Pharmacology Study of the Neutral Myocardial Imaging Agent Technetium-99m-N(NOEt)$_2$

Ping Fang, Bo-Cheng Wang, Chun-Ying Wu, Wei-Xing Wan, Ning-Yi Jiang, Xiang Zhou and Zheng-Ping Chen

State Key Laboratory of Nuclear Medicine, Jiangsu, and Department of Nuclear Medicine, Sun Yishian Memorial Hospital, Guangdong, Peoples Republic of China

Objective: The biological properties of a new neutral myocardial imaging agent ^{99m}TcN(NOEt)$_2$ were evaluated.
Methods: Blood clearance in rabbits, biodistribution in rats, and initial myocardial imaging in dogs were performed.
Results: Radiochemical purity of ^{99m}TcN(NOEt)$_2$ was more than 90% and stable for 6 h at room temperature. Blood disappearance was analyzed with a biexponential model and $T_{1/2(\alpha)}$ = 2.53 min, $T_{1/2(\beta)}$ = 330 min and Cl = 378 ml/h were obtained. Biodistribution studies demonstrated that ^{99m}TcN(NOEt)$_2$ localized selectively in the rat myocardium. Cardiac uptakes were 4.69, 4.20, 3.95 and 3.43% ID/g at 5, 30, 60 and 90 min postinjection, respectively. The mean heart-to-lung activity ratios were 1.69, 2.40 and 2.55 at 10 min, 30 min and 60 min postinjection, respectively.
Conclusion: Technetium-99m-N(NOEt)$_2$ exhibited favorable stability and biological properties. Further study in humans is required.
Key Words: myocardial imaging; technetium-99m-N(NOEt)$_2$; biodistribution

J Nucl Med Technol 1999; 27:54–56

Use of the metastable isomer ^{99m}Tc for labeling imaging agents in nuclear medicine has caused development of specific radiopharmaceuticals. Several ^{99m}Tc complexes have proven useful as perfusion imaging agents in diagnostic nuclear medicine.

Pasqualini (*1*) described a new efficient method for preparing the [Tc ≡ N] tracer in sterile and apyrogen conditions, and ^{99m}TcN(NOEt)$_2$ (NOEt: N-ethoxy, N-ethyl dithiocarbamate) shows high uptake in normal myocardial tissue (*2*,*3*) and redistribution has been characterized (*2–4*). We report the results of a pharmacology study of ^{99m}TcN(NOEt)$_2$.

MATERIALS AND METHODS

Ligands

N-methyl S-methyl dithiocarbazate [H_2N-N(CH_3)-C(= S)SCH_3, MDCZ] and N-ethoxy, N-ethyl dithiocarbamate [EtO(Et)N-C(= S)SNa, NOEt] were synthesized in our laboratory. Stannous chloride (AR grade) is commercially available. Details of the synthesis of the MDCZ and NOEt have been reported (*5*).

Preparation of Technetium-99m-N(NOEt)$_2$

The ^{99m}TcN(NOEt)$_2$ was prepared in two steps based on the reaction equations:

$$^{99m}TcO_4^- + HCl + Sn^{2+} + MDCZ \rightarrow [^{99m}TcN]_{int}$$

$$[^{99m}TcN]_{int} + NOEt \rightarrow {}^{99m}TcN(NOEt)_2$$

In the first step, the synthesis of [^{99m}Tc ≡ N] intermediate, 1–4 mL of ^{99m}Tc-pertechnetate were added to a lyophilized kit A containing 0.1 mg of $SnCl_2 \cdot 2H_2O$, 1.0 mg MDCZ and excipient, heated at 100°C for 15 min, then cooled to room temperature.

In the second step, preparation of ^{99m}TcN(NOEt)$_2$, the mixture of intermediate from the first step was added to kit B, containing 10.0 mg NOEt and excipient, and allowed to stand at room temperature for 10 min.

Determination of Radiochemical Purity of Technetium-99m-N(NOEt)$_2$

The radiochemical purity (RCP) of ^{99m}TcN(NOEt)$_2$ was evaluated by high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). The HPLC system used a Micro PAK MCH 5N cap (300 mm × 4 mm) reverse-phase column, using MeOH:H_2O = 80:20 as mobile phase, with a flow rate of 1.0 mL/min, at room temperature. TLC was performed on a polyamide film strip eluted using ascending chromatography with acetonitrile.

