Original Article

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Technetium-99m-Tetrofosmin Uptakes on Dipyridamole-Stress Planar Imaging : Comparison with Those in Excised Rat Organs

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ABSTRACT

We studied how precisely images reflect tracer uptake in the myocardium, and examined what degree technetium-99m-tetrofosmin (^{99m}Tc-tetrofosmin) in the liver and gastrointestine gave the effect to a myocardial image. After administering dipyridamole and ^{99m}Tc-tetrofosmin to normal rats, we compared the myocardial uptakes obtained using a gamma camera with the actual uptakes in the excised organs. **Methods** : Thirty-seven rats were used. Following imaging the anterior view at 5, 10, 15, 30, 60 and 90 min after administration of the tracer, uptakes in the heart, lung, liver and blood were estimated with a well-type scintillation counter (WC) and represented as percentage of the injected dose per gram of tissue (%ID/g). The regions of interest (ROIs) were placed on planar images (PI) and the uptake in each organ was estimated as percentage of the injected dose per pixel (%ID/pixel). The ratios of PI-to-WC and heart-to-organ were also evaluated. **Results** : Cardiac uptake with WC was 1.688%±0.395% at 10 min post-injection. On the other hand, that with PI was 1.855%±0.965% at 10 min post-injection. There were particularly great differences from 15 min post-injection between both measurements (PI/WC ratio : about 1.2 times). Pulmonary uptake with WC was the maximum at 15 min (0.861%± 0.387%) post-injection, and there was hardly a variation in the activity later than 15 min. However, PI measurement showed the maximum value at 15 min (0.777%±0.163%), and decreased gradually. Hepatic uptake with WC was the maximum at 5 min (1.545%±0.563 %), and rapidly decreased. On the other hand, PI measurement showed higher value than WC as the whole. **Conclusion** : PI measurement showed higher uptakes in each organ than WC measurement. There were great differences between both measurements, at late phase particularly. As one of these causes, it was considered that the decrease of activity in blood was different from that in each organ with increasing time.

KEY WORDS

^{99m}Tc-tetrofosmin, Organ uptake, Planar image, Dipyridamole, Rat

INTRODUCTION

External imaging of regional myocardial perfusion during pharmacological coronary vasodilatation for the detection of coronary stenosis were reported initially in experimental animals[1] and in man[2] and subsequently in patients[3]. These studies demonstrated the physiologic basis for assessing the severity of coronary stenosis in terms of restricted coronary flow reserve and sharply separated normal subjects from patients with significantly impaired coronary flow.

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Then basic physiologic requirement of any method for assessing coronary flow reserve is that the myocardial uptake of the imaging agent is proportional to flow at high coronary flow rates of at least 4 times of resting levels. An agent whose uptake is not related to flow will be insensitive to detect mild stenosis, whereas an agent whose myocardial uptake is linearly related to flow will be sensitive. The myocardial uptake of thallium-201 is linearly proportional to flow at resting control levels down to zero[4)-6)], but at higher flow levels which are necessary to assess coronary flow reserve thallium uptake is not proportional to flow. As the other causes of discrepancy between myocardial uptake and coronary flow, this does lead to significant amounts of tracer in the liver, duodenum and small intestine, and as reflux of these contents is not uncommon, these can lead to interference with the myocardial uptake[7)-9)]. For an accurate quantification, we are necessary to solve problems about the physical factor including absorption or Compton scatter of gamma ray. Some solution methods have been already devised, and these methods have been tried with the clinical cases[10)-12)]. Nevertheless we cannot understand how they were accurate because we can not measure the actual uptake in patients' organs. Recently some documents have been reported about various time intervals of the distribution and uptake in each organ of animals[13)-15)] and patients[16),17)] using Tc-99m myocardial perfusion agent. However, they only evaluated the uptake in excised organs, and they did not estimate in detail the relation between uptakes obtained with a gamma camera and those in the excised organs.

The purpose of this study was to compare with uptakes obtained with a gamma camera and the actual values obtained with a well-type scintillation counter in each organ, and to examine how the uptakes in the lung, liver and blood gave the effect to the dipyridamole-stress myocardial imaging using ^{99m}Tc-tetrofosmin.

