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Effects of Ischemic-like Insult on Myocardial Thallium-201 Accumulation

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SUMMARY Despite extensive clinical use of thallium-201 (201Tl) for myocardial imaging, the effect of ischemia on myocardial accumulation and release of 201Tl independent of flow has not been fully defined. Therefore, myocardial accumulation of 201Tl in response to ischemic-like myocardial injury was assessed in vitro using the cultured fetal mouse heart preparation. Cultured fetal mouse hearts (n = 311) were subjected to injury simulating ischemia by deprivation of oxygen and oxidizable substrates for periods ranging from 15 minutes to 10 hours. The extent of irreversible injury was determined by the percentage of lactate dehydrogenase (LDH) lost from the hearts to the culture medium during recovery from injury. Injury was essentially reversible at 1 hour of insult. The fraction of 201Tl content in injured compared with control hearts was not significantly lower after 1 hour of insult. By 3 hours of insult, irreversible injury as assessed by loss of LDH was detectable and the extent of injury increased progressively through 10 hours. During the 3–10-hour period of irreversible injury, 201Tl accumulation within injured hearts compared with controls was related in a monotonically decreasing fashion to the loss of LDH as described by a mathematical kinetic model that fit the observations closely (R2 > 0.99). These results indicate that in this organ culture preparation, in which there is effectively an unlimited reservoir of 201Tl and no confounding effects of perfusion, the time-dependent 201Tl accumulation is determined by the extent of irreversible injury.

THALLIUM-201 (201Tl) is widely used as a myocardial perfusion imaging agent for the detection of coronary artery disease and myocardial infarction.1-6 Nevertheless, because of the confounding effect of perfusion on myocardial 201Tl availability, the direct impact of ischemic damage on myocardial accumulation of 201Tl has not been fully defined. Studies of myocardial 201Tl accumulation in dogs and man do not allow differentiation between the effects of reductions in coronary blood flow and the intrinsic ability of the myocardium to extract the tracer.7, 8

We used the cultured fetal mouse heart preparation to study the direct impact of ischemic-like injury on accumulation of 201Tl by diffusion. This organ culture preparation does not depend upon blood flow to provide metabolic substrate.9,10 Instead, each heart is placed on a stainless-steel grid at a gas-medium interface and is supplied with nutrients by diffusion from the culture medium. Thallium-201 availability is essentially unlimited because the hearts are bathed in a relatively large volume of thallium-containing culture medium. Therefore, myocardial 201Tl accumulation

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During ischemic-like injury can be studied independent of coronary blood flow.

Under control conditions, the hearts are incubated in 95% oxygen and 5% carbon dioxide with standard culture medium. To simulate ischemia, the fetal mouse heart is deprived of oxygen and oxidizable substrates.\textsuperscript{11-14} With this insult to the myocardial tissue, the effects of graded injury can be investigated without the confounding effects of changes in coronary blood flow that occur during ischemic injury in intact animals. Irreversible injury as indicated by LDH release can be induced in fetal mouse hearts after 3 hours of incubation in 95% nitrogen and 5% carbon dioxide with culture medium deprived of the oxidizable substrates. The extent of ischemic-like injury can be increased simply by increasing the duration of deprivation of oxygen and oxidizable substrates.

In the present study, we evaluated the impact of varying periods of ischemic-like insult on myocardial 201\textsuperscript{TI} accumulation. We compared 201\textsuperscript{TI} accumulation during ischemic-like injury with the extent of LDH release after recovery from simulated myocardial ischemia as an index of myocardial necrosis. This comparison was facilitated by a mathematical model of 201\textsuperscript{TI} ion kinetics in myocardial tissue, which allows a continuous description of the relationship between 201\textsuperscript{TI} accumulation and LDH loss as a function of time.

