The Interpretation of Thallium-201 Cardiac Scintigrams

Studies in Experimental Ischemic Heart Disease in Dogs

A.P. Selwyn, E. Welman, T.A. Pratt, J. Clark, C. McArthur, and J.P. Lavender

SUMMARY Twenty-one anesthetized and thoracotomized dogs were studied. Scintigrams of the heart were recorded using a continuous infusion of krypton-81m into the aortic sinuses as well as intravenous injections of thallium-201. A gamma camera linked to a digital computer was used to record the myocardial distribution of these tracers. The distribution of 201Tl was similar to that of 81mKr when myocardial blood flow was normal (seven dogs). In 14 dogs, the left anterior descending coronary artery (LAD) was narrowed to produce a regional decrease in myocardial blood flow. Blood flow changes were measured with an electromagnetic flowprobe. When the epicardial ECG was normal in seven dogs, the 201Tl scintigram showed no regional decreases in activity when the tracer was delivered after LAD narrowing. In contrast, a decrease in the activity of 81mKr was observed in the region supplied by the LAD. When the decrease in blood flow was associated with ECG signs of ischemia in seven dogs, both 81mKr and 201Tl scintigrams showed decreased activity in the ischemic area. The cardiac distribution of 201Tl was determined in five dogs while myocardial blood flow and metabolism were normal. LAD narrowing then produced 24 hours of severe myocardial ischemia. The distribution of creatine kinase activity in the left ventricle (U/mg DNA) was similar to the distribution of 201Tl (counts/mg DNA), \( r = 0.83; P < 0.001. \) These studies suggest that 201Tl scintigraphy of the heart can demonstrate decreases in regional myocardial perfusion only when metabolism is disturbed.

THALLIUM-201 is being widely investigated and used to image the heart in the assessment of patients with coronary artery disease.1-3 Following a peripheral venous injection, the regional distribution of this radionuclide in the heart has been used to obtain information about myocardial ischemia and infarction.4,5 201Tl cardiac scintigrams are reported as demonstrating abnormalities of regional myocardial perfusion.6 Experimental research has suggested a close relationship between the distribution of 201Tl in the heart and regional myocardial blood flow.7 These experiments have made certain assumptions about the energy-dependent cell membrane extraction of the indicator.4,6 The purpose of this study was to test whether 201Tl scintigrams of the heart can provide an assessment of changes in regional myocardial perfusion. The relative importance of coronary blood flow and myocardial metabolic processes in the interpretation of 201Tl cardiac scintigrams is discussed.

Methods

Twenty-one mongrel dogs weighing between 32 and 51 kg were anesthetized with intravenous sodium thiopental (Pentothal, 16 mg/kg). Anesthesia was maintained by the intermittent intravenous injection of pentabarbital (Saggital, 2 mg/kg). Respiration was maintained via a cuffed endotracheal tube with a mechanical ventilator. A left thoracotomy was performed and the heart supported in a pericardial cradle. A reversible snare was placed around the left anterior descending coronary artery (LAD) approximately 2 cm from where this vessel emerges from under the left atrial appendage. A reversible snare was placed around the left anterior descending coronary artery (LAD) approximately 2 cm from where this vessel emerges from under the left atrial appendage. This snare consisted of 4 silk strands pulled through an 8-cm length of 3 French cardiac catheter tubing.

An electromagnetic flow probe (e.m.f.) of suitable size was positioned around the LAD immediately proximal to the snare. Most commonly a 3- to 5-mm probe was used. Pulsatile regional coronary flow was recorded by connecting the probe to a Systems Electronic for Medicine flowmeter (type 275). This has a carrier frequency of 285 Hz and the response is nominally flat (less than 3 dB down) to 80 Hz. Phasic and mean tracings were recorded on a multichannel instrument (Hewlett Packard 7788A). At the end of the experiments, calibration was carried out by tying off all the branches of the left coronary artery except the one of interest and perfusing the artery with the dog's own blood with a constant infusion pump. The accuracy of the probe and flowmeter was tested during each calibration by repeatedly measuring 20 values for flow between 10 and 150 ml/min. The probe showed a systematic and random error of less than 5% when compared to the pump and absolute measurement. The absolute measurements were made by taking timed collections into tared glass containers and weighing. The diastolic notch on an arterial pressure wave was used as the
beginning of diastolic coronary flow. Planimetry was used to calculate areas under the phasic tracings and the calibration data were used to calculate mean and phasic regional coronary flow (ml/min).

