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Biological Dosimetry of Bone Marrow for Incorporated Yttrium-90

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The biological response of bone marrow to incorporated radionuclides depends on several factors such as absorbed dose, dose rate, proliferation and marrow reserve. The determination of the dose rate and absorbed dose to bone marrow from incorporated radionuclides is complex. This research used survival of granulocyte-macrophage colony-forming cells (GM-CFCs) as a biological dosimeter to determine experimentally the dose rate and dose to bone marrow after administration of ^{90}Y-citrate. **Methods:** The radiochemical ^{90}Y-citrate was administered intravenously to Swiss Webster mice. Biokinetics studies indicated that the injected ^{90}Y quickly localized in the femurs (0.8% ID/femur) and cleared with an effective half-time of 62 hr. Subsequently, GM-CFC survival was determined as a function of femur uptake and injected activity. Finally, to calibrate GM-CFC survival as a biological dosimeter, mice were irradiated with external ^{137}Cs gamma rays at dose rates that decreased exponentially with a half-time of 62 hr. **Results:** Femur uptake was linearly proportional to injected activity. The survival of GM-CFCs was exponentially dependent on both the initial ^{90}Y femur activity and the initial dose rate from external ^{137}Cs gamma rays with 5.1 kBq/femur and 1.9 cGy/hr, respectively, required to achieve 37% survival. Thus, ^{90}Y-citrate delivers a dose rate of 0.37 cGy/hr to the femoral marrow per kBq of femur activity and the dose rate decreased with an effective half-time of 62 hr. **Conclusion:** Survival of GM-CFCs can serve as a biological dosimeter to experimentally determine the dose rate kinetics in bone marrow.

Key Words: radionuclide therapy; biological dosimetry; bone marrow; dose rate

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The goal of radionuclide therapies such as radioimmunotherapy is to deliver a sufficiently large dose to the tumor without unduly affecting the critical normal organs such as bone marrow. The biological response of the bone marrow encountered in such therapies is likely to depend on several variables, such as initial absorbed dose rate to the marrow, dose rate decrease half-time, the cumulative absorbed dose received by the marrow, repair and proliferative capacities of the marrow, and bone marrow reserve (*1–4*). No clear correlation has been observed between cumulated absorbed dose and biological response of the marrow. This is not surprising because it is unlikely that one can predict marrow response with a single variable when the response depends on a complex array of variables whose relationship to the response is poorly understood. The prediction of the biological response of bone marrow to radiation insults from incorporated radionuclides will ultimately rely heavily on both biological factors (e.g., prior exposure to chemotherapeutic agents, bone marrow reserve) and physical factors (e.g., temporal dependence of the dose rate, cumulated absorbed dose to the marrow) (*2–4*). Therefore, accurate dosimetric information is a prerequisite for understanding and predicting the marrow response.

The complications in bone marrow dosimetry arise primarily from difficulties in obtaining accurate quantitative biodistribution data. Early dose estimates for bone marrow after administration of therapeutic activities assumed that the blood activity concentration (readily measurable) was the same as the bone marrow activity concentration (*5*). However, Eary et al. (*6*) subsequently showed that in dogs, the concentrations of activity in the blood and bone marrow are not the same but differ by a factor of about 5. Consequently, other investigators have implemented a marrow-to-blood activity concentration ratio of 0.2:0.4 (*7,8*) in their bone marrow dose calculations. The adequacy of these assumptions and the theoretical models used to calculate marrow absorbed dose and dose rate kinetics have not been experimentally verified. Experimental verification of calculated absorbed doses to bone marrow with devices such as thermoluminescent dosimeters and MOSFET dosimeters are impractical for obvious reasons. Therefore, one must rely on biological dosimeters to obtain information on the absorbed dose rate profiles and cumulated absorbed doses to the marrow.

Several biological dosimeters have been used over the years

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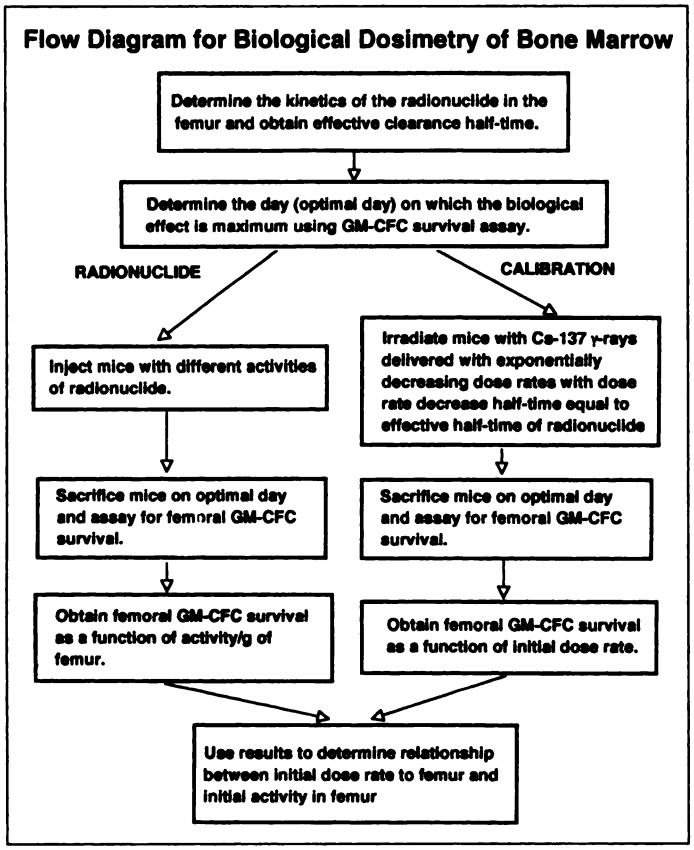

