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The improved biological properties in mice of ^{99m}Tc–TBI for myocardial perfusion imaging

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Summary

The aim of this study was to improve the biological properties of ^{99m}Tc–TBI complex for myocardial perfusion imaging. The effect of Tween-80 on the biodistribution in mice of ^{99m}Tc–TBI was reported. The experimental results show that liver and blood uptakes of Tween-80 added (TA) complex are significantly lower than those of corresponding non-Tween-80 added (NTA) complex. Also, the clearance rate from blood of TA complex is faster than that of NTA complex. At 60 min post-injection time, the ratios of heart-to-liver, heart-to-lung and heart-to-blood were 1.29, 137.75 and 4.73, respectively, for the TA complex; however, the corresponding ratios were 0.57, 13.07 and 2.65, respectively, for NTA complex. The biodistribution in mice of the Tween-80 added ^{99m}Tc–TBI (^{99m}Tc–TBI(T)) is comparable with the widely used myocardial perfusion imaging agent ^{99m}Tc–MIBI in some aspects; especially, the heart-to-liver ratios of ^{99m}Tc–TBI(T) at various post-injection times are better than those of ^{99m}Tc–MIBI. The biological properties of ^{99m}Tc–TBI complex for myocardial imaging were improved significantly by using Tween-80 as an auxiliary agent. It is worthy of further studies.

Key Words: ^{99m}Tc–TBI; myocardial perfusion imaging agent; tween-80; biodistribution; radiopharmaceuticals

Introduction

^{99m}Tc complexes are the most widely used radiotracers for non-invasive diagnosis and assessment of functional and anatomical abnormalities in

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patients.[1,2] Monocationic hexakis (isonitrile) complex: ^{99m}Tc–*t*-butylisonitrile (^{99m}Tc–TBI) was reported as the first technetium-labeled myocardial perfusion imaging agent used in clinics.[3] This imaging agent has very high cardiac uptake and the liver uptake is also marked. It is not perfect because of its high uptake in liver and slow washout, so the patients should wait a long time post-injection for imaging. Subsequent development of the isonitrile concept gave rise to a new cation, ^{99m}Tc–hexakis–methoxy isobutyl isonitrile (MIBI) which is being applied for myocardial perfusion imaging,[4] but the synthesis of MIBI ligand is more difficult than that of TBI ligand. We have made great efforts to improve the biological properties of ^{99m}Tc–TBI. Here we report on the effect of Polyoxyethylene (20) sorbitan mono-oleate (Tween-80) on the biodistribution of ^{99m}Tc–TBI.

Experimental

Synthesis of ^{99m}Tc-complex

The ligand TBI was obtained from commercial sources (97%, Acros). ^{99m}Tc as sodium pertechnetate was obtained from commercial generator systems. The synthesis of ^{99m}Tc–TBI was carried out as follows: 1 mg of TBI, 100 µl of ethanol, and 50 µg of $SnCl_2 \cdot 2H_2O$ were added to a sterile 10 ml vial. The vial was then sealed and 0.5 ml of generator eluate containing about 18.5 MBq of $^{99m}TcO_4^-$ was injected. The mixture was shaken and immediately placed in a boiling water bath for 10 min. The product was allowed to cool to room temperature and samples were taken for TLC analysis before subsequent use. The radiochemical purity of ^{99m}Tc–TBI was evaluated by TLC and ranged between 95% and 99%.

The complex with Tween-80 (about 20 mg) added (TA) was expressed as ^{99m}Tc–TBI(T), corresponding to the complex with non-Tween-80 added (NTA).

Animal distribution studies

In vivo distribution studies of ^{99m}Tc–TBI were carried out in mice (average weight about 20 g, obtained from Animal Center of Beijing Medical University). ^{99m}Tc–TBI complex (about 740 KBq in 50 µl solution) was injected through the tail vein. The mice were sacrificed at

different times (5–120 min post-injection) and the organs of interest were weighed and counted in a NaI well-type counter. The %ID/g in each organ and blood was calculated by comparing its activity with appropriate standards of injected dose (ID).

