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Detection of Adriamycin-induced cardiotoxicity in cultured heart cells with technetium 99m-SESTAMIBI

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Abstract. Adriamycin, a broad-spectrum cytotoxic agent useful in cancer chemotherapy, is limited by a dose-dependent cardiomyopathy mediated in part by disruption of mitochondrial energetics. Hexakis(2-methoxyisobutyl isonitrile)technetium(I) (^{99m}Tc-SESTAMIBI) is a gamma-emitting radiopharmaceutical with myocellular accumulation properties dependent on mitochondrial membrane potential. To test the hypothesis that ^{99m}Tc-SESTAMIBI could monitor Adriamycin-induced alterations in cardiac energetics, cultured chick heart cells were treated with Adriamycin and ^{99m}Tc-SESTAMIBI tracer kinetics were determined. Concentration- and time-dependent depression of ^{99m}Tc-SESTAMIBI accumulation was evident within 60 min of treatment. The apparent K_i for acute Adriamycin inhibition of tracer accumulation was 82 μM. After 24 h of treatment, Adriamycin concentrations as low as 0.1 μM demonstrated detectable inhibitory effects. The apparent K_i for this subchronic Adriamycin inhibition of ^{99m}Tc-SESTAMIBI accumulation was 18 μM. Subchronic concentration-dependent increases in adriamycin-induced myocellular injury as reflected by lactate dehydrogenase (LDH) release correlated inversely with decreases in ^{99m}Tc-SESTAMIBI accumulation. These data further support a contribution from altered mitochondrial energetics to Adriamycin-induced injury and establish a pharmacological foundation for pursuing the possibility of noninvasive imaging of chronic Adriamycin cardiotoxicity in cancer patients using ^{99m}Tc-SESTAMIBI.

Introduction

Adriamycin is an anthracycline antibiotic with antitumor activity against a broad spectrum of human tumors [54, 61]. The mechanisms of its antimitotic action have been attributed to its ability to intercolate between DNA base pairs and inhibit DNA, RNA, and protein synthesis in rapidly dividing cells [58, 62]. However, the chemotherapeutic use of Adriamycin is limited clinically by its well-known dose-dependent cardiotoxicity [24, 31]. Because myocardial cells essentially cease to divide shortly after birth, cardiotoxicity is thought to operate via mechanisms other than the antimitotic effects of Adriamycin or its metabolites [6, 39, 52]. Adriamycin has been reported to generate oxygen free radicals and increase lipid peroxidation [19, 27, 36, 40, 56, 60], to interfere with arachidonic acid metabolism and alter prostaglandin levels [48], to alter Ca^{2+}-release pathways in the sarcoplasmic reticulum [20], and to inhibit membrane-transport adenosine triphosphatases (ATPases) [39]. The mechanism of the toxic action of Adriamycin has also been attributed to Adriamycin-induced alterations in mitochondrial membrane function and energetics. Studies have shown disruption of Ca^{2+} fluxes across mitochondrial membranes [34, 38], inhibition of mitochondrial ATPases and coenzyme Q_{10} [14, 16, 21, 30], and inhibition of mitochondrial creatine kinase reassociation with mitochondrial membranes [37, 50]. At concentrations exceeding 90 μM, Adriamycin, which binds avidly to cardiolipin [13], the membrane receptor for mitochondrial creatine kinase [4, 35], has also been recently reported to block the import of precursor proteins into the matrix of isolated mitochondria [8].

Hexakis(2-methoxyisobutyl isonitrile)technetium(I) (^{99m}Tc-SESTAMIBI, Fig. 1) is a low-valence organotechnetium complex useful in myocardial perfusion and viability imaging [18, 43, 49, 53, 57]. Biophysical analysis has demonstrated that the unique lipophilic cationic properties of ^{99m}Tc-SESTAMIBI renders the complex capable of responding in a Nernstian manner to transmembrane potentials generated across the plasma membrane and mitochondrial inner membrane of living cells [5, 43, 44]. ^{99m}Tc-SESTAMIBI has been found associated with the mitochondrial compartment in a variety of cultured cell preparations in vitro [5, 7, 43] and in mammalian hearts in vivo [3]. In the steady state, ^{99m}Tc-SESTAMIBI is concentrated up to 1000-fold into the mitochondrial inner matrix

