Comparison of the Myocardial Uptake of a Technetium-Labeled Isonitrile Analogue and Thallium

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The myocardial transmicrovascular transport of thallium-201 (\(^{201}\text{Tl}\)) and technetium-99m hexakis(2-methoxyisobutylisonitrile) (MIBI) were compared during variable blood flow levels in nine blood-perfused, isolated rabbit hearts. Seventeen injections of radiolabeled albumin and EDTA as well as \(^{197}\text{Tl}\) and MIBI were performed by indicator-dilution techniques. When coronary flow was varied from 0.52 to 3.19 ml/g/min, myocardial extraction for MIBI averaged 0.38±0.09 (SD) whereas \(^{201}\text{Tl}\) myocardial extraction averaged 0.73±0.10 (p<0.001). Net extraction, which was calculated using end points of 1.8–4.9 minutes, averaged 0.41±0.15 for MIBI and was less than the \(^{201}\text{Tl}\) net extraction of 0.57±0.13 (p<0.001). The mean capillary permeability-surface area product for MIBI (0.44±0.13 ml/g/min) was one third of \(^{201}\text{Tl}\) (1.30±0.45 ml/g/min; p<0.001). However, parenchymal cell permeability-surface area product for MIBI (47.58±25.85 ml/g/min) was much higher than \(^{201}\text{Tl}\) (6.52±6.51 ml/g/min; p<0.0001), and apparent cellular volume of distribution for MIBI (15.15±3.31 ml/g) was also higher than \(^{201}\text{Tl}\) (10.19±4.00 ml/g; p<0.01). These data suggest that capillary permeability for \(^{201}\text{Tl}\) is greater than MIBI, but the reverse is true at the parenchymal cell wall. In addition, a new blood-perfused preparation is used for indicator-dilution techniques, and previously developed modeling analyses are also extended to these experiments. (Circulation Research 1989;65:632-639)

Several technetium-99m (\(^{99m}\text{Tc}\))-labeled agents have been proposed for myocardial perfusion imaging.\(^1\)–\(^4\) These compounds may serve as an alternative to thallium-201 (\(^{201}\text{Tl}\)) perfusion studies because of better photon statistics, Anger camera imaging properties, cost, and clinical availability. Although many synthetic compounds of \(^{99m}\text{Tc}\) appear to be promising myocardial agents, only the isonitrile compounds have achieved clinical imaging studies that are similar to those obtained with thallium.\(^3\)–\(^5\) Specifically \([^{99m}\text{Tc}]\)hexakis(t-butylisonitrile) (TBI) and its ether derivative \([^{99m}\text{Tc}]\)hexakis(2-methoxyisobutylisonitrile) (MIBI; RP-30, E.I. du Pont de Nemours, North Billerica, Massachusetts) have shown good, clinical potential in these preliminary studies. MIBI might prove useful for myocardial imaging by intravenous injection since Williams et al\(^6\) found its extraction on passage through the lungs to be less than the extraction of another isonitrile, TBI.

The interpretation of perfusion images of cardiac uptake for these agents is clearly dependent on the capillary exchange process. However, no previous studies have quantified the transcapillary transport of both \(^{201}\text{Tl}\) and MIBI in the same hearts having physiological coronary flow. Accordingly, the goal of this report is to compare the myocardial uptake of these two perfusion agents at variable flow levels by standard paired-indicator dilution techniques. Another prime goal is to demonstrate that a blood-perfused rabbit heart\(^7\) can be adapted to study cardiac isotope transport by a previously developed computer model analysis.\(^8\)–\(^10\)

Materials and Methods

All experiments used an isolated isovolumetrically contracting rabbit heart as previously described,\(^1\) but the apparatus was modified to permit whole-blood perfusion instead of a buffer perfusate.\(^7\) The hearts (n=9, from New Zealand White rabbits) were quickly removed and mounted on a perfusion apparatus (Figure 1), which was already primed with 70–100 ml heparinized (600 IU/kg) rabbit blood. A constant-flow pump was set at a rate that pro-
produced a mean coronary perfusion pressure of 100-125 mm Hg. A thermistor and pacing catheter were placed in the right ventricle via the right atrium to monitor tissue temperature (37±1°C) and maintain a heart rate of at least 180 beats/min. A vinyl catheter was also placed in the right ventricle via the pulmonary artery to collect coronary sinus drainage for all isotope sample determinations and to measure coronary flow. A fluid-filled catheter was also inserted into the left ventricle via the left atrium. Coronary perfusion and left ventricular pressures were continuously measured and recorded. In addition, the first derivative of left ventricular pressure (dP/dt) was obtained by electronic differentiation of the pressure signal.