Results and discussion

Biological distribution results in mice for ^{99m}Tc–TBI and ^{99m}Tc–TBI(T) are shown in Tables 1 and 2. Both these complexes exhibit significant heart uptake and have good target/non-target ratios. Tween-80 has great effects on the biodistribution in mice, especially the effect on liver uptake are significant. The liver uptakes of TA complexes are about 50% of that of NTA complexes. At the same time, Tween-80 has an obvious effect on the accumulation of radioactivity in blood, it not only decreases the uptake but also increases the rate of clearance from blood. The effects of Tween-80 on the uptake of myocardium and lungs are not significant.

The ratios of heart-to-liver, heart-to-lung and heart-to-blood at 5 and 60 min are shown in Table 2. Comparing the ratios, the TA complexes heart-to-liver and heart-to-blood ratios are significantly greater than that of the corresponding NTA complexes. On the other hand, the heart-to-lung ratio of TA complexes is not significantly different from that of NTA complexes. Based on the results of the biological studies, Tween-80 has a notable effect on the biodistribution of lipophilic ^{99m}Tc–TBI complex and improved its properties distinctly for myocardial imaging.

Nowadays, ^{99m}Tc–MIBI is the most widely used myocardial perfusion imaging agent. In order to compare the biological properties of ^{99m}Tc–TBI and ^{99m}Tc–TBI(T) with that of ^{99m}Tc–MIBI, the biodistribution data referenced from the literature are also listed in the tables. From Table 1, we can see that the uptake of ^{99m}Tc–MIBI in liver at 5 min post-injection is higher than that of ^{99m}Tc–TBI(T), but the uptake of ^{99m}Tc–MIBI in lung at 5 min post-injection is obviously lower than that of ^{99m}Tc–TBI(T). So the ratios shown in Table 2 are different between ^{99m}Tc–MIBI and ^{99m}Tc–TBI(T); the improved ^{99m}Tc–TBI(T) complex may be suitable for instant imaging at early post-injection time.

In 1995, Bouquillon *et al.*[6] reported a new complex ^{99m}Tc-trimethylsilylmethylisonitrile (TMSiMI). Biodistribution studies of this new complex in rat indicated that it was mainly taken up in the liver and

Table 1. Biodistribution of ^{99m}Tc complex in mice (%ID/g, $n = 4$)[a]

Tissue	Complex	Post-injection time/min				
		5	10	20	60	120
Heart	A	21.78 ± 2.15	29.42 ± 3.37	23.69 ± 3.35	21.83 ± 1.66	21.39 ± 2.20
	B	32.20 ± 2.76	31.30 ± 1.24	32.54 ± 2.59	33.06 ± 3.46	26.95 ± 1.98
	C	32.14 ± 0.75	27.72 ± 2.61	24.93 ± 1.56	26.83 ± 4.78	—
Liver	A	40.67 ± 4.93	42.28 ± 3.11	39.12 ± 2.95	38.25 ± 2.87	39.33 ± 4.48
	B	18.40 ± 1.82	22.24 ± 0.37	23.66 ± 3.34	25.59 ± 2.85	22.13 ± 1.43
	C	34.09 ± 0.37	36.44 ± 0.81	27.77 ± 6.84	25.88 ± 1.51	—
Lung	A	50.07 ± 2.27	34.56 ± 1.76	16.32 ± 3.34	8.23 ± 1.21	5.01 ± 0.46
	B	65.04 ± 7.22	40.35 ± 5.46	19.98 ± 4.88	6.48 ± 1.23	5.70 ± 0.52
	C	11.21 ± 1.43	4.56 ± 0.26	2.75 ± 0.18	2.19 ± 0.35	—
Kidney	A	31.59 ± 1.55	36.21 ± 5.08	32.87 ± 1.92	37.03 ± 2.76	34.18 ± 3.94
	B	25.04 ± 1.60	26.18 ± 1.55	28.45 ± 3.24	34.25 ± 1.78	31.55 ± 1.41
	C	88.85 ± 14.79	53.49 ± 2.81	52.52 ± 8.33	42.41 ± 0.35	—
Brain	A	0.43 ± 0.08	0.36 ± 0.04	0.35 ± 0.05	0.21 ± 0.04	0.14 ± 0.01
	B	0.22 ± 0.05	0.21 ± 0.05	0.18 ± 0.03	0.15 ± 0.03	0.12 ± 0.04
	C	0.48 ± 0.02	0.29 ± 0.07	0.18 ± 0.04	0.15 ± 0.03	—
Muscle	A	6.77 ± 1.67	8.33 ± 1.59	6.52 ± 0.35	5.56 ± 0.62	5.85 ± 1.16
	B	4.23 ± 0.53	5.36 ± 1.33	4.89 ± 0.93	5.84 ± 1.66	5.91 ± 0.48
	C	7.28 ± 0.28	6.46 ± 0.86	5.02 ± 0.56	4.89 ± 0.87	—
Blood	A	5.66 ± 1.11	3.65 ± 0.25	2.63 ± 0.25	1.67 ± 0.20	0.95 ± 0.17
	B	2.32 ± 0.20	1.09 ± 0.25	0.47 ± 0.04	0.24 ± 0.02	0.20 ± 0.03
	C	2.51 ± 0.48	0.79 ± 0.05	0.28 ± 0.03	0.20 ± 0.01	—

