Preclinical pharmacology study of neutral myocardial imaging agent $^{99m}TcN(NOEt)_2$ *

FANG Ping, WU Chunying, WAN Weixing, ZHOU Xiang, CHEN Zhengping
(*State Key Laboratory of Nuclear Medicine, Wuxi 214063*)
JIANG Ningyi
(*Sun Yishian Memorial Hospital, Guangzhou 510120*)

Abstract To explore the biological properties of a new neutral myocardial imaging agent $^{99m}TcN(NOEt)_2$, preparation and characterization of $^{99m}TcN(NOEt)_2$, kinetics of blood-drug clearance in rabbits, biodistribution in rats, test of undue toxicity in mice and myocardial imaging in dogs were performed and volunteer imaging. Radiochemical purity of $^{99m}TcN(NOEt)_2$ was over than 90% and stable for 6 hours at room temperature. Blood disappearance was analyzed with biexponential model, $T_{1/2}(\alpha)$=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 myocardium of rats. Cardiac uptake were 2.79, 2.25, 2.00 and 1.88%ID/organ at 5, 30, 60 and 90 min of postinjection, respectively. The heart-to-lung activity ratio was 1.16 at 60 min. Images showed that pulmonary uptake decreased faster than cardiac uptake in a dog. The mean heart-to-lung activity ratios in a dog were 1.69, 2.40 and 2.55 at 10, 30 and 60 min of postinjection, respectively. The heart was distinguishable on scans at 30 min. Whole body imaging showed that cardiac uptake was 2.82%ID at 90 min, but hepatic uptake was 30%ID and remained constant. The test of undue toxicity showed that the dose received by mice was 614 times as by human. Volunteer imaging suggests $^{99m}TcN(NOEt)_2$ redistribution with time. $^{99m}TcN(NOEt)_2$ exhibited favorable stabilities, biological properties and safety. It is worth for further studying in human.

Keywords Myocardial imaging agent, $^{99m}TcN(NOEt)_2$, Biodistribution, Radionuclide imaging

1 Introduction

Utilization of the metastable isomer ^{99m}Tc as imaging agents in nuclear medicine has caused rapid development of the inorganic chemistry of technetium, principally as a consequence of the need to design more powerful and specific radiopharmaceuticals. A number of complexes of ^{99m}Tc containing $[Tc{=}O]^{3+}$ and $[O{=}Tc{=}O]^{+}$ cores have proved useful in diagnostic nuclear medicine as perfusion imaging agents and this has demonstrated the wide role of technetium(V) chemistry.

The $[Tc{\equiv}N]^{2+}$ core constitutes a group isoelectronic with $[Tc{=}O]^{3+}$. Pasqualini[1] described a new efficient method for preparing $[Tc{\equiv}N]$ group at tracer level under sterile and apyrogen conditions. $^{99m}TcN(NOEt)_2$ (NOEt: N-ethoxy, N-ethyl dithiocarbamate) showed a high uptake in normal myocardial tissue[2,3] and redistribution characterization.[2~4] In this paper, we reported the results of preclinical pharmacology study of $^{99m}TcN(NOEt)_2$.

2 Materials and methods

2.1 Materials

Lyophilized kit A contains 1.0mg MDCZ[5] [N-methyl S-methyl dithiocarbazate, H_2N-$N(CH_3)$-$C(=S)SCH_3$], 0.1 mg of $SnCl_2{\cdot}2H_2O$ and excipient; Lyophilized kit B contains 10.0 mg NOEt[5] [N-ethoxy, N-ethyl dithiocarbamate [EtO(Et)N-C(=S)SNa] and excipient. MDCZ and NOEt was synthesized in our laboratory, stannous chloride (AR grade) is commercially available.

2.2 Instruments

PACKARD COBRA auto γ counter, Siemens DIACAM SPECT.

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2.3 Preparation of ^{99m}TcN(NOEt)$_2$

The preparation of ^{99m}TcN(NOEt)$_2$ was carried out in following two steps:

(1). Synthesis of [^{99m}Tc≡N] intermediate

One to four milliliters of generator eluted pertechnetate was added to a lyophilized kit A, heated at 100°C for 15 min, cooled to room temperature.

(2). Preparation of ^{99m}TcN(NOEt)$_2$

The mixture of intermediate was added to kit B, stood for 10 min at room temperature.

2.4 Determination of radiochemical purity (RCP) of ^{99m}TcN(NOEt)$_2$

RCP of ^{99m}TcN(NOEt)$_2$ was evaluated by TLC. TLC was carried out on polyamide film strip and was eluted using ascending chromatography with acetonitrile.