**Methods**

**Fetal Mouse Heart Organ Culture**

Albino mice (strain CR-1 [ICR]BR, Charles River Breeding Laboratories) that were 17 days pregnant were killed by cervical dislocation. Each litter contained six to 14 fetuses, which were removed aseptically and decapitated. The lower half of the body was removed at the level of the diaphragm. Through a right anterior thoracotomy, hearts that weighed 1.5–3.0 mg (wet weight) were removed, dissected from pericardium and great vessels, and placed on stainless-steel grids in Falcon organ culture dishes as described by Wildenthal\textsuperscript{9} and Ingwall et al.\textsuperscript{10,15} Approximately 0.5 ml of minimal essential medium (MEM) (no. 320–1095, Grand Island Biological Co.) was placed in the medium well of each culture dish.

**Experimental Protocol**

The experimental protocol is outlined in figure 1. Media containing 201\textsuperscript{TI} (3–15 \(\mu\)Ci/ml) were prepared for the control and injury groups of fetal mouse hearts. The control medium was MEM and the injury medium was a nitrogen-equilibrated, substrate-deprived MEM that did not contain the oxidizable substrates glucose, valine, leucine and isoleucine. The substrate-deprived medium does contain 292 mg/l of L-glutamine, which has been shown to be actively metabolized into carbon dioxide\textsuperscript{16-19} and which may provide significant quantities of energy for cell function, especially in glucose-depleted culture medium.\textsuperscript{20} For each solution, two aliquots each of 2.5 \(\mu\)l, 5 \(\mu\)l and 10 \(\mu\)l were set aside as standards. After overnight equilibration, MEM was removed and either MEM containing 201\textsuperscript{TI} (for controls) or substrate-deprived MEM containing 201\textsuperscript{TI} (for insulated hearts) was substituted. Control hearts were incubated with MEM, 95% oxygen and 5% carbon dioxide. To simulate ischemic injury, hearts were incubated with substrate-depleted MEM, 95% nitrogen and 5% carbon dioxide for 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8 and 10 hours. At the conclusion of the experiment, culture medium was removed and the hearts were blotted and weighed. The hearts and standard solutions were each counted for 1 minute in a gamma well scintillation counter (Searle 1195).

Immediately after simulated ischemia, the lactate concentration, measured with an Automatic Clinical Analyzer (Dupont), was 0.6 mEq/l using the method of Marbach and Weil;\textsuperscript{21} control media contained 0.1 mEq/l. The pH of the medium measured immediately after simulated ischemia was 7.37 (Corning pH meter 125); the pH of the control medium was 7.38.

In previous studies, the beating response of fetal mouse hearts has been assessed quantitatively during injury and after recovery from injury. Normal beating function has been classified as more than 60 ventricular beats/min.\textsuperscript{22} During ischemic-like injury, Roeske et al.\textsuperscript{23} showed that heart rate decreases 50% in 3 minutes and 90% in 6 minutes, and asystole occurs within 8 minutes. After recovery from as much as 10 hours of ischemic-like injury, fetal mouse hearts resume normal beating.

The ratio (R) of net accumulation of isotope in the heart relative to the concentration in the medium was calculated as cpm/mg wet weight divided by cpm/\(\mu\)l of culture medium (cpm/mg wet wt/\(\mu\)mol medium). The water content of injured and control hearts has been studied in this organ culture preparation. For each data point, 10 fetal mouse hearts were weighed, dried to constant weight at 55°C, and reweighed. The mean values (±sd) for water (mg) per mg wet weight are: controls, 0.775 ± 0.066 (n = 3); 5 hours of ischemic-like injury, 0.858 ± 0.003 (n = 3); 8 hours of ischemic-like injury, 0.866 ± 0.022 (n = 5); 10 hours of ischemic-like injury, 0.868 ± 0.007 (n = 5).
Thus, after 10 hours of insult, there is a 12% increase in the water content. However, the use of an isotope ratio rather than absolute counts/min avoids the overestimation of counts per minute at 10 hours of injury.

