99m}Tc-sestamibi, introduced in the late 1980s and now widely used (*8*). Sestamibi is retained in the myocardium for a long period of time; this property, combined with the moderately energetic gamma emission of ^{99m}Tc, makes feasible SPECT, which has been demonstrated to be more effective than planar imaging in clinical applications (*9–11*). However, liver accumulation of sestamibi can interfere with the correct evaluation of heart images, and also imposes a time delay between injection and acquisition. More recently ^{99m}Tc-teboroxime, an MPIA characterized by very rapid washout from the myocardium has been proposed (*12,13*). With this agent, planar imaging is possible only within a few minutes after injection, and tomographic images are possible only with multiple-headed gamma camera systems. Again, liver accumulation of this agent is very high and interferes with heart images, particularly in planar views. Tetrofosmin is a newer ^{99m}Tc MPIA which exhibits more rapid hepatobiliary clearance, and in turn allows for an earlier acquisition time (*14,15*). Tetrofosmin also undergoes myocardial washout, although at a much slower rate than is observed for teboroxime.

We have been developing a new class of ^{99m}Tc MPIAs that is designed to clear rapidly and extensively from the liver. This new class of ^{99m}Tc MPIAs is based upon a cationic ^{99m}Tc(III) center which has been configured so that it cannot suffer in vivo reduction to a neutral ^{99m}Tc(II) form which has been shown to wash out of the heart (*16,17*). This resistance to in vivo reduction means that these agents can be designed to be retained in the myocardium for long periods of time, similar to the myocardial retention observed for sestamibi. The prototypical member of this class has been designated ^{99m}Tc-Q3, and in both animal and human studies this prototype has shown good clinical potential (*18–20*). Most importantly, ^{99m}Tc-Q3 exhibits rapid and extensive clearance from the liver, and good myocardial images can be obtained at rest within 15 min after injection (*21*). We now report on an improved

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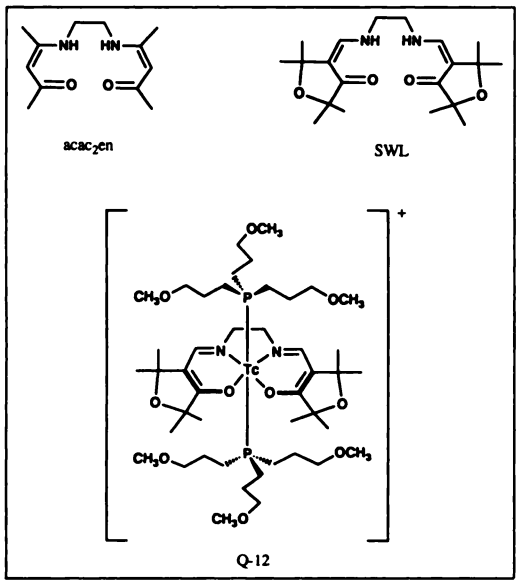


FIGURE 1. Structures of the Schiff base ligands $acac_2en$ and SWL and structure of the Tc(III)-Q12 cation.

member of this class, designated ^{99m}Tc-Q12: trans-(1,2-bis(dihydro - 2,2,5,5 - tetramethyl - 3(2H)furanone - 4 - methyleneimino)ethane)bis(tris(3 - methoxy - 1 - propyl)phosphine) technetium(III)-99m (Fig. 1) (*1,22–25*).

Initial in vitro experiments with ^{99m}Tc-Q12 demonstrated that this agent is chemically very stable and that it can be prepared with high radiochemical purity. Studies in animal models showed no signs of toxicity (acute or subacute), as well as the expected high and stable myocardial uptake (*26*). More recent studies in dogs have shown a good correlation between blood flow and ^{99m}Tc-Q12 myocardial uptake (*24*).

The aim of the present study was to characterize the behavior of ^{99m}Tc-Q12 in humans. To this end we first assessed the biodistribution, dosimetry and safety of ^{99m}Tc-Q12 after intravenous injection at rest or during exercise in normal volunteers. We then evaluated the clinical use of this tracer by assessing organ uptake ratios under fasting or feeding conditions, at different acquisition times after injection, both at rest and following exercise. The results of these initial human studies are reported herein.

MATERIALS AND METHODS

Chemistry

The metastable isotope ^{99m}Tc, as sodium pertechnetate in aqueous NaCl (0.15 *M*) was obtained from a commercial generator (Sorin Biomedica, Italy or DuPont de Nemours, Germany) eluted with saline that was provided by the manufacturer. Proton and ^{13}C-NMR spectra were obtained on a Varian Gemini-300 FT NMR spectrometer. Phosphorous-31-NMR spectra were obtained on a JEOL FX90Q spectrometer. Infrared spectra were recorded on a Perkin-Elmer 283B spectrophotometer. Fast atom bombardment (FAB) and electron impact (EI) mass spectra were obtained on a Finnigan TSQ 700 spectrometer while inductively coupled plasma (ICP) mass spectroscopic measurements were made using a Sciex Elan Model 250 instrument. Elemental analyses were determined by Atlantic Microlab, Inc., (Norcross, GA). TLC analyses of organic compounds were carried out with Analtech glass-backed 250-μ silica gel GF plates. TLC analysis for reduced hydrolyzed technetium in ^{99m}Tc-Q12 was carried out on paper strips using 80% acetone, 20% saline as solvent.