Epicardial electrocardiographic (ECG) complexes were recorded, using a saline-soaked cotton wick electrode attached to the chest lead of a Hewlett Packard 7788A ECG amplifier (input impedance, 50 M Ω).7 The standardization used throughout was 1 mm = 1 mV.

A 7 french cardiac catheter was inserted into the inferior vena cava via a right femoral venotomy. A specially designed no. 3 french catheter was inserted via a left femoral arteriotomy and seated in the aortic sinuses. 81mKr was continuously eluted in 5% dextrose from its cyclotron-produced parent compound rubidium-81 held in a portable generator. 8 81mKr (7–10 mCi/min) was delivered into the aortic sinuses at a constant rate of between 5 and 10 ml/min of 5% dextrose.

Each dog was positioned so that the chest was within the field of a gamma camera (Toshiba GCA 202). A digital computer was used to record the total and regional activity of any gamma emissions from the heart as quantitative high spatial resolution images (Deltron-Nova 1220).

Throughout these experiments a high resolution collimator (parallel holes) was used (Toshiba RDH 660). The overall resolution of collimator and camera was 7 mm for 81mKr and 17 mm for 201Tl. This was determined by calculating the full-width half-measure from a line source positioned within the left ventricular cavity of an asytoptic dog heart. The source was 5 cm from the camera face.

Seven dogs were studied while control regional coronary flow varied by less than ± 5% and the epicardial ECG showed no abnormalities. 81mKr cardiac scintigrams were recorded by collecting 250,000 counts in 30 seconds with the camera detection window at 190 keV ± 20%. The epicardial ECG complexes were recorded from eight positions within the area supplied by the LAD. The camera detection window was changed to 80 keV ± 20%, and 1 mCi of thallium-201 (Duphar) was given as a single intravenous injection. After 10 minutes, quantitative 201Tl scintigrams of the heart were recorded by the computer and Polaroid film by collecting 250,000 counts.

The LAD was then completely occluded in five of these seven dogs. The pericardium and chest were drained and closed and the anesthesia continued to 24 hours. The chest was opened and each heart surrounded with crushed ice. The hearts were excised and washed with sucrose solution (0.25 mol/liter). The right atrium and ventricle, left atrium, pulmonary artery, and aorta were removed by dissection. An incision along the junction between the interventricular septum and the posterior free wall of the left ventricle (LV) allowed the specimen to be laid flat on a cooled Perspex surface. The specimen was held in place by a metal grid. This divided the epicardial LV surface into 1 cm² regions. The outline of the LV specimen, the epicardial coronary vessels, the snare, electrode positions and the area of discoloration were marked on a Perspex sheet held over the grid and specimen.

The following procedures were carried out at 4°C. Up to 30 tissue samples (0.3 to 0.5 g) of the full thickness of the specimen were taken from sites in the ventricular muscle supplied by the LAD, left circumflex coronary artery, and right coronary artery. The position of each sample site was noted with reference to the overlaid grid. The tissue samples were placed in tared tubes and weighed. Homogenization was then carried out in 10 volume sucrose (0.25 mol/liter) containing mercaptoethanol (10⁻³ mol/liter) with a Polytron homogenizer by 3 x 5 sec at setting 5. A sample (0.5 ml) of each homogenate was removed for determination of DNA. The remaining homogenate was centrifuged at 600 g for 10 minutes to sediment the nuclei, myofibrils, and membrane fragments. Samples of the post-nuclear supernatant extract were taken for the assay of creatine kinase (CK) activity. These samples were first diluted 1 in 500 vol/vol with sucrose-mercaptoethanol solution.

