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Interaction of Technetium-99m-N-NOET with Blood Elements: Potential Mechanism of Myocardial Redistribution

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Technetium-99m-N-NOET is a new ^{99m}Tc-labeled, neutral cardiac perfusion imaging agent which has been shown to undergo apparent redistribution in animal models and in humans. The purpose of this study was to investigate the interaction of ^{99m}TcN-NOET with red blood cells (RBCs) and to determine the effects of these interactions on myocardial uptake and clearance of ^{99m}TcN-NOET. **Methods:** After bolus administration, myocardial ^{99m}TcN-NOET clearance was monitored for 1 hr using a sodium iodide detector in 22 isolated, buffer-perfused rat hearts. Hearts were perfused as follows: seven controls with Krebs-Henseleit (KH) buffer (Group 1), five hearts with KH containing RBCs (Group 2), five hearts with KH containing RBCs and albumin (Group 3), five hearts with KH containing RBCs and dextran (Group 4). In a separate protocol, RBCs were incubated in ^{99m}TcN-NOET and then perfused through five hearts (Group 5). **Results:** Technetium-99m-N-NOET myocardial uptake (%ID) was significantly lower in all RBC groups (RBCs = 5.0% ± 1.7%; RBCs+albumin = 8.2% ± 2.1%; RBCs + dextran = 4.0% ± 0.8%; incubated RBCs = 8.8% ± 1.5%) compared with controls (72.2% ± 2.8%; $p < 0.05$). Retention (99.4% ± 0.6%) was near linear in the KH control group with virtually no fractional clearance at 60 min. Retention in groups whose perfusates contained RBCs (RBCs = 62.2 ± 4.2%; RBCs+albumin = 29.9 ± 4.3%; RBCs + dextran = 69.3 ± 3.6%) were all significantly lower than control. Addition of albumin to RBC perfusate resulted in significantly lower retention (29.9% ± 4.3%; $p < 0.01$) than was observed in RBC perfusate alone (62.2% ± 4.2%). Substitution of dextran for albumin produced retention similar to RBCs alone (69.3% ± 3.6%; p = ns). In a separate protocol, RBC binding of ^{99m}TcN-NOET was high (64.4% ± 8.6%) in triple-washed RBCs. Technetium-99m-N-NOET bound to RBCs was subsequently extracted from red cells by the myocardium when those cells were infused into hearts. **Conclusion:** Technetium-99m-N-NOET has high binding affinity to blood elements and transfers bidirectionally between myocardium and blood. The interaction of ^{99m}TcN-NOET with blood elements represents a potential mechanism of redistribution.

Key Words: technetium-99m-N-NOET; myocardium redistribution; erythrocytes

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Technetium-99m-N-NOET (bis(N-ethoxy, N-ethyl dithiocarbamato) nitrido technetium(V)) is a novel myocardial perfusion imaging agent (*1,2*) which is currently under preliminary clinical evaluation (*3*). It is a member of a class of neutral myocardial imaging agents,^{99m}Tc-nitrido dithiocarbamates (*1,2*), characterized by the presence of the Tc-N triple bond group $(Tc\text{-}N)^{2+}$. It is lipophilic and has been shown to be efficiently extracted by the myocardium in preliminary studies using rats, rabbits, dogs, monkeys and humans (*4,5,6*). Technetium-99m-N-NOET has demonstrated favorable biodistribution in animals (*4*) and in humans (*7*). The relationship of microsphere-determined myocardial blood flow and ^{99m}TcN-NOET myocardial activity in dogs was found to be linear over a wide range of flows induced by dipyridamole (*8*). Good diagnostic accuracy in comparison with ^{201}Tl and coronary arteriography has been demonstrated (*3,9*). This is the first neutral ^{99m}Tc myocardial perfusion imaging agent showing

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long retention times in normal myocardial tissue (*2,7,9*). Excellent quality images in canine studies (*8*) and man (*3,7*) have demonstrated myocardial redistribution (*3,8*). These observations are important because no previously reported technetium-based myocardial perfusion imaging agent has demonstrated this thallium-like behavior to any significant degree.

Studies performed in vitro by our laboratory and others (*10,11*) have demonstrated high uptake and retention with little clearance in crystalloid buffers. However, studies in vivo have shown myocardial clearance over time (*8*) and apparent redistribution on scans (*3,8*). The reconciliation of these findings may be related to the perfusion medium. Binding to blood elements such as albumin and red cells could account for the disparities observed between studies in vitro and in vivo and represent a potential mechanism of redistribution.