MATERIALS AND METHODS

Animals

Thirty-seven male Donryu rats (222g~340g, mean 279g±8.54g, Charles River, Japan) were adopted, and every animals were freely given food and water until administration of ^{99m}Tc-tetrofosmin.

Preparation of ^{99m}Tc-tetrofosmin

^{99m}Tc-tetrofosmin was prepared from a freeze-dried kit (Nihon Medi-physics, Tokyo, Japan) by reconstitution with approximately 2 mL of a sterile pertechnetate solution containing 296MBq. Preparation of this tracer does not require heating but only a 15-min incubation at room temperature.

Biodistribution and planar imaging of dipyridamolestress ^{99m}Tc-tetrofosmin

Rats were anesthetized with ether or pentobarbital 4.0mg/100g intraperitoneally, and administered dipyridamole with a rate of 0.28mg/kg/min over 2 min and ^{99m}Tc-tetrofosmin 20-30MBq/0.2mL into the caudal vein. An anesthetized rat was placed supine on the face of a field of a gamma camera (Sigma 410S, Aloka, Tokyo) equipped with low-energy, high-resolution parallel-hole collimators, and planar images (PI) were acquired using the anterior view at 5, 10, 15, 30, 60 and 90min post-injection by 5-8 each. Data were obtained for 2 min using 256 x 256 matrix (pixel size 1.0mm) and a 20% (126-154keV) energy window. Image data were obtained by a computer (MCS560, Aloka, Tokyo). Using both the anterior and the posterior view we investigated the difference of total counts and average counts per pixel in each

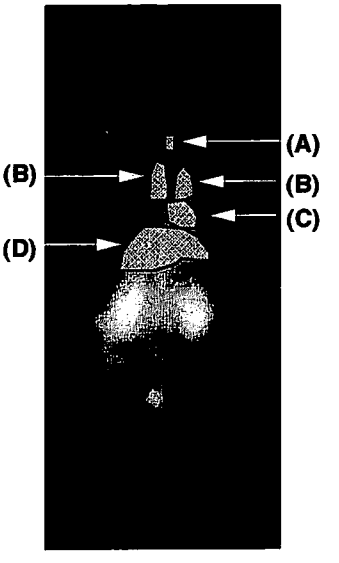

Figure 1 Regions of interest (ROIs) of the mediastinum (A), lung (B), heart (C), and liver (D) in the anterior view. From these ROIs, the total counts and average counts per pixel are obtained for each organ.

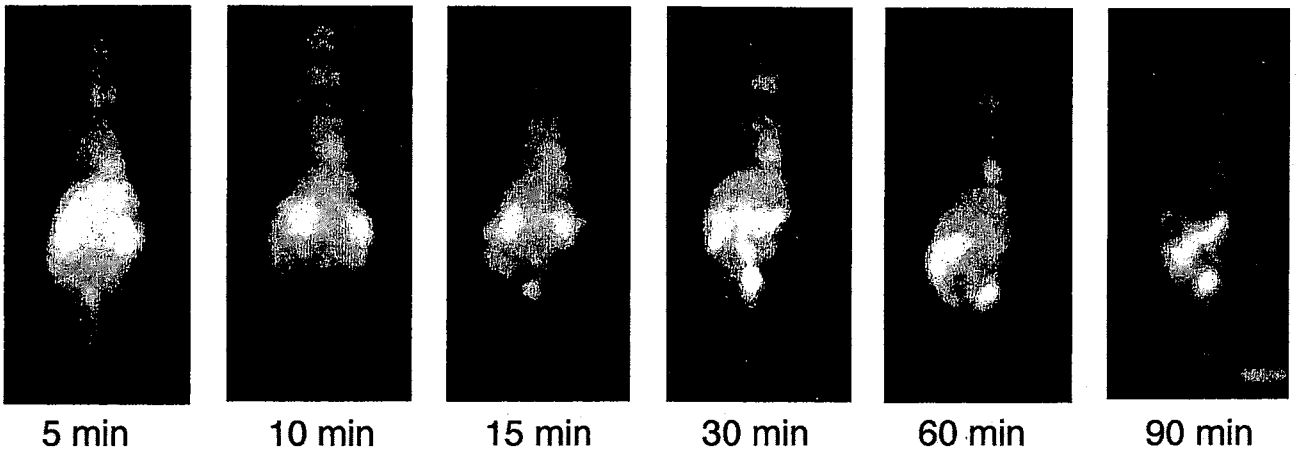

Figure 2 Serial images at 5, 10, 15, 30, 60 and 90 min after the injection. Hepatic radioactivity at early phase is more than cardiac radioactivity. Small intestinal radioactivity increases at 60min, whereas hepatic radioactivity decreases.