The tetradentate ligand 1,2-bis[dihydro-2,2,5,5-tetramethyl-3(2H)-furanone-4-methyleneamino]ethane (SWL) was prepared via the Schiff base condensation of ethylenediamine with the appropriate dicarbonyl compound. A solution of dihydro-2,2,5,5-tetramethyl-3(2H)furanone (32.1 g, 226 mmole) in 50 ml of diethyl ether was added dropwise to a stirred suspension of sodium hydride (18.1 g 60%, 453 mmole) in 400 ml of ethyl ether containing two drops of ethanol and 36.5 ml (453 mmole) of ethyl formate at 0°C. After stirring overnight at room temperature, the reaction mixture was taken up into water, washed with additional ethyl ether, acidified with 6N HCl and extracted with ethyl ether. The combined ether layers were washed with water and brine, dried over magnesium sulfate, decolorized with charcoal, filtered through celite and evaporated. The residue was recrystallized from ethyl ether-hexanes to afford 30.4 g (79%) of 4-hydroxymethylene-dihydro-2,2,5,5-tetramethyl-3(2H)furanone as an off-white solid. Freshly distilled ethylenediamine (1.15 ml, 17.4 mmole) was added to a solution of 4-hydroxymethylene-dihydro-2,2,5,5-tetramethyl-3(2H)furanone (5.9 g, 34.7 mmole) in 40 ml of tetrahydrofuran. After refluxing this solution for 1 hr, the solvents were removed by evaporation. The residue was triturated with ice-cold ether (50 ml) and the solid was collected by filtration. Recrystallization from tetrahydrofuran (50 ml) followed by drying at 70°C under high vacuum (1 mm Hg) afforded 5.3 g (76%) of 1,2-bis[dihydro-2,2,5,5-tetramethyl-3(2H)furanone-4-methyleneamino]ethane (SWL) as a white solid ^{1}H-NMR (300 MHz, 1/1 CD_3OD/D_2O + 1 drop 1% KOD) δ 1.25 (s, 12H), 1.40 (s, 12H), 3.55 (s, 4H), 7.17 (s, 2H); ^{13}C-NMR (75 MHz, CD_3NO_2) δ 27.00, 32.64, 50.90, 80.02, 82.05, 109.39, 150.17, 204.13; IR (KBr) 1670, 1590, 1470, 1440 cm^{-1}; TLC (silica, EtOAc (5% TEA)) R_f = 0.56; FAB mass spectrum m/z = 365 (M + H); Anal. Calculated for $C_{20}H_{32}N_2O_4$ C, 65.93; H, 8.79; N, 7.69. Found C, 65.68; H, 8.90; N, 7.65.

Tris(3-methoxy-1-propyl)phosphine (TMPP) was prepared by slowly adding a solution of 1-chloro-3-methoxypropane (20.0 g, 184 mmole) in 50 ml of tetrahydrofuran to magnesium metal (4.48 g, 184 mmole) in order to maintain a gentle reflux. Refluxing was continued for 2 hr postaddition to afford a solution of the Grignard reagent, 3-methoxy-1-propyl magnesium chloride. The solution was filtered to remove traces of magnesium metal, then cooled to −78°C in a dry ice-acetone bath under argon. Dichloroethoxyphosphonite (3.50 ml, 30.7 mmole) in 10 ml of tetrahydrofuran was added dropwise to the Grignard reagent over 1 hr. The solution was warmed to room temperature gradually, then heated to reflux for 2 hr. The reaction was quenched by the addition of deaerated water (20 ml). The tetrahydrofuran solution was dried over sodium sulfate, cannula transferred to a dry flask and distilled (bp = 154 − 155°C @ 2.4 mmHg) to afford 3.80 g (49%) of TMPP as a colorless oil: ^{1}H-NMR (benzene-d_6) δ 3.29 (t, J = 6.5 Hz, 6H), 3.21 (s, 9H), 1.51 − 1.64 (m, 6H), 1.28 − 1.36 (m, 6H); ^{13}C-NMR (benzene-d_6) δ 73.3 (J_{PC} = 12.1 Hz), 58.2, 25.6 (J_{PC} = 13 Hz), 23.0 (J_{PC} = 12.5 Hz); ^{31}P-NMR (benzene-d_6) δ − 32.9; EI mass spectrum m/z = 251 (M + 1).

Radiochemical Preparation

One synthesis of ^{99m}Tc-Q12 was based on the two-step procedure used in the preparation of ^{99m}Tc-Q2 and ^{99m}Tc-Q3 (*18,20*). In

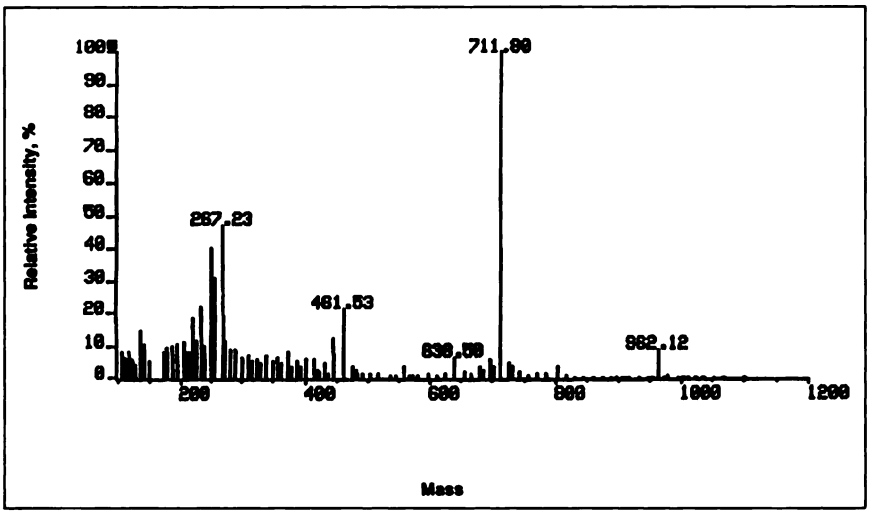


FIGURE 2. Fast atom bombardment mass spectrum (FAB-MS) of ^{99g}Tc-Q12.

the first step, $^{99m}TcO_4^-$ (20–200 mCi, 0.74–7.4 GBq, 1 ml) was added to a vial containing 15 mg of SWL in 0.1 ml of ethanol. The solution was deaerated for 15 min with a vigorous stream of argon; 15 μg of $SnCl_2$ (in degassed ethanol) and 0.03 ml of 1 *M* NaOH were then added to this solution of SWL and ^{99m}Tc-pertechnetate. The preparation was then incubated for 5 min at 100°C to yield the $[^{99m}Tc^{V}(O)(SWL)]^+$ intermediate. Radiochemical purity was determined by reversed-phase HPLC to be >95% (PRP-1 column: 150 × 4.1 mm, 10 μ; 45% acetonitrile/0.1 *M* NH_4OAc, 2.0 ml/min, t_r = 3.0 min).

In the second step, 0.1 ml of a TMPP · HCl solution (1 g TMPP · HCl in 10 ml ethanol) was added to the $[^{99m}Tc^{V}(O)(SWL)]^+$ preparation and the solution heated for 15 min at 100°C to yield the desired $[^{99m}Tc^{III}(SWL)(TMPP)_2]^+$ (^{99m}Tc-Q12) complex. Separation of the radiolabeled complex from reagents was performed by diluting the preparation to 20 ml with water, loading it onto a prewet C18 Sep-Pak and washing the loaded Sep-Pak with water (20 ml) and ethanol:water (80:20, 4 ml); the product was then eluted in ca. 60% yield with 2 ml of 80:20 ethanol:saline. Quality control effected by reverse-phase HPLC (same conditions as above, t_r = 3.5 – 4.0 min) showed >95% radiochemical purity. This eluate was diluted with normal saline to generate an ethanol concentration <5%, and the diluted eluate was then passed through a sterile 0.2-μ Flow Pore D-26 filter to provide a preparation suitable for human use. The apyrogenicity of this preparation was evaluated by standard Lymulus Amebocite Lysate testing on decayed samples.

