Annals of Nuclear Medicine Vol. 10, No. 4, 375–381, 1996

Comparison of cationic myocardial perfusion agents: Characteristics of accumulation in cultured smooth muscle cells

Kayoko NAKAMURA, Toshikazu SAMMIYA, Jun HASHIMOTO, Ryochi ISHIBASHI, Kazuhiro MATSUMOTO and Atsushi KUBO

Department of Radiology, Keio University School of Medicine

The uptake and washout kinetics of two cationic lipophilic ^{99m}Tc-labeled myocardial perfusion agents, ^{99m}Tc-methoxyisobutylisonitrile (^{99m}Tc-MIBI) and ^{99m}Tc-1,2-bis[bis-(2-ethoxyethyl)-phosphino]ethane (^{99m}Tc-Tetrofosmin), were studied in cultured smooth muscle cells and compared to the conventional myocardial perfusion agent, ^{201}Tl. Both ^{99m}Tc-MIBI and ^{99m}Tc-Tetrofosmin had a 4-fold greater uptake than ^{201}Tl, and they were washed out of cells through similar kinetics which had slower rates than ^{201}Tl. Incubation with metabolism inhibitors had a modest influence on the uptake of these two ^{99m}Tc-labeled agents, although their extent and inhibited sites were slightly different. Ion transport inhibitors did not affect the uptake of ^{99m}Tc-MIBI, although the ^{99m}Tc-Tetrofosmin uptake was slightly inhibited when the Ca^{2+} channel was blocked. Our studies indicate that ^{99m}Tc-MIBI and ^{99m}Tc-Tetrofosmin were taken up by smooth muscle cells in similar pharmacokinetic patterns, but their accumulation reflected a different meaning for cell viability.

Key words: ^{99m}Tc-MIBI; ^{99m}Tc-tetrofosmin; ^{201}Tl, smooth muscle cells

INTRODUCTION

NEW ^{99m}Tc-LABELED myocardial perfusion agents, which may replace ^{201}Tl, have been introduced during the last few years. Among them, ^{99m}Tc-methoxyisobutylisonitrile (^{99m}Tc-MIBI) and ^{99m}Tc-1,2-bis[bis(2-ethoxyethyl)-phosphino]ethane (^{99m}Tc-Tetrofosmin), are both potential for routine clinical studies of myocardial perfusion. ^{99m}Tc-MIBI is one of the cationic isonitrile ^{99m}Tc-complexes; that is, it has a net charge of + 1 and a coordination number of six with six isonitrile ligand groups. On the other hand, ^{99m}Tc-Tetrofosmin is a lipophilic complex with two diphosphone ligands surrounding a technetium dioxo core; it too has a + 1 charge. Several laboratories, including ours, evaluated the biodistribution of both ^{99m}Tc-agents in normal volunteers.[1,2] It is interesting that both of them showed similar heart uptake and retention and blood clearance kinetics, in spite of their different chemical structures. Extensive studies have been carried out to evaluate the detection of myocardial disease by either of these two ^{99m}Tc-labeled agents. There have been no significant differences observed between the two agents.

On the other hand, the uptake mechanism for either of the two ^{99m}Tc-labeled compounds remains unclear. By using erythrocytes,[3] myocyte cells,[3–9] and tumor cells,[10–16] the accumulation mechanism of ^{99m}Tc-MIBI was exclusively studied. It was also clinically reported that ^{99m}Tc-MIBI localizes in several tumors in patients.[17] On the other hand, there have been few reports estimating the accumulation mechanism of ^{99m}Tc-Tetrofosmin in cells,[18] while the comparison of ^{99m}Tc-MIBI with other ^{99m}Tc-labeled compounds has been studied in some tumor cells.[11,12] It is important to clarify the mechanism of uptake of myocardial perfusion agents to interpret the biochemical and physiological characteristics of tumors as well as heart disease and to compare ^{99m}Tc-labeled compounds with regard to these points.

The uptake of ^{99m}Tc-labeled agents in the heart involves many factors such as blood flow, vascularity, plasma proteins, and so on. To compare the two ^{99m}Tc-labeled agents equivalently, we need a very simple *in vitro* system such as a cell culture model. Myocytes are generally used in most experiments, but they have to be prepared for each

Received March 28, 1996, revision accepted July 10, 1996.

