Subcellular Distribution of Technetium-99m-N-NOEt in Rat Myocardium

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The aim of this study was to determine the subcellular distribution of bis(N-ethoxy N-ethyl)dithiocarbamato nitrido technetium(V) (^{99m}TcN-NOEt) in rat heart by differential centrifugation techniques. Extraction of the activity from homogenized rat heart tissue was also performed to assess whether myocardial retention might induce changes in the chemical identity of the complex. **Methods:** Anesthetized rats were intravenously injected with ^{99m}TcN-NOEt, the heart tissue was extracted and homogenized and tissue fractions were obtained by differential centrifugation. The efficiency of organelle separation was determined by assay of each centrifugal fraction using enzyme markers. Lactate dehydrogenase (LDH), acid phosphatase (ACP), alkaline phosphatase (ALP) and 5'-nucleotidase (5'ND) activities were assayed using standard spectrophotometric methods. Succinic dehydrogenase (SDH) activity was determined using a *p*-iodonitrotetrazolium-linked assay. Severe cell membrane and organelle disruption were induced by prolonging the homogenization time and their effect on the subcellular distribution of ^{99m}TcN-NOEt was studied. The activity from homogenized heart tissue was extracted using the Folch technique and analyzed by TLC and HPLC. **Results:** Most of the ^{99m}TcN-NOEt activity was found to be associated with the hydrophobic components of the cell. No evidence of specific association of activity with the cytosolic and mitochondrial components was observed. Organelle and membrane cleavage did not cause release of activity into the cytosol. Approximately 90% of ^{99m}TcN-NOEt activity was extracted from ventricular tissue and the chemical nature of ^{99m}TcN-NOEt was not altered by uptake by myocardium. **Conclusion:** Cell membranes are the most apparent site of localization of ^{99m}TcN-NOEt in heart tissue.

Key Words: technetium-99m-N-NOEt; subcellular distribution and extraction

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Bis(N-ethoxy, N-ethyl)dithiocarbamato nitrido technetium-99m (^{99m}TcN-NOEt) (*1,2*) is a new imaging agent currently under preliminary clinical evaluation as a tracer for myocardial perfusion (*3,4*). This radiopharmaceutical possesses a neutral charge and its chemical structure is characterized by the presence of a terminal Tc≡N multiple bond (*1*) (Fig. 1). Experiments in animal models (rats, dogs and monkeys) and in humans have demonstrated that ^{99m}TcN-NOEt is rapidly extracted by the myocardium with slow washout from the heart (*2–4*). Myocardial retention in cultured rat myocites has been shown to be higher than that of ^{201}Tl, ^{99m}Tc-BATO and ^{99m}Tc-MIBI (*5*). Studies in dogs showed that the relationship between microsphere-determined blood flow and ^{99m}TcN-NOEt activity is linear over a wide range of flows induced by dipyridamole and that this tracer undergoes redistribution in ischemic-reperfused heart (*6*). This latter result has also been observed in humans (*3,4*).

Technetium-99m-N-NOEt is the first reported neutral ^{99m}Tc complex showing long retention times in normal myocardial tissue (*2,3*). Determination of its subcellular localization would therefore be a relevant step to fully understanding the kinetics of this myocardial perfusion agent. The purpose of this study was to determine the subcellular distribution of ^{99m}TcN-NOEt in the rat heart by standard differential centrifugation techniques. In particular, we followed methodologies described in two previous papers concerning determination of the subcellular localization of ^{99m}Tc-MIBI (*7,8*). In these works, subcellular fractionation techniques were used and the efficiency of organelle separation was ascertained with enzyme markers. Since a number of studies on ^{99m}Tc-MIBI have been reported (*7,8*), the subcellular distribution of ^{99m}Tc-MIBI was also assessed using the same experimental procedures and used as a control. We further studied the effect on the subcellular distribution of ^{99m}TcN-NOEt of producing extensive membrane cleavage and disruption of the internal organelles by prolonging the homogenization time. Finally, the chemical integrity of ^{99m}TcN-NOEt, after the uptake process, was determined by chromatographic analysis of the activity extracted from heart tissue using the Folch procedure (*9–11*).