The Sep-Pak purified product is obtained in 30%–50% overall yield (based on ^{99m}Tc-pertechnetate). The final radiopharmaceutical formulation (5% ethanol in saline) is stable for at least 8 hr; no loss in radiochemical purity is observed over this time period. Inductively coupled plasma mass spectroscopy (ICP-MS) experiments were performed on decayed radiopharmaceutical preparations to ensure that the Sep-Pak purification removed all detectable TMPP. In all cases, the purified preparations contained less phosphorus than can be detected by this technique (ca. 1 ppm total phosphorus). Phosphorus contents were calculated from a calibration curve wherein the ^{31}P intensity signal from a series of standard solutions was plotted as a function of known ^{31}P concentration. In addition, for each experiment, the solvent matrix of the sample was also subject to ICP-MS analysis so that any signal due to the solvent matrix could be distinguished from the sample signal.

The majority of patient studies were conducted with ^{99m}Tc-Q12 prepared from a one-step "instant kit" suitable for human use (*24*). The chemistry of this one-step formulation will be described in a subsequent publication. All ^{99m}Tc-Q12 prepared in this manner was of >95% radiochemical purity, as assessed by reverse-phase HPLC (same conditions as above).

Chemical Characterization of Technetium-99m-Q12

The Q12 complex was chemically characterized by fast atom bombardment mass spectroscopy (FAB-MS) after adding "carrier" ^{99g}Tc to the ^{99m}Tc preparation. In this experiment, the ^{99m}Tc-Q12 complex was prepared with generator eluate that had been spiked with 2.5 mg of $Na^{99g}TcO_4$. The "carrier-added" preparation was purified by reverse-phase HPLC, and the collected Q12 preparation analyzed by positive ion FAB-mass spectrometry. The FAB-MS spectrum shows a parent peak at m/z = 962 amu which is consistent with the Q12 cation, $[Tc(SWL)(TMPP)_2]^+$ (calculated average mass is 962.1 amu). Fragment ions corresponding to losses of the phosphine ligands and phosphine R groups are also observed. The predominant peak in the FAB mass spectrum of Q12 occurs at m/z = 712 amu, which corresponds to the loss of one TMPP ligand. The FAB mass spectrum of Q12 is shown in Figure 2.

Protocols

All protocols involving humans were approved by the Institute H. S. Raffaele Ethics Committee. Each subject or volunteer signed an informed consent statement; the attending cardiologist agreed to the protocol and consent form, according to the Ethic Committee guidelines.

Safety

The clinical safety of ^{99m}Tc-Q12 was evaluated in normal volunteers by monitoring vital signs, as well as blood and urine chemistries, immediately before and 24 hr after tracer administration; the details of these evaluations are provided below. Also, in all human studies an attending physician monitored the subject for possible adverse reactions, and the presence or absence of such adverse reactions was recorded.

Kinetics, Biodistribution and Dosimetry

Ten normal volunteers, nine males and one female (age 22 to 42 yr) were studied to assess dosimetry by acquiring radiobiodistribution data. Seven subjects were studied at rest and three were studied by injecting the tracer under exercise conditions. Each volunteer was enrolled after a general clinical evaluation including blood pressure, heart rate, blood chemistry (hemoglobin, hematocrit, complete blood count, sodium, potassium, urea, calcium, creatinine, chloride, SGOT, SGTP) and urine chemistry; the female volunteer also had a test to establish that she was not pregnant at the time of the study. Blood and urine chemistry, blood pressure and heart rate were also monitored up to 24 hr postinjection. Data were acquired with a 40-cm circular gamma camera system (7500 Orbiter-Siemens; equipped with a general-purpose, parallel-hole collimator) using a 64 × 64 matrix size and Zoom 1.

Before tracer administration, either at rest or under exercise, whole-body transmission images were recorded to determine the fraction of attenuated activity for each organ. In this procedure a homogeneous flood source (a circular flood phantom; diameter 50 cm, thickness 1 cm) filled with ca. 10 mCi of ^{99m}Tc aqueous solution was used as an external source. First, 4,000 Kcounts were acquired from the flood source and the elapsed time registered. Using this time, four sequential planar views (head, chest, upper abdomen and lower abdomen) were then acquired by positioning the volunteer equidistant between the flood phantom and the gamma camera. In order to ensure the same body position during the biodistribution imaging, each view was registered through the use of reference markers.

For rest studies, a volunteer who had fasted for over 12 hr was positioned supine on the gamma camera bed. A plastic catheter (20 gauge) was inserted in the left antecubital vein for intravenous administration of the tracer and a catheter of the same size was inserted in the contralateral vein for collection of blood samples.

After the transmission scans, approximately 12 mCi of ^{99m}Tc-Q12, in a volume of 1–5 ml for the purified preparation and <1 ml for the kit formulation, were injected as a bolus through the indwelling catheter. Radiochemical purity was assessed by HPLC analysis before the injection, and the syringe residual was similarly analyzed; in all cases, both analyses showed a radiochemical purity of greater than 95%. A dynamic acquisition of the upper body (chest and upper abdomen) was started simultaneously with the injection and was continued for 60 min (0.5 sec/frame for a total of 1 min and then 1 min/frame for 59 min). Planar views (LAO 45°, anterior, LAO 70°) were subsequently acquired. Approximately 1 hr after injection, subjects were repositioned with the use of the reference markers noted above (from the transmission study), and whole-body anterior and posterior projections were recorded in a preset time mode of 2 min. This same biodistribution acquisition protocol was repeated at 3 hr and 5 hr postinjection.

For the stress studies, after completion of the transmission acquisitions, catheters were inserted as for the rest studies. The subjects were then exercised on a treadmill (Marquette Case 12) following the Bruce protocol and a twelve-lead ECG was constantly recorded. The tracer was injected when the normal volunteers approached maximum fatigue; they were then encouraged to continue the exercise for 1 min longer in order to allow for complete tracer circulation. After the end of the exercise protocol, the subjects were monitored by ECG during the recovery period; the duration of recovery, heart rate and systolic blood pressure were also assessed. The subjects were then positioned supine on the gamma camera bed. The rest of the protocol was the same as described above for the rest studies.