The blood passed through a membrane oxygenator that was gassed with a 3% CO₂ and air mixture. Supplemental O₂ gas was available, if needed, and during the protocols, blood gas measurements were collected every 30-40 minutes. Appropriate adjustments were made to maintain blood pH, P⁰₂, and P⁰₂ in the physiological range, and the glucose level was also monitored and adjusted as needed to a level of 80-120 mg/dl.

Protocol

After stabilization of temperature, ventricular pressure, and coronary flow and pressure, samples of venous outflow were collected into an automated sample changer for isotope background determinations. A mixture of 20 µCi indium-111-labeled albumin, 6 µCi cobalt-58-labeled ethylenediaminetetraacetic acid (EDTA), 35 µCi ⁹⁹mTc and 90 µCi MIBI was dissolved in saline and rabbit blood and was then immediately injected as a bolus (0.2-0.3 ml) into the aortic inflow of the coronary arteries (n=17). The coronary venous effluent was collected into preweighed plastic tubes at 1.2-5-second intervals depending on the flow rate over a 2-5-minute collection time. When the sampling was completed, coronary flow was again determined. The full sample weights were measured and counted along with an aliquot (0.1 ml) of each injectate in a gamma well counter. Appropriate correction for energy crossover, time, background, and physical decay during the counting process was made for each curve. When multiple injections (at variable flow) were made in an individual heart, isotope background was noted to be less than 1% of peak activity in all instances. In addition, the wet weight of a portion of the left ventricular free wall was determined for subsequent estimation of the tissue water fraction.¹¹

MIBI Preparation and Analysis

The isonitrile salt (supplied by E.I. duPont) and formamidim sulfonic acid were prepared as a 4 mg/ml and a 0.4 mg/ml solution, respectively, in distilled water. The isonitrile and formamidim sulfonic acid solutions (0.5 ml each) were added to a nitrogen-purged vial along with approximately 0.2-0.4 ml ⁹⁹mTcO⁴⁻ (10 mCi); the vial was placed into a boiling water bath for 10-15 minutes and then allowed to cool to room temperature. Thin-layer chromatography was performed on each freshly prepared batch of MIBI. CH₃CN/CH₃OH/0.5 M NH₄(CH₃COO)₂/THF was the developing solvent, which was proportioned 40/30/20/10, and the MIBI was spotted on 2 cm×20 cm sections of JC-18 plates (Whatman, Maidstone, England). The MIBI peak was detected at an Rₚ of 0.52 and always contained greater than 95% of the total ⁹⁹mTc.

Myocardial Transport Analysis

For each injection, the plasma flow (Fₚ), the time of collection (t), the isotopic activity of each sample [C(t)], and the injected dose of each isotope (qₒ) was known. Consequently, the normalized dilution curves for albumin [hR(t)] and the diffusible tracers [hD(t)] could be calculated from the general equation:

\[ h(t) = F_p \cdot C(t)/q_o \] (1)

²⁰⁹TI and MIBI are diffusion-limited substances and capillary membrane permeability can be estimated from these normalized curves. Specifically, the instantaneous fractional extraction [E(t)] was calculated at each point assuming that albumin (reference tracer) does not leave the vascular space:

\[ E(t) = 1 - hD(t)/hK(t) \] (2)

If both tracers are equally dispersed in the arterial inflow and if tracer backflux from the extravascular to intravascular space is negligible, then fractional extraction can be used to determine the capillary permeability and surface area product (PSₕₕ) according to Crone:¹⁴

\[ PS_{kap} = -F_p \log_e(1-F_{max}) \] (3)

Eₚmax represents the peak value during the early plateau phase of E(t) up to the peak of the albumin curve and represents the best estimate of average
fractional extraction. An additional analysis was used to evaluate net extraction ($E_{net}$), which is an integral extraction, defined as

$$E_{net} = \frac{\int_0^T [h_R(T)-h_D(T)]dT}{\int_0^T h_R(T)dT}$$

In this $E_{net}$ analysis, $T$ was the time at which 99.99% of the albumin reference had emerged in the venous effluent and varied from 1.8 to 4.9 minutes. This function differs from $E_{max}$ and is used to estimate the net myocardial extraction or retention of each diffusible tracer.