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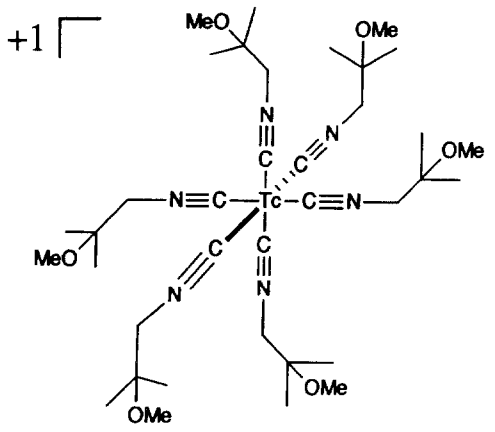

Fig. 1. Structure of hexakis(2-methoxyisobutyl isonitrile)-technetium(I)

as compared with the extracellular space by the combined effects of large negative plasma and mitochondrial membrane potentials [43], such that >95% of the whole cell activity originates from the mitochondrial compartment. The mitochondrial uncoupler carbonyl cyanide-*m*-chlorophenyl hydrazone (CCCP) releases >90% of myocellular ^{99m}Tc-SESTAMIBI [43], and depolarizing mitochondrial and plasma membrane potentials with high K buffer plus the ionophore valinomycin completely inhibit the myocellular accumulation of ^{99m}Tc-SESTAMIBI [43, 44].

The responsiveness of ^{99m}Tc-SESTAMIBI to mitochondrial energetics suggest that the imaging agent could detect mitochondrial alterations associated with Adriamycin toxicity. We tested this hypothesis using a well-characterized cultured chick heart-cell model [22, 50, 51]. Accumulation of ^{99m}Tc-SESTAMIBI was evaluated as a function of acute and subchronic exposure to Adriamycin. In addition to their established usefulness in studying the mechanisms of action of Adriamycin, cultured heart-cell preparations have been shown to be efficacious predictors of the biological behavior in vivo of this class of radiopharmaceutical [41], thereby justifying the utility of these model preparations.

Materials and methods

Tissue culture and cellular tracer studies. Techniques for obtaining monolayers of spontaneously contractile chick ventricular myocardial cells from 10- to 11-day-old chick embryo hearts disaggregated with trypsin have been described in detail elsewhere [43]. For tracer studies, cells were grown in culture media in the presence or absence of Adriamycin (as described below) in 100-mm-diameter plastic culture dishes containing seven coverslips (25-mm diameter) placed on the bottom of each dish to serve as a substrate for cell attachment and growth. Cells were maintained in a humidified atmosphere of 5% CO_2/95% air for 3 days, yielding a confluent layer of spontaneously contractile myocardial cells on each coverslip. For determination of lactate dehydrogenase (LDH) release, cells were incubated with culture media in identical 100-mm-diameter plastic culture dishes without coverslips.

Radiotracer accumulation methods have been described elsewhere [5, 43]. Briefly, coverslips with confluent cells were removed from culture media and preequilibrated for 40–60 s in tracer-free experimental buffer. Tracer uptake and retention experiments were initiated by immersion of coverslips in 60-mm glass Pyrex dishes containing 4 ml loading solution consisting of buffer with 0.1–1.0 n*M* [Tc-SESTAMIBI] (4–25 pmol/mCi; 50–100 μCi/ml). Cells on coverslips were removed at various times, rinsed three times in 25 ml ice-cold (2° C) isotope-free solution for 8 s each to clear extracellular spaces, and placed in 35-mm plastic petri dishes. Aliquots of the loading buffer and stock solutions were then obtained and placed in glass test tubes for standardizing cellular data with the extracellular concentration of ^{99m}Tc-SESTAMIBI [nM_o]. Preparations and aliquots were assayed for ^{99m}Tc activity in a well-type sodium iodide gamma counter (Omega 1; Canberra, Meriden, Conn.). Cell protein on each coverslip or culture dish was extracted in 1% sodium dodecyl sulfate with 10 m*M* Na-borate and quantified by the method of Lowry et al. [29] using bovine serum albumin as the protein standard. Data were combined with generator equilibrium equations [23], and results were expressed as femtomoles of cell-associated Tc-SESTAMIBI per milligram of protein per nanomolar extracellular [Tc-SESTAMIBI].