2.5 Biodistribution study of ^{99m}Tc N(NOEt)$_2$ in rats

0.1 mL(3.7MBq) of ^{99m}TcN(NOEt)$_2$ were injected in Sprague-Dawley rats (180~220 g) via tail vien. The rats were killed under anesthesia (diethyl ether) by cervical dislocation at 5, 30, 60 and 90 min of postinjection. The organs of interest were collected, weighed and counted. The radioactivities of each organ were expressed as %ID/organ and %ID/g respectively. The activity ratios of heart to liver and heart to lung were obtained.

2.6 Kinetics of blood-drug clearance of ^{99m}TcN(NOEt)$_2$ in rabbits

Four New Zealand rabbits, 2.2~2.8 kg. 0.4 mL(50 MBq) of ^{99m}TcN(NOEt)$_2$ was injected into each rabbit via ear-edge vein. The vein-blood samples were obtained from the another ear at a series time spots of postinjection. The radioactivities of samples were counted and expressed as the percentage of the injected dose (%ID). The curve of blood activity-time was plotted.

2.7 Imaging of ^{99m}TcN(NOEt)$_2$ in a dog

0.5 mL(148 MBq) of ^{99m}TcN(NOEt)$_2$ was injected into the lingual vein of a dog (8 kg). The messages were collected by SPECT immediately. The curves of activity-time of heart, liver and lung were plotted. The images were obtained at 30, 45, 60, 90 and 120 min. The whole body images were obtained at 90 min.

2.8 Undue toxicity test of ^{99m}TcN(NOEt)$_2$

According to the regulations of Pharmacopoeia of P.R.China (95 Edition), the undue toxicity of the injection of ^{99m}TcN(NOEt)$_2$ was determined by observing the death and survival of mice (18~20 g) within a period of 48 h with a certain injection dose of ^{99m}TcN(NOEt)$_2$.

2.9 Volunteer imaging of ^{99m}TcN(NOEt)$_2$

A 50-year old man with coronary disease, underwent exercise testing for assessment of coronary artery disease (CAD). At the peak exercise, 925 MBq of ^{99m}TcN(NOEt)$_2$ was injected intravenously. The initial images were obtained at 45 min of postinjection with a rotating gamma camera. The energy spectrometer was centered on the 140 keV photopeak with a 20% of spectral window. The head of the camera turned through a 180° arc in a circular orbit around the thorax of the patient at 6-degree increments for 30s each (32 projections). Delayed rest images were obtained at 3h postinjection using the same technique.

3 Results and discussions

3.1 RCP of ^{99m}TcN(NOEt)$_2$

The TLC analysis showed that the R_f values of ^{99m}TcN(NOEt)$_2$, and $^{99m}TcO_4^-$ were 1.0, and 0.3~0.4 respectively; the reaction solution of intermediate were mixtures, and the R_f values of [^{99m}Tc≡N]$_{int}$ were 0.0~0.1 and 0.7~0.8. We found that all the the mixtures of intermediate underwent easily substitution reactions with ligand NOEt to produce ^{99m}TcN(NOEt)$_2$ in high yields (over 90%), and the complex was stable for 6 h at room temperature.

3.2 Biodistribution of ^{99m}TcN(NOEt)$_2$

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

^{99m}TcN(NOEt)$_2$ showed a high cardiac uptake and a slow washout. Though the initial high pulmonary uptake, the rapid clearance was helpful to the improvement of activity ratio of heart/lung. The ratio of heart/liver kept reducing while the complex accumulated in the liver. Because of the lower concentration in the kidneys and higher in the liver, the complex was eliminated mainly through hepatobiliary system.

Due to the discrepancy of biodistributions of ^{99m}TcN(NOEt)$_2$ reported, we made a comparison and showed in Table 3. The results of biodistribution of $^{99m}TcN(NOEt)_2$ in this paper were similar to the results Pasqualini[7] and Guillaud[8] reported.

Table 1 Biodistribution of $^{99m}TcN(NOEt)_2$ in rats (%ID/organ $x \pm s$ n=4)

Organ	Time/min			
	5	30	60	90
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 T/NT of $^{99m}TcN(NOEt)_2$ in rats*

Time/min	Heart/Liver	Heart/Lung
5	1.44	0.59
30	1.39	0.92
60	0.88	1.16
90	0.79	1.43

* ratio of %ID/g

Table 3 Biodistribution comparison of $^{99m}TcN(NOEt)_2$ with references in rats at 30 min (%ID/organ)

Author	Heart	Liver	Lung	Heart/Liver*	Heart/Lung*
Pasqualini[6]	3.64	25.8	3.09		
Pasqualini[7]	2.26	29.4	2.40	1.24	1.61
Guillaud[8]	1.80	27.1	5.70		
This paper	2.25	19.9	5.26	1.39	0.92

* Ratio of %ID/g

3.3 Kinetics of blood-drug clearance of $^{99m}TcN(NOEt)_2$

Fig.1 is the curve of blood activity vs time of $^{99m}TcN(NOEt)_2$ in rabbits. The data were analyzed with biexponential model. The distribution half life of $T_{(1/2)\alpha}$=2.53 min, the elimination half life of $T_{(1/2)\alpha}$=330 min, and the clearance rate of Cl=378 mL/h were obtained, the results showed that the complex was washed out very slowly from blood. Biexponential equation is $C=0.036e^{-16.43t}+0.033e^{-0.126t}$.