Blood and Urine Clearances

To determine the blood clearance profile of ^{99m}Tc-Q12, venous samples were drawn from the arm contralateral to the injection site. During the resting studies, the first sample was drawn immediately after the bolus injection, and about 20 samples were taken within the first 10 min. During the stress studies, sampling was started 5 min after tracer injection. For both rest and stress studies, subsequent samples were collected at 30 min, 1 hr, 1.5 hr, 3 hr, 5 hr and 24 hr after injection. A portion (0.5 ml) of each whole-blood sample was assayed for ^{99m}Tc content; results were corrected for physical decay of the isotope, normalized for the circulating blood volume and expressed as percentage of injected dose/ml. The rest of each whole-blood sample was centrifuged to separate plasma from RBCs; 0.5 ml of each plasma sample was then assayed as above to estimate the plasma clearance which reflects the extravascular clearance.

The urine clearance profile was obtained by collecting urine over the following periods: 0–1, 1–3, 3–5 and 5–24 hr after injection; after volume measurements, 0.5 ml from each sample was assayed for ^{99m}Tc content; these data were corrected for physical decay of the isotope, normalized to the volume of urine collected and the resulting values expressed as percentage of injected dose.

Patient Studies

Seventy patients with suspected or proven coronary artery disease were studied with ^{99m}Tc-Q12 in order to evaluate the optimal time to wait between injection and imaging, as well as the influence of fasting conditions. All patients were previously studied with echocardiography, rest and exercise ECG. A detailed clinical history was collected from every subject, paying particular attention to the risk factors for coronary diseases; blood and urine chemistries were also evaluated. Subjects with evidence of liver or kidney disfunction were excluded. All patients discontinued cardioactive medications and maintained fasting conditions for the 12 hr preceding tracer injection. Blood pressure values were kept under control with diuretics and/or ACE inhibitors. The subjects were then randomly classified into four different groups according to protocols based on varying elapsed times between injection and image acquisition. These elapsed times were chosen in light of the organ and blood clearances observed in normal volunteers; for practical reasons, 60 min postinjection was the longest elapsed time studied. The groups were designated as follows: Group A had acquisition at 15 min postinjection, Group B had acquisition at 30 min postinjection, Group C had acquisition at 45 min postinjection, and Group D had acquisition at 60 min postinjection. Within groups B, C and D, ten subjects were studied under fasting conditions and ten subjects were studied after consuming a fatty meal subsequent to tracer administration. For Group A, the short elapsed time between injection and the first imaging (15 min postinjection) made it impractical for patients to eat before the first imaging procedure, and thus this group contains only the ten fasted patients.

Every subject was studied under the same conditions at rest and 48 hr later after treadmill exercise. During exercise, three ECG leads were continuously monitored and, at each minute during exercise and recovery, a twelve-lead ECG was also recorded. The endpoints were defined as angina of a least moderate severity, ≥2 mm ST segment depression, hypotension, frequent ventricular premature beats or ventricular tachycardia, excessive fatigue, shortness of breath or weakness. The exercise ECGs

were interpreted as positive if there was >1 mm ST depression of the flat or downsloping variety, or if there was a 1.5-mm upsloping ST segment depression. The exercise ECGs were interpreted as negative if there were no changes during exercise provided that the patient achieved >85% of maximal predicted heart rate. Tomographic studies over 360° (64 × 64 matrix, 64 views beginning from anteroposterior, 30 sec/view) were performed using the same gamma camera system specified above in the biodistribution studies. The tomographic studies were started at the indicated time after intervenous injection of 20–25 mCi of ^{99m}Tc-Q12.

Data Analysis: Kinetics

Time-activity curves were constructed for the period 10 to 60 min postinjection by positioning regions of interest (ROIs) over the entire liver, gallbladder, lungs and left ventricle of the heart as visualized in the anteroposterior dynamic images. The average counts per pixel of the relative ROIs were corrected for decay and normalized to the initial myocardial count density at 5 min postinjection. Organ activities were then plotted versus time after injection.

Data Analysis: Biodistribution and Dosimetry

Organ uptakes were calculated from the whole-body biodistribution studies using the simplified method described by Myres et al. (*27*) within the following formalism:

$$A = F'|(N_a \times N_p \times \exp(\mu l))^{1/2}|/A_0, \qquad \text{Eq. 1}$$

where A is the organ activity expressed as percentage of the injected dose, F′ is the cross-calibration factor (μCi/counts/min) obtained by positioning a source of known activity at 10 cm from the gamma camera and collecting counts for 2 min, N_a and N_p are the counts obtained from the emission scan in the anteroposterior and posteroanterior views respectively, $\exp(\mu l)$ is the body attenuation correction factor measured directly from transmission scans, and A_0 is the injected dose. The parameter $\exp(\mu l)$ is directly obtained from the ratio NF/NT where NT represents the counts within the ROIs drawn around the organ on the transmission image and NF represents the corresponding ROI counts obtained on the flood source image. For emission scans, ROIs were drawn around each organ and then counts and number of pixels within the ROI were recorded. When organs overlapped, the organ activity was determined by normalizing to full organ size the counts/pixel values obtained from nonoverlapped sites. This method was used for liver in the presence of gallbladder and right kidney, and for left kidney in the presence of activity within the intestine. For kidneys, twice the activity in the left kidney was used. Values obtained by application of formula 1 were corrected for the physical decay of ^{99m}Tc in order to determine the percent of injected dose (%ID) versus time for different organs (heart, lungs liver, gallbladder, kidneys and bladder). The bladder %ID was determined by summing the %ID estimated from imaging data (via Eq. 1) with the %ID excreted in the urine.

The mean absorbed dose $D(r_k)$ in a target organ r_k was calculated applying the standard MIRD formulation:

$$D(r_k)_{h=1}^{n} = \Sigma \quad \bar{A}_h S(r_k \leftarrow r_h), \qquad \text{Eq. 2}$$

where $\bar{A}_h$ is the source organ accumulated activity, $S(r_k \leftarrow r_h)$ is the mean dose to target organ r_h, and n is the number of source organs. In this study, bone marrow, heart, lungs, liver, gallbladder, kidneys and bladder were identified as source organs. Absorbed dose normalized to injected dose and expressed as mRad/mCi was assessed for the following target organs: bone marrow, kidneys, bladder wall and testes or ovaries. Source organ accumulated activity was directly calculated as the integral of the estimated best fit functions applied to the %ID versus time curve. Bone marrow accumulated activity was estimated as 25% of the blood accumulated activity (*28*). Bladder accumulated activity was integrated over the interval 0 to 5 hr assuming no voided urine until 5 hr postinjection. All absorbed dose calculations included the physical decay of ^{99m}Tc.