For reprint contact: Kayoko Nakamura, Ph.D., Department of Radiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, JAPAN.

experiment, and their biochemical and physiological characteristics are not always uniform. Normalization of the cell characteristics is necessary to compare the two labeled compounds. The cultured cells have homogeneous characteristics, but the establishment of a normal cell line is not easy. Recently smooth muscle cells isolated from Caucasian blood vessels have been able to be cultured in a monolayer for a few generations under definitive conditions and they have been commercially available. The cultured normal cell is an adequate and simple model to use for comparing the two ^{99m}Tc-labeled agents and ^{201}Tl, although the results are not directly comparable with those obtained in myocytes.

This paper deals with comparative studies of the three myocardial perfusion agents, ^{201}Tl, ^{99m}Tc-MIBI, and ^{99m}Tc-Tetrofosmin, with regard to their uptake and washout pharmacokinetics. We also investigated the effect of some metabolism and ion transport inhibitors on the uptake of ^{201}Tl and ^{99m}Tc-labeled compounds. The purpose of this study was to differentiate between the two ^{99m}Tc-labeled agents in an *in vitro* system, which difference has not been observed clinically.

MATERIALS AND METHODS

^{99m}Tc-Tetrofosmin (^{99m}Tc-1,2-bis[bis(2-ethoxyethyl)-phosphino]ethane)

This ligand was formulated into a freeze-dried kit. The agent was prepared from a freeze-dried kit (Myoview, Amersham International plc) by reconstitution with approximately 3 m*l* of a sterile sodium pertechnetate solution containing 190–370 MBq of ^{99m}Tc. The vial was shaken gently to ensure complete dissolution of the lyophilized powder, and the solution was allowed to stand at room temperature for 15 minutes.

^{99m}Tc-MIBI (^{99m}Tc-methoxyisobutylisonitrile)

This agent was prepared from a freeze-dried kit (Cardiolite, Daiichi Radioisotopes Inc.) by reconstruction with approximately 3 m*l* of a sterile sodium pertechnetate solution containing 190–370 MBq of ^{99m}Tc. The vial was heated at 100°C for 10 min and allowed to cool to room temperature.

All preparations were tested by thin-layer chromatography for quality control according to the manufacturer's recommendations and were used within 2 hours of reconstitution. The radiochemical purities of ^{99m}Tc-Tetrofosmin and ^{99m}Tc-MIBI ranged from 95 to 99%.

^{201}Tl was obtained as a sterile, pyrogen free, clinically injectable radiopharmaceutical from Amersham International plc.

Smooth cell-AO; Aortic Smooth Muscle Cells (AOSMC 751 3°)

These cells were purchased from Clonetics, Inc. through Kurabo, Inc. Primary cells were isolated from a male Caucasian on 03/27/92. The cells are continually observed and evaluated for characteristic smooth muscle cell morphology. The cells were maintained in a stationary phase in a Normal Human Smooth Muscle Cells Growth Medium (S-BM) medium supplemented with 5% fetal calf serum, human endothelial growth factor (EGF), recombinant fibroblast growth factor (r-FGF-B) and antibiotics.

Cellular uptake and retention of myocardial perfusion agents

Cells harvested from the culture were suspended in S-BM medium containing 5% of fetal calf serum (incubation buffer) with a concentration of approximately 10^6 cells/m*l*. Cell suspensions (500 μl) were incubated with 50 μl of ^{201}Tl, ^{99m}Tc-Tetrofosmin or ^{99m}Tc-MIBI (c.a. 3.7 KBq/m*l*) in the 1.5-m*l* tubes for various times at 37°C. Measured samples of the incubation buffer were withdrawn to standardize the cellular data to the concentration of extracellular activity. The incubation buffer was completely removed from the tube by centrifugation followed by rinsing the cells twice with cold fresh incubation buffer to clear the extracellular spaces. The radioactivities of the aliquots of cell suspension in the tubes were then counted. The nonspecific binding of radioactivity to the blank tube, which was pretreated with 1% BSA (bovine serum albumin)/PBS (phosphate bufferized saline), was 10–15% and 2% of the added activity for ^{99m}Tc-labeled agents and for ^{201}Tl, respectively.

The results are expressed as the radioactivities of ^{201}Tl and ^{99m}Tc per cell and are normalized to a constant incubating solution concentration. This quantification allowed a direct comparison of data for the various experiments and cultures.