Accordingly, in the present study, we used an isolated, buffer-perfused rat heart preparation to examine the role of erythrocytes and albumin on subsequent myocardial clearance of ^{99m}TcN-NOET. In addition, ^{99m}TcN-NOET uptake in erythrocytes was studied separately. Finally, myocardial extraction of ^{99m}TcN-NOET from erythrocytes incubated with ^{99m}TcN-NOET was examined. Demonstration that blood components have an affinity for ^{99m}TcN-NOET and can extract it from myocardium coupled with extraction of ^{99m}TcN-NOET from erythrocytes by the myocardium would consitute evidence of a mechanism that could explain the redistribution. Thus, the purposes of this study were to determine: (a) the effects of erythrocytes and albumin on myocardial uptake and clearance of ^{99m}TcN-NOET, (b) the extent of ^{99m}TcN-NOET uptake by erythrocytes and (c) the myocardial extraction of ^{99m}TcN-NOET from erythrocytes.

MATERIALS AND METHODS

Isolated, Perfused Heart Preparation

Male Sprague-Dawley rats (weighing 375–400 g) were anesthetized with 65 mg i.p. sodium pentobarbital. After deep anesthesia was achieved, 400 units heparin were administered intravenously and the heart was removed through a rapid parasternal thoracotomy. The aortic stump was then attached by suture to the cannula of the perfusion apparatus and perfused retrogradely. A Masterflex pump (Parmer Instruments, Burlington, IL) controlled the perfusion rate of the buffer and removed sinus drainage. Flow was held constant without recirculation. The temperature of the perfusate was maintained at 37°C by a waterbath. Coronary perfusion pressure (CPP) was also measured throughout each experiment by a similar Statham P23D pressure transducer connected to the perfusion line just before the cannula connected to the aortic stump. A left ventricular vent was placed at the apex in order to allow drainage of thebesian flow. Hearts were atrially paced at 300 bpm. Temperature and oxygen saturation of the perfusate were monitored in line by a probe. A separate in-line probe was used to monitor pH. To document left ventricular performance, a small saline-filled, latex balloon-tipped catheter was passed through a slit in the left atrial appendage and into the left ventricle. The balloon was inflated to achieve a diastolic pressure of 5–10 mmHg at the beginning of each experiment. Left ventricular developed pressure was measured by a Statham P23D pressure transducer connected to the left ventricular catheter throughout each experiment. Left ventricular developed pressure, coronary perfusion pressure, temperature, pH and oxygen levels were continuously recorded on a physiological recorder interfaced to a personal computer for visual monitoring. Cardiovascular parameters such as peak systolic pressure, minimum diastolic pressure, developed pressure and heart rate were calculated on-line using software (Labtech, Wilmington, MA).

Preparation of Perfusates

1. KH. A modified Krebs-Henseleit buffer containing (mmol/liter): 1.25 KH_2PO_4, 0.56 $MgSO_4$, 1.51 $CaCl_2$, 4.88 KCl, 0.833 EDTA, 127 NaCl, 20 $NaHCO_3$ and 5.77 dextrose. In KH experiments, this buffer was continuously bubbled with 95% O_2-5%CO_2 to maintain pH at 7.4 ± 0.05 and O_2 at > 250% saturation.

2. KH + RBCs. Whole bovine blood was collected at a local abbatoir, heparinized (15,000 U/liter) and maintained at 5° C during transport to the laboratory. This blood was centrifuged at 3000 rpm for 15 min. The plasma and buffy coat were then aspirated from the packed cells. After centrifugation, cells were resuspended in normal saline. This procedure was repeated three times and the isolated, washed cells stored at 5° C. Each day all RBCs were washed once and either used in an experiment or refrigerated at 5° C. Gentamycin (40 mg.) was added to stored cells as an antibacterial agent. Stored RBCs were not used beyond 1 wk. Hematocrit was 35%–40%. A silastic, gas-permeable lung was used to oxygenate the RBCs. Leukocytes were removed from the perfusate by filtration with a leukocyte filter just before each experiment. An identical filter was placed in line between the tank containing the perfusate with red cells and the isolated, perfused heart.

3. KH + RBCs + Albumin. Albumin (BSA fraction V, Sigma Chemical, St. Louis, MO) was added to KH perfusate containing RBCs. Albumin (100 g/500 ml KH) was dialyzed against a large volume of KH buffer using a membrane (132682, SpectraPor) for 24 hr before use. The albumin solution was filtered first with an 8.0-micron filter followed by a final filtration with a 1.2-micron filter just before use on the day of the experiment. The final concentration of albumin was 3 gm%.