DNA was measured by the method described by Peters; CK activity was assayed in a Cecil spectrophotometer by the method of Oliver, using the modifications described by Hearse.11 The CK activity in units per milliliter was then calculated and expressed per milligram of DNA.

Samples (0.2 ml) of the post-nuclear supernatant extract from each homogenate were placed in counting tubes. The 201Tl activity (80 keV gamma emissions) in each sample was measured in an automated well counter (Nuclear Enterprise). This result was then expressed per milligram of DNA and compared to the regional depletion of CK activity, using a linear regression analysis.

A constant infusion of 81mKr was delivered to the aortic sinuses in an additional seven dogs. Images of the myocardial distribution of this tracer were recorded by collecting 250,000 counts on Polaroid film and on the digital computer. During this time heart rate, blood pressure, and regional coronary flow were stable and the epicardial ECG showed no S-T segment or T wave changes. The 81mKr activity showed a stable decrease with variations of < ± 7%. In each dog of this group, the epicardial ECG, heart rate, and blood pressure were all stable. The LAD snare was tightened until mean coronary flow was diminished as required and the 81mKr activity showed a stable decrease with variations of < ± 7%. In each dog of this group, the epicardial ECG complexes in the area supplied by the LAD showed
changes within 60 seconds. Krypton-81m cardiac scintigrams were recorded as described above and the energy detection window of the camera was changed to 80 keV ± 20%. Seven minutes after LAD narrowing, 1 mCi of ²⁰¹Tl was given intravenously and images recorded after 10 minutes.

After each experiment the quantitative images of the heart obtained with ⁸¹mKr were displayed on the computer oscilloscope visual display unit within a 64 x 64 matrix of squares. The whole image was then enclosed within seven rectangular areas of interest. The activity (in counts per minute) in each area was expressed as a ratio of the total myocardial activity. The ²⁰¹Tl cardiac scintigram from the same experiment was then displayed within the same areas of interest and the corresponding seven ratios calculated. Throughout each experiment care was taken that the orientation between the heart and the gamma camera did not change. The dogs were held in position throughout each experiment and the images on the visual display were checked to ensure that they had not moved within the 64 x 64 matrix during the course of each experiment.

No computer processing, image enhancement, or background subtraction techniques were used.

At the end of each experiment the snared LAD was selectively injected with 5 ml of patent blue 5 dye, and the area of myocardium stained by the distribution of that vessel was dissected out and weighed.12

The experimental procedure for each group of dogs is summarized in Table 1. Paired t-tests were used to compare the regional myocardial distribution of ²⁰¹Tl and ⁸¹mKr. Linear regression analysis was used to compare the regional myocardial distribution of creatine kinase activity and ²⁰¹Tl.

Results

The regional myocardial distribution of ⁸¹mKr was similar to that of ²⁰¹Tl in the cardiac scintigrams from those experiments with no LAD stenosis and normal epicardial ECG complexes (Fig. 1, a and c). The ratios demonstrated that there were no significant differences in the relative myocardial distribution of the two indicators (Fig. 1c). Paired t-tests showed \( P > 0.50 \).

In all the experiments the percentage decreases in the regional myocardial activity of ⁸¹mKr produced by LAD narrowing were closely related to the percentage decreases in regional coronary flow using the flow probe (\( r = 0.91, P < 0.001, n = 21 \) observations, linear regression analysis).

Twenty-four hours after LAD occlusion, the regional myocardial distribution of CK activity showed marked decreases in the area affected by the LAD occlusion. The activity in the affected areas was \( 0.56 \pm 0.062 \) U/mg DNA and in the unaffected areas it was \( 1.30 \pm 0.13 \) U/mg DNA (mean ± SD). The regional myocardial activity of ²⁰¹Tl in remote regions of the heart unaffected by the LAD snare was treated as 100% in each dog. The percentage decrease in the regional myocardial activity of ²⁰¹Tl at each site was calculated from the measured activity within the region affected by the LAD snare. The relationship between the regional myocardial decrease in the activities of CK and ²⁰¹Tl was \( Y = 0.918X + 8.45, r = 0.93, P < 0.001, n = 80 \) myocardial samples.