Cellular washout

Cellular washout experiments were conducted by first incubating the cells for one hour at 37°C in incubation buffer containing ^{201}Tl, ^{99m}Tc-Tetrofosmin or ^{99m}Tc-MIBI. For stepwise washout experiments, the cells were rinsed with 100 μl of incubation buffer and the radioactivities of the cell pellets were determined after spinning the cell suspensions. This procedure was repeated three times. For the washout-kinetic studies, the cells were twice rinsed consecutively in 100 μl of ice-cold incubation buffer and incubated in fresh incubation buffer at 37°C for various lengths of time. The cells were processed as above to quantify the retained activity. The data values represent the mean for four or more individual observations. The error bars on the graphs were not shown when the SEM did not exceed 15% of the mean value.

Effects of inhibitors on the uptake of myocardial perfusion agents

Tests for the effects of metabolism inhibitors were performed by preincubating cells at 37°C with the drug for 30

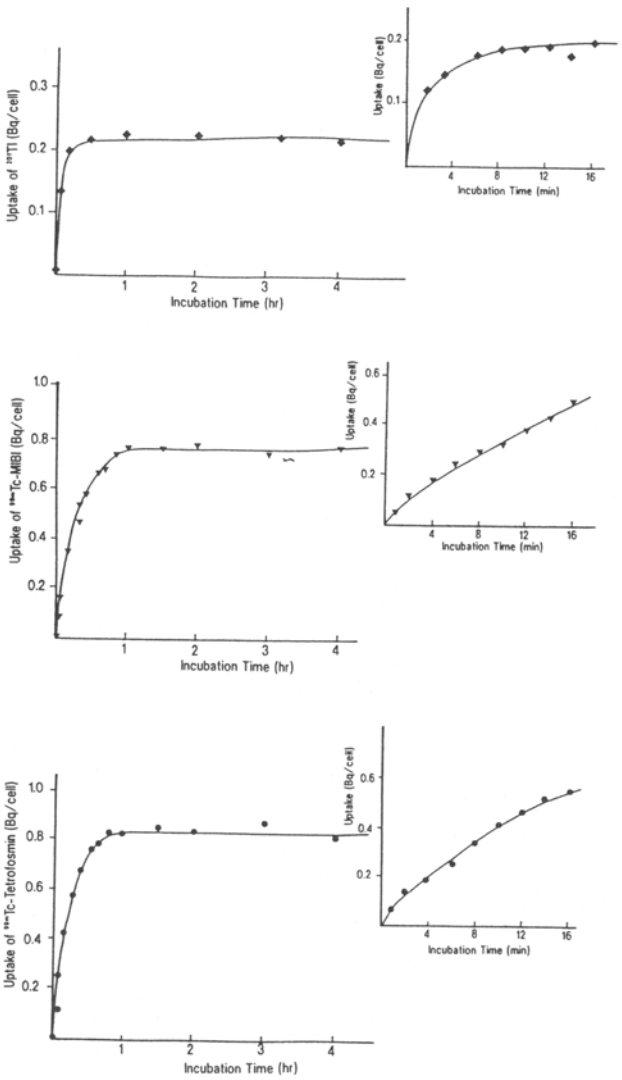

Fig. 1 Accumulation of myocardial perfusion agents in smooth muscle cells.

Top: ^{201}Tl (---◆---); Middle: ^{99m}Tc-MIBI (---▼---); Bottom: ^{99m}Tc-Tetrofosmin (---●---)

Right figure shows uptakes of ^{201}Tl, ^{99m}Tc-MIBI, and ^{99m}Tc-Tetrofosmin at initial phases. Cell associated counts are normalized to constant loading-solution concentration.

min followed by a 30 min ^{201}Tl, ^{99m}Tc-Tetrofosmin, or ^{99m}Tc-MIBI uptake. The following inhibitors were tested: rotenone (10 μM; inhibition of electron transfer); dinitrophenol (DNP, 10 μM; uncoupling of phosphorylation from electron transfer); and iodoacetate (IAA, 1 mM; inhibition of glycolysis). The following cationic membrane transport inhibitors were also evaluated for a possible effect on the uptake of the perfusion agents in this model: ouabain (10 μM; inhibition of Na^+/K^+ ATPase activity); amiloride (100 μM; blocking a Na^+/H^+ exchange); and verapamil (1 μM; blocking a Ca^{2+} channel). The effects on the uptake were monitored by exposing the cells to the inhibitors for 30 min followed by a 30-min isotope incubation in the presence of the drug. The cellular viability was visually examined by the trypan blue dye exclusion technique.