4. KH + RBCs + Dextran. This perfusate consisted of KH + RBCs as described above with dextran of similar molecular weight substituted for the albumin.

Red Cell Incubation Studies

Washed RBCs were incubated with 37 MBq/ml ^{99m}TcN-NOET for 15 min after which they were subjected to a series of three consecutive washes. Pilot studies using incubation times up to 1 hr were conducted with no further RBC extraction of ^{99m}TcN-NOET at this concentration measurable beyond 15 min. Thus, 15 min was chosen as the maximum length of the incubation period. Each wash consisted of suspension of the cells in KH followed by centrifugation. After centrifugation, the supernatant was decanted to separate it from the packed cell volume. Each fraction was assayed for radioactivity separately and the packed cells were resuspended in KH. Cells were centrifuged for 10 min at 1500 rpm (approximately 500 × g) in a DYNAC centrifuge (Clay Adams, Parsippany, NJ). After the final wash and assay of activity, cells containing 37 MBq ^{99m}TcN-NOET activity were then injected into the side arm of the apparatus designed to flush the bolus through the heart in the isolated perfused heart apparatus described above. Therefore, ^{99m}TcN-NOET incubated RBCs of known activity were perfused through hearts monitored by a sodium-iodide crystal and myocardial uptake was determined. After uptake measurements, the hearts were removed from the perfusion apparatus and assayed for radioactivity in a dose calibrator.

Technetium-99m-N-NOET Preparation

Kits for the preparation of ^{99m}TcN-NOET (*12*) were supplied by CIS bio international of France. Technetium-99m-sodium pertechnetate (925 MBq) was introduced into an intermediate vial containing 1,2 diaminopropane N, N, N′,N′ tetra-acetic acid (PDTA), S-methyl, N-methyl dithiocarbazate (DTCZ) and stannous chloride

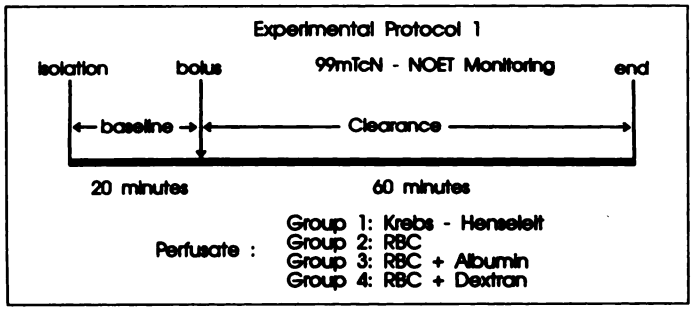

FIGURE 1. Experimental Protocol 1 for isolated perfused heart study.

dihydrate and heated in boiling water for 15 min. After the intermediate vial cooled to < 50°C, the ligand solution containing N-ethoxy, N-ethyl dithiocarbamate sodium salt monohydrate was combined with water, ^{99m}TcN-NOET. Finally, gamma cyclodextrin (10 mg in 1 ml H_2O), which served as a solubilizing agent for the lipophilic ^{99m}TcN-NOET, was added to the final complex in the perfused heart studies. No cyclodextrin was used in the RBC uptake studies since the purpose of those studies was to maximize RBC uptake. Quality control was performed using thin-layer chromatography with Silicagel and dichloromethane. The ^{99m}TcN-NOET has an R_f of approximately 0.8, and impurities did not travel. Radiochemical purity was greater than 90% in each experiment.

Tracer Kinetic Monitoring

To study tracer clearance kinetics, a bolus of ^{99m}TcN-NOET was loaded into the side arm of the perfusion line constructed of teflon tubing. It was not possible to flush all of the measured activity through the heart because the amount of ^{99m}TcN-NOET bound to the side-arm apparatus varied with each experiment. Thus, the percent injected dose = peak uptake/(injected dose − amount remaining in apparatus).

At the end of the heart stabilization phase, the bolus was introduced into the heart by turning stopcocks so that flow would be shunted smoothly through the side arm without interrupting flow to the heart. The entire side arm was completely shielded from the heart cannula and sodium iodide crystal by lead shielding. Background counts were obtained at the end of the experiment after removal of the heart to ensure that recorded activity was derived only from the heart. In KH-perfused control hearts, the bolus consisted of 3.7 MBq ^{99m}TcN-NOET. In all other groups, the bolus was 37 MBq ^{99m}TcN-NOET. The amount of activity was higher in these groups to compensate for the lower order of magnitude of peak activities due to RBC and albumin binding of ^{99m}TcN-NOET.