**Model Estimate Analysis**

For more rigorous analysis of the indicator-dilution curves, the heart was defined as an aggregate of capillary-tissue units in parallel, described previously in more detail. Briefly, each unit was a three-region (capillary, interstitial fluid, and cell), two-barrier, axially distributed convection-diffusion model. This model is an extension of the one-barrier model of Bassingthwaighte and is similar in concept to the two-barrier model of Rose et al. The parameters of the model for fitting the dilution curves are as follows:

1. $F_s$ (ml/g/min), average flow of the solvent delivering the solute = coronary blood flow (hematocrit, 1)
2. $PS_c$ (ml/g/min), capillary permeability-surface area product. This is a flow-independent estimate of the model and differs from $PS_{cap}$ derived from equation 3.
3. $V'_i$ (ml/g), interstitial fluid-volume distribution (estimated from the EDTA dilution curve and held constant for the MIBI and $^{201}$Tl fittings)
4. $PS_{pc}$ (ml/g/min), permeability-surface area product of parenchymal cell (sarcolemma of myocytes)
5. $V'_{pc}$ (ml/g), volume of tracer distribution within parenchymal cell

An automated, parameter adjustment program was used to optimize the parameter values to maximize the goodness of fit by using sensitivity functions as previously described. The coefficient of variation was used only as a measure of the overall goodness of fit and in the adjustment of parameter estimates. The heterogeneity of flows (distribution of regional flows) was represented by five or seven regions as previously determined in rabbit heart experiments. The greatest accuracy in estimation of the parameters results from a reduction in the degrees of freedom, given a physiologically appropriate model. Therefore, $PS_c$ for albumin and $PS_{pc}$ for EDTA were set at zero, which is appropriate for the relatively short observation times. Consequently, the large vessel transport function could be defined by the albumin curve, the measured average flow, the distribution of regional flow, and an assumed capillary volume of 0.035 ml/g.

For MIBI and $^{201}$Tl, transport through endothelial capillary cells and consumption of tracer in the parenchymal cell were not included. $PS_c$, $PS_{pc}$ and $V'_i$ were the parameters that were free in the fitting of the diffusible tracer curves, and $V'_{pc}$ was set to the value estimated from the EDTA transport function.

All data are expressed as mean ± SD. Comparisons between groups of a single numeric variable were performed by an ANOVA and appropriate $t$ statistic.

**Results**

Coronary blood flow varied from 0.56 to 3.19 ml/g/min for all experiments. The mean rabbit heart weight ($n=9$) was 4.51 ± 0.62 g, the hematocrit was 29 ± 7%, the fractional water content was 0.79 ± 0.02, and the heart rate was 199 ± 14 beats/min. The average aortic pressure was 97 ± 37 mm Hg, and end-diastolic pressure was 7 ± 3 mm Hg. The positive dP/dt averaged 1,590 ± 661 mm Hg/sec, and the negative dP/dt was 1,097 ± 450 mm Hg/sec.