organ. Since the results were almost equal, then we used only the anterior view. Immediately after acquisition, rats were killed by exsanguination under ether or pentobarbital anesthesia, and the organs were removed by dissection. Samples of a myocardium, lung, liver and blood were measured in pre-weighed containers and assayed for radioactivity in a well-type scintillation counter (ARC-500, Aloka, Tokyo, WC).

Data analysis

Percent uptake with WC measurement. Percent uptake was expressed as a percentage of the injected dose per gram of tissue (%ID/g) in each organ, and the ratios of heart-to-lung, heart-to-liver and heart-to-blood were obtained. Each standard sample was measured for 1 min.

Percent uptake with PI measurement. The regions of interest (ROIs) were determined on the mediastinum, heart, lung and liver of a planar image (Fig.1), and the counts and pixel numbers in the ROIs obtained. Percent uptake was expressed as a percentage of the injected dose per pixel (%ID/pixel) in each organ. The PI-to-WC ratios were also obtained as well as ratios of heart-to-lung, heart-to-liver and heart-to-mediastinum. Percent uptake in blood with PI measurement was obtained using the counts of upper mediastinal region. One ml-syringe containing approximately 0.2mL-standard was placed at the location corresponding to the center of rat body and imaged for 15 seconds.

Statistical analysis

Data was presented as mean$\pm$s.d.. Differences between PI and WC were compared using Paired t-test. Organ differences were evaluated using Two-factor factorial ANOVA and Scheffe's F. Statistical significance was defined as $p<0.05$.

RESULTS

Comparison of tissue activity obtained with WC and PI

Figure 2 shows an example of serial images with increasing time. Hepatic activity at early phase is much more than cardiac activity. At late phase, small intestinal activity increased with decreasing hepatic activity. The gastrointestinal activity at 60 min post-injection became markedly greater than the cardiac activity. Table 1 and Table 2 show tissue activity and PI-to-WC ratio, and heart-to-organ ratios, respectively.

Percent uptake in the heart. Cardiac uptake with WC increased until 10 min (1.668%$\pm$0.395%) post-injection, and there was a tendency of almost constant uptakes later than 30 min. PI measurement showed the maximum uptake at 10 min (1.855%$\pm$0.965%) with subsequent gradual decrease (Fig.3A). The difference between both measurements was almost constant with PI/WC ratio of about 1.15 (Fig.3B).

Percent uptake in the lung. Pulmonary uptake with WC was the maximum at 15 min (0.861%$\pm$0.387%) post-injection, and there was a tendency of decrease later than 30 min. PI measurement showed the

Table 1 Tissue activity obtained with WC and PI measurement after injection of ^{99m}Tc-tetrofosmin

Tissue	Weight(g)	5 min	10 min	15 min	30 min	60 min	90 min
Heart	1.262±0.046						
WC		1.084±0.253	1.668±0.395	1.346±0.504	1.068±0.129	1.196±0.385	1.218±0.172
PI		1.360±0.320	1.855±0.965	1.631±0.504	1.214±0.291	1.149±0.192	0.916±0.345
PI/WC		1.069±0.168	1.049±0.390	1.157±0.293	1.163±0.127	1.200±0.254	1.112±0.242
Lung	1.229±0.098						
WC		0.550±0.221	0.693±0.338	0.861±0.387	0.339±0.140	0.224±0.042	0.154±0.041
PI		0.574±0.145	0.636±0.289	0.777±0.163	0.543±0.121 †	0.587±0.200**	0.533±0.151 †
PI/WC		1.125±0.312	1.067±0.456	1.517±0.619	1.672±0.585	2.421±0.951	3.666±1.061
Liver	12.56±0.924						
WC		1.545±0.563	1.456±0.671	1.006±0.454	0.807±0.413	0.559±0.127	0.263±0.070
PI		2.331±0.484 †	1.984±0.418	1.656±0.713	1.416±0.572 †	1.057±0.227***	0.622±0.254 †
PI/WC		1.710±0.284	1.872±0.649	1.679±0.517	1.773±0.420	1.993±0.645	2.772±0.551
Blood							
WC		0.039±0.015	0.045±0.030	0.031±0.019	0.018±0.011	0.020±0.013	0.010±0.003
PI		0.546±0.217**	0.478±0.223 †	0.496±0.225**	0.397±0.147***	0.470±0.148***	0.298±0.108**
PI/WC		15.87±2.532	7.416±2.747	12.48±2.623	18.62±9.036	42.99±9.376	34.06±8.405