Data Analysis: Patient Organ Uptake Ratios

From the anteroposterior view (i.e., the first of the 64 steps taken during the tomographic studies), average counts/pixel values were calculated for liver, lungs and heart; for the normal heart, the ROI was at least 16 pixels away from the pathologically affected area. The resulting values were corrected for background activity by subtracting the average counts/pixel value obtained by placing an ROI next to the organ being considered. Heart-to-liver and heart-to-lungs ratios were then calculated. The values of these ratios obtained under different conditions (rest/stress, fasting/fed and time postinjection) for Groups B, C and D were then statistically analyzed. To determine if the varying conditions generated statistically significant differences, an ANOVA was applied with two grouping factors (fasting/fed and time) and one within factor (rest/stress); this is also known as a SPLIT-PLOT design (*29*). Student's t-test was used to compare Group A (which was studied at 15 min postinjection only under fasting conditions) to the group studied at 30 min postinjection under the same conditions.

Image Quality

For the 70 patient studies, rest/stress tomograpic images obtained between 15 min and 60 min postinjection as described above, were analyzed by three independent observers experienced in nuclear cardiology. Observers were asked to assess whether or not the images had adequate diagnostic value.

RESULTS

Chemistry

The radiochemical purity of ^{99m}Tc-Q12, as assessed by HPLC, is always above 95%, both before injection and immediately after injection (as measured in the syringe residual). All radioactive impurities elute before ^{99m}Tc-Q12 in the reversed-phase HPLC system, and thus appear to be more hydrophilic than ^{99m}Tc-Q12. Moreover, these radioactive impurities appear to be rapidly excreted through the renal system and do not add to the heart or liver uptakes. The Sep-Pak purification procedure provides 30%–50% overall yield of a radiopharmaceutical with low phosphorus content (<1 ppm by ICP-MS) in a sterile and apyrogenic form suitable for intravenous administration to humans.

Safety

Blood and urine chemistries were normal in the 10 volunteers for 24 hr after injection of ^{99m}Tc-Q12. None of the 70 patients studied reported any subjective adverse reactions within 48 hr of tracer administration. Three normal volunteers and eight patients reported a transient metallic taste immediately after injection of ^{99m}Tc-Q12.

Kinetics

For the resting studies, time-activity curves generated from ROIs over the entire heart, lungs, liver and gallblad-

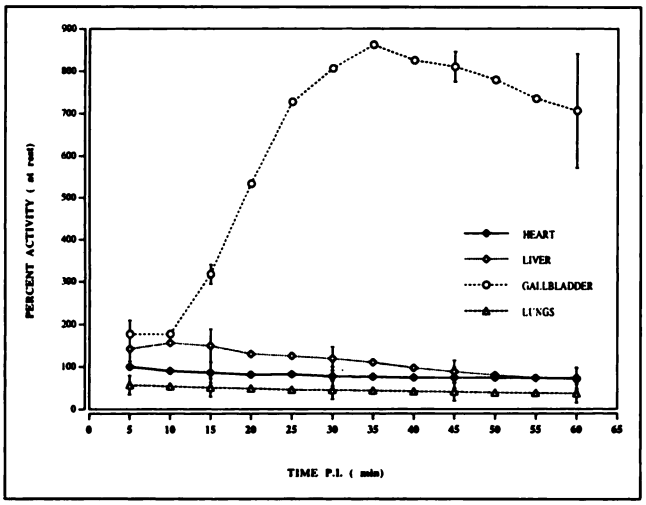


FIGURE 3. Time-activity curves for various organs. Technetium-99m-Q12 injected at rest in normal volunteers (mean of n = 5; error bars indicate ±1 s.d.). The data are normalized to cardiac activity at 5 min postinjection.

der from 10 to 60 min postinjection show the highest concentration of activity in the gallbladder. The activity in the gallbladder peaks at 35 min postinjection (Fig. 3) and then decreases, although the activity in the gallbladder always remains higher than the activity in the other organs. The initial liver activity is 160% that of the heart; at 60 min postinjection the liver activity drops 44% and becomes only 90% that of the heart. The heart activity generated by ^{99m}Tc-Q12 remains substantially constant at all times after 5 min postinjection, with no evidence for washout or redistribution. The lungs exhibit intravascular activity in the first 2 min postinjection; after this intravascular activity washes out, the lung activity remains stable and relatively low. Once the contributions from activity in the gallbladder and major biliary ducts are subtracted, the liver activity is seen to decline steadily from 10 min postinjection, the first time at which meaningful measurements could be made.

Biodistribution and Dosimetry

In normal volunteers, when ^{99m}Tc-Q12 is injected at rest at 1 hr postinjection, the highest observed ^{99m}Tc accumulations (expressed as percent of injected dose after application of decay and attenuation correction) are 14.6% ± 4.1% in the bladder, 6.3% ± 3.0% in the liver and 7.3% ± 3.1% in the gallbladder. The %ID in the heart is 2.2% ± 0.4% while the two lungs accumulate 3.9% ± 0.4% ID. A limited ROI (16 pixel) over the quadricipitis, taken as background, shows 0.17% ± 0.04% of ID (Table 1).

From 1 to 3 to 5 hr postinjection, the heart activity remains substantially stable; the uptakes measured at these times, 2.2% ± 0.4%, 1.9% ± 0.5% and 1.7% ± 0.7% respectively, are not statistically different ($p \geq 0.05$, paired t-test with 12 degrees of freedom). Figure 4 shows the whole-body biodistribution images obtained for one volunteer at 1, 3 and 5 hr postinjection.

After injection of ^{99m}Tc-Q12 under stress conditions, the biodistribution pattern is quite similar to that observed at rest; the average heart uptake is higher under stress conditions, although the difference observed in these studies is not statistically significant (Table 2).

The results of the dosimetric calculations based on these data are given in Table 3.

Blood and Urine Clearances

In both the rest and stress exercise studies in normal volunteers, clearance of activity from the blood can adequately be described by a dual exponential function. At rest, the maximum observed whole-blood pool activity is 40% ID at about 2 min postinjection, this then decreases to 10% ID within 10 min postinjection and to <5% ID at 20 min postinjection. The blood pool activity remains negligible from 20 min postinjection to the end of the study (24 hr postinjection). In the exercise studies, the first blood sample was collected at 5 min postinjection. The blood-pool activity had already decreased to the same level observed in the rest studies at approximately 10 min postinjection (i.e., to 10%).