**Indicator-Dilution Curves**

An example of the experimental normalized dilution curves for one heart is shown in Figure 2. The paired $^{201}$Tl and MIBI curves were quite different, and the calculated instantaneous extraction curves were also disparate. The $E(t)$ curve for $^{201}$Tl is typical for cation extraction with an early peak and rapid fall after plateau, but MIBI extraction has a very early rise and prolonged plateau stage. This suggests fundamental differences in the transport mechanisms for these two agents. The tail portion of the normalized dilution curve for MIBI was always flatter and lower than the albumin curve, which accounts for the observed plateau phase of MIBI extraction. The return of tracer to the venous effluent from the extravascular space dominates the tail portion of these dilution curves. MIBI has more prolonged extraction and less reflux from the extravascular region, which may be attributable to intracellular retention or, conceivably, binding to interstitial structures. The h(t) data from each individual experiment is shown in Table 1. The mean $E_{max}$, $PS_{cap}$, $PS_c$, and $E_{net}$ values for MIBI were all significantly less than the corresponding $^{201}$Tl determinations. However, mean $PS_{pc}$ and $V'_{pc}$ values for MIBI were both significantly greater than corresponding $^{201}$Tl measurements. The Crone estimate of $PS_{cap}$ was always less than the $PS_c$ estimate, and the disparity was greater for $^{201}$Tl (which demonstrates a higher $E_{max}$ than MIBI).

Figure 3 shows the individual ($n=16$) values of $E_{max}$ and coronary blood flow for both $^{201}$Tl and MIBI. There is a negatively sloped linear relation between $E_{max}$ and blood flow for each tracer, and MIBI values are always less than corresponding $^{201}$Tl determinations. The least-squares best-fit linear regression line is also shown for both isotopes:
E_max MIBI = -0.09 flow + 0.51 (r = -0.80) and E_max 201Tl = -0.11 flow + 0.91 (r = -0.85). The mean E_max for MIBI was 0.39 ± 0.09 over a flow range of 0.52–3.19 ml/g/min, which was significantly less (p < 0.001) than the mean E_max for 201Tl of 0.73 ± 0.10.

It should be noted that the average E_est difference between 201Tl and MIBI was 0.15 ± 0.10 at 1.8–4.9 minutes postinjection, which represents a much smaller disparity in net myocardial extraction than would have been estimated from the capillary permeability or E_max determinations. It is also important to note that both E_max and E_est are inversely related to blood flow, and both assess the fraction of tracer extracted by the myocardium. In addition, myocardial uptake of 201Tl and MIBI can be estimated by the product of E_est and flow plotted versus flow (graph not shown). If net extraction were 100%, then the slope of these curves would be 1.0 and an "ideal" flow-limited perfusion marker would be identified. However, this function shows a slope of 0.30 for 201Tl (r = 0.80) that is larger than the MIBI slope of 0.12 (r = 0.50), which implies that 201Tl more closely resembles a flow-limited tracer than MIBI.

An estimate of myocardial tracer residence time can be made from the model parameter estimates. Tracer retention is related to the reciprocal of the PS_c/(V'p_c + V') value (minutes) that relates the capillary exchange rate with the apparent volume of distribution. When the mean estimates for PS_c, V'p_c, and V' were used, MIBI retention was 20.6 minutes compared with 3.1 minutes for thallium. This should not be confused with a t_1/2 value but, rather, expresses a sixfold to sevenfold higher organ retention of tracer that has left the vascular space for MIBI compared with 201Tl over this physiological flow range.

Discussion

This report demonstrates that a modified, blood-perfused, isolated heart preparation can be used to
evaluate cardiac transport of isotopes by standard indicator-dilution techniques. This preparation shows that myocardial capillary exchange of \(^{203}\text{TI} \) is greater than MIBI, but the intracellular distribution of the isonitrile compound is much greater than \(^{203}\text{TI} \). The mechanism for cardiac transport of these two isotopes is clearly different, and clinical implications for myocardial perfusion imaging can be seen.

**Experimental Model**

The use of this experimental model to study cardiac transport of multiple tracer represents a modification of previously reported techniques.\(^1\) The blood-perfused model initially described by Grice and coworkers\(^7\) has been modified to permit tracer kinetic studies with indication-dilution techniques previously developed for buffer-perfused hearts.\(^1\)

It is of interest to note that \(^{203}\text{TI} \) \(E_{\text{max}} \) in these buffer-perfused hearts averaged 0.70 at control and was 0.73 in this present report. Other investigators\(^16,19\) have also used blood-perfused, isolated heart preparations to evaluate tracer permeability, and these types of determinations provide reliable estimates of cardiac isotope transport.