MATERIALS AND METHODS

Preparation of Technetium-99m-N-NOEt

The compound ^{99m}TcN[Et(OEt)NCS$_2$]$_2$ (^{99m}TcN-NOEt) was prepared using both liquid and freeze-dried formulation as previ-

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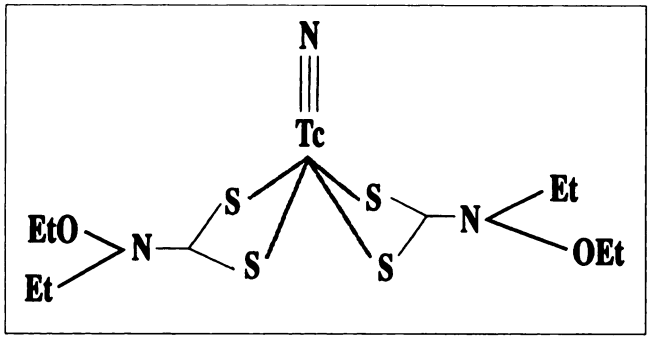

FIGURE 1. Structure of ^{99m}TcN-NOET.

ously described (*2*). Liquid formulation involves addition of [^{99m}Tc]pertechnetate (0.6 GBq–1.0 GBq) to a vial containing 1.0 mg S-methyl N-methyl dithiocarbazate [$H_2NN(CH_3)C(=S)SCH_3$], 3.0 mg tris(*m*-sulfophenyl)phosphine sodium salt [$P(m\text{-}C_6H_4SO_3)_3$]Na_3 dissolved in 1.0 ml HCl (0.10 *M*). The mixture was heated at 100°C for 15 min and then cooled at room temperature, and the pH was raised to 8.0 by adding 1.0 ml sodium phosphate buffer (0.20 *M*). Finally, 1.0 ml of a water solution containing 10 mg N-ethoxy N-ethyl dithiocarbamate [Et(OEt)NCS_2]Na was added at room temperature and the formation of the final product was completed within 5 min. The lyophilized formulation was carried out as follows. Pertechnetate (0.6 GBq–1.0 Gbq) was added to a vial containing 1.0 mg S-methyl N-methyl dithiocarbazate [$H_2NN(CH_3)C(=S)SCH_3$], 0.1 mg $SnCl_2 \cdot 2H_2O$ and 10 mg 1,2-diaminopropane-N,N,N′,N′-tetraacetic acid (DPTA) in a freeze-dried form. The mixture was heated at 100°C for 15 min and then cooled to room temperature. Ten milligrams [Et(OEt)NCS_2]Na dissolved in 1 ml water were finally added to the reaction vial and the solution was left to stand for 5 min at room temperature. To avoid activity loss as a consequence of absorption by the syringe walls, 20 mg of gamma-cyclodextrine were added to both formulations.

The radiochemical purity (RCP) of ^{99m}TcN-NOEt has been evaluated by HPLC analysis or by using a previously described method (*2*). The final yield was always >97%. Figure 2 shows the HPLC profile of ^{99m}TcN-NOEt activity (dashed line) eluted through HPLC, System Gold equipped with programmable solvent Module 126, sample injection valve 210A, analog interface Module 406, scanning detector Module 166 (Beckman Instruments, Inc., San Ramon, CA) and radioisotope Detector Model 170 (Beckman Instruments, Inc., Fullerton, CA). The HPLC system included a C18 ultrasphere Reversed-phase precolumn (length, 45 mm, diameter 4.6 mm) and a C18 Ultrasphere Reversed-phase column (length, 250 mm, diameter 4.6 mm) (Beckman Instruments). The flow rate was 1 ml min^{-1} and the mobile phase was A = water and B = methanol (gradient: time 0 min 100% A for 2 min; at time 2 min 0–100% B in 3 min; time 5 min 100% B for 11 min; at time 16 min 0–100% A in 3 min; time 19 min 100% A for 6 min). Retention time of ^{99m}TcN-NOEt was 10.88 min.