The plasma clearances at rest and stress follow the same profiles observed for the respective blood clearances. However, the peak activity observed in plasma is lower than that observed in whole blood (20% versus 40% ID).

The amount of activity excreted in the urine at 1 hr postinjection is somewhat higher in the resting studies than in the studies conducted under stress (Fig. 5): 14.6% ± 4.1% versus 10.2% ± 2.4%. Similarly, at 24 hr postinjection the excreted amounts are 26% ± 4% at rest and 23% ± 5% at stress.

TABLE 1
Technetium-99m-Q12 Biodistribution at Rest (%ID, mean ± s.d., n = 7)

	1 hr postinjection	3 hr postinjection	5 hr postinjection
Heart	2.20 ± 0.40	1.87 ± 0.50	1.72 ± 0.70
Lungs	3.98 ± 0.42	3.07 ± 0.48	3.10 ± 0.50
Liver	6.30 ± 2.98	4.60 ± 2.51	3.82 ± 1.80
Gallbladder	7.31 ± 3.10	8.01 ± 3.39	6.80 ± 2.50
Bladder	14.59 ± 4.1	18.6 ± 3.80	20.7 ± 5.06
Kidney	3.8 ± 1.02	2.7 ± 0.82	2.4 ± 0.35
Muscle (16 pixels)	0.17 ± 0.04	0.092 ± 0.003	0.085 ± 0.005

TABLE 2
Technetium-99m-Q12 Biodistribution at Stress (%ID, mean ± s.d., n = 3)

	1 hr postinjection	3 hr postinjection	5 hr postinjection
Heart	2.39 ± 0.4	2.11 ± 0.5	1.87 ± 0.23
Lungs	4.1 ± 0.6	4.0 ± 0.43	3.8 ± 0.43
Liver	5.33 ± 0.3	4.23 ± 0.7	3.2 ± 0.5
Gallbladder	7.55 ± 0.32	11.2 ± 4.5	9.64 ± 3.5
Bladder	10.2 ± 2.4	15.96 ± 1.9	20.59 ± 2.5
Kidney	5.6 ± 0.9	3.2 ± 1.01	2.1 ± 0.46
Muscle (16 pixels)	0.13 ± 0.047	0.093 ± 0.01	0.09 ± 0.026

Patient Organ Uptake Ratios

Average heart-to-lung and heart-to-liver ratios in patients are given in Table 4. An ANOVA shows that the administration of a fatty meal did not significantly improve the heart-to-liver ratio. However, significantly ($p \leq 0.05$) better heart-to-liver ratios were observed at stress relative to those observed at rest; this presumably results from the higher blood-flow rates encountered during exercise. As expected, lengthening the time elapsed between tracer administration and image acquisition significantly ($p \leq 0.05$) improves the heart-to-liver ratio since longer elapsed times allow for more extensive clearance of the tracer through the hepatobiliary system.

The heart-to-lung ratio is positive at 15 min postinjection, allowing facile visualization of the myocardial uptake.

Image Quality

The three independent observers considered the rest/stress images in all 70 patient studies to be of good quality, providing adequate diagnostic value independent of acquisition time (even for the 15 min postinjection studies) and fasting conditions.

DISCUSSION

Chemistry

The chemistry of the "Q series" of ^{99m}Tc complexes is now well established (*16,18,19*). The octahedral coordination sphere of these cationic, nonreducible Tc(III) complexes is comprised of a tetradentate Schiff base ligand (*L4*) and two monodentate phosphine ligands which are situated trans to one another. Both ^{99m}Tc-Q3 and ^{99m}Tc-Q12 contain the same phosphine ligand (TMPP), but they differ in the identity of the *L4* Schiff base. Figure 1 shows the structures of the *L4* ligands used to prepare ^{99m}Tc-Q3 (*L4* = $acac_2en$) and ^{99m}Tc-Q12 (*L4* = SWL) (*26,30*). The cyclic ether moieties of SWL make this L4 ligand easier to label with ^{99m}Tc. The greater rigidity of SWL also appears to impart greater chemical stability to ^{99m}Tc-Q12 (relative to ^{99m}Tc-Q3), and this may account for the more extensive hepatobiliary clearance of ^{99m}Tc-Q12.

TABLE 3
Technetium-99m-Q12: Estimated Radiation Absorbed Dose (rest, 5 hr postinjection)

Organs	Dose (mRad/mCi)
Breasts	1.8
Heart wall	25.1
Gallbladder wall	290.0
Kidney	37.6
Liver	21.5
Lungs	13.8
Ovaries	5.9
Testes	3.4
Bladder	97.1
Red marrow	3.2

Source organs: heart, gallbladder, liver, lungs, kidney, red marrow and bladder.

Given the well-documented chemistry of the "Q-series," the chemical identity of $^{99m/g}$Tc-Q12 is easily established by FAB mass spectroscopy conducted in the cationic mode (Fig. 2). The observed parent peak at 962 amu is consistent with the proposed formulation and cationic charge of Q12. In addition, the predominant peak observed at 712 amu corresponds to loss of one TMPP ligand from the Q12 complex; this fragmentation pattern is characteristic for the "Q series" of mixed ligand Tc(III) complexes, and is consistent with the known trans labilizing effect of phosphine ligands.

The two-step labeling procedure used to prepare ^{99m}Tc-Q12 involves initial stannous reduction of $^{99m}TcO_4^-$ to generate the Tc(V) intermediate $[^{99m}Tc^{V}(O)(SWL)]^+$, and then subsequent application of TMPP in a substitution/reduction reaction to form the product $[^{99m}Tc^{III}(SWL)(TMPP)_2]^+$, ^{99m}Tc-Q12. This procedure has been designed to generate an agent which contains >95% ^{99m}Tc-Q12 and <5% of radiolabeled impurities, all of which exhibit reversed-phase HPLC retention times less than that of ^{99m}Tc-Q12. This means that the radiolabeled impurities are all more hydrophilic than is ^{99m}Tc-Q12, and thus these impurities do not accumulate in the liver. Rather, these impurities appear to be rapidly cleared through the renal system and do not degrade the heart to nontarget organ ratios. The one-step kit preparation also provides >95% radiochemically pure ^{99m}Tc-Q12 with the same ensemble of hydrophilic impurities.

A simple C18 Sep-Pak purification was developed to remove the excess ligands (SWL, TMPP) from the final formulation. The efficiency of this purification was checked by determining the amount of total phosphorus, which is a

TABLE 3
Technetium-99m-Q12 Organ Ratios in Patients (Average Counts, n = 70 mean ± s.d.)