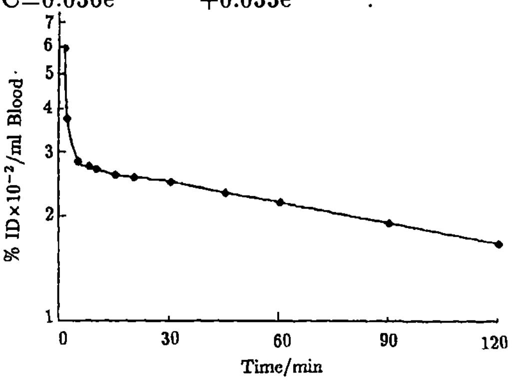

Fig.1 Curve of blood radioactivity-time of $^{99m}TcN(NOEt)_2$ in rabbits

3.4 Imaging of $^{99m}TcN(NOEt)_2$ in dogs

Fig.2 is the time-activity curves for the heart, liver and lung within 30 min of postinjection. It exhibits significant heart uptake which is constant. The initial lung uptake is high, but the clearance is rapid. So it is helpful to improve the ratio of heart to lung. The hepatic uptake remains in high level.

The distinct images was obtained at 45 min of postinjection (Fig.3), the ratios of heart to liver and heart to lung at certain time spots are listed in Table 4. At 90 min, heart and liver uptakes were 2.82 and 30.13%ID, respectively. The activity of lung was close to background.

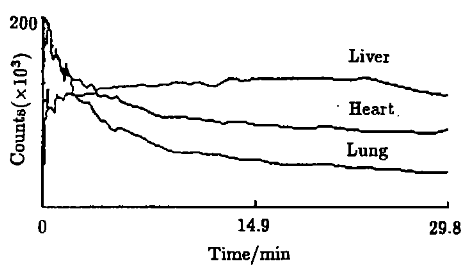

Fig.2 Curve of time-activity of heart, liver and lung

Table 4 Activity ratios of T/NT of $^{99m}TcN(NOEt)_2$ in dog

Time/min	10	15	30	45	60	90	120
Heart/Liver	0.69	0.65	0.59	0.46	0.44	0.38	0.35
Heart/Lung	1.69	1.78	2.40	2.35	2.55	2.01	2.16

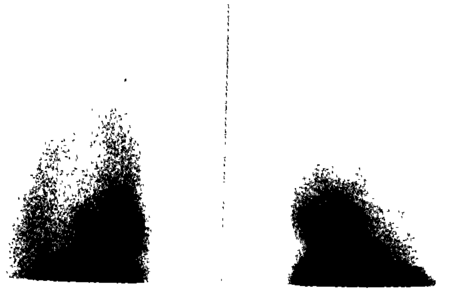

Fig.3 Dog planar gamma images at 45 min

3.5 Test of undue toxicity of ^{99m}TcN$(NOEt)_2$

Mice were injected with 0.23 times the human's dose of ^{99m}TcN$(NOEt)_2$. The dose received by mice was 614 times as human received per kg when assuming a person weight is 50 kg. So it was very safe.

3.6 Volunteer imaging of ^{99m}TcN$(NOEt)_2$

Fig.4 shows the stress and delayed images of a patient with coronary artery disease. The

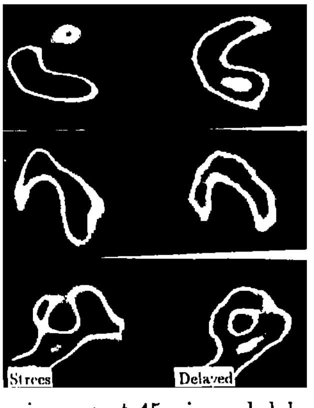

Fig.4 Stress images at 45 min and delayed images at 3 h of ^{99m}TcN$(NOEt)_2$ postinjection in patient

defect observed on the stress images showed normalization at 3 h postinjection. This suggested that ^{99m}Tc$(NOEt)_2$ has a redistributes with time.

4 Conclusion

^{99m}Tc$(NOEt)_2$ can be conveniently prepared with high radiochemical purity and high stability. Animal experiments showed a high myocardium uptake, long retention time in heart and low activity ratio of heart to liver of ^{99m}TcN$(NOEt)_2$. Preliminary clinical study showed the pharmaceutical has a redistribution characterization. It is worth to further study in human.

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