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Preparation and primary biological evaluation of novel nitrido-^{188}Re complexes/lipiodol

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Abstract Two new nitrido-^{188}Re complexes were prepared by a modified method in high yield. These complexes were stable *in vitro.* The biodistribution in normal mice showed that these nitrido-^{188}Re complexes could accumulate in liver and dissipate quickly from almost all organs. TAE was performed with the use of lipiodol solutions of two complexes to rabbit VX2 liver tumor models. SPECT images showed that the two lipiodol solutions could remain in tumor for about 9 h (^{188}ReN-NEPTDD/lipiodol) and 12 h (^{188}ReN-NEMMPTDD/lipiodol), respectively.

Key words Nitrido, TDD derivatives, Lipiodol, Liver cancer

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1 Introduction

Liver cancer, in particular, hepatocellular carcinoma (HCC), is a common malignant tumors[1]. Hepatectomy and liver transplantation are the main curative treatment. However, most patients are not eligible for surgery because of their liver dysfunction or considerable tumor size. For such situation, transcatheter arterial embolization (TAE) is a good alternative for HCC treatment[2].

Blood supply of liver cancer cells is mainly obtained from the hepatic artery, while that of normal hepatic cells is mainly obtained from the portal vein. Consequently, embolic agents could accumulate in liver cancer cells by embolization through the hepatic artery. Lipiodol is just an excellent embolic agent. In recent years, many attempts have been made to label lipiodol with therapeutic radioisotopes, including ^{131}I[3,4], ^{90}Y[5,6], and ^{188}Re[7-9] to develop promising TAE agent. Among the radioisotopes, ^{188}Re is an excellent candidate for radionuclide therapy. It possesses suitable nuclear characteristics ($E_{\beta max}$=2.1 MeV, 71%, $t_{1/2}$=16.9 h) and its preparation is easy. $^{188}ReO_4^-$ could be obtained from ^{188}W/^{188}Re generator like the ^{99}Mo/^{99m}Tc generator currently in use and its γ-rays can be used to monitor biodistribution and calculate the dose.

Researchers have made great efforts to develop new labeled compounds of ^{188}Re, such as ^{188}Re-TDD, ^{188}Re-HDD, ^{188}Re-SSS, etc. Their lipiodol solutions could remain in liver tumor for hours, but the labeling yields and stability of ^{188}Re-L were not satisfying. The research presented herein was inspired by recent researches on ^{99m}Tc≡N core complexes for their better stability than ^{99m}Tc=O core complexes[10-12]. Rhenium (Re) and technetium (Tc) belong to the same group in the periodic table, hence similar chemical behavior of the two elements. Therefore, the method for preparing ^{99m}Tc labeling compounds from $^{99m}TcO_4^-$ could be modified for preparing ^{188}Re labeling compounds from $^{188}ReO_4^-$. The difficulty of reduction and the instability of reduced ^{188}Re compounds are however the main challenge[13]. The modification used in this research was based on an improved two-step method used to prepare ^{99m}Tc≡N core compound[14]. $[^{188}ReN]_{int}^{2+}$ and

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^{188}ReN-L were obtained in the presence of oxalate and acetic hydrazide (AH) in high yield. The stability of ^{188}ReN-L *in vitro* and the biodistribution in normal mice were then studied. Furthermore, rabbit VX2 liver tumor models were established and TAE was performed to evaluate primary biological characters of ^{188}ReN-L/lipiodol solutions.

2 Materials and methods

2.1 Materials

Acetic hydrazide (AH), succinic dihydrazide (SDH), oxalic dihydrazide (ODH) and stannous chloride dihydrate were purchased from Aldrich Chemical Co., USA. Polyamide strip was from Sijia Biochemistry Plastic Factory, China. ^{188}W/^{188}Re generator was from Shanghai Institute of Applied Physics, Chinese Academy of Sciences. All other chemicals were of reagent grade and were used without further purification.

2.2 Analysis methods

Radiochemical purity (RCP) was measured by thin layer chromatography (TLC) and HPLC. TLC was performed on polyamide strip and was eluted with saline and acetonitrile (CH_3CN). R_f values for some selected moieties are shown in Table 1.