Experimental solutions and protocols. The control buffer for tracer studies was a modified Earle's balanced salt solution (MEBSS) with the following composition: Na^+, 145 m*M*; K^+, 5.4 m*M*; Ca^{2+}, 1.2 m*M*; Mg^{2+}, 0.8 m*M*; Cl^-, 152 m*M*; $H_2PO_4^-$, 0.8 m*M*; SO_4^{2-}, 0.8 m*M*; dextrose, 5.6 m*M*; HEPES, 4.0 m*M*; and bovine calf serum, 1% (v/v); pH, 7.4 ± 0.05. All experiments were performed in a humidified-air atmosphere maintained at 37° C. Synthesis of the radiolabeled compound ^{99m}Tc-SESTAMIBI was performed using a one-step kit formulation (kindly provided by T. R. Carroll, Cardiolite, E. I. DuPont, Medical Products Division, Billerica, Mass.); excess starting materials and reducing agent were removed by reverse-phase chromatography, and radiochemical purity was documented to be >97% by thin-layer chromatography [43].

To assay the acute effects of Adriamycin on ^{99m}Tc-SESTAMIBI accumulation, preparations were transferred to MEBSS loading buffer containing Adriamycin (1–100 μ*M*) for 60 min and cell-associated ^{99m}Tc activity was determined. For ^{99m}Tc-SESTAMIBI retention experiments, preparations were preequilibrated for 40 min in loading buffer. Adriamycin (final concentration, 20 μ*M*) was then added directly to the radiotracer loading buffer, and preparations were removed at various times for analysis of cell-associated activity. To assay the subchronic effects of Adriamycin on ^{99m}Tc-SESTAMIBI accumulation, first preparations were grown for 2 days in standard culture media, then Adriamycin (0.1–60 μ*M*) was added, and cells were incubated for an additional 24 h. The 60-min accumulation of ^{99m}Tc-SESTAMIBI was determined in MEBSS loading buffer containing the same concentration of Adriamycin as the culture conditions. The effect of subchronic Adriamycin exposure on ^{99m}Tc-SESTAMIBI unidirectional washout kinetics was evaluated by preequilibration of preparations for 60 min in tracer loading buffer followed by three rinses in ice-cold MEBSS to clear extracellular spaces and, finally, incubation of preparations in isotope-free control buffer (37° C) for the times indicated. For all experiments, fresh stocks of Adriamycin or verapamil (Sigma Chemical Co., St. Louis, Mo.) were prepared with dimethylsulfoxide (DMSO). The final DMSO concentration was <0.35% for all experiments, levels shown to have no effect on contractile activity [26], action potential configuration [26], or ^{99m}Tc-SESTAMIBI kinetics in cultured heart cells [43].

LDH release. LDH released by cells into media was measured spectrophotometrically [45] with a commercial LDH kit (340-UV, Sigma). Monolayers of cells were first grown for 2 days with standard culture media in 100-mm culture dishes. Standard media was then replaced with 4 ml media containing Adriamycin (1–60 μ*M*) or an equal amount of drug carrier (DMSO) alone, and cells were incubated for an additional 24 h. Two aliquots of 250 μl media were obtained for LDH determination and the remaining media was removed for protein determination. Cells were then disrupted in 3 ml distilled water for 15 min at 37° C. Two 250-μl aliquots of the resulting supernatant were removed for LDH analysis and cell residual was scraped from the culture dish for protein determination. Each LDH sample was diluted into 2.65 ml 0.1 *M* potassium phosphate buffer (pH 7.5) and allowed to sit for 20 min (25° C), after which sodium pyruvate (100 μl of a 2.27-m*M* stock) was added.