In seven of the 21 dogs, LAD narrowing produced a fall in coronary blood flow of 37-56% (mean = 49%). The epicardial ECG showed no changes and the myocardial distribution of ⁸¹mKr demonstrated regional decreases in activity in the area supplied by the snared vessel (Fig. 2a). The ratios in Figure 2b demonstrate the changes in the distribution of this isotope when regional coronary flow was diminished. The ²⁰¹Tl cardiac scintigrams and the ratios shown in Figure 2, a and b, did not demonstrate any regional defect when this isotope was delivered while the LAD was narrowed in these seven dogs.

In seven dogs the LAD was narrowed until mean coronary flow decreased by 35-60% (mean = 51%). The

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Groups of Dogs and Experiments using Thallium-201 Scintigraphy</th>
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</thead>
<tbody>
<tr>
<td>LAD flow* (ml/min)</td>
<td>²⁰¹Tl image</td>
</tr>
<tr>
<td>---------</td>
<td>--------------</td>
</tr>
<tr>
<td>Group 1 (n = 7) Control</td>
<td>53 ± 5.0</td>
</tr>
<tr>
<td>24 hr after LAD narrowed</td>
<td></td>
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<tr>
<td>Group 2 (n = 7) Control</td>
<td>48 ± 7.0</td>
</tr>
<tr>
<td>After LAD narrowed</td>
<td>24 ± 9.0</td>
</tr>
<tr>
<td>Group 3 (n = 7) Control</td>
<td>52 ± 8.0</td>
</tr>
<tr>
<td>After LAD narrowed</td>
<td>26 ± 11.0</td>
</tr>
</tbody>
</table>

* Measured by electromagnetic flowmeter.
† Mean ± SD.
‡ Linear regression analysis: CK U/mg DNA and thallium-201 counts/mg DNA.
FIGURE 1  a: Scintigrams of the heart using $^{81m}$Kr and $^{201}$Tl are shown. The regional myocardial distribution of these isotopes were similar when the coronary circulation was intact. The $^{201}$Tl image is shown in a; the $^{81m}$Kr image is shown in b. c: The $^{81m}$Kr and $^{201}$Tl scintigrams in each experiment were divided into six areas of interest on the visual display unit. The relationship between these areas and the cardiac anatomy is shown. d: In each experiment, the $^{81m}$Kr and $^{201}$Tl images were positioned within the same six areas of interest. The ratios of the regional to the total activity in each area show, with imaging, that the regional myocardial distribution of the tracers was similar.

epicardial ECG showed planer S-T segment depression (<2 mm) and T wave inversion in two, and characteristic S-T segment elevation (<2 mm) in the other five. The $^{81m}$Kr cardiac scintigrams and the ratios of the myocardial distribution of $^{81m}$Kr showed a regional decrease in activity after LAD narrowing (Fig. 3, a and b). The $^{201}$Tl cardiac scintigrams and the ratios of activity in these dogs showed a corresponding regional decrease in activity (Fig. 3, a and b).

The segments of myocardium supplied by the snared LAD weighed between 29 and 43 g.

The results are summarized in Table 1.

Discussion

In these experiments regional coronary flow in the snared vessel was measured with the electromagnetic probe and changes in regional myocardial perfusion were observed by imaging the steady rate equilibrium of krypton-81m in the heart. During a continuous infusion of $^{81m}$Kr into the aortic sinuses, a proportion of this tracer reaches the coronary circulation. This inert and freely diffusing indicator has a half-life (13 sec, decay constant 3.2/min) that is faster than normal and reduced values for the turnover of myocardial flow per unit volume. The
steady state equilibrium of $^{81m}$Kr in the myocardial water space in these circumstances depends mostly on arrival of the tracer by blood flow and the constant radioactive decay. The $^{81m}$Kr myocardial signal will arise mostly from the extracellular fluid space and myocardial tissues. The short half-life will prevent washout of the tracer from being important.\textsuperscript{13, 14}