	15 min postinjection fasting (n = 10)	30 min postinjection fasting (n = 10)	30 min postinjection with fatty meal (n = 10)	45 min postinjection fasting (n = 10)	45 min postinjection with fatty meal (n = 10)	60 min postinjection fasting (n = 10)	60 min postinjection with fatty meal (n = 10)
Heart-to-lung							
Rest	1.33 ± 0.25	1.54 ± 0.16	1.59 ± 0.16	1.81 ± 0.20	1.75 ± 0.25	1.57 ± 0.46	1.90 ± 0.36
Stress	1.46 ± 0.20	1.70 ± 0.19	1.59 ± 0.12	1.84 ± 0.12	1.83 ± 0.22	1.73 ± 0.29	2.08 ± 0.16
Heart-to-lung							
Rest	0.63 ± 0.05	0.60 ± 0.29	0.61 ± 0.16	0.94 ± 0.15	1.01 ± 0.37	1.40 ± 0.46	1.50 ± 0.35
Stress	0.67 ± 0.11	0.78 ± 0.46	0.69 ± 0.20	1.07 ± 0.14	1.43 ± 0.31	1.65 ± 0.33	1.55 ± 0.35

measure of TMPP concentration, in the purified preparation. Inductively coupled plasma mass spectroscopy was used to measure these very low concentrations of phosphorus. This very sensitive technique shows that the amount of phosphorus in the final formulation is always <1 ppm, which is the level of trace phosphate in the normal saline used to prepare the final formulation. Thus, the C18 Sep-Pak procedure removes more than 99.9% of the TMPP used in the original reaction.

The C18 Sep-Pak purification procedure provides a 30%–50% overall yield of ^{99m}Tc-Q12 in a matrix of normal saline which contains no more than 5% ethanol. After filtration, this solution is apyrogenic and sterile. Thus, the described procedure generates a radiopharmaceutical of high radiochemical purity (>95%) and high chemical purity (total phosphorus content <1 ppm) in a form suitable for intravenous administration to humans. However, because of the necessity of diluting the ethanol content to less than 5%, the volume of injectate is correspondingly larger (1 to 5 ml) than normal, and thus this Sep-Pak purified formulation is not useful for first-pass radioangiography which currently requires <1 ml injected volume.

Human Studies

The studies reported herein establish that the injection of ^{99m}Tc-Q12 into ten normal volunteers generates no subjective or objective adverse reactions under the described experimental conditions. No substantive subjective reactions were reported by any of the 70 patients during the 48 hr after tracer administration.

Technetium-99m-Q12 exhibits rapid whole-blood and plasma clearances as well as rapid heart uptake, similar to what is observed for other ^{99m}Tc based MPIAs (*8,13,14*). The dosimetry results presented in Table 3 indicate that the critical nontarget organ for ^{99m}Tc-Q12 is the gallbladder; at rest, an injected dose of 20 mCi ^{99m}Tc-Q12 would deliver about 6 rad to the wall of the gallbladder. The doses delivered to the sex organs and bone marrow at rest are in the range of those delivered by the other ^{99m}Tc MPIAs (*8,13,14*). During exercise, the dose delivered to most organs is markedly reduced because of the generally more rapid clearance of the agent. Moreover, ^{99m}Tc-Q12 undergoes renal, as well as hepatobiliary clearance; this rapid initial renal clearance, comparable to that observed for tetrofosmin (*14*), reduces the overall dose absorbed by the patient. There is very little lung or thyroid uptake of ^{99m}Tc-Q12, although the salivary glands exhibit significant uptake (Fig. 4).

Technetium-99m-Q12 generates favorable heart/liver ratios at short times postinjection. A comparison of the data obtained herein for ^{99m}Tc-Q12 to literature data for ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin, shows that at 60 min postinjection, at rest under fasting conditions, the heart-to-liver ratio is 0.6 ± 0.1 for sestamibi (*8*), 1.2 ± 0.8 for tetrofosmin (*14*) and 1.4 ± 0.5 for Q12. The peak of activity in the gallbladder is reached at 36 min postinjection for Q12 (Fig. 4) versus >60 min postinjection for sestamibi (*8*) (both values at rest under fasting conditions), further indicating the lower hepatobiliary activity of Q12 relative to sestamibi at early times postinjection. This faster hepatobiliary clearance of ^{99m}Tc-Q12 from the principle nontarget organ system translates directly into earlier image acquisition times.

The myocardial uptake of ^{99m}Tc-Q12 is significantly higher than that reported for ^{99m}Tc-tetrofosmin (*14*): 2.2% ± 0.4% versus 1.2% ± 0.4% (% injected dose at rest, 1 hr postinjection). Moreover, the myocardial uptake of ^{99m}Tc-Q12 remains constant with time, whereas ^{99m}Tc-tetrofosmin washes out of the heart with time (*14*). Thus, ^{99m}Tc-Q12 combines the relatively high myocardial uptake and prolonged myocardial retention of ^{99m}Tc-sestamibi with the relatively rapid hepatobiliary clearance of ^{99m}Tc-tetrofosmin.

The data in Table 4 show that heart-to-lung and heart-to-liver ratios observed for ^{99m}Tc-Q12 are consistently higher at stress than at rest, and that this result is independent of image acquisition time and independent of whether the patient is fasted or fed after tracer administration. This result is compatible with the higher blood flows encountered under exercise conditions, and with previously reported animal studies which demonstrate that the myocardial uptake of ^{99m}Tc-Q12 is proportional to blood flow (*24*).

The optimal time for imaging with a MPIA results from a balance between the need to have good target to nontarget contrast (and most specifically, good heart-to-liver contrast), and the need to have sufficient heart activity (assuming that the heart uptake remains constant through

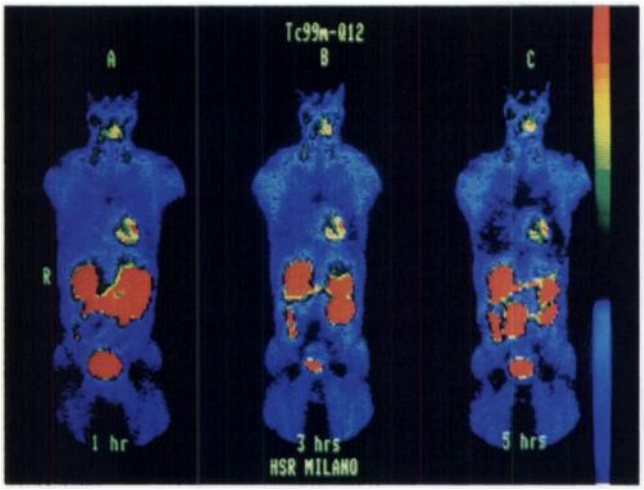


FIGURE 4. Anteroposterior whole-body images at 1 (A), 3 (B) and 5 (C) hr after injection of ^{99m}Tc-Q12 at rest in a normal volunteer.

the end of the acquisition). For ^{99m}Tc-Q12, good image quality is achievable as early as 15 min postinjection (Fig. 6A, 6B) although a longer delay would be recommended for patients with compromised biliary function; between 15 min postinjection and 60 min postinjection, the time elapsed before image acquisition does not affect image interpretation or diagnosis. With such an early acquisition time it may be feasible to develop a one-day rest/stress imaging protocol for ^{99m}Tc-Q12.