Determination of Partition Coefficient of Technetium-99m-N(NOEt)$_2$

The partition coefficient of ^{99m}TcN(NOEt)$_2$ was determined in octanol and 0.1 mol/L phosphate buffer (PB, pH 7.0 and pH 7.4). For each pH, 10 µL ^{99m}TcN(NOEt)$_2$ (approximately 1,000,000 cpm) were mixed with 3.0 mL PB and 3.0 mL octanol, vortexed for 3 min, and centrifuged for 5 min at 4000 rpm. One milliliter octanol was transferred to another tube

For correspondence or reprints contact: Ping Fang, MSc, Associate Research Professor, 20 Qian Rong Rd., Wuxi, Jiangsu, P.R. China.

containing 3.0 mL PB and 2.0 mL octanol, and the above procedure was repeated. One milliliter octanol and 1.0 mL PB were counted and the partition coefficient was calculated as cpm of octanol per cpm of PB.

Determination of In Vitro Stability of Technetium-99m-N(NOEt)$_2$

Technetium-99m-N(NOEt)$_2$ was kept standing for 6 h at 25°C and the RCP was determined at regular intervals. The solution also was diluted with saline and the RCP was determined for multiple dilutions.

Biodistribution Study of Technetium-99m-N(NOEt)$_2$ in Rats

Sixteen Sprague-Dawley rats (180–220 g) were injected with 3.7 MBq (0.1 mL) ^{99m}TcN(NOEt)$_2$ each in the tail vien. The rats were killed under anesthesia (diethyl ether) by cervical dislocation at 5, 30, 60 and 90 min postinjection, the organs of interest were collected, weighted and counted. The radioactivity of each organ was expressed as percent injected dose (% ID) per organ and percent injected dose (% ID) per gram of tissue. The activity ratios of heart-to-liver and heart-to-lung were obtained.

Blood Clearance of Technetium-99m-N(NOEt)$_2$ in Rabbits

Each of four New Zealand rabbits (2.2–2.8 kg) was injected with 50 MBq (0.4 mL) ^{99m}TcN(NOEt)$_2$ in the ear-edge vein. Blood samples were obtained from the other ear at a series of times postinjection. The radioactivity of the samples was counted and expressed as a percentage of the injected dose (% ID). The blood time-activity curve was plotted.

RESULTS AND DISCUSSION

RCP of Technetium-99m-N(NOEt)$_2$

The HPLC retention times (t_R) of ^{99m}TcN(NOEt)$_2$ and $^{99m}TcO_4^-$ were 8.7 min and 2.4 min, respectively. All the mixtures of the intermediate underwent substitution reactions easily with the NOEt ligand to produce ^{99m}TcN(NOEt)$_2$ in high yields.

The TLC analysis showed that the R_f values of ^{99m}TcN(NOEt)$_2$, $^{99m}TcO_4^-$ and $[^{99m}Tc \equiv N]_{int}$ were 1.0, 0.3 ~ 0.4, 0.0 ~ 0.1 and 0.7 ~ 0.8, respectively. The RCP was greater than 90% for all preparations.

TABLE 1
Biodistribution of Technetium-99m-N(NOEt)$_2$ in Rats

	Time (% ID/organ × ± s)			
Organ	5 min (n = 4)	30 min (n = 4)	60 min (n = 4)	90 min (n = 4)
Brain	0.88 ± 0.10	0.51 ± 0.07	0.33 ± 0.06	0.27 ± 0.03
Heart	2.79 ± 0.36	2.25 ± 0.13	2.00 ± 0.14	1.88 ± 0.11
Liver	17.52 ± 2.35	19.92 ± 2.12	31.32 ± 2.37	28.73 ± 1.85
Spleen	1.27 ± 0.23	1.16 ± 0.17	0.93 ± 0.14	0.73 ± 0.06
Lung	9.00 ± 1.61	5.26 ± 0.56	3.89 ± 0.30	2.57 ± 0.13
Kidney	4.34 ± 0.17	4.89 ± 0.34	4.97 ± 0.89	4.95 ± 0.40
Muscle	23.57 ± 5.10	25.16 ± 1.80	26.56 ± 1.73	23.38 ± 4.41
Bone	5.06 ± 0.42	5.21 ± 0.18	5.55 ± 0.96	4.93 ± 0.15

TABLE 2
Activity Ratios of Technetium-99m-N(NOEt)$_2$ in Rats

Time	Heart/liver ratio	Heart/lung ratio
5 min	1.44% ID/g	0.59% ID/g
30 min	1.39% ID/g	0.92% ID/g
60 min	0.88% ID/g	1.16% ID/g
90 min	0.79% ID/g	1.43% ID/g

Partition Coefficient of Technetium-99m-N(NOEt)$_2$

The partition coefficients of ^{99m}TcN(NOEt)$_2$ were 434 and 427 at pH 7.0 and 7.4, respectively. Because of the lack of charge and high lipophilicity, ^{99m}TcN(NOEt)$_2$ will be adsorbed into the vial wall or into the wall of the syringe to a great extent. To avoid this adsorption, solubilizing agents were added to kit B.