The $^{201}$Tl content of insulted and control hearts relative to medium (R) was plotted against time (fig. 2A). For each time point and condition, accumulation ratios were expressed as the mean ± SEM. The values of $R(t)$ in injury were divided by $R(t)$ for control conditions to yield $R$-INJURY/$R$-CONTROL as a function of time (fig. 2B).

Analysis of Thallium Accumulation Data

A three-compartment model was used to describe the time dependence of $R$, the $^{201}$Tl content of hearts relative to that of culture medium. The two mathematical functions resulting from this model contain three parameters in the case of the injured hearts and two parameters in the case of the control hearts. These parameters were then estimated from the observations of $R$ by a nonlinear least-squares program. These five parameters provide a summary description of $R$ in injury vs control in terms of net ion transport rates into and out of the myocytes and allow a smooth function to be fitted to the data (fig. 2A).

The Appendix explains in detail the compartmental model used in this work. For simplicity, it is assumed that thallium is compartmentalized in the culture medium, in the extracellular space, and in myocardial cells. Transport between medium and extracellular space is considered to be infinite in comparison to transport into and out of the intracellular space. The rate constants $K_i$ and $K_o$ govern the accumulation and release of thallium from the intracellular space.

In compartmental analysis, it is conventional and usually necessary to assume the system under study is in steady state with respect to the tracer transport mechanisms. The steady-state assumption causes the differential equations governing tracer kinetics to be linear, with constant coefficients. Because the current experiments were carried out during the varying periods of tissue injury, it was necessary to allow for non-steady state conditions. Non-steady state conditions were included in the model by allowing the transport rate $K_o$ to be time-dependent.

Lactic Dehydrogenase Experiments

In another group of cultured hearts, the percentage of lactic dehydrogenase (LDH) loss from the hearts into the culture medium was measured after 20 hours of recovery from 0, 1, 3, 5, 6, 8 and 10 hours of injury with 95% nitrogen, 5% carbon dioxide and substrate-deprived MEM. For this calculation, LDH in the medium (the numerator) is divided by the sum of LDH in the medium plus LDH in the heart (the denominator). For each LDH determination, two hearts were homogenized in 0.1 M phosphate buffer, pH 7.4, with 1 mM EDTA and 1 mM beta-mercaptoethanol. LDH activity was measured in duplicate by the method of Bernstein and Everse. The percentage of LDH loss from hearts to culture medium was expressed as mean ± SEM and was plotted against time (fig. 3A). To account for unequal variances, the percent LDH loss from hearts to culture medium was transformed by the
logit function both to linearize this injury function of time and to stabilize the variances in the LDH measurements, which were markedly different for different time points. Means and standard errors of logit (%LDH LOSS/100) were computed and plotted as a function of time (fig. 3B). The logit function is considered an appropriate transformation for measurements that have the character of percentages, and is defined as:

$$\text{logit}(x) = \ln\left(\frac{x}{1 - x}\right).$$

The equations describing the curves shown in figures 2B and 3B were used to eliminate the common parameter of time, yielding % LDH loss as a function of R-injury/R-control (fig. 4).

**Statistical Methods**

All statistical hypothesis tests were performed with the BMDP 1981 Series of Statistical Programs. The mean and SEM of all measurements made at multiple time points were computed with the BMDP7D analysis of variance program that also tests for unequal variances. With this test, the logit transformation was confirmed as a variance stabilizing transformation for the determinations of %LDH LOSS (Levene test for equal variances \(p < 0.001\) before transformation, \(p = \text{NS}\) after transformation). The linear regression of logit

**Figure 3.** (A) Percentage of LDH released from myocardium to medium 20 hours after recovery from 0–10 hours of myocardial injury. (B) Logit of percentage of LDH released from myocardium to medium 20 hours after recovery from 0–10 hours of myocardial injury. At the discrete experimental points, means of the logit (%LDH loss/100) values are plotted (squares). Error bars (computed for logit values) indicate SEM about the mean logit value. The roughly equal heights of these error bars display the variance equalizing effect of the logit transformation on these percentage data. The regression equation is Logit(%LDH loss/100)(t) = −3.167 + 0.3344(t).