Myocardial ^{99m}TcN-NOET activity was monitored at 1-min intervals during ^{99m}TcN-NOET myocardial clearance by a lead-collimated sodium iodide scintillation detector. Time-activity curves were recorded for each experiment. These curves were displayed on a computerized multichannel analyzer. Myocardial clearance curves were corrected for background and ^{99m}Tc decay at the end of each study. At the end of the experimental protocol, all hearts were placed in a dose calibrator and the activities were recorded. Activity for each heart at peak uptake could then be back calculated using the clearance curves.

Experimental Protocols

Protocol 1. In 22 experiments, rat hearts were isolated, mounted on the perfusion apparatus and perfused for 20 min to ensure stabilization of all perfusate and cardiovascular performance parameters. The hearts were then divided into four groups and subjected to the protocol shown in Figure 1.

For Group 1 (KH control, n = 7), the radiotracer was introduced to the hearts by bolus injection followed by 1 hr of clearance. Bolus injection was chosen because no significant difference in peak uptake or retention was demonstrated between infusion lasting 30 min and bolus injection in pilot experiments. Flow remained constant at 12 ml/min throughout the protocol. For Group 2 (KH + RBCs, n = 5), washed bovine RBCs were added to the KH perfusing the hearts. In Group 3 (KH + RBCs + albumin, n = 5), the conditions were exactly the same as in Group 2, except that albumin was added to the perfusate. For Group 4 (KH + RBCs + dextran, n = 5), the conditions were the same as in Group 3, except that the dextran of similar molecular weight was substituted for albumin. The control flow was 6 ml/min for all RBC groups.

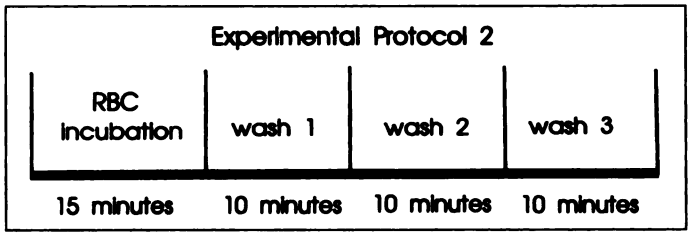

FIGURE 2. Experimental Protocol 2 for RBC incubation study.

Protocol 2: RBC Extraction

Washed RBCs were incubated with 1 mCi ^{99m}TcN-NOET and subsequently subjected to three washes (Fig. 2). These cells were then perfused through isolated hearts (Group 5, n = 5) at a flow of 12 ml/min while radioactivity was monitored. After uptake measurements, the hearts were removed from the perfusion apparatus and assayed for radioactivity.

Statistical Analysis

A one-way analysis of variance (ANOVA) procedure was used to analyze group differences. ANOVA with repeated measures was used to assess differences in hemodynamic parameters over time. Tests of mean differences were conducted by t-test analyses using the Bonferroni correction for multiple tests. Data were reported as mean ± s.e.m. All experimental animals were handled in accordance with the position of the AMA on research animal abuse and with the approval of the IACUE of the UOHSC.

RESULTS

Protocol 1: Isolated Perfused Hearts

Hemodynamic Parameters. A summary of the hemodynamic results is presented in Table 1. The data represent hemodynamics at the end of baseline and over 1 hr during the clearance phase of the protocol. All 22 hearts had normal coronary perfusion pressure and left ventricular diastolic, peak systolic and developed pressures at baseline, which remained stable over the course of the experiment. All hearts were paced at 300 bpm, and there were no significant differences among the groups at baseline or during the experiment. There were no significant differences in pH, temperature or oxygenation of the perfusate among the four groups studied which could account for any of the kinetic differences reported.

Peak Technetium-99m-N-NOET Uptake. Remaining ^{99m}Tc activity in each heart was measured in a dose calibrator at the end of the clearance phase. Activity at peak uptake was calculated from end activity and probe clearance rate as shown in Figure 3. Percentage of injected dose administered was calculated from these peak uptake values as shown in Table 2. The percent injected dose was significantly lower in all four groups containing RBCs compared to the control group.