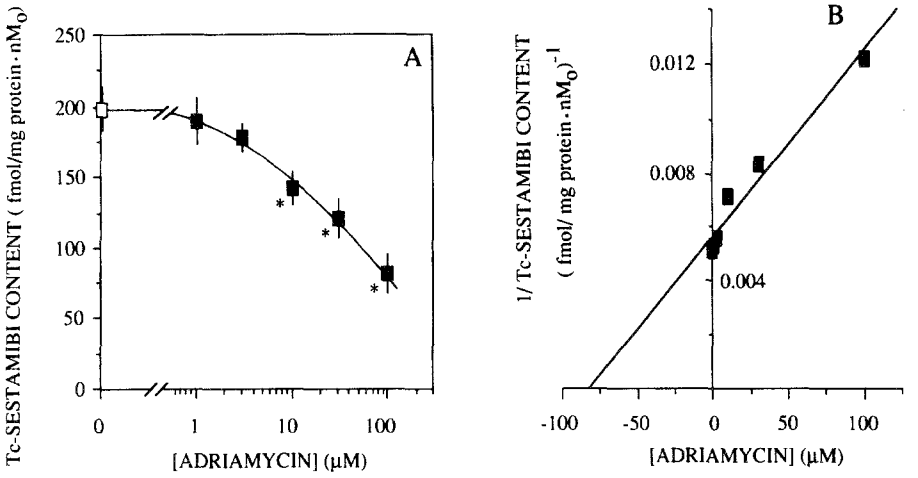

Fig. 2 A, B. Concentration-effect curve for acute Adriamycin inhibition of ^{99m}Tc-SESTAMIBI accumulation into cultured chick heart cells. **A** Preparations were incubated for 60 min in MEBSS buffer containing tracer ^{99m}Tc-SESTAMIBI in the absence (□) or presence (■) of Adriamycin at the concentrations indicated. Each point represents the mean value ± SEM for three determinations. The *asterisks* denote $P \leq 0.05$. **B** Dixon plot of the data. The apparent K_i is 82 μ*M* and the correlation coefficient is 0.957

The rate of absorbance change at 340 nm was determined spectrophotometrically. Control experiments revealed no interference from Adriamycin (30 μ*M*) on the absorbance assay. The amounts of protein and LDH extracted from media alone were determined by identical methods and subtracted from the values obtained from cultured preparations to yield the total cell-associated values. By this method, the total LDH content (both intracellular and released) normalized to the milligrams of cell protein on each dish was determined at each Adriamycin concentration.

Statistical analysis. Data are presented as mean values ± SEM for 3–4 determinations unless otherwise indicated. Statistical significance was determined by one-way analysis of variance or Student's two-tailed, unpaired *t*-test [11]. All data in each figure or panel were obtained from the same culture; in statistical analysis, experimental points were always evaluated relative to control preparations from the same culture. Data similar or identical to those illustrated in all figures and in Table 1 were obtained with preparations from at least one additional culture.

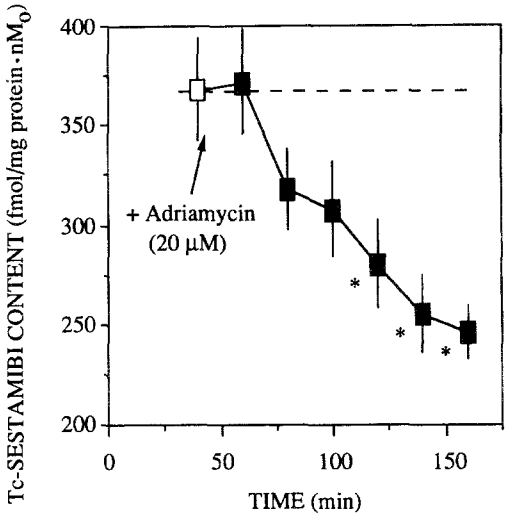

Fig. 3. Time course of Adriamycin-induced release of preequilibrated ^{99m}Tc-SESTAMIBI in cultured heart cells. Preparations were loaded with ^{99m}Tc-SESTAMIBI in control MEBSS for 40 min, then Adriamycin (20 μ*M*) was added directly to the loading buffer, and the ^{99m}Tc-SESTAMIBI retained by cells was determined at the times indicated. Each point represents the mean value ± SEM for four determinations. The *asterisks* denote $P<0.05$

Results

Under control conditions, cultured chick ventricular myocytes have been shown to accumulate ^{99m}Tc-SESTAMIBI in a membrane-potential-dependent manner to a plateau of 200–250 fmol (mg protein)$^{-1}$ (nM_0)$^{-1}$ within 40–60 min [5, 43, 45]. The mitochondrial uncoupler carbonyl cyanide-*m*-chlorophenyl hydrazone (CCCP) releases >90% of myocellular ^{99m}Tc-SESTAMIBI [43]. Incubation for 60 min in the presence of Adriamycin also reduced the steady-state accumulation of ^{99m}Tc-SESTAMIBI in a concentration-dependent manner (Fig. 2A). Acute treatment with Adriamycin concentrations of ≥10 μ*M* significantly reduced ^{99m}Tc-SESTAMIBI accumulation. A Dixon plot of the data resulted in ân apparent K_i of 82 μ*M* for acute Adriamycin-induced inhibition of the net ^{99m}Tc-SESTAMIBI uptake (Fig. 2B).