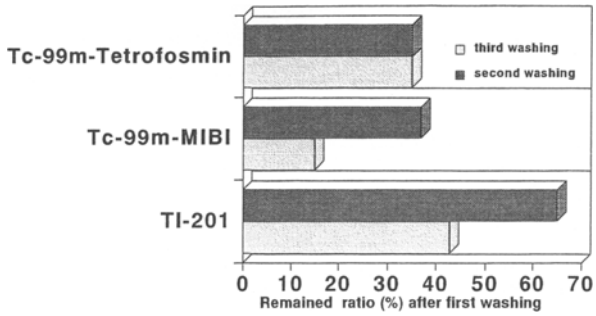

Fig. 2 Step-wise washout of myocardial perfusion agents from smooth muscle cells.

Each bar represents of mean of 4 individual cells in the tube. The coefficient of variation was always < 15% and is not shown in the figure for reasons of clarity.

Table 1 Uptake-kinetic comparison among ^{201}Tl, ^{99m}Tc-MIBI, and ^{99m}Tc-Tetrofosmin

	^{201}Tl	^{99m}Tc-MIBI	^{99m}Tc-Tetrofosmin
Rate (MBq/cell/min)			
early phase	30 ± 3 (4)	28 ± 5 (4)	38 ± 5 (4)
late phase	1.4 ± 0.2 (4)	7.3 ± 1.0 (4)	7.1 ± 1.5 (4)
Plateau value* (MBq/cell)	222 ± 20 (4)	750 ± 101 (4)	824 ± 93 (4)

Data are means ± SD (number of experiments).

*2–15% of non-specific adsorption to the tubes were included.

The results, in bar graph form, are expressed as the pooled mean for four individual tubes.

RESULTS

Cellular uptake and retention of myocardial perfusion agents

Incubation of cultured smooth muscle cells with one of the three agents tested at 37°C resulted in an exponential accumulation of activity up to an apparent plateau or equilibrium condition. After normalizing the results to a uniform extracellular loading-solution concentration, a comparison of time-dependent uptake could be made for the different agents. ^{201}Tl and ^{99m}Tc-labeled agents were accumulated in smooth muscle cells at a different rate and to a distinctly different plateau value (Fig. 1). The data gave a good fit to the biexponential curve. Note that extraction of ^{201}Tl within the first few minutes is almost equal for the ^{99m}Tc-labeled agents, but at later times ^{99m}Tc-labeled agents were accumulated to a greater extent. The maximum uptake of ^{99m}Tc-Tetrofosmin was almost 4-fold greater than that of ^{201}Tl and higher ratios persisted for accumulation times greater than 3 hours. Kinetic comparisons among the three agents are summarized in Table 1.

Cellular washout

After incubation to equilibrium, the cells were incubated in isotope-free incubation buffer. Figure 2 compares the three agents with respect to the stepwise washouts with incubation buffer. The first washout was due, at least in part, to the loose extracellular matrix. ^{99m}Tc-Tetrofosmin was washed out by the first rinsing with 65% of the initial apparent uptake value, in spite of ^{201}Tl only being washed out at 35%. Only a small amount of ^{99m}Tc-Tetrofosmin was washed out by the next, second and third rinsing steps, while ^{99m}Tc-MIBI and ^{201}Tl were released from the cells step by step.

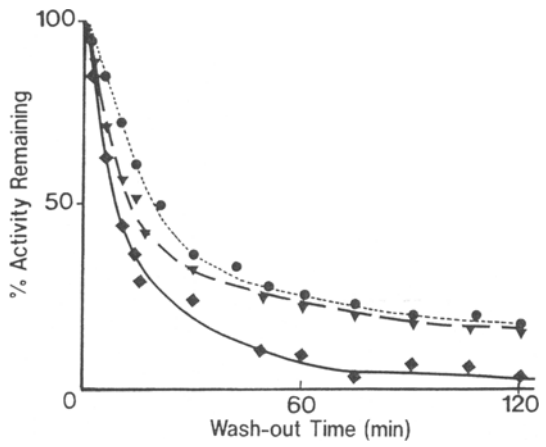

Fig. 3 Relative washout rates of myocardial perfusion agents from the smooth muscle cells.