The results showed that when coronary flow was not obstructed, the regional myocardial distribution of $^{81m}$Kr and $^{201}$Tl were similar, using images recorded with a gamma camera. However, when regional perfusion was diminished as shown by the flow probe and $^{81m}$Kr scintigrams, the $^{201}$Tl images of the heart did not demonstrate the regional decrease in perfusion while the ECG was normal. When the LAD narrowing and decreases in myocardial perfusion were accompanied by epicardial ECG evidence of some cellular abnormality, the $^{201}$Tl scintigrams demonstrated regional decreases in activity corresponding to the defects seen in the $^{81m}$Kr images. The regional decreases in myocardial perfusion in these experiments may or may not be accompanied by manifestations of tissue ischemia. This will depend on the available collateral blood flow and metabolic demand (MVO\textsubscript{2}) at the time of LAD narrowing.

The regional myocardial depletion of CK activity has been used to assess ischemic damage after coronary occlusion.\textsuperscript{15} These experiments showed that the distribution and severity of the myocardial depletion of $^{201}$Tl and CK activity 24 hours after LAD occlusion were similar. This suggested that in these circumstances $^{201}$Tl was acting as a tissue marker of ischemic damage.

The affected regions of myocardium were positioned on the edge of the images of $^{81m}$Kr and $^{201}$Tl activity. The segments of tissue affected by the coronary narrowing were dissected and weighed. The use of patent blue 5 dye overestimates the size of the ischemic area; however, this technique was used in an attempt to ensure that the areas of interest were big enough to resolve as defects using $^{201}$Tl and $^{81m}$Kr.

Strauss et al.\textsuperscript{2} and others have used experimental models to show that the regional distribution of $^{201}$Tl in the heart is related to myocardial blood flow. Those experiments did not separate the energy-dependent cellular mechanisms that dominate the extraction of this tracer from the blood. Poe\textsuperscript{6} has shown that the proportion of $^{43}$K and related compounds extracted by the myocardium is inversely related to blood flow. Buja et al.\textsuperscript{16} have shown that ischemic myocardium has a limited ability to

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{The myocardial distribution of $^{81m}$Kr is shown before (a) and after (b) partial occlusion of the LAD. The regional defect in activity that appears was not accompanied by epicardial ECG changes. The $^{201}$Tl scintigrams showed no such defect in activity (c). d: The ratios showing the regional activity of $^{81m}$Kr demonstrate the loss of activity in the area supplied by the LAD. The ratios for $^{201}$Tl show a normal distribution when the epicardial ECG was normal. Paired t-tests showed that the change in the regional myocardial distribution of $^{81m}$Kr were significant ($P = < 0.01$) and the changes in $^{201}$Tl were not ($P = < 0.20$) for areas 1 and 2.}
\end{figure}
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1.50
1.25
1.00
0.75
0.50
0.25

Kr before LAD narrowing.
Kr after LAD narrowing.
201 Tl given after LAD narrowing.

FIGURE 3 The myocardial distribution of 81mKr is shown before (a) and after (b) partial occlusion of the LAD. The regional defect in activity that appeared was accompanied by epicardial ECG changes in these dogs. The 201 Tl cardiac scintigrams showed a similar defect in activity (c). d: The ratios showing the regional activity of 81mKr demonstrates the loss of activity in the area supply by the narrowed LAD. The ratios for 201 Tl showed a similar regional loss in activity (compare to Figure 1b). Paired t-tests showed that the changes in the regional myocardial distribution of 81mKr and 201 Tl were significant (P < 0.01) for areas 1 and 2.

take up 201 Tl, and this is followed by a redistribution of the indicator resulting from rediffusion and recirculation and also collateral flow. Hamilton et al.17 have demonstrated that ischemic but not infarcted myocardium can take up 201 Tl during the complex kinetics of this tracer that follows the initial distribution of an intravenous injection.