Table 1 R_f value for some selected moieties

Samples	$^{188}ReO_4^-$	$^{188}ReO_2 \cdot nH_2O$	$[^{188}ReN]_{int}^{2+}$	^{188}ReN-complexes
Saline	0.1	0.1	0.8~1.0	0.1~0.2
CH_3CN	0.3~0.4	0.1	0.1	0.7~0.8

HPLC was carried out on a System Gold instrument equipped with a solvent Module 110B, and a radioisotope detector Module 170 (Beckman Instruments). HPLC solvents consisted of solvent A, trifluoroacetic acid (TFA) (0.1% v/v in water), and solvent B, CH_3CN. TLC analyses were carried out using a C-18 reversed-phase column (250×4.6 mm, Diamonsil^{TM}) at a flow rate of 1 mL·min^{-1} with a gradient elution: 0~7 min, 100% B; 7~18 min, 20% A and 80% B; 18~20 min, 100% A; 20~25 min, 100% B.

2.3 Preparation of ^{188}ReN-NEPTDD and ^{188}ReN-NEMMPTDD/lipiodol

NEPTDD(2,2,9,9-tetramethyl-4,7-diaza-4-ethyl-piperidinyl-1,10-decanedithiol) and NEMMPTDD(2,2,9,9-tetramethyl-4,7-diaza-4-ethyl-(3,5-dimethyl)-piperidnyl-1,10-decanedithiol), the two TDD derivatives, were synthesized by six steps according to procedures in Refs.[7,15-17] and were stored as hydrochloride salts.

The nitrido-^{188}Re complex was prepared by an improved two-step synthesis:

(1) $^{188}ReO_4^-$ + AH + $SnCl_2 \cdot 2H_2O$ + $(HOOC)_2$ → $[^{188}ReN]_{int}^{2+}$

(2) $[^{188}ReN]_{int}^{2+}$ + NEPTDD/NEMMPTDD → ^{188}ReN-NEPTDD/NEMMPTDD

Generator-eluted [$^{188}ReO_4^-$] solution (approximately 74 MBq) were added to the mixture of AH, oxalate and $SnCl_2 \cdot 2H_2O$ in a test tube. The mixture was adjusted to pH 2.0 before a 30-min reaction at room temperature to obtain $[^{188}ReN]_{int}^{2+}$. NEPTDD or NEMMPTDD was then added to intermediate system and increased the pH value to 7.0. It was subsequently heated at 70℃ for 30 min to prepare ^{188}ReN-L. The yields were measured by TLC. All the mixtures were also measured by HPLC.

Stability of the nitrido-^{188}Re complexes was studied by measuring the RCP using TLC at different time intervals after preparation.

Lipiodol was added to the prepared system of ^{188}ReN-L and the mixture was shaken properly to extract the nitrido-^{188}Re complexes into lipiodol. The mixture was centrifuged for 10 min in a centrifuge tube to layer the water and the lipiodol phase. The lipiodol phase containing ^{188}ReN-L was washed by normal saline and collected by a syringe with a long needle.

2.4 *In vitro* reaction with cysteine, histidine, glutathione and bovine serum albumin (BSA)

The prepared system of nitrido-^{188}Re complexes was added to a test tube containing PBS solution of cysteine(1:4 v/v, labeled product: solution of Cysteine). The mixture was incubated at 37℃ for 12 h. The RCP was measured at 30 min, 1 h, 2 h, 6 h and 12 h by TLC.

The same procedure was applied to three separate experiments, using BSA, PBS solution of histidine, and glutathione respectively.

PBS solution used above contained 116 mg of NaOH and 680.4 mg of KH_2PO_4 per 100 mL, with pH of 7.4.

2.5 Biodistribution study

All biodistribution studies were carried out in compliance with the national laws related to the conduct of animal experimentation.

The nitrido-^{188}Re complex (0.1 mL, approximately 740 kBq) was intravenously injecteded via the tail to normal Kunming mice (18.0±2.0 g). The mice were sacrificed at 15 min, 1 h, 3 h, 5 h and 24 h post-injection (PI). The organs of interest, blood, bones, and muscles were collected, weighed and counted. The results were expressed as percentage of injected dose per gram (ID%/g) of organs.