Fractional Technetium-99m-N-NOET Myocardial Clearance. The myocardial clearance curves produced by the computerized multichannel analyzer were background-subtracted and decay-corrected. They were then normalized to peak uptake

TABLE 1
Hemodynamic Data

	Baseline	15 min	30 min	45 min	60 min
CPP (mmHg)					
Group 1	59.6 ± 4.2	59.1 ± 4.1	61.1 ± 3.6	61.3 ± 3.8	61.4 ± 3.9
Group 2	60.6 ± 1.2	60.0 ± 0.9	60.0 ± 1.1	60.0 ± 1.0	60.0 ± 1.2
Group 3	61.8 ± 1.5	64.4 ± 2.3	65.4 ± 2.6	65.6 ± 2.5	65.8 ± 2.4
Group 4	62.6 ± 2.7	62.4 ± 2.5	62.4 ± 2.5	62.4 ± 2.5	62.6 ± 2.7
Group 5	62.0 ± 1.2	62.0 ± 1.2	64.0 ± 1.9	64.0 ± 1.9	64.0 ± 1.9
Left ventricular systolic pressure (mmHg)					
Group 1	96.4 ± 2.9	95.9 ± 2.4	94.7 ± 3.9	94.9 ± 2.1	93.1 ± 3.0
Group 2	103.8 ± 3.3	98.8 ± 1.6	98.6 ± 1.4	99.4 ± 1.8	99.0 ± 1.7
Group 3	104.2 ± 2.5	100.8 ± 2.6	101.4 ± 3.0	101.2 ± 3.2	102.0 ± 3.1
Group 4	96.4 ± 4.2	96.2 ± 5.9	97.6 ± 5.4	98.0 ± 5.3	96.4 ± 4.8
Group 5	90.4 ± 4.3	93.4 ± 3.3	95.2 ± 1.9	95.2 ± 1.9	95.0 ± 1.8
Left ventricular diastolic pressure (mmHg)					
Group 1	6.6 ± 0.5	6.7 ± 1.0	6.9 ± 0.5	6.4 ± 0.5	6.6 ± 0.6
Group 2	6.6 ± 0.4	6.2 ± 0.5	5.8 ± 0.5	6.0 ± 0.4	5.8 ± 0.5
Group 3	7.6 ± 0.7	7.0 ± 0.6	7.0 ± 0.6	7.0 ± 0.6	7.0 ± 0.6
Group 4	6.8 ± 0.4	6.8 ± 0.4	6.8 ± 0.4	6.8 ± 0.4	6.8 ± 0.4
Group 5	6.8 ± 0.4	6.8 ± 0.4	6.8 ± 0.4	6.8 ± 0.4	6.8 ± 0.4
Heart rate (bpm)					
Group 1	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0
Group 2	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0
Group 3	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0
Group 4	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0
Group 5	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0

Data are mean ± s.e.m. CPP = coronary perfusion pressure; Group 1 = KH control (n = 7); Group 2 = KH + RBC (n = 5); Group 3 = KH + RBC + albumin (n = 5); Group 4 = KH + RBC + dextran (n = 5); Group 5 = KH + RBC (NOET incubation) (n = 5).

for each experiment and averaged for each group to produce mean curves. Figure 3 quantitatively demonstrates mean decay-corrected ^{99m}TcN-NOET clearance from the control and RBC groups plotted as a percentage of the activity at the beginning of the clearance phase. There was almost no clearance of ^{99m}TcN-NOET in the KH control group. Total 1 hr fractional clearance was less than 1% of peak uptake. Myocardial clearance in the RBC-alone group demonstrated significant clearance compared with controls at every time beyond one minute. Addition of albumin to RBC perfusate resulted in significantly more clearance than the control groups at every time beyond 1 min and RBCs-alone at every time from 15 min onward. Substitution of dextran for albumin resulted in clearance that was not significantly different than that of RBCs-alone but was significantly slower than RBCs + albumin and significantly faster than control at every time beyond 1 min.