Synthesis of ^{99m}Tc-MIBI was carried out from a lyophilized kit formulation (Cardiolite®, E.I. Du Pont, Billerica, MA). Kits were reconstituted and tested for radiochemical purity by previously described methods (*12*).

Subcellular Distribution Studies

Female rats (Sprague Dawley) weighing 250–300 g were anesthetized with an intraperitoneal injection of a mixture of ketamine (15 mg kg^{-1}) and xilazine (18 mg kg^{-1}). A jugular vein was

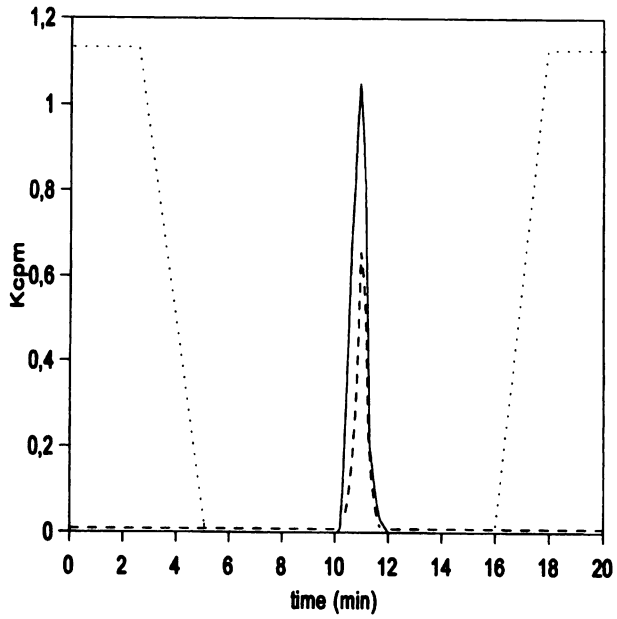

FIGURE 2. HPLC profile of ^{99m}TcN-NOET activity after extraction from heart tissue. (——) = extracted ^{99m}TcN-NOET activity; (----) = reference ^{99m}TcN-NOET activity; (···) = concentration of solvent A. kcpm = 10^3 × counts per minutes.

surgically exposed and 100 μl (100 μCi) of a solution containing the radioactive complex (^{99m}TcN-NOEt or ^{99m}Tc-MIBI) were injected. The animals were killed by cervical dislocation (5 min after injection for ^{99m}TcN-NOEt and 10 min after injection for ^{99m}Tc-MIBI), the hearts extracted and washed with cold water to remove the residual blood. The atrial tissue was dissected and discarded, while the remaining septal and ventricular tissue were grossly minced on ice with a scalpel and placed in a centrifuge tube containing 10 volumes of a homogenization medium (Tris HCl, 0.020 mol dm^{-3}, pH = 7.4, 4°C,) and protease inhibitors [phenylmethyl-sulfonyl fluoride (PMSF) (1 m*M*) and leupeptins (1 μg/ml), benzamidin hydrochloride hydrate (1 m*M*), iodoacetamide (1 m*M*) and pepstatin A (1 μ*M*)] dissolved in the same Tris HCl buffer. The tissue sample (0.8–1.2 g) was homogenized using an Ultra-Turrax Model T50 homogenizer (Janke & Kunkel KG, Staufen, Germany) for 30, 60 and 180 sec (n = 5 for each time point) and the activity was measured in a gamma counter. The sample tubes were kept on ice during the homogenization procedure. The homogenate was subjected to differential centrifugation (4°C) at 1000 × g (10 min) (P1) in a Minifuge GL centrifuge (Heraus-Christ GmbH, Osterode am Harz, Germany) and at 10,000 × g (10 min) (P2), 50,000 × g (30 min) (P3) and 100,000 × g (60 min) (P4 and S) in a Beckman Model L8-50M/E ultracentrifuge (Fig. 4). Each set of pellets was mixed with 10 volumes of cold homogenization buffer and gently solubilized with a IKA Vibro-Fix VF-1 instrument (Janke & Kunkel, Staufen, Germany). Technetium-99m activity in each pellet and supernatant was determined using a Cobra gamma counter (Table 1). The efficiency of organelle separation was determined by assay of each centrifugal fraction with enzyme markers (Table 1). Lactate dehydrogenase, acid phosphatase, alkaline phosphatase and 5′-nucleotidase activities were assayed from 0.02 to 0.20 ml aliquots of each fraction using spectrophotometric assay kits (Sigma Chemical Co., St. Louis, Mo). Succinic dehydrogenase activity was determined using a *p*-iodo-