Tumor implantations were executed to establish rabbit VX2 liver tumor models using New Zealand white rabbits weighing 2.5±0.3 kg. Three weeks after implantations, the tumors were 2.0~3.0 cm in diameter as anticipated, and TAE was performed. Briefly, the femoral artery was exposed under general anesthesia, and a 5-F-in-diameter dilator and a 3-F-in-diameter microcatheter were inserted under X-ray observation. The hepatic artery which supplies the blood flow to the tumor was catheterized, and 0.4 mL of ^{188}ReN-L/lipiodol solution of about 37 MBq was injected. The catheter was removed and the femoral artery was ligated. SPECT was performed to evaluate distribution of radioactivity at 1 h, 5 h and 24 h after TAE. The scans were obtained using a dual-head γ-camera with medium-energy general-purpose collimators. The energy window was centered for about 155 keV. Images were acquired for 10 min using a 64×64 matrix (for ^{188}ReN-NEPTDD) or 128×128 matrix (for ^{188}ReN-NEMMPTDD). Regions of interest (ROI) were drawn manually on a 1-h image around areas of interest. ROIs of the same size and shape were applied to 5-h and 24-h images. The radioactivity counts of each ROI (C_{ROI}) were noted and the curves of C_{ROI} *vs.* t were drawn to estimate the biological half life of the complexes in ROIs.

3 Results and discussion

3.1 Effects of donor of nitride nitrogen atoms

The preparation method for nitrido-^{188}Re complex in Ref.[14] could produce $[^{188}ReN]_{int}^{2+}$ in high yield under mild conditions by adding oxalate, which changed the standard reduction potential of the reaction involving $^{188}ReO_4^-$ remarkably. Although the final nitrido-^{188}Re complexes obtained were simplexes, the intermediate $[^{188}ReN]_{int}^{2+}$ were two compounds as detected by HPLC. One possible reason for this difference is the different donor of nitrido nitrogen atoms. However this will hardly influence the formation of final nitrido-^{188}Re complex after adding appropriate ligand.

The effects of different donors of nitrido nitrogen atoms such as AH, SDH and ODH on the yield of intermediate $[^{188}ReN]_{int}^{2+}$ were studied. The results showed that using AH could result in a single intermediate in the highest yield comparing with SDH and ODH. The HPLC chromatogram of the intermediate $[^{188}ReN]_{int}^{2+}$ obtained in the presence of AH is shown in Fig.1a. The intermediate was eluted at 16.8 min. The yield of $[^{188}ReN]_{int}^{2+}$ was 98.2% measured by TLC. By adding corresponding ligand to the intermediate system, the nitrido-^{188}Re complexes were obtained, with the yields being 96.2% for ^{188}ReN-NEPTDD and 95.3% for ^{188}ReN-NEMMP-TDD measured by TLC. The HPLC chromatograms of the ^{188}ReN-L are shown in Fig.1b and Fig.1c, respectively. The retention time was 12.9 min for ^{188}ReN-NEPTDD and 12.7 min for ^{188}ReN-NEMMPTDD, respectively.