---◆---: ^{201}Tl; ---▼---: ^{99m}Tc-MIBI; ---●---; ^{99m}Tc-Tetrofosmin

Table 2 Washout-kinetic comparison among ^{201}Tl, ^{99m}Tc-MIBI, and ^{99m}Tc-Tetrofosmin

Rate [$t_{1/2}$ (min)]	^{201}Tl	^{99m}Tc-MIBI	^{99m}Tc-Tetrofosmin
first phase	9 ± 1 (4)	17 ± 2 (4)	13 ± 3 (4)
second phase	25 ± 3 (4)	37 ± 4 (4)	36 ± 3 (4)

Data are means ± SD (number of experiments).

The cells were rinsed with cold incubation buffer twice, and then they were incubated with fresh incubation buffer at various times at 37°C (Fig. 3). Analysis of the washout curve suggested that the process may be biexponential with two compartments with a rapid initial phase, and a slower secondary component that was released continuously to 120 min of incubation. It was interesting that 17% of the ^{99m}Tc-Tetrofosmin and 15% of the ^{99m}Tc-MIBI remained in the cells at 120 min, while 96% of the ^{201}Tl was washed out of the cells.

Comparisons of the washout kinetics of these three agents are summarized in Table 2. The release of the ^{99m}Tc-labeled agents was slower than that of ^{201}Tl.

Effects of inhibitors on the uptake of myocardial perfusion agents

To determine if the cellular accumulation of these myocardial perfusion agents occurred by a similar mechanism, their uptake was monitored after chemically inhibiting selective metabolic pathways and ion transport. Conditions were chosen that would produce the least effect on the smooth muscle cells. Estimates of cellular viability by the trypan blue exclusion method showed a small effect after 90 min exposure to the inhibitor. From the cell uptake results shown in Fig.1, the 30 min-incubation with ^{201}Tl or ^{99m}Tc-agents followed by the preincubation with inhibitors for 30 min may be enough to reach the

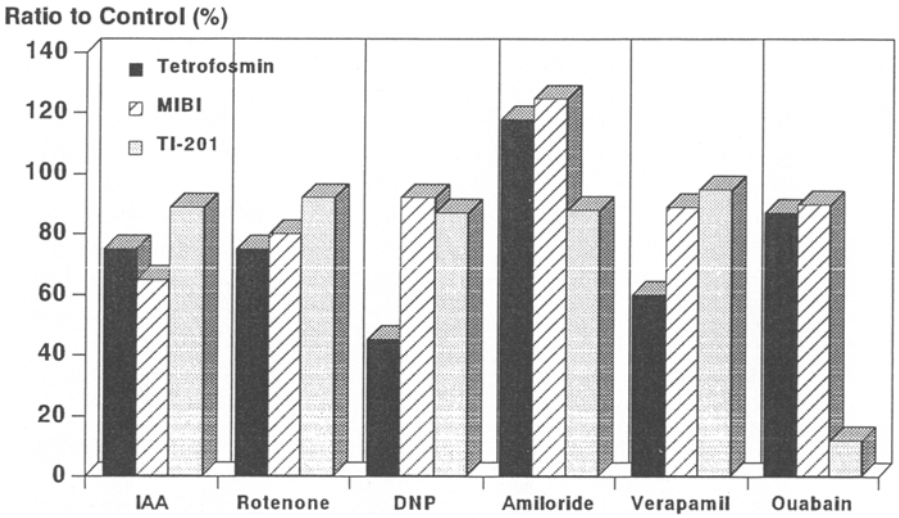

Fig. 4 Effect of metabolism or ion transport inhibitors on accumulation rate of myocardial perfusion agents in smooth muscle cells.

Each bar represents of mean of 4 individual cells in the tube. The coefficient of variation was always < 15% and is not shown in the figure for reasons of clarity.

plateau level. Following 30 min of preincubation in various metabolism inhibitors, ^{201}Tl did not demonstrate any effects. This behavior is in striking contrast to that of ^{99m}Tc-labeled agents. Figure 4 shows that DNP decreased the ^{99m}Tc-Tetrofosmin net uptake, while IAA decreased the ^{99m}Tc-MIBI net uptake ($p < 0.01$). To test for linkage to a specific transport system, uptake experiments on the three myocardial perfusion agents were performed in the presence of three different ion transport inhibitors (Fig. 4). A significant decrease ($p < 0.001$) occurred in the ^{201}Tl uptake with ouabain. The uptake of ^{99m}Tc-MIBI was not decreased by any ion transport inhibitors we investigated. On the other hand, verapamil inhibited the accumulation of ^{99m}Tc-Tetrofosmin slightly ($p < 0.05$). These effects were observed in a similar manner when the incubation medium did not contain growth factors or sodium bicarbonate.