Values were expressed as mean ± s.d.. WC : well scintillation counter, PI : planar imaging. (%)

Values of WC and PI were expressed as %ID/g and %ID/pixel, respectively.

There was no statistical difference (p>0.05 two group t-test) between WC and PI measurement except where indicated.

† p<0.05, *p<0.01, **p<0.005, ***p<0.001

Table 2 Activity ratios of heart versus lung or liver after injection of ^{99m}Tc-tetrofosmin

	5min	10min	15min	30min	60min	90min
Heart-to-Lung						
WC	2.167±0.711	2.578±0.502	2.635±0.833	3.666±1.081	5.063±1.201	6.891±2.107
PI	2.192±0.304	2.532±0.275	2.215±0.540	2.299±0.324*	2.204±0.367***	2.135±0.206*
Heart-to-Liver						
WC	0.781±0.321	1.241±0.427	1.286±0.622	1.824±0.591	2.199±0.497	4.048±1.224
PI	0.876±0.497	0.899±0.524	1.014±0.579	0.820±0.253**	1.215±0.351**	1.661±0.270 †
Heart-to-Blood						
WC	31.64±13.72	50.91±7.801	48.40±12.27	54.47±19.70	100.1±23.56	107.2 ±33.56
PI	2.411±0.496**	3.662±2.088***	3.172±0.755***	2.824±0.735***	2.749±0.471***	3.206±0.316*

Values were expressed as mean ± s.d.. WC : well scintillation counter, PI : planar imaging.

There was no statistical difference (p>0.05 two group t-test) between WC and PI measurement except where indicated.

† p<0.05, *p<0.01, **p<0.005, ***p<0.001

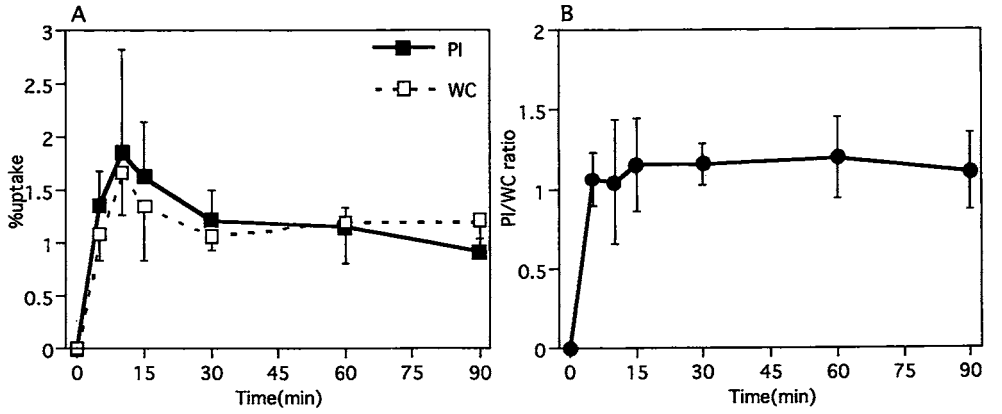

Figure 3 (A), cardiac uptake obtained with PI and WC ; (B), PI-to-WC ratio for the heart.