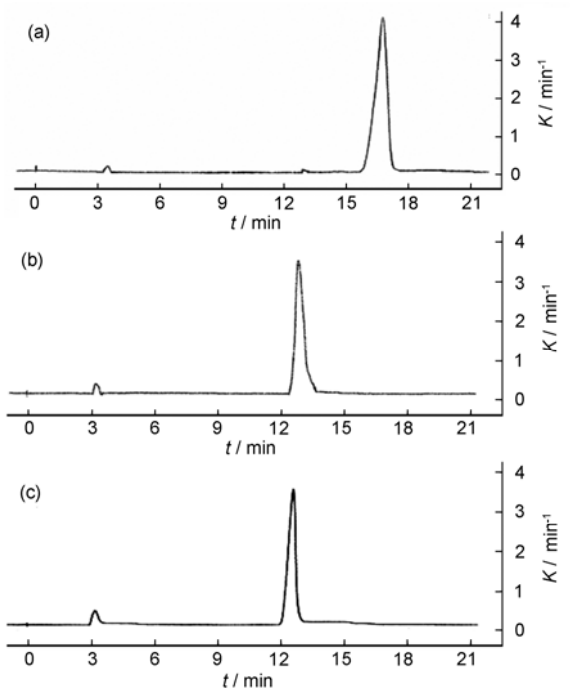

Fig.1 HPLC chromatogram of the mixture of intermediate $[^{188}ReN]_{int}^{2+}$ (a), ^{188}ReN-NEPTDD (b) and ^{188}ReN-NEMMPTDD (c).

3.2 *In vitro* and *in vivo* stability

The nitrido-^{188}Re complexes were placed in open air for 24 h at room temperature. The RCP measured by TLC at different hours are listed in Table 2. It can be seen that RCP changes were negligible, indicating a high *in vitro* stability of these complexes.

Table 2 RCP changes in 24 h (%)

Hours	0.5	1	3	6	24
^{188}ReN-NEPTDD	96.2	96.4	96.1	95.8	95.6
^{188}ReN-NEMMPTDD	95.3	95.8	95.5	95.2	95.0

The nitrido-^{188}Re complexes were incubated at 37℃ for 12 h with cysteine, histidine, glutathione and BSA respectively. RCP changes detected by TLC were minimal. This indicated that the nitrido-^{188}Re complexes would not transchelate with cysteine, histidine, glutathione and BSA, suggesting their high *in vivo* stability.

3.3 Animal experiments

Table 3 and Table 4 show biodistributions of ^{188}ReN-NEPTDD and ^{188}ReN-NEMMPTDD.

The results indicated that the two complexes behaved similarly *in vivo*. Concentration of the complexes in liver was higher than that in other organs. The ID%/g value of the complexes in liver was 14.75±0.72 for ^{188}ReN-NEPTDD and 13.65±0.77 for ^{188}ReN- NEMMPTDD at 15 min PI. The ID%/g value in every organ of interest decreased with time. For ^{188}ReN-NEPTDD, the ID%/g value in every organ at 5 h PI was less than 50% of that at 15 min PI. The trend was especially pronounced in liver, with the ID%/g at 5 h PI being 26.5% of that at 15 min PI. Comparably, ^{188}ReN-NEMMPTDD dissipated more quickly from the organs than ^{188}ReN-NEPTDD. The ID%/g value of ^{188}ReN-NEMMPTDD in every organ except liver at 3 h PI was far less than 50% of that at 15 min PI. The change of ID%/g value of the two complexes in liver was similar. The ID%/g value at 3 h PI was 50.1% for ^{188}ReN-NEPTDD and 56.2% for ^{188}ReN- NEMMPTDD of that at 15 min PI. At 24 h PI, the ID%/g values were very small. Most were far less than 1.0 except those in liver. All the data showed that the two complexes dissipated quickly from mice. These characters suggest that the two complexes might be extracted into an embolic agent such as lipiodol and administered through the hepatic artery as promising intraarterial administration agents for therapy of liver cancer. Quick dissipation from organs could reduce the damage to normal organs in case the complexes are unfortunately reextracted from embolic agents.

SPECT images (Fig.2) were taken to evaluate distribution of radioactivity at 1 h, 5 h and 24 h after the rabbit VX2 liver tumor models were executed by TAE. The SPECT images at 1 h after TAE showed that the ^{188}ReN-L/lipiodol solutions accumulated in tumor substantially, and only a few in normal liver. From the SPECT images at 5 h and 24 h after TAE, one can find that the lipiodol solution remained in tumor, but the radioactivity in normal liver reduced dramatically to almost background. These showed that the ^{188}ReN-L/lipiodol solutions remained longer in tumor than in normal liver. It is estimated that the biological half life of the ^{188}ReN-L/lipiodol was 9.0 h for ^{188}ReN-NEPTDD/lipiodol and 12 h for ^{188}ReN-NEMMPTDD/lipiodol in tumor, while 3.5 h for ^{188}ReN-NEPTDD/lipiodol and 4.0 h for ^{188}ReN-NEMMPTDD/lipiodol in normal liver.