DISCUSSION

Both ^{99m}Tc-Tetrofosmin and ^{99m}Tc-MIBI hold promise as potential myocardial perfusion ^{99m}Tc-labeled agents. Clinically they have behaved in a very similar manner although their accumulation and retention mechanism have remained obscure. We compared the characteristics of two ^{99m}Tc-labeled agents by using cultured smooth muscle cells. ^{201}Tl, a conventional myocardial perfusion agent, was also studied as a reference to compare with the two ^{99m}Tc-labeled agents.

The smooth muscle cells which we used were isolated from human aortic smooth muscle and could be maintained in a stationary phase for several generations. Myocytes were generally isolated from some experimental animals for every experiment. Cultured smooth muscle cells can be expected to have the same biochemical and physiological characteristics in any experiment. This led us to use the cultured smooth muscle cells as biological material to compare these three myocardial perfusion agents although smooth muscle cells are quite different from myocardial cells with regard to biochemical properties.

Both ^{99m}Tc-Tetrofosmin and ^{99m}Tc-MIBI are lipophilic and have a positive charge, which causes them to stick to the surface of plastic tubes. Referring to the report from Amersham plc (personal communication), we pretreated the tubes with 1% BSA/PBS. We noticed that 10–15% of the radioactivity was still stuck to the surface of the pretreated tubes. Because the non-specific adsorptions were much dependent on the incubation medium including inhibitors, we did not evaluate any differences with less than 15% deviation.

Both ^{99m}Tc-Tetrofosmin and ^{99m}Tc-MIBI exhibited very similar uptake kinetics, which were significantly different from ^{201}Tl. Both of the ^{99m}Tc-labeled agents were accumulated in the cell at a rate slower than ^{201}Tl in the early phase, but they took a little longer to reach the plateau than ^{201}Tl did. Consequently, the plateau levels of the ^{99m}Tc-labeled agents were four times that of ^{201}Tl.

The washout kinetics of ^{99m}Tc-labeled agents were also found to have similar patterns. Their washout rates were generally lower than those of ^{201}Tl. It is interesting that ^{99m}Tc-labeled agents still remained in the cell even after a 120 min-incubation period with fresh incubation buffer. A still more interesting observation was that ^{99m}Tc-Tetrofosmin was released from cells once, while ^{99m}Tc-MIBI and ^{201}Tl were washed out step-by-step by the fresh isotope-free buffer. It is well known that ^{201}Tl is washed out of the heart and redistributes again. Clinically it was also observed that very small amounts of ^{99m}Tc-labeled agents are washed out of the heart.[2] Our previous clinical comparison of the two ^{99m}Tc-labeled agents showed that ^{99m}Tc-MIBI appeared to be washed out of the heart more rapidly than ^{99m}Tc-Tetrofosmin,[1,19] but their precise pharmacokinetic values remain unclear. These results suggest that both of the ^{99m}Tc-labeled agents are retained in the cell in a different manner, while ^{201}Tl exists loosely in the cell similar to K^+.

How ^{99m}Tc-labeled agents are accumulated in the cell was also evaluated on the basis of the effect of chemical inhibitors on net uptakes. The effect of inhibitors on the ^{99m}Tc-MIBI uptake in ventricular chick myocytes was reported by Kronauge JF et al.[9] They showed that IAA and CCCP inhibited the uptake, but no effect was observed in the presence of DNP, which is an uncoupler of phosphorylation like CCCP, in our experiments. Platts EA et al. also reported the effect of cation channel and metabolism inhibitors on the uptake of ^{99m}Tc-Tetrofosmin in rat myocytes.[18] They showed that cation channel inhibitors, such as 100 μM of ouabain, 10 μM of bumetanide and 1,000 μM of amiloride, did not have any significant effect. Their report on the metabolic inhibition was partially inconsistent with our experimental results. They showed that CCCP inhibited cell uptake, indicating that ^{99m}Tc-Tetrofosmin was retained in the cell principally through the mitochondrial membrane potential. Our experimental results showed that mitochondrial electron transport inhibitors, such as rotenone, as well as DNP, uncoupler of phosphorylation, affected the cell uptake, suggesting that ^{99m}Tc-Tetrofosmin is taken up in smooth muscle cells partially by the mitochondrial electron transport energy and partially by its membrane potential. The effect of IAA on the ^{99m}Tc-Tetrofosmin net uptake was more significant in their myocytes than in our smooth muscle cells.