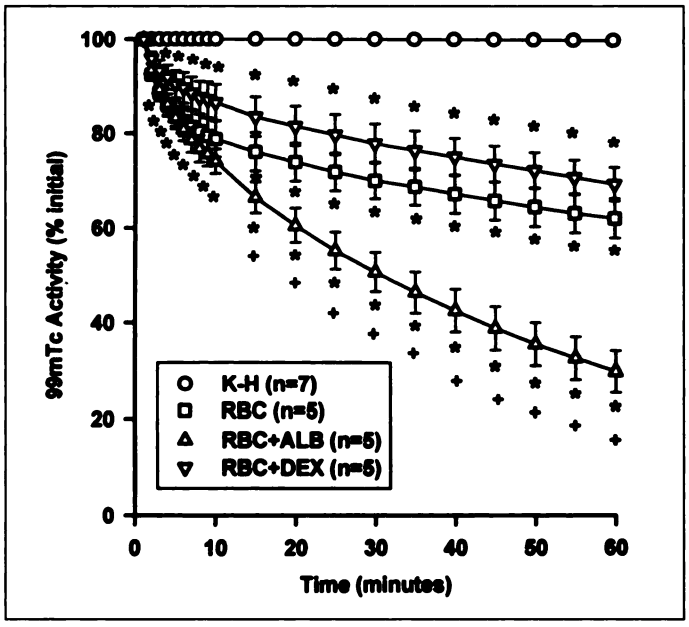

FIGURE 3. Quantitative demonstration of mean decay-corrected ^{99m}TcN-NOET clearance from the control, RBC, albumin and dextran-perfused groups plotted as a percentage of the activity at the start of the clearance phase. *p < 0.05 from control; +p < 0.05 from RBC.

Final myocardial retention in the KH control group (99.4% ± 0.6%) was significantly higher than that of the other three groups ($p < 0.05$). Final retention in the KH + RBCs + albumin (29.9% ± 4.3%) group was significantly lower ($p < 0.05$) than that of both the KH + RBCs (62.2% ± 4.2%) and KH + RBCs + dextran (69.3% ± 3.6%) groups. Retention in these latter two groups did not differ significantly.

TABLE 2
Myocardial Peak Uptake

Group no.	% ID
1	72.2 ± 2.8
2	5.0 ± 1.7*
3	8.2 ± 2.1*
4	4.0 ± 0.8*
5	8.8 ± 1.5*

*p < 0.05 from Group 1.

Data are mean ± s.e.m. Group 1 = KH control (n = 7); Group 2 = KH + RBC (n = 5); Group 3 = KH + RBC + albumin (n = 5); Group 4 = KH + RBC + dextran (n = 5); Group 5 = KH + RBC (NOET incubation) (n = 5).

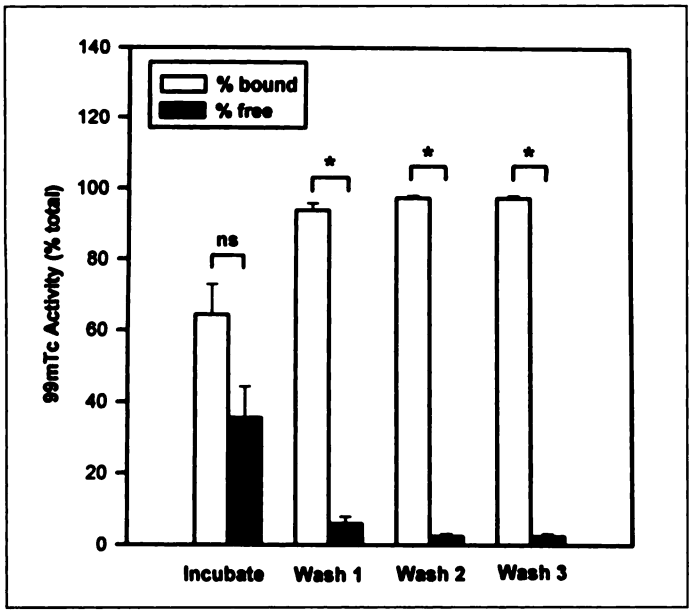

FIGURE 4. Effect of three washes on the percentage of free and bound ^{99m}TcN-NOET after incubation with RBCs. *$p < 0.05$.

Protocol 2. RBC Extraction

RBC retention of ^{99m}TcN-NOET after incubation is shown in Figure 4. RBC extraction of ^{99m}TcN-NOET was 64.4% ± 8.6%. After three washes, the percentage of activity remaining in the third supernatant was only 2.5% ± 0.7%. Peak myocardial activity at 1 min after perfusion with the incubated RBCs was 117.8 ± 7.5 μCi (Fig. 5). One minute later, after the unbound activity had cleared, peak myocardial activity was 88.3 ± 13.4 μCi. Both peak values were significantly greater than the maximum value for free ^{99m}TcN-NOET activity in the perfusate 27.5 ± 6.6 uCi ($p < 0.0002$ vs. peak; $p < 0.005$ vs. peak + 1 min).