**Figure 4.** Parametric plot of %LDH ultimately lost vs the fraction of control 201Tl accumulation remaining in injured hearts. The discrete data points shown were obtained from the time points when both LDH and 201Tl were measured (circles). For the experimental values, mean values are plotted. Error bars indicate SEM. Standard errors of the mean are shown for both the x-axis and the y-axis values. The smooth dashed curve relating LDH and 201Tl continuously was plotted by the computer program, using the kinetic model.
(\%LDH LOSS/100) vs time was performed by BMDP program BMDP1R, which also estimated standard errors of the regression coefficients. All computations were performed on a Digital Equipment Corp. VAX 11/780 computer running the VSM 2.4 operating system. Plots for figures 2A, 2B, 3B and 4 were made with the VERSAPLOT software on a VERSATEC 1200A printer/plotter, both from VERSATEC, Inc.

Results

Three hundred eleven fetal mouse hearts were cultured — 107 control and 204 insult preparations.

Thallium-201 Experiments

Thallium-201 accumulation was studied using 61 control and 149 insulted hearts (fig. 2A). Thallium-201 accumulated throughout the 10-hour control period in which cultured fetal mouse hearts were oxygenated and supplied with oxidizable substrates. The net isotope accumulation ratio increased from 1.49 ± 0.04 at 15 minutes to 22.00 ± 0.99 at 10 hours. During insult, 201Tl content of hearts relative to medium peaked at 4 hours and then decreased. The 201Tl accumulation ratio in injured hearts (fig. 2A) increased from 1.10 ± 0.04 at 15 minutes to 7.27 ± 0.29 at 4 hours, but thereafter decreased. Thus, after 4 hours of insult, as injury progressed, net 201Tl release occurred and progressed. Net 201Tl accumulation was significantly lower for injured than for control hearts at all time points (e.g., 1.10 ± 0.04 vs 1.49 ± 0.04 at 15 minutes) (fig. 2A).

At 1 hour of injury, the 201Tl content of insulted compared with control hearts was 66.9 ± 4.9%, compared with 73.8 ± 3.3% at 15 minutes (NS) (fig. 2B). From 3 to 10 hours, the 201Tl content was significantly lower in insulted than in control hearts. At 3 hours, 201Tl content in insulted hearts was 63.7 ± 3.8% of that in control hearts and continued to decrease as the duration of the insult increased. By 10 hours of ischemic-like injury, the amount of 201Tl remaining in injured hearts was 12.9 ± 1.2% of that in control hearts.

The parameters resulting from kinetic modeling of the data in figure 2A are shown in table 1. For the control hearts, these values can be read as follows: the Ki of 0.262 indicates that the myocytes take up 26.2% of the thallium in the extracellular space per minute while they release 0.329% of the intracellular thallium per minute. In this simpler case, we can infer an equilibrium value of R by substituting these values into equation 7 of the Appendix and observing that the time-dependent term in equation 7 disappears at equilibrium. Thus, R-equilibrium = 0.323 (1 + 0.262/0.00329) = 26.

For the injured hearts, Ki is lower. In this case, the myocytes take up 16.7% of the thallium in the extracellular space per minute. Kinetic modeling indicates a release of 0.348% of the intracellular thallium at the beginning of injury, essentially in agreement with control. Ko/(INJURY) = c2e2c1; thus, c1 = Ko (t = 0) for R-INJURY. The c2 describes the fractional rate of increase of Ko over time. The estimated value of c2 = 0.0036 indicates that Ko increases at 0.36% per minute. The larger one estimates c2 to be, the greater the rate of progression of injury per minute in terms of increase in rate of thallium release by the myocardial cells.