TABLE 1
Distribution of Technetium-99m-N-NOEt Activity and Subcellular Markers*

Marker*	Cell fraction	P1	P2	P3	P4	S
			60 sec Homogenization			
SDH	Mitochondria	82.0 ± 9.5	15.2 ± 6.2	0.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
ACP	Lysosome	5.8 ± 3.7	9.8 ± 6.6	3.4 ± 0.2	0.8 ± 0.2	78.2 ± 8.6
5'ND	Sarcolemma	16.6 ± 8.3	64.2 ± 13.4	3.2 ± 0.2	2.5 ± 1.1	7.5 ± 3.8
ALP	SR	30.9 ± 8.3	24.8 ± 7.8	20.1 ± 5.6	5.7 ± 2.7	10.2 ± 6.5
LDH	Cytoplasm	10.5 ± 0.7	0.7 ± 0.3	0.4 ± 0.1	0.2 ± 0.1	84.2 ± 1.6
^{99m}TcN-NOEt		15.8 ± 6.4	60.2 ± 2.9	12.1 ± 6.5	5.1 ± 2.8	2.9 ± 1.6
^{99m}Tc-MIBI		80.9 ± 10.7	15.3 ± 7.6	0.8 ± 0.4	0.6 ± 0.4	0.9 ± 0.3
			180 sec Homogenization			
SDH	Mitochondria	44.0 ± 4.5	36.6 ± 10.4	15.4 ± 10.0	0.0 ± 0.0	0.0 ± 0.0
ACP	Lysosome	4.8 ± 1.7	8.4 ± 2.9	9.4 ± 7.1	11.8 ± 6.0	64.2 ± 9.0
5'ND	Sarcolemma	15.6 ± 7.1	45.2 ± 10.4	17.1 ± 3.2	3.5 ± 2.1	17.2 ± 5.3
ALP	SR	28.4 ± 7.1	22.3 ± 5.8	20.6 ± 3.6	9.7 ± 1.4	18.3 ± 5.5
LDH	Cytoplasm	4.5 ± 3.0	0.5 ± 0.3	0.6 ± 0.3	0.4 ± 0.2	93.0 ± 1.3
^{99m}TcN-NOEt		14.2 ± 3.1	42.5 ± 5.8	28.1 ± 5.5	5.3 ± 3.8	1.3 ± 0.8
^{99m}Tc-MIBI		19.9 ± 2.7	4.3 ± 0.9	1.8 ± 0.1	0.9 ± 0.7	70.6 ± 8.3

*Values are expressed as percent of the total homogenate activity (n = 5/centrifugation time). SDH = succinate dehydrogenase; ACP = acid phosphatase; 5'N = 5'-nucleotidase; ALP = alkaline phosphatase; LDH = lactate dehydrogenase; P1 = 1000 × g; P2 = 10 000 × g; P3 = 50 000; g; P4 = 100 000 × g; S = sumatant.

nitrotetrazolium-linked assay (*13*). Spectophotometric measurements were carried out using a Beckman DU-40 spectrophotometer.