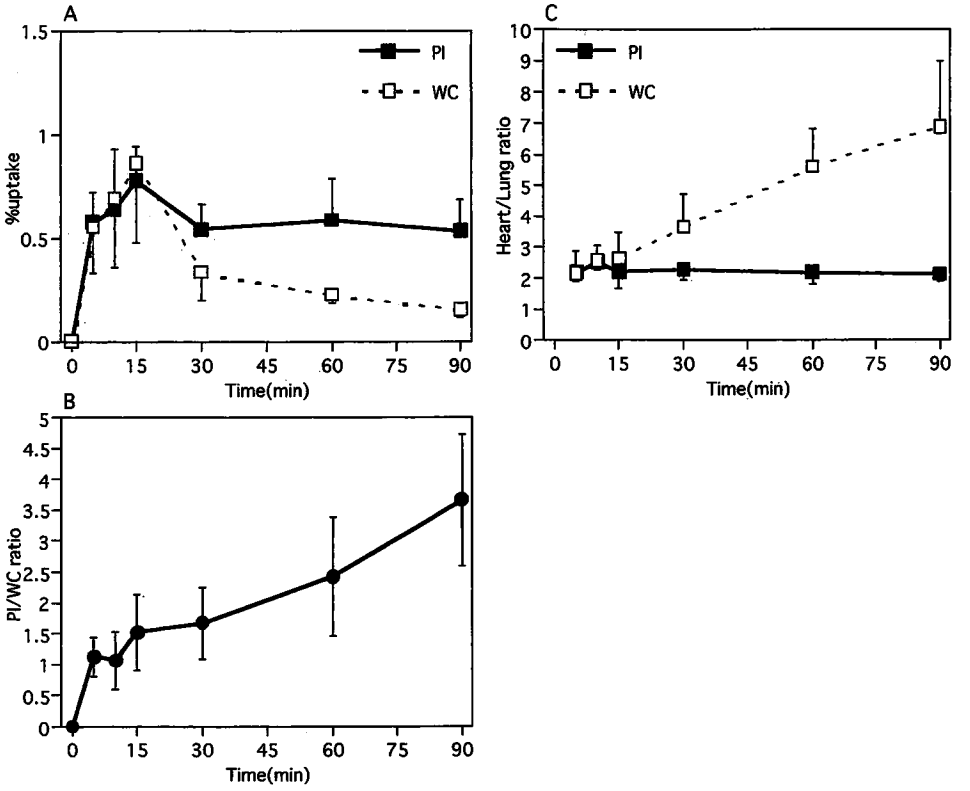

Figure 4 (A), pulmonary uptake obtained with PI and WC ; (B), PI-to-WC ratio for the lung ; (C), Heart-to-Lung ratio.

maximum uptake at 15 min (0.777%±0.163%), and the variation in uptake was hardly shown later than 30 min (Fig.4A). The PI-to-WC ratio was approximately 1.1 at 10 min and increased significantly later than 15 min (Fig.4B, $p<0.0001$). Compared the heart-to-lung ratios between WC and PI, both were 2.578 ±0.502 and 2.532±0.275 at 10 min, respectively, and there was no significant difference, but the values in WC measurement increased significantly later than 30 min (Fig.4C, $p<0.01$).

Percent uptake in the liver. Hepatic uptake with WC was the maximum at 5 min (1.545%±0.563%) post-injection with subsequent decrease. On the other hand, the uptake with PI was the maximum at 5 min (2.331%±0.484%, PI/WC ratio : about 1.7times). There was significantly higher than that with WC as a whole ($p<0.05$) (Fig.5A-B). The heart-to-liver ratio with WC increased with increasing time, with the values of 0.781±0.321 and 2.199±0.497 at 5 and 60 min, respectively, while the heart-to-liver ratios with PI were hardly more variable than those with WC (Fig.5C).

Percent uptake in the blood. Compared the heart-to-blood ratios between WC and PI, the heart-to-blood ratios with WC increased with increasing time, with the values of 31.64±13.72 and 100.1±23.56 at 5 and 60 min, respectively. The heart-to-blood ratios with PI were less variable than those with WC (Fig.6C).

DISCUSSION

The activity in the liver and gastrointestine deteriorates the imaging quality of myocardial scintigraphy. The procedures of improvement including absorption or Compton scatter correction have recently been devised[7), 8)]. However, we can not understand how the method is accurate because the myocardial uptake can not evaluate in patients. Therefore, we compared values obtained using a gamma camera with the actual values obtained using a well-type scintillation counter in rat organ.