LDH Loss

After 20 hours of recovery from the ischemic-like insult, the percentage of LDH loss from hearts to culture medium was determined. LDH loss was measured in 46 control cultures and in 55 cultures with graded injury. LDH loss during simulated ischemia was similar to loss from control hearts at 1 hour, but exceeded control values at 3 hours (p < 0.001) (fig. 3A). Between 3 and 10 hours of insult, the loss of LDH from hearts to medium increased progressively. These values of ultimate %LDH loss vs time were well fitted (multiple R² > 0.96) by a linear regression model when the %LDH loss was transformed by the logit transformation to account for unequal variances. The resulting linear regression equation is:

\[
\text{logit} \left(\%\text{LDH LOSS}/100\right) (\text{TIME}) = -3.167 + 0.3344 (\text{TIME})
\]

The standard errors of these regression coefficients are ± 0.12 for the intercept of – 3.167 and ± 0.03 for the slope of 0.3344. In this equation, TIME is in units of hours.

Percentage of Control 201Tl Accumulation vs LDH Loss

The amount of 201Tl remaining in hearts during ischemic-like injury was related in a monotonically decreasing fashion to the quantity of LDH loss, which provided a measure of the extent of irreversible injury. By 1 hour of insult, there was no significant difference in either thallium content compared with 15 minutes of insult or LDH loss compared with control studies. During the 3–10-hour period of injury, the 201Tl content in injured hearts relative to control hearts decreased monotonically as LDH loss from hearts to culture medium increased (figs. 2B, 3A and 4).

Discussion

The results of this study of cultured fetal mouse hearts demonstrate that reduction in myocardial 201Tl content is monotonically related to the extent of myocardial necrosis as measured by LDH release. At 1
hour of insult, LDH release from hearts to culture medium was not significantly different from control. At this time, however, $^{201}$TI accumulation in insulted hearts was somewhat reduced compared with control hearts. From 3 to 10 hours of insult, there was a progressive increase of LDH release into the culture medium. LDH release during this time demonstrated a close, monotonically inverse relationship with the $^{201}$TI content in injured compared with control hearts.

The reduction in $^{201}$TI content to 74% of control by 15 minutes of insult may be related to changes induced by even a brief period of reversible ischemic-like injury. For 15 minutes to 1 hour of insult, the $^{201}$TI content relative to control (fig. 2B) decreased only slightly. The present study, however, focuses primarily on the period of insult from 3 to 10 hours, when irreversible myocardial injury occurs. At 10 hours of insult, there is an approximately 10-fold decrease in thallium accumulation in insulted hearts compared with control hearts. The thallium accumulation ratios for insulted and control hearts can be fit closely to a relatively simple kinetic model that requires only three parameters for insulted hearts and two parameters for control hearts (figs. 2A and 5). Our observations indicate that the $^{201}$TI content in insulted compared with control fetal mouse hearts decreases in a predictable manner when an ischemic-like insult causes irreversible myocardial injury.

The mathematical description of the time course of thallium accumulation during injury is surprisingly simple and may define the direction of thallous ion movement during injury. $K_i$ does not change with time in either injured or control hearts, but the correlation of $K_0$ with %LDH loss is consistent with known potassium efflux from injured myocardium. Thus, according to this model, the dominant component of net thallium accumulation in injured cells is the rate of efflux, not the rate of influx. These observations suggest that as the duration of insult increases, thallium influx does not change but the cells become increasingly leaky.

When interpreting thallium accumulation data in this organ culture preparation, the influence of beating should be considered. Because the control hearts are beating and the injured hearts become asystolic within 8 minutes of insult, the injured hearts might accumulate less thallium, in part because of a diffusion boundary layer. The diffusion boundary layer exists because convex movement of the culture medium approaches zero very close to the cell surface, as does convex movement of any fluid close to a surface. When the rate at which cells remove essential factors from this stagnant layer becomes equal to the rate at which these factors diffuse from the bulk of the medium, it is possible that the diffusion boundary layer could impede thallium accumulation. However, this seems unlikely in the fetal mouse heart organ culture preparation because the extracellular space equilibrates within 1 minute. The effective time constant for diffusion is at least an order of magnitude greater than the rates measured in these experiments for thallium accumulation in the myocardial cells. Thus, the diffusion boundary layer is probably not limiting.