Analysis of Technetium-99m-N-NOEt

Extraction of ^{99m}TcN-NOEt from rat heart tissue, injected intravenously with the radiopharmaceutical, was performed using the standard Folch technique (*9*). The rats were treated as described above and the hearts removed, washed in buffer, weighed and counted in a gamma counter to obtain the percent activity per heart and the percent activity per gram values. The heart tissue was then homogenized in a 2:1 chloroform-to-methanol mixture at a 20:1 volume with a Ultra-Turrax Model T50 homogenizer. After filtering the homogenate to remove the particulate, a volume of 0.29% NaCl solution was added (20% of the original volume) and the organic and aqueous phases were separated. The two phases and the residual particulate matter were counted to determine the percent activity per fraction. Initial ^{99m}Tc activity, 92% ± 3.4%, was recovered in the chloroform phase. The organic phase was taken to dryness and redissolved in a small amount of chloroform for TLC as described above. The results revealed that the extracted activity migrated as a single peak on silica gel or reversed-phase TLC plates and showed the same R_f values (0.89 silica gel; 0.48, reversed-phase) corresponding to ^{99m}TcN-NOEt (*2*) (Fig. 3). The HPLC peak of the extracted activity was eluted using the same procedure detailed above and was found to overlap with the control peak corresponding to ^{99m}TcN-NOEt reference activity (Fig. 2). The recovery of the extracted activity from the HPLC column was 98.8%. The chemical nature of the extracted activity was further investigated by electrophoresis carried out on a Whatman 3.0 paper sheet at ΔV = 200, 250, 350 V for 1 and 2 hr for each potential difference. The radioactive tracer was found to remain at the origin, indicating that the uptake process did not affect its neutral character.

RESULTS

The subcellular distribution of ^{99m}TcN-NOEt was evaluated using the protocol illustrated in Fig. 4. Two critical parameters in this procedure are the homogenization time and centrifugation rates. We kept the centrifugation procedure for separating the various subcellular fractions fixed. The homogenization time was extended from 30 to 60 sec or 180 sec (n = 5 for each time point). Table 1 reports the distribution of subcellular markers and of ^{99m}TcN-NOEt activity. The values obtained for the homogenization times 30 and 60 sec were not significantly different, and Table 1 includes only the results corresponding to 60 sec. The homogenization procedure appeared to disrupt most of the

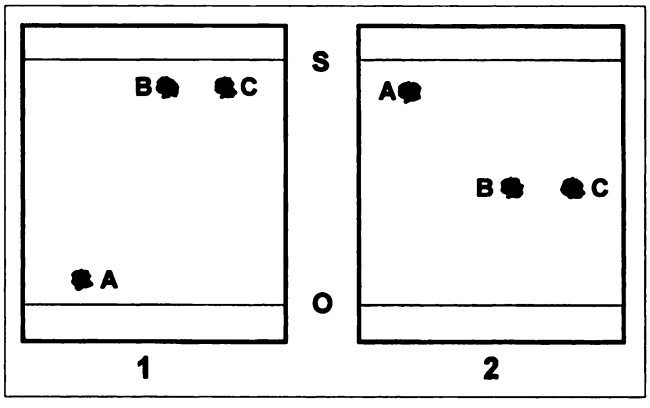

FIGURE 3. TLC chromatography of ^{99m}TcN-NOET activity after extraction from heart tissue. (1) silica gel plates, mobile phase, CH_2Cl_2; (2) reversed phase C18 plates, mobile phase, methanol-acetonitrile-tetrahydrofuran-ammonium acetate (0.5 mole dm^{-3}) (3:3:2:2). (A) $^{99m}TcO_4^-$ reference; (B) extracted ^{99m}TcN-NOET activity; (C) reference ^{99m}TcN-NOET activity; (O) = origin; (S) = solvent front.