Stability of Technetium-99m-N(NOEt)$_2$

The RCP of ^{99m}TcN(NOEt)$_2$ remained over 90% for 6 h at room temperature. The complex also was stable following up to 32 serial dilutions with saline.

Biodistribution of Technetium-99m-N(NOEt)$_2$

The results of biodistribution of ^{99m}TcN(NOEt)$_2$ in rats are shown in Table 1 . The activity ratios of % ID per gram of T/NT are shown in Table 2.

Technetium-99m-N(NOEt)$_2$ showed a high cardiac uptake with a slow washout. Rapid clearance of the initial high pulmonary uptake improved the heart/lung ratio over time. As the complex accumulated in the liver the heart/liver ratio declined over time. The complex was eliminated mainly through the hepatobiliary system.

Comparison of reported biodistributions of ^{99m}TcN(NOEt)$_2$ are shown in Table 3. Our biodistribution results of ^{99m}TcN(NOEt)$_2$ were similar to the results reported by Pasqualini (*6*) and Guillaud (*8*).

Kinetics of Blood Clearance of Technetium-99m-(NOEt)$_2$

The data for the blood time-activity curve for ^{99m}TcN(NOEt)$_2$ in rabbits were analyzed with a biexponential model. The

TABLE 3
Reference Comparison of Biodistribution of Technetium-99m-N(NOEt)$_2$ in Rats at 30 Minutes (% ID/organ)

	% ID/organ			Heart/ liver ratio	Heart/ lung ratio
Source	Heart	Liver	Lung		
Pasqualini (*6*)	3.64	25.8	3.09	—	—
Pasqualini (*7*)	2.26	29.4	2.40	1.24% ID/g	1.61% ID/g
Guillaud (*8*)	1.80	27.1	5.70	—	—
This paper	2.25	19.9	5.26	1.39% ID/g	0.92% ID/g

distribution half-life $T_{(1/2)\alpha}$ = 2.53 min, the elimination half-life $T_{(1/2)\beta}$ = 330 min, and the clearance rate Cl = 378 mL/h were obtained. The complex washed out very slowly from blood. The biexponential equation is $C = 0.036\ e^{-16.43t} + 0.033\ e^{-0.126t}$.

CONCLUSION

Technetium-99m-$N(NOEt)_2$ can be prepared conveniently with high radiochemical purity and high stability. Animal experiments showed high myocardium uptake, long retention time in the heart, and a low heart-to-liver activity ratio of $^{99m}TcN(NOEt)_2$. Further evaluation of this radiopharmaceutical as a myocardial imaging agent is justified.

ACKNOWLEDGMENT

This work was supported by the Ministry of Public Health, Peoples Republic of China (P.F. 96Q-077), the Nature Science Foundation of Jiangsu Province (P.F. BK95120313), and the Department of Health of Jiangsu Province (P.F. H9534).

REFERENCES

1. Pasqualini R, Comazzi V, Bellande E, et al. A new efficient method for the preparation of ^{99m}Tc radiopharmaceuticals containing the Tc ≡ N multiple bond. *Appl Radiat Isot.* 1992;43:1329–1333.
2. Fagret D, Marie PY, Brunotte F, et al. Myocardial perfusion imaging with technetium-99m-NOET: comparision with thallium-201 and coronary angiography. *J Nucl Med*. 1995;36:936–943.
3. 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.
4. Johnson G III, Nguyen KN, Pasqualini R, et al. Interaction of technetium-99m-N- NOET with blood elements: potential mechanism of myocardial redistribution. *J Nucl Med*. 1997;38:138–143.
5. Fang P, Zhou X, Wu C, et al. Preparation and biodistribution of new neutral myocardial imaging agent $^{99m}TcN(NOEt)_2$ (in Chinese). *Zhonghua Heyixue Zazhi*. 1997;1:20.
6. 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.
7. Pasqualini R, Magon L, Bardy A, et al. Radiopharmaceutical product more particularly having a cardiac tropism incorporating a nitride complex of a transition metal and its preparation process. U.S. Patent 5,288,476. 1994.
8. Guillaud C, Comazzi V, Joubert F, et al. Metabolite analysis of the neutral technetium-99m nitrido dithiocarbamate complex TcN-NOEt after injection in rats (Abstract). *J Nucl Med*. 1996;37:188p.