Comparison with WC and PI measurement

Heart. Cardiac uptake with PI was higher than that

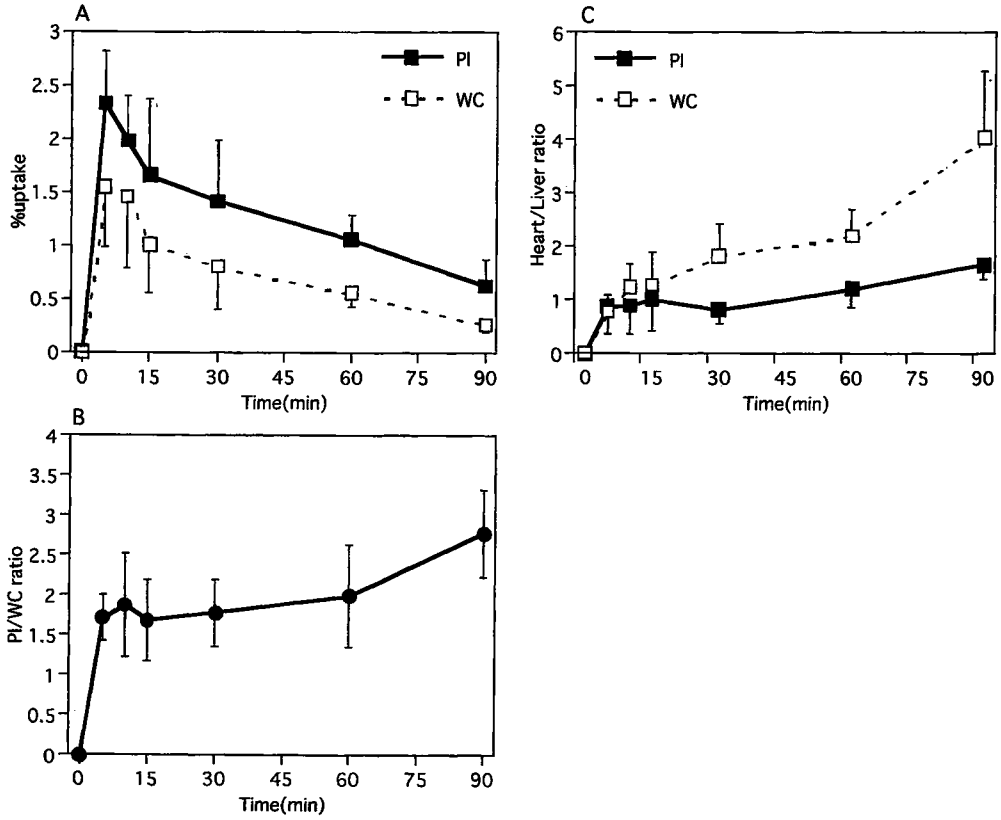

Figure 5 (A), hepatic uptake obtained with PI and WC ; (B), PI-to-WC ratio for the liver ; (C), Heart-to-liver ratio.