Based on these, the two ^{188}ReN complexes showed better stability than other ^{188}ReO complexes[7-9], and the ^{188}ReN-L/lipiodol is of potential application in TAE for the HCC treatment, so as to substitute ^{131}I/lipiodol that are in use at present with poor stability[3,4].

Table 3 Biodistribution (ID%/g) of ^{188}ReN-NEPTDD in normal mice (x±s, n=3)

Organs	Time / h				
	0.25	1	3	5	24
Blood	6.33±0.65	5.22±0.38	4.68±0.03	3.73±0.21	0.55±0.00
Heart	2.98±0.32	2.35±0.31	1.77±0.35	1.35±0.07	0.60±0.01
Liver	14.75±0.72	8.07±0.77	7.80±0.78	3.91±0.27	0. 91±0.03
Spleen	5.49±0.64	3.87±0.21	3.80±0.32	2.07±0.04	0. 60±0.16
Lung	4.09±0.41	3.83±0.61	3.59±0.22	2.83±0.28	0.71±0.03
Kidney	4.00±0.24	3.23±0.28	3.06±0.36	2.19±0.18	0.74±0.11
Brain	0.49±0.04	0.37±0.02	0.32±0.07	0.25±0.03	0.23±0.00
Muscle	2.21±0.10	1.25±0.21	1.20±0.02	0.99±0.13	0.36±0.01
Bone	3.15±0.40	2.89±0.31	2.41±0.44	0.50±0.29	0.27±0.05

Table 4 Biodistribution (ID%/g) of ^{188}ReN-NEMMPTDD in normal mice (x±s, n=3)

Organs	Time / h				
	0.25	1	3	5	24
Blood	10.51±0.23	7.23±1.05	1.91±0.41	1.57±0.10	0.15±0.04
Heart	6.00±0.43	3.78±0.52	1.06±0.12	0.94±0.10	0.14±0.03
Liver	13.65±0.77	10.68±0.79	6.84±0.54	4.52±0.47	1.07±0.14
Spleen	8.77±1.14	5.54±0.68	1.68±0.11	1.20±0.05	0.14±0.06
Lung	10.82±0.73	6.83±0.57	2.26±0.24	2.15±0.32	0.10±0.04
Kidney	9.10±1.08	4.87±0.83	1.73±0.54	1.62±0.24	0.60±0.14
Brain	0.73±0.13	0.27±0.02	0.08±0.01	0.08±0.03	0.03±0.00
Muscle	2.28±0.69	1.36±0.40	0.46±0.07	0.35±0.22	0.08±0.01
Bone	3.50±0.14	2.70±0.31	0.47±0.08	0.46±0.05	0.35±0.13