DISCUSSION

The aim of the current study was to determine the effects of RBC interaction with ^{99m}TcN-NOET on myocardial uptake and clearance kinetics in vitro and to determine whether these interactions of ^{99m}TcN-NOET with blood elements could explain the redistribution observed in vivo.

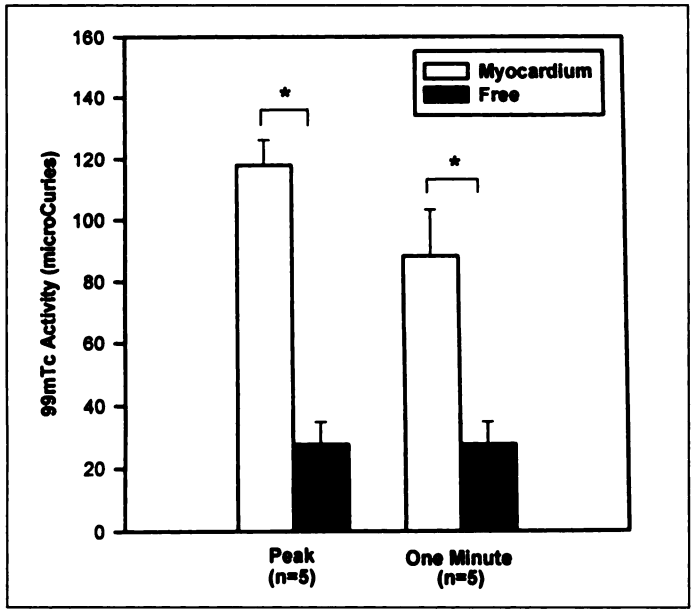

FIGURE 5. Myocardial activity of ^{99m}TcN-NOET at peak uptake and 1 min postpeak compared with free activity in hearts exposed to a bolus of RBCs incubated with ^{99m}TcN-NOET. *$p < 0.05$.

Hemodynamic Parameters

The summary of the hemodynamic parameters presented in Table 1 demonstrates that all hearts had hemodynamic values at baseline which were not significantly different and remained stable over the course of the experiment. Thus, there were no hemodynamic alterations among the four groups of isolated hearts studied which could account for any of the kinetic differences reported.

Myocardial Uptake

Peak uptake was highest in the KH-perfused hearts and was substantially lower in all of the RBC groups, despite use of a 10-fold higher injected activity, indicating significant binding to both RBCs and albumin. The percent injected dose values indicate that albumin may additionally increase myocardial extraction of ^{99m}TcN-NOET compared with RBCs alone. The percent injected dose in the albumin group was nearly twice that for the RBCs-alone and RBCs + dextran groups. Thus, exposure to albumin and RBCs significantly reduced myocardial uptake of ^{99m}TcN-NOET compared with KH alone. The addition of dextran, a polysaccharide, to the RBC perfusate had no significant effect on uptake compared with RBCs-alone and produced significantly lower uptake compared to RBCs + albumin. This is evidence of protein binding as the specific mechanism of uptake for ^{99m}TcN-NOET and rules out increased oncotic pressure as a mechanism for this effect.

This specific mechanism of uptake was previously suggested to occur through linkage of the radiotracer to proteins bound in the lipid membrane of isolated myocytes (*5*). In cell fractionation studies in which rat myocytes were first homogenized and then subcellular organelles were separated by differential centrifugation, activity in the cellular fractions was present in all fractions except in the cytosol (*13*). In additional cell homogenate studies of rat myocytes, ^{99m}TcN-NOET was found to be associated predominantly with the membrane protein content (*5*).

Myocardial Retention

Myocardial clearance of ^{99m}TcN-NOET from normal myocardium is less than 1% at the end of 1 hr in KH perfusate. The clearance curves in Figure 3 illustrate significant decreases in ^{99m}TcN-NOET myocardial retention induced by both RBCs and albumin when compared with the KH control groups. The addition of dextran to the RBC perfusate did not accelerate clearance as did albumin. Therefore, properties of albumin such as molecular weight and generation of oncotic pressure may be ruled out as resulting in the observed increased ^{99m}TcN-NOET binding, which supports the hypothesis that proteins are attractive sites for ^{99m}TcN-NOET binding.