The acute inhibitory effect of a modest concentration of Adriamycin was time-dependent (Fig. 3). After loading to plateau (40 min) in control buffer containing ^{99m}Tc-SESTAMIBI, the addition of Adriamycin (20 μ*M*) gradually reduced cell-associated ^{99m}Tc-SESTAMIBI during the subsequent incubation. The net tracer content was 67% ± 4% of control values (P <0.01) after 2 h.

Subchronic treatment with Adriamycin for 24 h also reduced the steady-state accumulation of ^{99m}Tc-SESTAMIBI in a concentration-dependent manner (Fig. 4A). Incubation for 24 h in Adriamycin concentrations as low as 0.1 μ*M* significantly inhibited the 60-min accumulation of ^{99m}Tc-SESTAMIBI [control: 236.6 ± 19.4 (n = 4) vs 0.1 μ*M* Adriamycin: 191.9 ± 3.9 (n = 4) fmol (mg protein)$^{-1}$ (nM_0)$^{-1}$; P = 0.05]. An apparent K_i of 18 μ*M* for subchronic Adriamycin-induced inhibition was determined by a Dixon plot of the data (Fig. 4B).

Full kinetic analysis after 24 h of treatment with 1 μ*M* Adriamycin showed accumulation and washout half-times ($t_{1/2}$) of ~10 and ~5 min, respectively (Fig. 5). These data confirmed the lack of a significant difference in the overall uptake and unidirectional efflux rates of ^{99m}Tc-SESTAMIBI as compared with the previously published control $t_{1/2}$ values of 9.3 ± 1.5 and 8 ± 2 min, respectively [42]. In addition, ^{99m}Tc-SESTAMIBI has recently been found to be recognized as a transport substrate by the multidrug-resistance P-glycoprotein [46], a plasma mem-

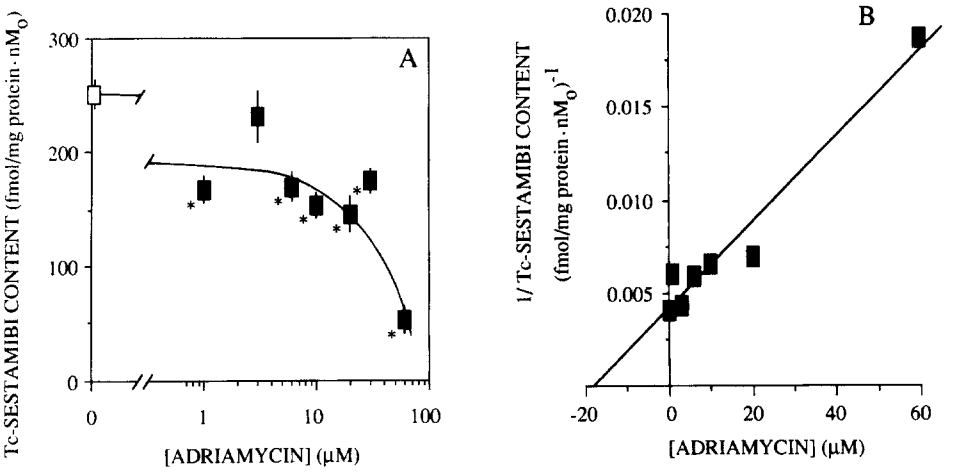

Fig. 4 A, B. Concentration-effect curve for subchronic Adriamycin inhibition of ^{99m}Tc-SESTAMIBI accumulation. **A** Preparations were grown for 24 h in culture media in the absence (□) or presence (■) of Adriamycin at the concentrations indicated, and then 60-min ^{99m}Tc-SESTAMIBI accumulation was determined. Each point represents the mean value ± SEM for four determinations. The *asterisks* denote $P<0.001$. **B** Dixon plot of the data. The apparent K_i is 18 μ*M* and the correlation coefficient is 0.954

brane efflux transporter blocked by calcium-channel inhibitors [10]. In cultured fibroblast cell lines, overexpression of P-glycoprotein has been induced by serial selection in toxic levels of Adriamycin [15]. Under our culture protocols, the addition of verapamil (10 μ*M*) had no significant effect on ^{99m}Tc-SESTAMIBI net uptake and washout kinetics (P >0.2; data not shown), suggesting that 24 h in Adriamycin (1 μ*M*) was not altering ^{99m}Tc-SESTAMIBI transport by selecting for P-glycoprotein expression in heart cells.