This discrepancy may be due to the different properties of the cells that were used in the two studies: myocardial cells contain a greater density of mitochondria than smooth muscle cells. The net uptake of ^{99m}Tc-Tetrofosmin in smooth muscle cells in our experiments was approximately 1/100 of that in myocytes in Platts' report.[18] Involvement of mitochondrial energy in the transport system would also be different from cell type to cell type,

but the precise reason is unclear.

As far as the smooth muscle cells we used are concerned, ^{99m}Tc-Tetrofosmin is taken up partially through the mitochondria by way of mitochondrial site electron transport and its membrane potential. On the other hand, the uptake of ^{99m}Tc-MIBI in the cells involves mitochondrial electron transport potentials rather than its membrane ones. The mitochondrial function plays an important role in the uptake and retention of both ^{99m}Tc-labeled agents as other reports have described, and the extent of its role was greater with ^{99m}Tc-Tetrofosmin than with ^{99m}Tc-MIBI. Furthermore, ^{99m}Tc-MIBI uptake would be more sensitive to cell glycolysis than that of ^{99m}Tc-Tetrofosmin.

Considering our experimental results together with other reports[9,18] we assume the uptake and retention mechanism of the ^{99m}Tc-labeled agents in smooth muscle cells to be as follows:

(1) ^{99m}Tc-Tetrofosmin is taken up by both mitochondrial electron transport systems and membrane potential. It is also affected by the calcium ion channel. It is partially held in the mitochondrial fraction with relatively strong affinity.

(2) ^{99m}Tc-MIBI is taken up mainly by the mitochondrial electron transport potential to a lesser extent than ^{99m}Tc-Tetrofosmin. Its retention in the cell is relatively loose.

These results suggest that ^{99m}Tc-Tetrofosmin can predict a mitochondrial viability unlike that with ^{201}Tl. On the other hand, ^{99m}Tc-MIBI uptake could be used to predict the density of mitochondria in cells rather than the mitochondrial and cell viabilities. ^{99m}Tc-Tetrofosmin is retained in the cell with a stronger affinity than ^{99m}Tc-MIBI and ^{201}Tl. Consequently, ^{99m}Tc-MIBI and ^{99m}Tc-Tetrofosmin are taken up by the cell in a similar pharmacokinetic pattern, but their accumulation reflects a different form of cell viability.

These assumptions are based on our experimental model using an established smooth cell line with homogeneous characteristics. They would not work for heart tissue which contains a higher density of mitochondria, or for a tumor of which the characteristics are far different from those of the normal cell. Furthermore, generally speaking, it is very difficult to correlate the experimental cell results *in vitro* with the clinical behavior of myocardial perfusion agents. We believe, however, that our *in vitro* pharmacokinetic data and speculation would be helpful in interpreting the accumulation of ^{99m}Tc-labeled agents in very basic biological cells.

ACKNOWLEDGMENTS

The authors thank Daiichi Radioisotopes Laboratories Ltd. and Amersham Medical Ltd. for their cooperation in making Cardiolite and Myoview available for us to use, and Dr. E.A. Platts, Dr. J.D. Kelly and Dr. R.D. Pickett for helpful and critical discussions.

