Mechanism of uptake of technetium-tetrofosmin. I: Uptake into isolated adult rat ventricular myocytes and subcellular localization

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**Background.** 99mTc-labeled tetrofosmin is a new myocardial imaging agent that gives stable heart uptake. However, little is known about the mechanism of uptake in heart tissue.

**Methods and Results.** Uptake of 99mTc-labeled tetrofosmin has been examined in isolated adult rat ventricular myocytes. The time course of uptake, efflux rate, and the effect of metabolic and cation channel inhibitors have been assessed. The subcellular localization of radioactivity in ex vivo rat heart tissue was examined by differential centrifugation of ventricular homogenate. Uptake into rat myocytes was found to be rapid and plateaued at ~1.5 pmol/10^6 cells/nmol extracellular Tc-labeled tetrofosmin after 60 minutes of incubation. Uptake was temperature dependent but independent of extracellular Tc-labeled tetrofosmin concentration. Uptake at 30 minutes was inhibited by the metabolic inhibitors iodoacetic acid and 2,4-dinitrophenol protein but was not affected by cation channel inhibitors. Cells previously incubated with 99mTc-labeled tetrofosmin and then placed into fresh medium were found to have a slow efflux of activity; after 1 hour, 65% of activity was still cell associated. The localization of radioactivity in subcellular fractions indicated that the majority of activity was recovered with the cytosol. However, examination of the distribution of two mitochondrial enzymes indicated that this may have been artifactual. Use of carbonyl cyanide m-chlorophenyl-hydrazone or oligomycin to perturb mitochondrial membrane potential decreased or increased recovery in the mitochondrial fraction, respectively.

**Conclusions.** 99mTc-labeled tetrofosmin uptake by myocytes is by a metabolism-dependent process that does not involve cation channel transport. The most likely mechanism for this is by potential driven diffusion of the lipophilic cation across the sarcolemmal and mitochondrial membranes. (J Nucl Cardiol 1995;2:317-26.)

Key Words: technetium 99m-labeled tetrofosmin · myocytes · mitochondria · metabolic inhibitors · cation transport · membrane potential

[99mTc(1,2-bis[bis(2-ethoxyethyl)phosphino]ethane)O_2]^+ (99mTc-labeled tetrofosmin [Myoview]) is a new myocardial imaging agent that has been shown to localize in myocardial tissue. In experimental systems, uptake is correlated with flow, and in clinical use 99mTc-labeled tetrofosmin (99mTc-tetrofosmin) has been used to image the myocardia of patients with coronary artery disease. Myoview images are characterized by rapid heart uptake and stable retention. Good pictures may be obtained as early as 5 minutes after injection and for several hours thereafter. In addition, there is rapid clearance of background activity from the blood and surrounding organs. Despite the blood clearance, the fraction of activity retained by the heart remains stable with no evidence of redistribution in either normal myocardium or, in the case of patients with reversibly ischemic segments, during 3 to 4 hours of imaging. A number of observations suggest that 99mTc-tetrofosmin may be retained by the cellular structure of the myocardium; however, there are little data in elucidation of the mechanism of action of the cation.

Although not structurally related, other technetium cationic complexes that localize in myocardium have been shown to act in a manner different from that of thallium. Uptake of the isonitrile complex 99mTc-labeled sestamibi (99mTc-sestamibi) is believed to be due to nonspecific transfer of the lipophilic cation across the plasma membrane in response to a charge gradient.

In this study we have examined the mechanism of uptake in isolated myocytes. In addition, we have examined subcellular distribution in ex vivo myocardial tissue.
from the perfusion apparatus, chopped finely with a blade, minutes of perfusion). The ventricular tissue was then cut pressure dropped below 40 mm Hg (normally 20 to 25 perfused with a recirculating perfusate containing 0.1% remove residual blood components, the heart tissue was and incubated, with shaking, in fresh medium containing hearts excised from male Wistar rats (200 to 300 gin) were calcium-tolerant rat ventricular myocytes were prepared incubation medium after loading the cells with activity was termined by removal of duplicate 2 ml aliquots of cell suspension into a final volume of 25 ml JMEM at room temperature. The cells were then separated by centrifugation at 1000 rpm for 3 minutes, the supernatant was removed, and cell-associated activity was assessed by counting the residual pellet for radioactivity in an automatic molar concentration of \( \text{Tc-tetrofosmin} \) was assumed to be the same as the total technetium concentration. Uptake was the total technetium concentration was approximately 0.1 to 0.5 nmol/L. Because RCP was greater than 95%, the total \( \text{Tc-tetrofosmin} \) complex concentration was taken as being the same as the total technetium concentration. Uptake was counted as the uptake studies. The cell pellet was resuspended in 2 ml fresh medium and diluted further into approximately 30 ml immediately, at a time nominated as \( t = 0 \). The activity content of 2 ml aliquots of cells removed from this cell suspension was then assessed with time. Preparation of Adult Rat Ventricular Myocytes. Calcium-tolerant rat ventricular myocytes were prepared according to the method of Geisbuhler and Rovetto.\(^{11}\) Preparations of \( 99mTc \)-tetrofosmin was prepared by reconstitution of vials of Myoview (Amersham International, Buckinghamshire, U.K.) with sodium pertechnetate eluted from an Amertec II generator (Amersham International) at a radioactive concentration of approximately 27 to 135 mCi/ml 1 to 5 GBq/ml). Radiochemical purity (RCP) was determined by thin-layer chromatography as detailed in the Myoview pack leaflet and found to be greater than 95%. The molar concentration of the medium was then adjusted to 50 1% bovine serum albumin but without collagenase. Rod-shaped cells were found to settle to the bottom of the culture tube within 5 minutes. The supernatant containing round and damaged cells was removed and fresh medium was added. The settling procedure was repeated twice. The cell yield and viability were then assessed by visual inspection of the cell suspension with an inverted light microscope and hemocytometer. Viable cells characteristically showed a rod-shaped morphology with clearly defined cell edges and did not contract spontaneously. Nonviable cells were found to be either shortened, square-shaped cells that contracted spontaneously or rounded, hypercontracted cells.

**Uptake of \( 99mTc \)-Tetrofosmin into Isolated Myocytes.** The cells were resuspended at concentrations of 0.25 to 5 \( \times 10^5 \) cells/ml in JMEM plus 0.1% bovine serum albumin. All cell incubations were carried out at 37°C except for the study of the effect of temperature on uptake. The cell suspension (approximately 30 ml) was incubated with 100 \( \mu l \) \( 99mTc \)-tetrofosmin preparation at concentrations such that the total technetium concentration was approximately 0.1 to 0.5 nmol/L. Because RCP was greater than 95%, the total \( \text{Tc-tetrofosmin} \) complex concentration was taken as being the same as the total technetium concentration. Uptake was

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**Washout of Activity From Myocytes.** To examine whether activity was loosely associated or tightly bound or sequestered by the cells, the effect of replacing the incubation medium after loading the cells with activity was studied. Cells were incubated with \( 99mTc \)-tetrofosmin for 30 minutes at 37°C. The cell suspension was then diluted fivefold by addition of warm oxygenated medium and the cells were recovered by centrifugation in the same manner as the uptake studies. The cell pellet was resuspended in 2 ml fresh medium and diluted further into approximately 30 ml immediately, at a time nominated as \( t = 0 \). The activity content of 2 ml aliquots of cells removed from this cell suspension was then assessed with time.

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**Figure 1.** Differential centrifugation protocol used to isolate rat heart homogenate subcellular fractions.