Maublant et al. (*10*) studied cell cultures from newborn rat myocytes and found relatively high washin rates (18 min) and long half times (>120 min) for washout of ^{99m}TcN-NOET in normal myocytes. Concordant results of extremely high retention in isolated cardiac myocytes (*10,14*) and crystalloid perfused rat hearts (*11*) seem to differ from data obtained in canine (*8*) and human (*5,7,9*) studies. Ghezzi et al. (*8*) found that myocardial ^{99m}TcN-NOET retention at 90 min postinjection on canine images was approximately 50% of the activity at 5 min postinjection. The percentage of maximal activity in blood was higher for ^{99m}TcN-NOET than sestamibi at every time from 2 min up to 240 min postinjection. Thus, the differences in these data appear to be related to interactions with blood elements, which provide a residual circulating pool of ^{99m}TcN-NOET

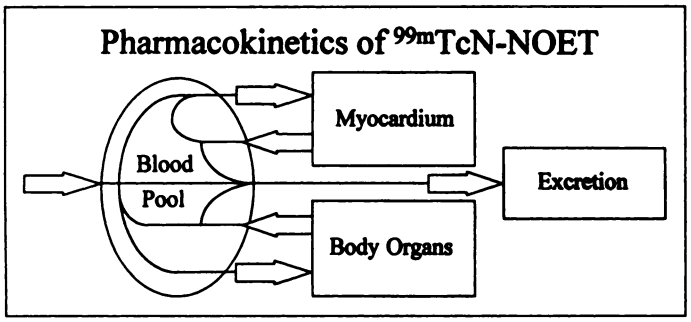

FIGURE 6. Proposed pharmacokinetics of ^{99m}TcN-NOET.

activity. It is also possible that species specific differences accounted for the discordance.

Red Blood Cell Extraction

Data from the current study indicate that RBCs avidly extract ^{99m}TcN-NOET (64.4 ± 8.6%) from aqueous media and maintain retention of this extracted activity despite repeated washings. Myocardial clearance of ^{99m}TcN-NOET was affected to approximately the same extent by RBCs (37.8%) and albumin (32.3% more than RBCs-alone). However, the percentage of RBCs (hematocrit = 35%–40%) was much greater than that of albumin (3 gm%) in perfusate. Therefore, albumin would seem to have the higher affinity for binding to ^{99m}TcN-NOET than RBCs in the current study.

Pharmacokinetics of ^{99m}TcN-NOET

Figure 6 illustrates the proposed pharmacokinetics of ^{99m}TcN-NOET. Technetium-99m-N-NOET is distributed in blood to the heart in proportion to flow (*6,8*). Based on the findings of the current study, we postulate that uptake results from passive diffusional mechanisms and continues as long as concentration gradients between tissue and blood exist. Equilibrium between tissue and blood occurs such that residual activity is maintained over time in blood and is available for further tissue exchange (*8*) with recirculation. Multiple circulatory passes are required to establish equilibrium over time. Thus, redistribution is possible wherever a persistent concentration gradient exists, as in chronically underperfused myocardial areas. Technetium-99m-N-NOET is available for uptake in underperfused myocardial areas due to persistence of tracer in blood.

Myocardial Redistribution

Thallium-201 is the only myocardial radiotracer, before ^{99m}TcN-NOET, which undergoes significant redistribution. For ^{201}Tl, evidence of redistribution implies viability in the area demonstrating delayed uptake due to the energy requirement for uptake. The clinical interpretation of apparent redistribution on images is markedly different depending on the tracer and mechanism (*15*). Caution must be maintained in the clinical interpretation of ^{99m}TcN-NOET redistribution on scans until the pathophysiologic significance of this phenomenon has been fully investigated due to the notable differences in molecular size, shape and charge between ^{201}Tl and ^{99m}TcN-NOET. Technetium-99m-N-NOET does appear to possess favorable characteristics for clinical imaging and further studies are warranted.

CONCLUSION

In normal buffer-perfused rat myocardium, extraction and retention of ^{99m}TcN-NOET is exceptionally high. However, significant affinities for binding to both albumin and RBCs were demonstrated. The interaction of ^{99m}TcN-NOET with RBCs results in reduced myocardial uptake and increased clearance in a nonrecirculating perfused heart model. Bidirectional transfer of ^{99m}TcN-NOET between RBCs and the myocardium was demonstrated. Therefore, the phenomenon of ^{99m}TcN-NOET redistribution, previously reported in canine and clinical studies, may be explained in part by binding characteristics of ^{99m}TcN-NOET to RBCs and other blood elements.

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