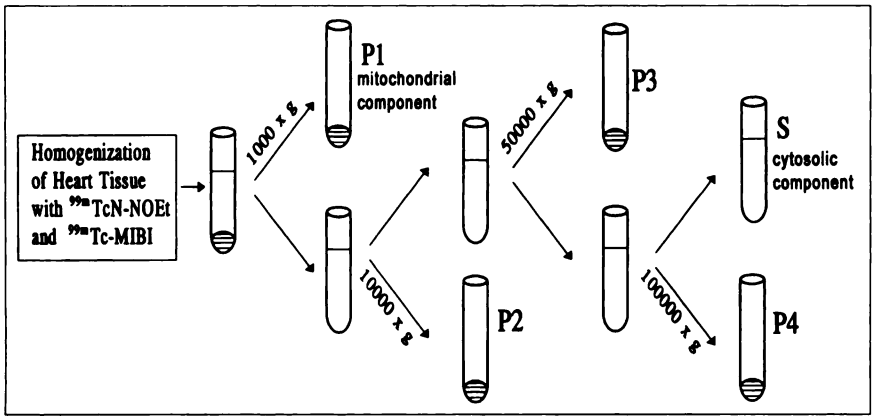

FIGURE 4. Schematic diagram of the subcellular fractionation procedure (P = pellet, S = supernatant).

heart cells, as evidenced by the quantitative release of LDH into the supernatant. When the homogenization time was 30 or 60 sec, ^{99m}Tc activity was primarily recovered in the 1000 × g and 10,000 × g pellets, which also exhibited the highest concentration of 5′ND and ALP. The highest concentrations of SDH, ACP and LDH were found in the first pellet and in the supernatant, respectively. Association of activity was approximately 15% with SDH and 3% with ACP and LDH, indicating that no specific localization into mitochondria, lysosomes or cytosol was observed. Extension of the homogenization time to 180 sec, caused ^{99m}TcN-NOEt activity to be distributed over the 1000 × g, 10,000 × g and 50,000 × g pellets, while significant amounts of the membrane markers 5′ND and ALP were found in almost all five centrifugal fractions. SDH was also spread over the P2 and P3 pellets. These results suggest that the prolonged homogenization period produced disruption of most of the internal organelles and cleavage of cellular membranes in shorter segments. In this situation, ^{99m}TcN-NOEt activity was associated with the first three pellets which contain the most consistent amount of the membrane components. Presently, it is impossible to establish clearly whether the association of the activity with the enzyme marker ALP corresponds to an actual in vivo localization of tracer in the sarcoplasmic reticular membranes or whether it is simply the result of the transfer of the lipophilic ^{99m}Tc complex from one hydrophobic component to another during the fractionation procedure.

A parallel experiment for determining the subcellular distribution of ^{99m}Tc-MIBI was carried out using the same fractionation procedure. The results are reported in Table 1. They show that ^{99m}Tc-MIBI activity was mostly recovered in the 1000 × g pellet, when the homogenization time was 30 or 60 sec, which was enriched with the mitochondrial component. When the homogenization time was extended to 180 sec, ^{99m}Tc-MIBI activity was released in the supernatant as a consequence of the disruption of the mitochondrial organelles. These findings are in agreement with previously published results (*7,14*).

Extraction of the activity localized in the heart tissue after injection of ^{99m}TcN-NOEt was accomplished with the standard Folch procedure (*9*), which is particularly suitable for highly lipophilic substances. Approximately 90% of the initial activity localized in the ventricular tissue was recovered in the organic phase ($CHCl_3$) and analyzed by HPLC and TLC to investigate the influence of the uptake process on the chemical identity of ^{99m}TcN-NOEt. The chromatographic profile of the eluted activity is shown in Figure 2. It exhibits a single peak which was found to be perfectly superimposable with the control peak corresponding to added ^{99m}TcN-NOEt reference activity. The correspondence between the extracted and the reference activity was also evidenced by TLC, as illustrated in Figure 3. These findings clearly demonstrates that the uptake process does not involve a change in the chemical nature of the tracer.