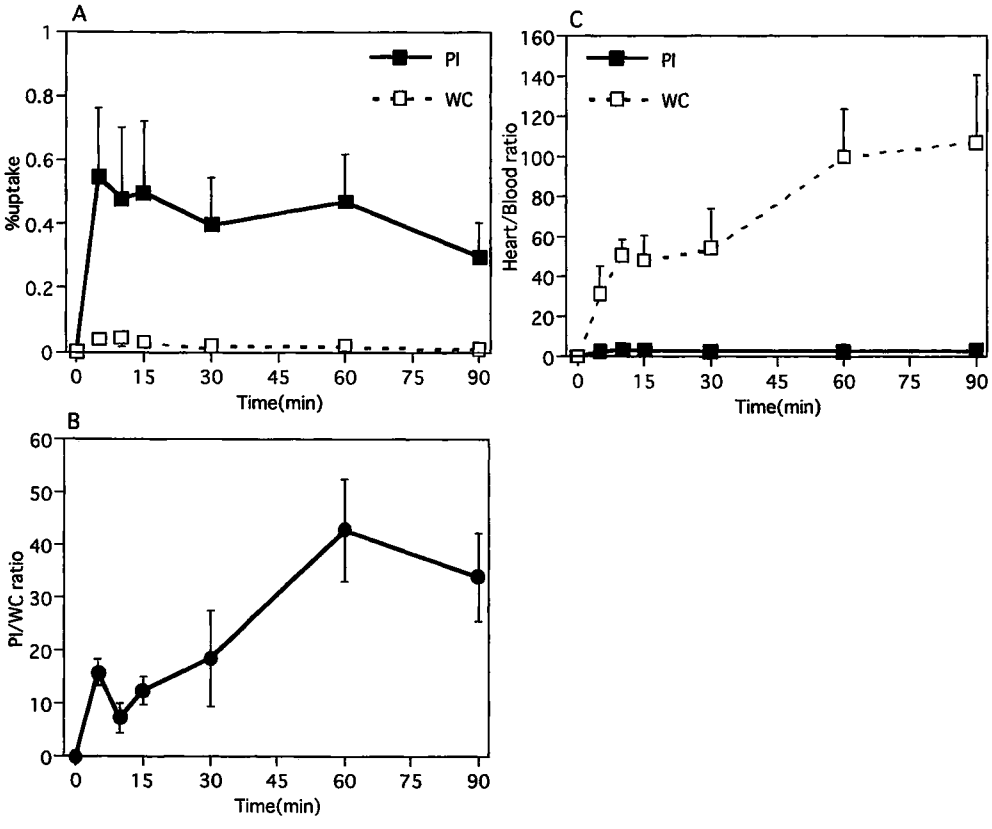

Figure 6 (A), background uptake obtained with PI and WC ; (B), PI-to-WC ratio for the background ; (C), Heart-to-background ratio. Mediastinum and blood are used as background with PI and WC, respectively.

with WC as a whole. Cardiac uptake (%ID/g) with WC changed significantly later than 15 min. On the other hand, %uptake with PI gradually decreased with increasing time. It was considered that one of these causes was due to the difference of activity between heart and blood because %uptake in blood at early phase was high but these values gradually decreased with increasing time.

Lung. Pulmonary uptake (%ID/g) with WC markedly decreased with increasing time. On the other hand, a change of %uptake with PI was hardly observed later than 30 min. The heart-to-lung ratio with WC was 3.666±1.081 and 5.063±1.201 at 30 and 60 min, respectively and these values increased with increasing time. Therefore, these results showed the rapid clearance of ^{99m}Tc-tetrofosmin from the lung. The heart-to-lung ratio with PI was 2.299±0.324 and 2.204±0.367 at 30 and 60 min, respectively. Jain et al.[19] have reported that the heart-to-lung ratio of ^{99m}Tc-tetrofosmin at normal stress studies hardly changed from 5 min to 180min post-injection and the values were 1.9±0.3, 2.2±0.5 and 2.1±0.4 at 5, 30 and 60 min, respectively. Similarly, Munch et al.[20] have reported that the heart-to-lung ratio of ^{99m}Tc-tetrofosmin at stress studies hardly changed from 5 min to 60min (about 2.5). In our study, the heart-to-lung ratio with PI hardly changed with increasing time, and our results were similar to their results. As one of the causes which the heart-to-lung ratio was different between PI and WC, the rapid clearance of ^{99m}Tc-tetrofosmin from the lung and the error in the setting of ROIs were considered, because a difference of activities between the lung and the blood decreased with increasing time and a boundary in the fort, the lung and the mediastinum occasionally became unclear.