The marked influence of beating in the control hearts, in contrast to asystole in the injured hearts, may be due to the periodic rapid alterations in cardiac cell membrane cation kinetics characteristic of depolarization. This periodic disruption of microscopic steady-state ion fluxes would be present only in the beating hearts and may be reflected directly in the significant difference in $K_i$ for injury and control conditions (table 1). This difference in $K_i$ was not observed in the earlier study, where both injured and control hearts beat.

This preparation derives its nutrient supply from the culture medium and utilizes deprivation of oxygen and oxidizable substrates rather than true ischemia to produce injury. Unlike ischemia in intact hearts, exogenous neural or humoral factors are not present. Despite the disadvantage of modeling ischemia incompletely, this preparation allows the study of the effects of injury on tracer kinetics in myocardium independent of blood flow.

The $^{201}$TI content in injured hearts relative to that in control hearts is measured during the acute period of injury, when the insult begins to lead to necrosis. In contrast, enzymatic and histologic markers indicate necrosis only after a relatively long period of recovery from injury (approximately 20 hours). In a recent series of experiments, we demonstrated that the thallium accumulation ratio was an earlier marker than LDH loss or histopathologic analysis in quantifying the salvage of fetal myocardium with inosine during ischemic-like injury. Insulted hearts incubated with inosine and tracer thallium demonstrated a 38% increase in the thallium accumulation ratio immediately after 10 hours of insult compared with insulted hearts not supplied with inosine. Confirmation of these results with LDH and histopathologic analysis required a 20-hour recovery period after the ischemic-like injury.

In the fetal mouse heart preparation, altering the duration of insult results in variations in the extent of damage, analogous to different regions of the focally ischemic human heart in vivo. Clinically, myocardial damage ranges from marginally deprived areas capable of full recovery to areas undergoing necrosis in the center of the most severely ischemic zone. The time course of ischemic-like injury is longer in the fetal mouse heart than in man. Therefore, it is difficult to correlate precisely the time course in these experiments with the time course of clinical infarction. Nevertheless, our data may provide insight into the application of serial quantitative imaging during evolving

**Figure 5.** Compartment model for thallium transport in the cultured fetal mouse heart preparation. The transport rate between medium and extracellular space is considered to be infinite in comparison to the transport rates into and out of myocardial cells.
myocardial infarction. Figure 2B shows that during the first 3 hours of injury, initial 201TI accumulation in the insulted myocardium remains approximately 70% of that in the control myocardium. This suggests that in a clinical setting, initial differences in 201TI content during early, evolving infarction remain largely dependent on myocardial perfusion and are minimally related to flow-independent cellular factors. At an intermediate time during infarction, the thallium image may be difficult to interpret accurately. Later, the content of 201TI in the injured relative to control myocardium begins to decrease (fig. 2B). This suggests that later in the course of infarction, cellular extraction factors become increasingly important in the generation of a 201TI defect. At 10 hours of injury, only one-tenth as much thallium remains in insulted hearts compared with control hearts.

In this preparation, the time course of thallium accumulation completely describes the outcome from ischemic-like injury and is correlated with the number of cells that ultimately die. During interventions to preserve infarcting myocardium, serial thallium accumulation data, when interpreted by kinetic modeling, may provide a useful early marker of changes in myocardial cellular viability.