DISCUSSION

The subcellular distribution of ^{99m}TcN-NOEt, determined by standard differential centrifugation techniques, strongly supports the view that this tracer can diffuse and localize in the hydrophobic components of myocardial cells. No specific association with mitochondria or cytosol was observed in this study. Such a picture is consistent with the high lipophilic character of ^{99m}TcN-NOEt (logP = 3.95) (*15*). Since data obtained using subcellular fractionation procedures should always be viewed cautiously, due to the dependence of the results on two crucial parameters (e.g., homogenization times and centrifugation rates), we carried out a parallel experiment to determine the subcellular distribution of ^{99m}Tc-MIBI with the same procedure used for ^{99m}TcN-NOEt. The main difference between these two complexes lies in their charges. In particular, ^{99m}TcN-NOEt is a neutral complex, while ^{99m}Tc-MIBI carries a monopositive charge. A sharp distinction between the subcellular distributions of the two tracers would therefore be expected. Moreover, many studies on the determination of the subcellular localization of ^{99m}Tc-MIBI have been documented (*16,17*), thus allowing a careful control of the results obtained here. Our findings on the subcellular distribution of ^{99m}Tc-MIBI (Table 1) were in satisfactory agreement with literature data and indicate that, as previously suggested (*8*), mitochondria are the most probable localization site of ^{99m}Tc-MIBI. When the homogenization

time was extended to 180 sec, approximately 70% of ^{99m}Tc-MIBI activity was released into the cytosolic fraction as a result of the disruption of mitochondria and extraction of the activity into the aqueous medium. This result agrees with that of Crane et al. (*7*). The comparison of the subcellular fractionation results obtained with ^{99m}TcN-NOEt and ^{99m}Tc-MIBI was therefore particularly revealing. The fact that ^{99m}TcN-NOEt activity was not released into the cytosol after membrane and organelle disruption strongly suggests that this neutral, lipophilic tracer remains tightly bound to the hydrophobic components of the cell. In contrast, ^{99m}Tc-MIBI, in the same situation, is efficiently removed from the disrupted mitochondria and transferred to the aqueous cytosolic phase as a result of the monocationic character of this complex.

The observed behavior of ^{99m}TcN-NOEt is also in agreement with a previous study on ^{99m}TcN-NOEt carried out using cultured heart cells of newborn rats (*5*). In this model, ^{99m}TcN-NOEt uptake was found to be high and no significant washout was detected during the time of observation (approximately 120 min). This stable trapping could be explained satisfactorily by supposing that, after crossing cell boundaries, ^{99m}TcN-NOEt is unable to return to the surrounding aqueous environment as a result of its vanishing solubility in water and high affinity for the lipophilic components of cultured heart cells.

CONCLUSION

Based on the results of the present study, it is reasonable to assume that cell membranes are the most probable subcellular localization site of ^{99m}TcN-NOEt. The nature of the interaction linking ^{99m}TcN-NOEt to the membrane substrate is still to be elucidated. The fact that the chemical identity of the tracer was not found to be altered by the localization process seems to indicate that its strength is low and rules out any description of the uptake mechanism involving ligand substitution or redox reactions. Such a weak interaction, which is presumably related to the high lipophilic character and neutral charge of ^{99m}TcN-NOEt, might also account in part for the redistribution of the tracer in ischemic-reperfused heart (*3,4,6*). In particular, it was generally observed that cationic technetium complexes do not undergo redistribution (*16,18,19*) or can only redistribute partially in the presence of an elevated blood concentration as a result of their stable trapping into myocardium (*20,21*). This result seems to originate from the interaction of the monopositive charge with large negative transmembrane potentials within mitochondria (*12*). In contrast, the assumption of a weak strength for the interaction of the neutral ^{99m}TcN-NOEt complex with the subcellular substrate entails that this tracer could move across cell boundaries following a concentration gradient. As a result, ^{99m}TcN-NOEt would be relatively free to diffuse from perfused regions towards areas of low blood concentration, a fact that has been experimentally verified both in humans (*3,4*) and in animal models (*6*).