Visual inspection of cardiac myocytes after 24 h of treatment with Adriamycin at concentrations exceeding 30 μ*M* revealed altered contractile activity, arrhythmias, and many rounded cells. A more quantitative measure of cell damage and membrane disruption was obtained by determination of LDH release at each Adriamycin concentration (Fig. 6, Table 1). The total protein content per dish was not significantly diminished by the 24-h exposure to Adriamycin at any concentration. The total LDH content per dish was maximal at 10 μ*M* Adriamycin (Table 1). However, subchronic Adriamycin produced a graded increase in LDH release into the media at concentrations exceeding 1 μ*M*. Adriamycin at 60 μ*M*, the maximal concentration tested, resulted in 33% LDH release (Fig. 6). A readily apparent inverse relationship was demonstrated between LDH release (Fig. 6) and ^{99m}Tc-SESTAMIBI accumulation (Fig. 4 A) at progressively increasing concentrations of Adriamycin during subchronic treatment.

Discussion

Adriamycin and its derivatives are among the most efficacious antimitotics used in clinical chemotherapy [54, 61]. However, as the total dose in treated patients exceeds 500 mg/m^2, the incidence of severe cardiomyopathy increases dramatically [24]. Endomyocardial biopsy, although imperfect, may be the most direct method for monitoring Adriamycin-induced myocardial damage [2]; however, sampling errors and the invasiveness of the procedure are burdensome for serial evaluation. Thus, simpler, less costly, noninvasive methodologies have been sought.

In this regard, echocardiography and electrocardiography, although widely available, have been found to be unreliable predictors of Adriamycin-induced left ventricular (LV) failure [32]. Significant clinical experience has been gained with radionuclide angiography as a noninvasive technique to monitor patients receiving Adriamycin. However, evaluation of the LV ejection fraction (LVEF) has been criticized as a specific indicator of drug-induced cardiomyopathy because of the insensitivity of rest LVEF, the nonspecificity of exercise LVEF, and the lack of correlation with the Adriamycin dose [1, 33, 47]. Recent studies

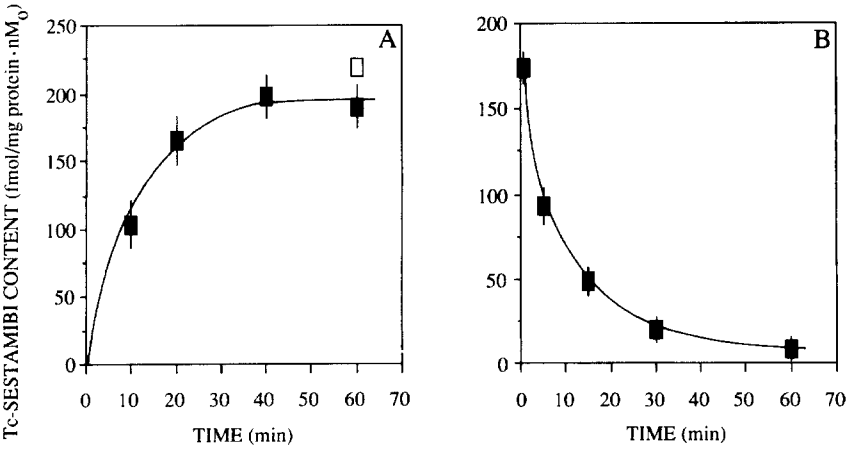

Fig. 5 A, B. Time course of ^{99m}Tc-SESTAMIBI accumulation (**A**) and unidirectional washout (**B**) after subchronic treatment with Adriamycin. Preparations were grown for 24 h in media in the absence (□) or presence (■) of Adriamycin (1 μ*M*) before determination of ^{99m}Tc-SESTAMIBI net uptake in MEBSS buffer at the times indicated. For washout experiments, preparations were preequilibrated in ^{99m}Tc-SESTAMIBI MEBSS for 60 min, then transferred to isotope-free buffer for the times indicated. Each point represents the mean value ± SEM for four determinations