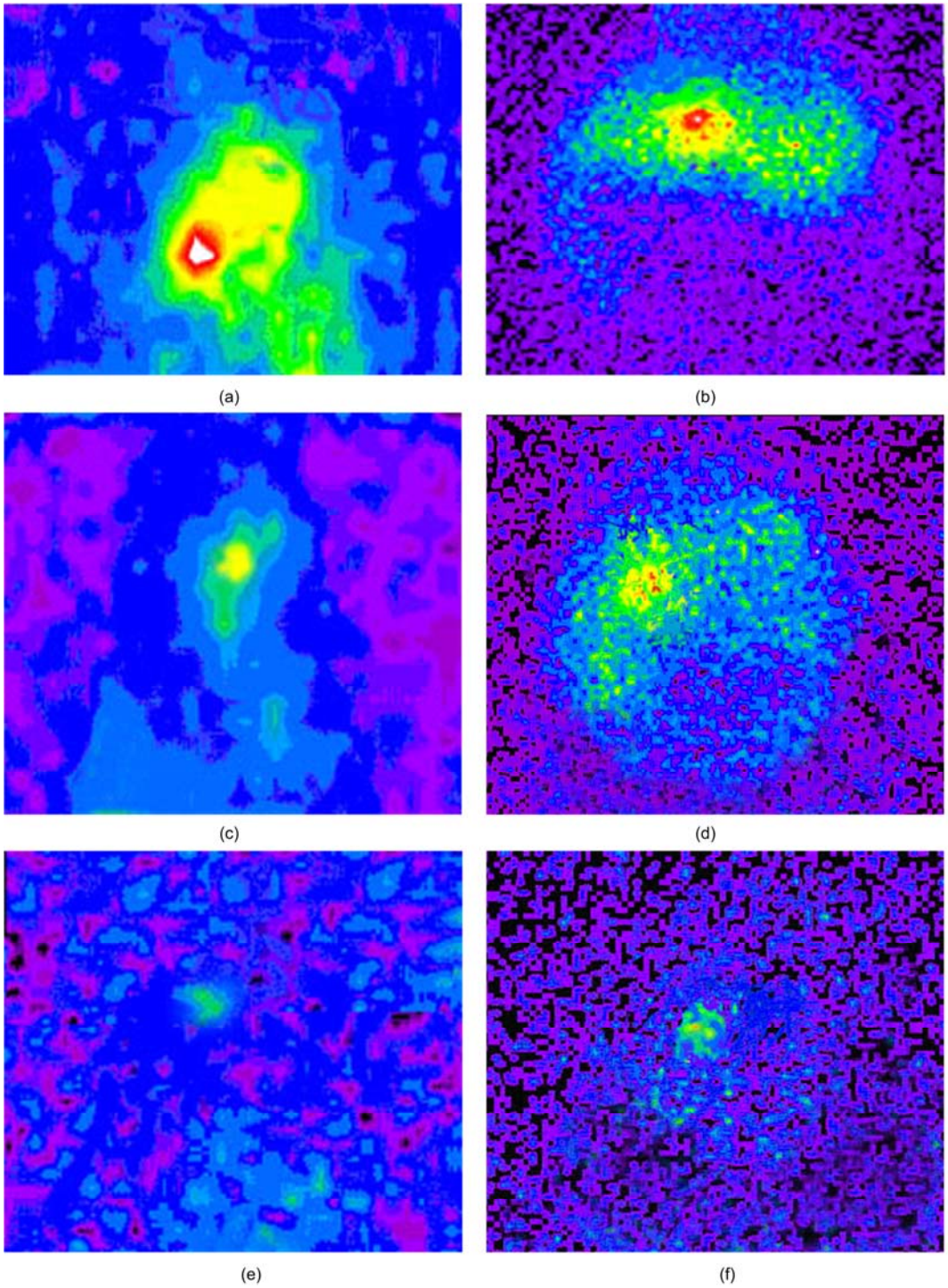

Fig.2 SPECT images at different hours after TAE: 1 h, ^{188}ReN-NEPTDD/lipiodol (a), 1 h, ^{188}ReN-NEMMPTDD/lipiodol (b), 5 h, ^{188}ReN-NEPTDD/lipiodol (c), 5 h, ^{188}ReN-NEMMPTDD/lipiodol (d), 24 h, ^{188}ReN-NEPTDD/lipiodol (e) and 24 h, ^{188}ReN-NEMM -PTDD/lipiodol (f).

4 Conclusions

Two new nitrido-^{188}Re complexes were prepared by a modified method in high yield. This method may be potentially applied to produce a wide range of Re-188 radiopharmaceuticals under mild conditions in high yield. These nitrido-^{188}Re complexes were stable *in vitro*. The biodistribution in normal mice showed that these ReN complexes dissipated quickly from almost all organs *in vivo*.

Rabbit VX2 liver tumor models were established and primary biological characters of ^{188}ReN-L/lipiodol solutions executed by TAE were evaluated. The results showed that the two lipiodol solutions remained much

longer in tumor than in normal liver. From the SPECT images and the biological half life in tumor, ^{188}ReN-NEMMPTDD/lipiodol solution is better than ^{188}ReN-NEPTDD/lipiodol solution in terms of their retention in tumor. It suggests that ^{188}ReN-NEMMPTDD/lipiodol may be developed to be a promising therapy radiopharmaceutical for liver cancer.

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