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Appendix

The mathematical model used here is simply a variant of a model used with good fit previously. The earlier experiment addressed thallium accumulation in myocardial tissue after establishment of fixed ischemic-like injury of varying degree. Thallium was added to the medium after recovery from the insult, and time-dependent accumulation of thallium was observed without further insult.
In figure 5, the rate constant for thallium transport out of the cells, Ko, is shown as time-dependent, Ko(t). In unjured tissue and in tissue with a fixed, nonprogressing degree of injury, the only difference from the model shown here is that Ko is constant. Cm is the concentration of 201Tl in the culture medium and the relative volume of this compartment is so large that it is assumed to be effectively unlimited. (We ignore a slight time-dependence of Cm caused by depletion of tracer from the medium by the hearts.) The transport rates of tracer into and out of the extracellular space in the fetal mouse hearts are shown as infinite. This simplification ignores diffusion into the interstitial spaces in the hearts and means that the concentration of tracer in the extracellular space can also be represented by the same constant term, Cm. Ve is the volume of the extracellular space in the cultured fetal mouse hearts; this extracellular space is represented by the middle box in the diagram. The intracellular space is represented by the right-hand box. The volume of this space is constant, Vc. The time-dependent concentration of tracer inside the myocytal cells is Cc(t) and hence the time-dependent amount of tracer inside the cells is Ac(t) = Cc(t) Ve. Because of the assumed very rapid equilibration of tracer concentration in the extracellular space with that in the medium, the amount of tracer in the extracellular space of the hearts is the constant product, CmVe.

The two lines connecting the extracellular compartment with the intracellular compartment represent communication between these two lumped spaces, which are assumed homogeneous in compartmental modeling. This method does not attempt to separate the distinct effects of membrane permeability, active transport through ion exchange pump mechanisms, and three-dimensional diffusion within the two spaces. Instead, the approximating assumption is made that all these mechanisms can be lumped into two simplified rate parameters — Ki, the rate of flux of tracer mass into the cellular compartment from the extracellular space per unit of mass of tracer in the extracellular compartment, and Ko, the rate of flux of tracer mass out of the cellular space per unit of mass of tracer in the cellular space.

When Ki and Ko were estimated from this model in the earlier study,11 Ki was independent of injury while Ko increased with injury, as assessed by %LDH ultimately lost. This finding — that the net cellular release rate but not the net cellular uptake rate of tracer is related to the extent of irreversible injury — has allowed us to assume a constant Ki and a time-dependent Ko(t) for purposes of the present model, where injury is ongoing during the accumulation of thallium in the hearts. Under these assumptions, the amounts of tracer in the extracellular space and in the intracellular space, Ae and Ac, respectively, can be described for a compartmental model by two equations, the first a constant relationship and the second a first-order differential equation:

\[ A_e = C_m V_e \]  
\[
\frac{d(Ac)}{dt} = K_i A_e - K_o(t) A_c. 
\]

The solution to equation 2 for zero tracer in the intracellular compartment at time zero (Ac(0) = 0) is

\[ A_c(t) = \frac{K_o(t)}{K_i A_e} - \int_0^t K_o(u) du. \]

The general expression in equation 3 useful in a description of the time dependence of R, a specific form for the time dependence of Ko must be adopted. Because in the present study the logit function of %LDH loss was very closely linear in time (fig. 3B), the Ko values estimated in the earlier study were fitted to the logit of the %LDH losses found in that study. The fit was even better than the linear relationship reported previously.11 These two general relationships can be summarized as

\[ \log(\%LDH loss/100) = a + bt, \]

where the logit function is defined as

\[ \log(x) = \log_e(x/(1-x)) \]

and

\[ \log(K_o) = c + d(\log(\%LDH loss/100)), \]

where a, b, c, and d are constants and t is time. By combining these two relationships to eliminate %LDH loss, one obtains the following linear relationship between \( \log(K_o) \) and time:

\[ \log(K_o) = a + c t, \]

where a = c + da and c2 = db, both just constants. Raising e to the power of each side of this equation results in:

\[ K_o(t) = c_1 e^{c_2 t}, \]

where c1 = e\(^c\), another constant.