While evaluation of diagnostic accuracy using ^{99m}Tc-Q12 was not within the scope of this work, the image quality obtained during our SPECT studies allowed delineation of both rest and stress perfusion defects. A preliminary correlation between the grade of coronary stenosis and the presence of perfusional defects as assessed by ^{99m}Tc-Q12 was performed for the 47 patients in whom such a correlation was feasible (*31*); these included the five patients studied at 15 min postinjection. In this preliminary study, readings by three independent observers established

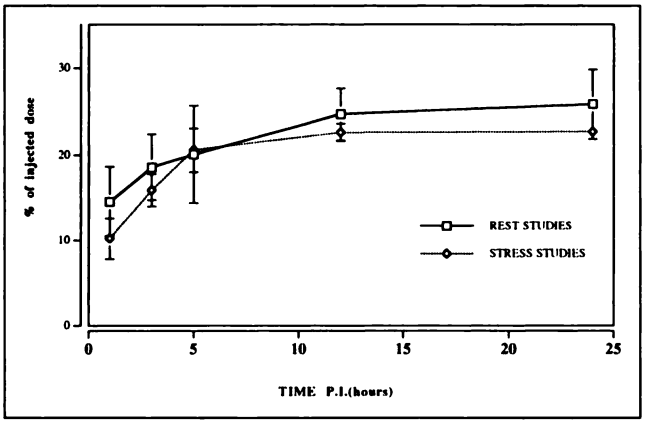
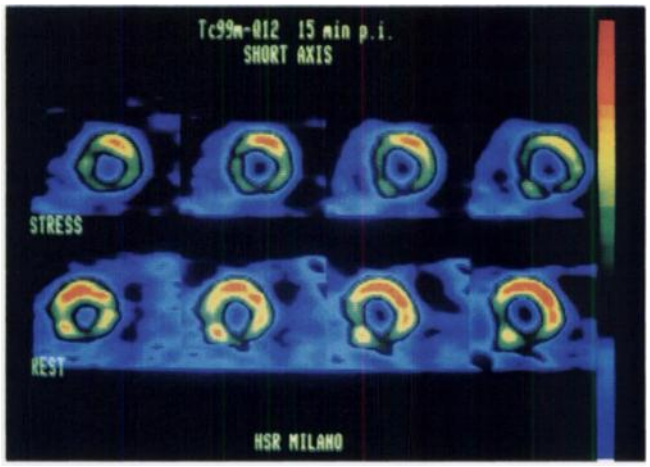


FIGURE 5. Cumulative urinary clearance of ^{99m}Tc-Q12 after injection at rest (mean of n = 5) and during exercise (mean of n = 3). Error bars indicate ±1 s.d.



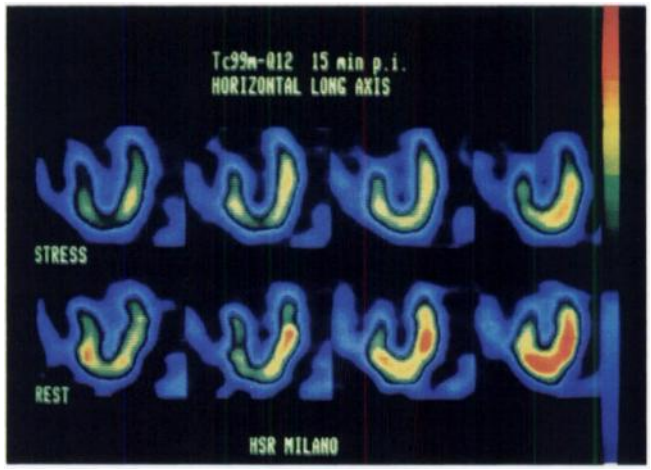


FIGURE 6. Technetium-99m-Q12 myocardial SPECT images obtained 15 min postinjection at rest and during exercise in a patient with significant coronary artery disease. The perfusion images along the short (A) and horizontal long (B) axes demonstrate ischemia involving anterolateral segments.

that 46/47 patients with angiographically demonstrated CAD had perfusional defects demonstrated by ^{99m}Tc-Q12 imaging.

Finally, it should be noted that the advent of ^{99m}Tc-Q12 in an "instant kit" formulation (*25*) would allow for injection of this tracer in a small volume (<1 ml) suitable for assessment of right and left ventricular ejection fraction by first-pass radionuclide angiography. Further studies are clearly warranted in order to establish the clinical efficacy of this new agent.

SUMMARY

Technetium-99m-Q12 is a radiotracer developed for myocardial perfusion imaging. It shares the same advantages as do the other myocardial perfusion imaging agents (MPIAs) based on ^{99m}Tc; the most important being the 141-keV photon, which is optimal for gamma camera imaging, the relatively low dosimetry and the ready availability of the isotope. The in vivo kinetics of ^{99m}Tc-Q12 were studied in ten normal volunteers. To assess the potential clinical use of this agent, 70 patients were evaluated. Safety parameters measured in this population up to 24 hr postin-

jection demonstrated no clinically significant, drug-related adverse reactions. Technetium-99m-Q12 exhibits good heart uptake (2.2% ID at 1 hr postinjection under resting conditions) with no significant myocardial washout or redistribution up to 5 hr postinjection. The tracer clears rapidly from blood and from the liver via the hepatobiliary system. This rapid hepatobiliary clearance minimizes the major disadvantage encountered with ^{99m}Tc-sestamibi and ^{99m}Tc-teboroxime and allows effective myocardial imaging at times as short as 15 min postinjection.

On the basis of the studies reported herein, ^{99m}Tc-Q12 appears to be a promising MPIA. Further studies will be required in order to establish the clinical efficacy of this agent.

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