REFERENCES

1. Kubo A, Nakamura K, Sammiya T, Shimizu S, Hashimoto S, Iwanaga S, et al. Phase I clinical study of ^{99m}Tc-MIBI. *KAKU IGAKU (Jpn J Nucl Med)* 28: 1133–1142, 1991.
2. Wackers FJT, Berman DS, Maddahi J, Watson DD, Beller GA, Strauss HW, et al. Technetium-99m hexakis 2-methoxyisobutyl isonitrile: Human biodistribution, dosimetry, safety, and preliminary comparison to thallium-201 for myocardial perfusion imaging. *J Nucl Med* 30: 301–310, 1989.
3. Sands H, Delano ML, Gallaghe BM. Uptake of hexakis (t-butylisonitrile) technetium(I) and hexakis-(isopropyl-isonitrile) technetium(I) by neonatal rat myocytes and human erythrocytes. *J Nucl Med* 27: 404–408, 1986.
4. Chiu ML, Herman ZW, Kronauge JF, Piwnica-Worms D. Comparative effects of neutral dipolar compounds and lipophilic anions on Technetium-99m-hexakis(2-methoxy-isobutyl isonitrile) accumulation in cultured chick ventricular myocytes. *Invest Radiol* 27: 1052–1058, 1992.
5. Maublant JC, Moins N, Gachon P, Renoux M, Zhang Z, Veyre A. Uptake of Technetium-99-teboroxime in cultured myocardial cells: Comparison with Thallium-201 and Technetium-99m-sestamibi. *J Nucl Med* 34: 255–259, 1993.
6. Piwnica-Worms D, Chiu ML, Kronauge JF. Divergent kinetics of ^{201}Tl and ^{99m}Tc-SESTAMIBI in cultured chick ventricular myocytes during ATP depletion. *Circulation* 85: 1531–1541, 1992.
7. Piwnica-Worms D, Chiu ML, Knonauge JF. Detection of adriamycin-induced cardiotoxicity in cultured heart cells with technetium-99m-SESTAMIBI. *Cancer Chemother Pharmacol* 32: 385–391, 1993.
8. Crane P, Laliberte R, Heminway S, Thoolen M, Orlandi C. Effect of mitochondrial viability and metabolism of technetium-99m-sestamibi myocardial retention. *Eur J Nucl Med* 20: 20–25, 1993.
9. Kronauge JF, Chiu ML, Cone JS, Davison A, Holaman BL, Jones AG, et al. Comparison of neutral and cationic myocardial perfusion agents: Characterization of accumulation in cultured cells. *Nucl Med Biol* 19: 141–148, 1992.
10. Cordobes MD, Starzec A, Delmon-Moingeon L, Blanchot C, Kouyoumdjian J-C, Prevost G, et al. Technetium-99m-sestamibi uptake by human benign and malignant breast tumor cells: Correlation with *mdr* gene expression. *J Nucl Med* 37: 286–289, 1996.
11. Delmon-Moingeon LI, Piwnica-Worms D, Abbeele AD Vd, Holman BL, Davidson A, et al. Uptake of the cation hexaxis(2-methoxyisobutylisonitrile)-technetium-99m by human carcinoma cell ines *in vitro*. *Cancer Research* 50: 2198–2202, 1990.
12. Piwnica-Worms D, Rao CV, Kronauge JF, Croop JM. Characterization of multidrug resistance P-glycoprotein transport function with an organotechnetium cation. *Biochemistry* 34: 12210–12220, 1995.
13. Ballinger JR, Huan HN, Berry BW, Boxon I. ^{99m}Tc-sestamibi as an agent for imaging P-glycoprotein-mediated in a rat breast tumor cell line and its doxorubicin-resistant variant. *Nucl Med Commun* 16: 253–257, 1995.
14. Rao VV, Chiu ML, Kronauge JF, Piwnica-Worms D. Expression of recombinant human multidrug resistance P-

glycoprotein in insect cells confers decreased accumulation of technetium-99m-sestamibi. *J Nucl Med* 35: 510–515, 1994.

15. Piwnica-Worms D, Chiu ML, Budding M, Kronauge JF, Kramer RA, Croop JM. Functional imaging of multidrug-resistant P-glycoprotein with an organotechnetium complex. *Cancer Research* 53: 977–984, 1993.
16. Komori T, Matsui R, Adachi I, Shimizu T, Sueyoshi K, Narabayashi I. *In vitro* uptake and release of ^{201}Tl and ^{99m}Tc-MIBI in HeLa cell. *KAKU IGAKU (Jpn J Nucl Med)* 32: 651–658, 1995.
17. Sutter CW, Joshi MJ, Stadalnik RC. Noncardiac uptake of technetium-99m MIBI. *Seminars in Nuclear Medicine* 24: 84–86, 1994.
18. Platts EA, North TL, Pickett RD, Kelly JD. Mechanism of uptake of technetium-tetrofosmin I. Uptake into isolated adult rat ventricular myocytes and subcellular localization. *J Nucl Cardiol* 2: 317–326, 1995.
19. Kubo A, Nakamura K, Hashimoto J, Sammiya T, Iwanaga S, Hashimoto S, et al. Phase I Clinical Trial of a new myocardial imaging agent, ^{99m}Tc-PPN1011. *KAKU IGAKU (Jpn J Nucl Med)* 29: 1165–1176, 1992.