[a] A: ^{99m}Tc–TBI, B: ^{99m}Tc–TBI(T), C: ^{99m}Tc–MIBI.[5]

Table 2. The T/NT ratios for ^{99m}Tc complex in mice ($n = 4$)

Complex	Hear/liver		Heart/blood		Heart/lung	
	5 min	60 min	5 min	60 min	5 min	60 min
^{99m}Tc–TBI	0.54	0.57	3.85	13.07	0.43	2.65
^{99m}Tc–TBI(T)	1.75	1.29	13.88	137.75	0.50	4.73
^{99m}Tc–MIBI[5]	0.94	1.04	12.80	134.15	2.87	12.25

very low in the heart. In contrast, the ^{99m}Tc–TBI(T) complex reported here shows high heart uptake, retention and low liver uptake.

When compared with published data[7] on ^{99m}Tc–MIBI, ^{99m}Tc–tetrofosmin and ^{99m}Tc–Q12, ^{99m}Tc–TBI(T) shows similar heart uptake, retention. However, the blood clearance of ^{99m}Tc–TBI(T) was faster than that of ^{99m}Tc–Q12 and the lung uptake of ^{99m}Tc–TBI(T) was higher than that of all three ^{99m}Tc complexes. In brief, the biodistribution of all these complexes, which were introduced for myocardial imaging, was comparable for most organs.

Generally, the liver accumulations of high lipophilic complexes are greater than that of hydrophilic complexes. At the same time, the clearance rates of hydrophilic complexes from liver, lungs and blood are higher than those of high lipophilic complexes.[1,2] Tween-80, average M_n ca. 1312, is a surface-active agent. It can improve the solubility in water of lipophilic complexes. On the basis of these facts, we inferred that the Tween-80 and ^{99m}Tc complex combine to form a new compound, which can be stable in mice at least and the lipophilicity of this formative compound is less than that of the original ^{99m}Tc complex. So it has lower uptake and faster clearance from liver and blood. In addition, the lipophilic complex is mainly excreted by hepatobiliary system. ^{99m}Tc–TBI with high lipophilicity is excreted by the bile. Since the lipophilicity of ^{99m}Tc–TBI(T) and ^{99m}Tc–MIBI is less than that of ^{99m}Tc–TBI, they are mainly excreted by the hepatobiliary system, with a small renal excretion. These conclusions should be investigated further.

Conclusions

Based on the results of the experiments reported here, we have concluded that the auxiliary agent Tween-80 has an obvious effect on the biodistribution of ^{99m}Tc–TBI which is the first-reported ^{99m}Tc-labeled myocardial perfusion imaging agent for clinical use. The

biological properties of ^{99m}Tc–TBI for myocardial perfusion imaging were improved significantly. In addition, the biological properties of ^{99m}Tc–TBI(T) were compared with the widely used myocardial perfusion imaging agent ^{99m}Tc–MIBI; the heart-to-liver ratios of ^{99m}Tc–TBI(T) at various post-injection times were better than those of ^{99m}Tc–MIBI. Clinical trials are being conducted and their results will be reported in due course.

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