Liver. Percent uptake with WC was subsequently decreased but %uptake with PI was significantly higher than that with WC as a whole. The heart-to-liver ratios with WC were 0.781±0.321, 1.824±0.591 and 2.199±0.497 at 5, 30 and 60 min, respectively and their values increased with increasing time, that is, the rapid clearance from the liver was demonstrated. On the other hand, the heart-to-liver ratio with PI was 0.876±0.497, 0.820±0.253 and 1.215±0.351 at 5, 30 and 60 min post-injection, respectively and these values slightly increased but there was not a great change comparing with WC measurement. Sinusas et al.[14] have reported that the heart-to-liver ratio using dynamic pharmacological stress ^{99m}Tc-tetrofosmin planar imaging with both adenosine and dipyridamole in a canine was about 0.6 at 15 min post-injection. Jain et al.[19] have reported that the heart-to-liver ratio for ^{99m}Tc-tetrofosmin at normal stress studies was 1.3±0.4, 1.4±0.3 and 1.6±0.4 at 5, 30 and 60 min, respectively. Munch et al.[20] have reported that the heart-to-liver ratio for ^{99m}Tc-tetrofosmin at stress studies slightly increased from 5 min to 60 min and the values were 1.05, 1.15 and 1.45 at 5, 30 and 60min, respectively. Similarly, Flamen et al.[16] have reported that the heart-to-liver ratio for ^{99m}Tc-tetrofosmin at normal dipyridamole-stress studies increased at 30 min (0.78±0.09) and at 60 min (1.37±0.4), respectively. Our results were similar to their results. However, the heart-to-liver ratio obtained with WC in a rat was significantly higher than that obtained with PI. These results suggested the possibility of rapid clearance from human liver.

Blood. The counts of upper mediastinal region were used with PI measurement because we can not estimate the activity in blood directly. Expressing as percentage of the peak blood activity, blood clearances with WC were 40% and 22% at 30 and 90min, respectively but that with PI was 73% and 55% at 30 and 90min, respectively. These results suggested the possibility that the real clearance from the blood may be rapid than the value obtained with PI measurement.

A unit of %uptake was expressed as a percentage of the injected dose per gram of tissue (%ID/g) with WC and a percentage of the average counts per pixel (%ID/pixel) with PI, respectively. There may be problems in the comparison of absolute values between %ID/g and %ID/pixel, but the comparison of relative change will be possible. Since some authors[13),19),20)] have described the uptake in animal and patients using %ID/g and %ID/pixel, we adopted these units.

CONCLUSION

The PI measurement showed higher uptake in rat organ than WC measurement. There were particularly

great differences between both measurements at late phase particularly after the injection. As causes of the difference, it was considered that WC method measured only organ taken by dissection, whereas PI method measured an organ plus background including blood. There were certainly the interferences of scatter from a liver and a small intestine. It was concluded that uptakes or heart-to-organ ratio obtained clinically with PI were not always accurate.

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ラットを用いたジピリダモール負荷プラナー画像における ^{99m}Tc-tetrofosmin 摂取率：摘出臓器の摂取率との比較

小野口昌久，高山　輝彦，利波　紀久，水上　勇治
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要　旨

心筋画像に影響を及ぼす肝臓や腸管内の ^{99m}Tc-tetrofosmin（以下,TF）がどの程度取り込まれているのか，臨床では生体内の臓器摂取率を直接測定することができない。今回ラットを用い，TF のガンマカメラによる摂取率および心臓/臓器比がどの程度真の値を表現しているかをウェル型シンチレーションカウンタ（以下, WC）とプラナー像（以下, PI）の両者で比較検討した。正常な雄ラット37匹に，Dipyridamole 0.28mg/kg/min を２分間静注後，TF を投与し，５，10，15，30，60，90分後にガンマカメラで撮像した。心臓，肺臓，肝臓および血液を採取して，WC で投与量に対する臓器１g あたりの摂取率（%Injected dose/g）を測定した。PI の測定は各臓器に関心領域(以下，ROI)を設定し，投与量に対する１ピクセル当たりの摂取率(%Injected dose /pixel) を算出した。さらに，PI/WC 比，心臓/各臓器比を算出した。WC による心臓の摂取率は，投与10分後で1.688%±0.395%であったが，PI では1.855%±0.965%となった。特に15分以降で両者に差がみられた（PI/WC 比：約1.2)。WC による肺の摂取率は投与15分後で最大値（0.861%±0.387%）となり，その後ほとんど変化はみられなかった。一方，PI では投与15分後（0.777%±0.163%）で最大となったが，その後経時的に減少した。WC による肝臓の摂取率は投与５分後で最大（1.545%±0.563%）となり，速やかに減少したが，PI では WC に比し全体的に高い値を示した。各臓器において，PI による測定は WC に比し高値を示した。両者の差は，特に遅い時間で大きくなった。これらの原因の１つとして，各臓器と血液内の放射能の減少が経時的に異なることが示唆された。