This model for the increase in the rate of thallium release from the cells as a function of time is useful only over the time period of the experiments, i.e., 10 hours. For arbitrarily longer time periods, this function predicts an arbitrarily large increase in the rate of thallium release and hence may not be meaningful for much longer times. One may now integrate this function for the purpose of simplifying equation 3 and can give it a particular functional form with specific parameters:

\[ K_o(t) = \int_0^t K_o(x) dx = \int_0^t c_1 e^{c_2 x} dx = \frac{c_1}{c_2} (e^{c_2 t} - 1). \]

Notice that Ko is different from Ko; it is a specific integrated form of Ko with parameters c1 and c2. Substituting this expression for Ko into equation 3 gives:

\[ Ac(t) = Ki Ae e^{-Ko(t)e^{c_2 t}} \int_0^t K_o(x) e^{c_2 x} dx \]

because Ki and Ae are constant and come outside the integration in equation 3.

Calculating R from this model, let Vt = Ve + Vc, the total wet volume of the heart cells. Let Z be the counts per minute (CPM) of thallium-201 tracer per unit of mass of tracer and let D be the density of the solute volume, Vt. Then, from the definition of R:

\[ R(t) = Z(Ac(t) + Ae) / (DVt) = Ac(t) + Ae / CmDVt. \]

Substituting Ac from equation 1 and Ac from equation 5,

\[ R(t) = Ve / DVt + K_i Ve e^{-Ko(t)e^{c_2 t}} \int_0^t K_o(x) e^{c_2 x} dx. \]

The relative volume of the extracellular space as a proportion of the total volume Ve/Vt has been determined from measurements of uptake of labeled sorbitol,10 which is excluded from the intracellular space and is well distributed in the extracellular space.29 This proportion is Ve/Vt = 0.34, and the density of these fluid volumes is approximately 1.054. This gives Ve/(DVt) = 0.323 and substitution of equation 4 in the above expression for R(t) results in the model for R with ongoing injury. As shown, the model has three parameters to be determined from the data, Ki, c1 and c2:

\[ R-INJURY(t) = 0.323(1 + K_i e^{-Ko(t)e^{c_2 t}}) \int_0^t K_o(x) e^{c_2 x} dx. \]

In the much simpler case of the control hearts, there is no ongoing injury and both Ko and Ki are constants, the two parameters of the resulting model. Substitution of Ko as a constant in equation 4 gives Ko(t) = Ko for the control hearts, and substitution of this result in equation 6 yields the model for R previously employed12:

\[ R-CONTROL(t) = 0.323[1 + K_i e^{-Ko(t)e^{c_2 t}}]. \]

The nonlinear least-squares fit of functions R-INJURY(t) and R-CONTROL(t) was performed with the Levenburg-Marquardt subroutine ZXSSQ in the International Mathematical and Statistical Library.
(IMSL) of FORTRAN subroutines for the Digital VAX 11/780 computer. The two gross transport rates, $K_i$ and $K_o$, which are calculated from the mathematical models 6 and 7 and from the actual experimental observations of $R$, are not offered as physiologic constants of the fetal mouse heart tissue. They are based on grossly simplifying assumptions and cannot be used to infer such constants as membrane permeability and rates of diffusion. Only much more detailed treatments\textsuperscript{31, 32} can separate these physical processes and identify the exact mathematical functions that define the dynamics of tracer distribution even in the simplified physical setting offered by the fetal mouse heart preparation.

Nevertheless, the functions shown in equations 6 and 7 fit the observations exceedingly well when the relevant constants are estimated from these data by least-squares techniques. These good fits are obtained with a minimum number of parameters. Therefore, these functions can be used for a summary and continuous description of the dynamics of $R$ in control hearts and in hearts undergoing ischemic-like injury to calibrate observations of $R$ to degrees of injury inferred from determinations of LDH loss.
Effects of ischemic-like insult on myocardial thallium-201 accumulation.
S Z Goldhaber, J B Newell, N M Alpert, E Andrews, G M Pohost and J S Ingwall

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