The use of a blood-perfused preparation does raise questions of possible permeability disrupters released by blood cell components, but it is beyond the scope of this report to assess microvascular permeability by other independent techniques such as osmotic transients.\(^20,21\) However, Vargas et al\(^1\) and Grabowski and Bassingthwaighte\(^22\) independently performed isolated heart permeability studies that concluded that both indicator-dilution and osmotic transient methods provide adequate estimates of permeability in this type of experimental preparation. The assumption that albumin remains in the vascular space during instantaneous fractional extraction measurements has not been critically evaluated. However, according to data from Bean,\(^23\) the estimated extraction for albumin would be less than 2% (reflection coefficient, \(-0.85\)) and would not be expected to cause a significant error in parameter estimates.

In contrast to buffer-perfused experiments, a blood-perfused, isolated heart can be perfused at physiological levels of coronary perfusion, and interstitial edema is also prevented. Rabbit hearts (in vivo) typically receive 2–3.5 ml/min of coronary blood flow,\(^24\) but a hemoglobin-free, buffer-perfused heart requires a twofold to threefold increase in perfusion just to supply enough oxygen. Myocardial isotope transport should be evaluated at physiological blood flow because diffusible perfusion agents will be affected by the global perfusion rate.\(^18\) Tracer capillary exchange will also be affected by the presence of interstitial edema, which is increased in buffer-perfused hearts due to hyperemic perfusion and protein-free perfusate. As in normal in vivo hearts,\(^23\) the fraction of water content in blood-perfused hearts was 0.79±0.02 compared with a value of 0.83±0.02 noted in a similar preparation with a Krebs-Henseleit buffer.\(^11\) The mean model estimate for \(V_f \) was 0.26 ml/g, which is also lower than the 0.32 value (by the same model analysis) reported for Tyrode-perfused rabbit hearts.\(^10\) Using a continuously weighed isolated heart, Vargas et al\(^21\) have noted an approximate 19% increase in tissue water during buffer perfusion, which is similar to the 23% difference observed between blood- and buffer-perfused hearts in the present study. The presence of a high flow rate and increased interstitial volume would tend to underestimate \(E_{\text{max}} \) as well as the parenchymal cell volume distribution. Consequently, tracer clearance would be accelerated compared with normal flow levels and interstitial volume. These limitations would result in model parameter estimates that would poorly reflect tracer transport in the intact heart.

**Capillary Transport**

In these experiments, the capillary permeability for \(^{203}\text{TI} \) is clearly much higher than MIBI. The average \(E_{\text{max}}, P_{Scap}, \) and \(PS \) values for \(^{203}\text{TI} \) are twofold to fourfold higher than for MIBI, and all these parameters assess the tracer exchange process at the capillary level. Perfusion agents are typically expected to have relatively high transcapillary exchange rates to more accurately reflect variable flow rates. Therefore, based on capillary transport, \(^{203}\text{TI} \) would be expected to be a superior perfusion agent compared with MIBI because it demonstrates a greater \(E_{\text{max}} \) and \(P_{Scap} \) value at any given flow. The higher first-pass extraction and permeability of \(^{203}\text{TI} \) compared with MIBI probably reflect a different transport mechanism as has been previously reported.\(^2\) The overall molecular size and lipophilicity of these two agents is clearly different, and these factors must impact on the transcapillary exchange process. The size of the MIBI compound is probably a major factor in limiting its passage across the capillary barrier compared with \(^{203}\text{TI} \). In addition, the computer-modeling estimates of \(PS \) also confirm a much higher (fourfold) rate for \(^{203}\text{TI} \) compared with MIBI. The estimates of \(PS \) are flow independent and further substantiate the observation of a different transport mechanism for the two perfusion agents. The computer model estimates for \(PS \) were consistently greater than the \(P_{Scap} \) value determined by the Crone (Equation 3) method. This has been noted previously with several different tracers\(^9,10\) and also confirms earlier observations\(^16,19\) that \(P_{Scap} \) underestimation of capillary permeability is greater for tracers having relatively higher \(E_{\text{max}} \) values (\(^{203}\text{TI} \) vs. MIBI).