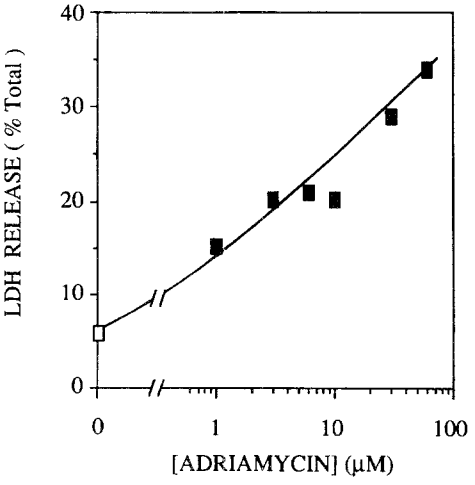

Fig. 6. LDH release during subchronic Adriamycin-induced myocellular injury. Preparations (2 days old) were washed and grown for an additional 24 h in fresh culture media in the absence (□) or presence of (■) of Adriamycin at the concentrations indicated. LDH release into the media and cell-associated LDH were determined spectrophotometrically (see Materials and methods). LDH release is presented as a percentage of total LDH. Each point represents the mean value for two independent determinations from preparations assayed in duplicate. A second independent culture showed the same results. $P<0.05$ for ≥ 3 μ*M* [Adriamycin] vs the control value

demonstrate that [^{111}In]-antimyosin may target drug-induced myocellular damage before mechanical derangement [9], and promising results have been obtained with [^{123}I]-/[^{125}I]-metaiodobenzylguanidine, the norepinephrine analog, for detection of the impaired cardiac adrenergic neuronal activity associated with Adriamycin-induced cardiomyopathy [59].

The present study targeted another component of Adriamycin-induced myocellular injury by testing the feasibility of noninvasively monitoring altered mitochondrial energetics with the lipophilic cationic radiopharmaceutical ^{99m}Tc-SESTAMIBI.

Adriamycin inhibits ^{99m}Tc-SESTAMIBI accumulation

The cultured heart-cell model revealed both an acute and a subchronic effect of Adriamycin on mitochondrial membrane potential as reflected by ^{99m}Tc-SESTAMIBI accumulation. Concentration- and time-dependent depression of mitochondrial membrane potential was evident within 60 min of exposure to the cytotoxic agent. Relatively high Adriamycin concentrations, however, were required to elicit acute effects; an apparent K_i of 82 μ*M* was found for acute inhibition of ^{99m}Tc-SESTAMIBI accumulation. On the other hand, 24 h of Adriamycin treatment inhibited tracer accumulation at more pharmacologically relevant concentrations. An apparent K_i of 18 μ*M* (10 μg/ml) for subchronic Adriamycin inhibition of ^{99m}Tc-SESTAMIBI accumulation was found. Adriamycin levels as low as 0.1 μ*M* (0.06 μg/ml) demonstrated detectable inhibitory effects. These concentrations are similar to those previously reported to produce structural cell damage such as distortion of myofibrils, swelling of mitochondria with disruption of mitochondrial cristae, vacuolar degeneration, and fragmentation and segregation of nucleoli in whole-organ and tissue-culture models of Adriamycin toxicity [17, 22, 38, 50].

We did not directly assess ultrastructural changes in this study, but rather documented myocellular membrane damage by measurement of LDH release into the supernatant. Under control conditions, 6% of the total LDH was found in the media, a value approximately 2- to 10-fold those previously reported from our laboratory [45]. However, this discrepancy can be attributed to the different experimental procedures used to determine released LDH. Prior protocols evaluated LDH release after cells had been washed and incubated in MEBSS for only 120 min, whereas the present study determined LDH released into the supernatant during a total incubation period of 24 h following a media change. In control preparations, this prolonged assay condition allowed more LDH to be spontaneously released into the media. Interestingly, the total LDH (cells and media) measured in the presence of 10 μM Adriamycin was >50% of the control values, with no change being noted in total cell protein. These data suggest that moderate Adriamycin-induced cell stress and repair mechanisms alter LDH synthesis. Nonetheless, readily demonstrated was a concentration-dependent increase in Adriamycin-induced LDH release (Fig. 6) that correlated inversely with the suppression of ^{99m}Tc-SESTAMIBI accumulation (Fig. 4 A).