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REFERENCES

1. Pasqualini R, Duatti A. Synthesis and characterization of the new neutral myocardial imaging agent [^{99m}TcN(NOEt)$_2$] (NOEt = N-ethyl N-ethoxydithiocarbamato). *J Chem Soc Chem Commun* 1992;1354–1355.
2. Pasqualini R, Duatti A, Bellande E, et al. Bis(dithiocarbamato) nitrido technetium-99m radiopharmaceuticals: a class of neutral myocardial imaging agents. *J Nucl Med* 1994;35:334–341.
3. Giganti M, Cittanti C, Colamussi P, et al. Biodistribution in man of bis[(N-ethyl, N-ethoxy) dithiocarbamate] nitrido technetium(V), a promising new tracer for myocardial perfusion imaging [Abstract]. *J Nucl Med* 1994;35: 155P.
4. Fagret D, Marie PY, Brunotte F, et al. Myocardial perfusion imaging with technetium-99m-NOET: comparison with thallium-201 and coronary angiography. *J Nucl Med* 1995;36:936–943.
5. Maublant J, Zhang Z, Ollier M, et al. Uptake and release of bis(N-ethoxy, N-ethyl dithiocarbamato) nitrido ^{99m}Tc(V) in cultured myocardial cells: comparison with ^{201}Tl, MIBI and teboroxime [Abstract]. *Eur J Nucl Med* 1992;19:597.
6. Ghezzi C, Fagret D, Arvieux CC, et al. Myocardial kinetics of TcN-NOET: a neutral lipophilic complex tracer of regional myocardial blood flow. *J Nucl Med* 1995;36:1069–1077.
7. Crane P, Laliberté R, Heminway S, Thoolen M, Orlandi C. Effect of mithocondrial viability and metabolism on technetium-99m-sestamibi myocardial retention. *Eur J Nucl Med* 1993;20:20–25.
8. Carvalho PA, Chiu ML, Kronauge JF, et al. Subcellular distribution and analysis of technetium-99m-MIBI in isolated perfused rat hearts. *J Nucl Med* 1992;33:1516–1521.
9. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957;226:497–509.
10. Uccelli L, Bolzati C, Comazzi V, et al. Subcellular distribution of bis(dithiocarbamato) nitrido Tc(V) radiopharmaceuticals in myocardium cells of male rats [Abstract]. *J Nucl Med* 1992;33:850.
11. Uccelli L, Pasqualini R, Duatti A, Giganti M, Piffanelli A. Uptake mechanism of the neutral myocardial imaging agent Tc99mN-NOEt [Abstract]. *J Nucl Med* 1993;34:18P.
12. Piwnica-Worms D, Kronauge JF, Chiu ML. Uptake and retention of hexakis(2-methoxyisobutylisonitrile)technetium (I) in cultured chick myocardial cells: mitochondrial and plasma membrane potential-dependence. *Circulation* 1990;82:1826–1838.
13. Pennington RJ. Biochemistry of dystrophic muscle. Mitochondrial succinate-tetrazolium reductase and adenosine triphosphatase. *Biochem J* 1961;80: 649–654.
14. Mousa SA, Williams SJ, Sands H. Characterization of in vivo chemistry of cations in the heart. *J Nucl Med* 1987;28:1351–1357.
15. Duatti A, Marchi A, Pasqualini R, Comazzi V, Bellande E. Preparation of technetium-99m nitrido dithiocarbamates: a new class of neutral, myocardial imaging agents [Abstract]. *J Nucl Med* 1991;32:925.
16. Beller GA, Watson DD. Physiological basis of myocardial perfusion imaging with technetium-99m agents. *Semin Nucl Med* 1991;21:173–181.
17. Saha GB, Go RT, Macintyre WJ. Radiopharmaceuticals for cardiovascular imaging. *Nucl Med Biol* 1992;19:1–20.
18. Rossetti C, Vanoli G, Paganelli G, et al. Human biodistribution and clinical use of technetium(III)-99m-Q12. *J Nucl Med* 1994;35:1571–1580.
19. Kelly JD, Forster AM, Higley B, et al. Technetium-99m-tetrofosmin as a new radiopharmaceutical for myocardial perfusion imaging. *J Nucl Med* 1993;34: 222–227.
20. Liu P, Dawood F, Riley R, Houle S, MacLaughlin PR. Could the myocardial tracer Tc-MIBI be made to redistribute by altering its blood concentration? *Circulation* 1988;78:384–387.
21. Ritcher WS, Cordes M, Calder D, Eichstaedt H, Felix R. Washout and redistribution between immediate and two-hour myocardial images using technetium-99m sestamibi. *Eur J Nucl Med* 1995;22:49–55.