The work of Adolph et al.18 supports the view that reversibly hypoxic myocardium has a limited capacity for 201 Tl uptake. Clinical research comparing 201 Tl scintigraphy and the ECG testing during exercise does not always show 201 Tl defects with regional ECG abnormalities.19 This experimental model will tend to oversimplify the clinical situation in man, but the specific techniques of 81mKr scintigraphy and graded coronary occlusion have been used to demonstrate a single principle in the use of 201 Tl.

In patients with segmental rigid coronary artery stenosis and functional disturbances of regional myocardial perfusion, 201 Tl scintigrams will demonstrate defects in activity on stress.4,19 However, the results of this study would suggest that 201 Tl cardiac scintigraphy may not detect regional decreases in myocardial perfusion due to coronary artery disease until these have progressed sufficiently to disturb myocardial metabolism at rest or during stress.

In conclusion, 201 Tl cardiac scintigrams could not detect experimental decreases in regional myocardial perfusion alone in these experiments. The myocardial distribution of this indicator did demonstrate decreases in regional myocardial perfusion when these were accompanied by disturbances of the epicardial electrocardiogram. These findings require further experiments that look at the cellular mechanisms of 201 Tl uptake and may be important in the interpretation of cardiac scintigrams by using this tracer in man.

Acknowledgments

The authors thank Guy’s Hospital, London, for supplying thallium-201 for part of this study.

References

Blood Pressure and Vascular Resistance in Genetically Hypertensive Rats Treated at Birth with 6-Hydroxydopamine

DAVID W.J. CLARK, DAVID R. JONES, E. LINTON PHELAN, AND CARRICK E. DEVINE

SUMMARY  Genetically hypertensive (GH) rats of the New Zealand strain and normotensive (N) rats were sympathectomized from birth with 6-hydroxydopamine (100 mg/kg, s.c., on alternate days, seven treatments). In adult treated rats from each strain (GHT and NTr), blood pressure was lower than normal. Functional tests and electron microscopy showed that denervation was virtually complete in mesenteric and hindlimb arteries; the innervation of the renal artery was little affected. Ganglionic blockade still caused a large fall in blood pressure in treated rats. Vascular resistance was higher in blood-perfused hindlimbs and tails of GH rats than in those of N rats; in contrast, resistance was similar in limbs and tails of GHT and NTr rats and was greater than that found in untreated N rats. Saline-perfused limb vessels had neither neurogenic nor myogenic tone and resistance was higher in GH limbs (whether these were from treated rats or not) than in untreated N limbs. In saline-perfused NTr limbs, there was paradoxical structural adaptation (probably luminal narrowing) of the hindlimb arteries and resistance vessels was higher than in untreated N rats. The resistance of saline-perfused GH and GHT limbs was similar. A high peripheral resistance appears to be the main mechanism sustaining genetic hypertension, and the integrity of the vasomotor sympathetic nerves is necessary for the development of this form of experimental hypertension.

A FEW DAYS after birth, the blood pressure of genetically hypertensive (GH) rats of the New Zealand strain is significantly higher than that of random-bred normotensive (N) rats. Blood pressure rises quickly in young rats, and pressures near those of the adult are present in 4-week-old N rats and 6-week-old GH rats. The discovery that injection of newborn rats with 6-hydroxydopamine hydrobromide (6-OHDA) permanently destroys or prevents the development of noradrenergic nerves has made possible a study of the etiological role of the sympathetic nervous system in genetic hypertension.

When mature rats are treated for the first time with 6-OHDA, destruction is confined to the terminal portions of the noradrenergic neuron and, as the perikaryon is unaffected, regeneration of the nerves occurs a few weeks after treatment is stopped. In newborn rats treated with high doses of 6-OHDA, the lesion is irreversible and affects the cell body and axons of many peripheral noradrenergic neurons, as well as some noradrenergic terminals in the brain. However, chronic sympathectomy
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