Limitations and clinical context of the model

Both acute and chronic effects have been reported for Adriamycin toxicity in animals and humans [31, 54]. Acute

Table 1. Effect of subchronic exposure to Adriamycin on LDH content in cultured chick heart cells

[Adriamycin] (μ*M*)	Total cell protein (mg)	Cell LDH (units/mg cell protein)	Media LDH (units/mg cell protein)	Total LDH (units/mg cell protein)
0	3.54	264	17.0	281
1	3.46	202	39.3	241
3	3.55	329	83.4	412
6	4.03	325	85.4	410
10	3.68	351	87.0	438
30	3.45	275	111.3	386
60	3.62	253	127.1	380

Data represent mean values for two independent determinations from preparations assayed in duplicate. A second culture showed the same results

clinical toxicity appears within hours to days and has even been reported after only a single dose. Acute toxicity can manifest as nonspecific EKG changes without clinical evidence of LV dysfunction or, at the other extreme, as pancarditis with clinical and EKG changes indicative of pericardial inflammation [12, 25]. Acute toxicity is rarely irreversible [25]. Chronic cardiomyopathy clinically manifests with a slower, often delayed onset, characterized by low-output congestive cardiomyopathy that is usually unresponsive to ionotropic agents. Prior cardiac irradiation appears to accentuate the risk of chronic Adriamycin toxicity [55]. In this regard, 24-h protocols of Adriamycin treatment in cultured heart-cell models are likely to simulate relevant clinical manifestations of chronic [22, 50] rather than acute toxicity as well as the subsequent effects on ^{99m}Tc-SESTAMIBI kinetics.

One relevant limitation of cultured heart-cell preparations is their inherent cellular inhomogeneity. Nonmuscle cells invariably are present in the preparation and cannot be completely eliminated. Using monoclonal antibodies directed to heart-muscle Na/K ATPase, nonmuscle-cell contaminants are found typically to represent 5%–20% of the cell population [28]. Ultrastructural assessment of human tissue and experimental models of chronic Adriamycin toxicity often show a heterogeneous pattern of myocellular damage and necrosis [17, 59]. Although differential responses of myocardial and nonmuscle cells to Adriamycin and changes in culture media have been demonstrated [22], the finding that nonmuscle cells accumulate 10 times less ^{99m}Tc-SESTAMIBI as compared with myocytes [5] implies that the majority of the detected radioactivity reflects the energetic status of the myocellular compartment.

It is unknown at this time whether planar or qualitative single-photon emission computerized tomographic (SPECT) imaging in humans could detect a heterogeneous depression in global myocardial accumulation of ^{99m}Tc-SESTAMIBI at the onset of toxicity. Strategies to grade myocardial tracer acccumulation relative to some internal reference, such as skeletal muscle or lung, or quantitative SPECT analysis with on-line attenuation corrections may prove worthy of pursuit. Although a clear concentration- and time-dependent effect of Adriamycin on mitochondrial energetics as revealed by ^{99m}Tc-SESTAMIBI kinetics was demonstrated in the cultured heart-cell model, the exact relationship of these energetic changes to the onset of human cardiomyopathy remains to be determined. Furthermore, because arrhythmic activity was noted at concentrations of Adriamycin exceeding 30 μM, a contribution from altered plasma membrane potential or additional Adriamycin-sensitive transport mechanisms to the net cell uptake of ^{99m}Tc-SESTAMIBI in this model cannot be excluded.

Conclusions

In summary, acute and subchronic treatment of cultured heart cells with Adriamycin produced a time- and concentration-dependent suppression of ^{99m}Tc-SESTAMIBI accumulation. Because ^{99m}Tc-SESTAMIBI net uptake and retention largely reflect mitochondrial membrane potential, these findings further suggest that alterations in mitochondrial energetic function and membrane integrity contribute to or are closely associated with Adriamycin-induced cardiotoxicity. These data obtained in vitro establish a pharmacological foundation for further pursuing noninvasive imaging of chronic Adriamycin cardiotoxicity in cancer patients using ^{99m}Tc-SESTAMIBI.

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