**Parenchymal Cell Transport**

Although \(^{203}\text{TI} \) has consistently higher transcapillary exchange rates than MIBI, myocardial tracer uptake includes both capillary and parenchymal cell transport. Capillary exchange dominates the first-
pass kinetics of extraction, but cellular exchange and distribution greatly affect the net uptake of perfusion agents. Our data show that MIBI has a significantly higher parenchymal cell permeability (PS\(_{\text{pc}}\)) and volume distribution (V\(\text{pc}\)) than \(^{201}\)TI (Table 1). This follows from our consistent observation that the transport curves \([h(t)]\) for MIBI consistently remained below those of albumin and always had a lower tail portion than \(^{201}\)TI curves. The tail portion of the transport curve dominates the PS\(_{\text{pc}}\) and V\(\text{pc}\) estimates and clearly demonstrates that cellular washout of MIBI is much slower than \(^{201}\)TI. Specifically, the MIBI transport curve shows a relatively low but nearly constant first-pass extraction with extremely rapid parenchymal cell membrane transport. When this relatively high V\(\text{pc}\) is combined with a relatively low PS\(_{\text{app}}\), myocardial residence time for MIBI is noted to be much higher than \(^{201}\)TI. The high lipophilicity of these isonitrile compounds may explain, in part, their relatively high cellular membrane uptake.\(^{26}\) In contrast, \(^{201}\)TI has relatively high capillary exchange, but its cellular permeability and distribution characteristics result in appreciable \(^{201}\)TI clearance from the parenchymal cell over the first 2–4 minutes. A previous report did note that net thallium clearance from cultured myocardial cells was 4.7-fold faster than MIBI.\(^{27}\) However, these investigators\(^{27}\) also noted a higher thallium cellular uptake in cultured cells than in MIBI, which is in contrast with our observations. This might be caused by impaired MIBI permeability or enhanced thallium permeability in cultured cells when compared with perfused whole-organ preparations. Overall, these authors concluded that myocardial uptake of MIBI was less than thallium, which is also our general observation, but the methodologies are quite different.

**Myocardial Fractional Retention**

Since external gamma camera imaging of perfusion is related to the continual effect of tracer washin and washout, the evaluation of myocardial uptake must combine both of these opposing transport mechanisms. The E\(_{\text{net}}\) function comes close to combining the summation of tracer washin and washout in a single value. The use of E\(_{\text{net}}\) permits a clinically relevant comparison of thallium's high capillary permeability with MIBI's higher cellular permeability. Specifically, the net amount of tracer that remains in the organ (as a fraction of the injected dose) is certainly an important factor in its ability to provide adequate perfusion images. The mean E\(_{\text{net}}\) for \(^{201}\)TI was significantly higher than...
MIBI, and the average difference was 0.15±0.10 functional units, which represents an average 25% reduction in the E_{net} function for MIBI compared with ^{201}Tl. It appears that the sixfold to sevenfold increase in MIBI cellular distribution somewhat compensates for the threefold to fourfold higher ^{201}Tl capillary permeability.

Based on this E_{net} analysis, ^{201}Tl would be expected to be a significantly better perfusion agent than MIBI. This study shows that MIBI lacks high extraction properties that are typically associated with cation perfusion agents like rubidium, thallium, potassium, and ammonia. However, our data suggest that MIBI capillary transport is accomplished by a different and slower mechanism compared with ^{201}Tl, and direct comparison to cation transport may not be completely appropriate. There are also differences in parenchymal cell transport that suggest a greater volume of distribution and slower washout for MIBI compared with ^{201}Tl. Additional MIBI may be extracted when recirculation occurs whereas further thallium extraction is limited by both a relatively faster washout and smaller cellular volume of distribution. Therefore, based on the modeling data during recirculation, MIBI may prove to be a better myocardial flow agent than ^{201}Tl because of its higher cellular retention.

In addition, we did not account for differences in photon imaging characteristics and statistics, disparities in tracer tissue stability (redistribution) during image collection times of 15–25 minutes, and potential differences in arterial input functions between MIBI and ^{201}Tl. Ultimately, more clinical comparisons will need to be performed to determine optimal MIBI imaging protocols for an imaging compound with relatively lower (compared with ^{201}Tl) peak extraction.

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