FIGURE 1. Flow diagram of protocol for using survival of granulocyte-macrophage colony-forming cells (GM-CFCs) in femoral bone marrow as a biological dosimeter for marrow irradiation with incorporated radionuclides.

to ascertain the absorbed dose received by bone marrow. Among them are induction of chromosome aberrations in lymphocytes and micronuclei in lymphocytes or reticulocytes (*9,10*). These biological dosimeters are generally useful at low doses (i.e., ~1 Gy). However, to measure higher absorbed doses to bone marrow that are encountered in radionuclide therapy (several grays), biological dosimeters that use survival endpoints are more appropriate. Therefore, our studies were undertaken to develop granulocyte-macrophage colony-forming cell (GM-CFC) survival as a biological dosimeter to elucidate the dose rate profiles and mean absorbed doses to the marrow after administration of radiochemicals.

MATERIALS AND METHODS

Experimental Model: Survival of Bone Marrow GM-CFCs

We used the survival of bone marrow GM-CFCs (*11,12*) as a biological dosimeter. GM-CFCs are progenitor cells within the bone marrow compartment. Therefore, the absorbed dose received by the bone marrow compartment can be determined by monitoring the response of these cells. To use survival of GM-CFCs as a biological dosimeter for bone marrow, several steps are required, the details of which are described later. Figure 1 illustrates the steps required to use GM-CFC survival to determine experimentally the absorbed dose received by femoral bone marrow in mice after intravenous administration of radionuclides.

Animals and Radionuclide Administration

Female Swiss Webster mice (5–6 wk of age and weighing 25 ± 2 g) (Taconic Farms, Germantown, NY), were used in our experiments. Carrier-free ^{90}Y as yttrium chloride in 0.05N HCl was obtained from DuPont NEN® Research Products (Boston, MA). The radiochemical was buffered with 0.1 *M* sodium citrate (pH = 7.0) and diluted with phosphate-buffered saline. Mice, in groups of four, were injected intravenously in the tail vein with 200 μl of solution containing the radiochemical.

Radionuclide Kinetics

Forty mice were injected intravenously with ^{90}Y-citrate (30 kBq/g body weight). At different times postadministration, the animals were killed in groups of four by an overdose of ether, and blood, femurs and muscle surrounding the femur were collected and transferred to preweighed scintillation vials. Sample weights were determined, and the vials were subsequently counted in a Beckman LS3800 liquid scintillation counter for Cerenkov radiation. After counting, the bone marrow was flushed from each femur by repeated aspiration in culture medium using a 3-ml syringe (see the section on survival of GM-CFCs), and the femur was dried, weighed and counted for radioactivity. The activities in bone and marrow compartments thus were determined.

Cerenkov Radiation Counting

Cerenkov counting was used as a nondestructive means to determine activity content in the femur, femoral bone and femoral bone marrow. Cerenkov counting is a well-known technique that has been used to ascertain activity in biological samples (*13,14*). Cerenkov radiation is generated by a charged particle such as a beta particle, traveling faster than the speed of light in a given medium (*13*). The threshold energy for a beta particle to produce Cerenkov radiation in water is about 263 keV (*13*). Therefore, radionuclides that emit high-energy beta particles such as ^{90}Y can be counted for Cerenkov radiation without destroying the tissue. The efficiency of the counting depends on the refractive index of the medium. The counting efficiency also depends on the color of the sample and the material of which the scintillation vial is made (e.g., glass, polypropylene).

To determine the counting efficiencies, we killed 10 animals, and blood (~300 μl), femurs and muscle surrounding the femurs were removed and transferred to 20-ml glass scintillation vials. The bone marrow was flushed from the femurs thoroughly with culture medium and the femurs were dried. A known activity of ^{90}Y (verified using liquid scintillation techniques) was injected into each dried femur with a microsyringe, and the same amount of activity also was added to the blood samples. For muscle samples, the same activity was divided into five 3-μl fractions and injected at different sites of the muscle. All samples were counted for Cerenkov radiation. The replicates for each type of tissue agreed to within 10%. The efficiencies for Cerenkov counting of these samples were determined by comparing the Cerenkov counts per second with the known activity injected into the samples. The Cerenkov counting efficiencies for ^{90}Y in blood, femoral bone and muscle were 0.21, 0.40 and 0.35, respectively, when the samples were placed in 20-ml borosilicate glass scintillation vials. The reliability of Cerenkov radiation counting was further examined using ^{114m}In, a radionuclide that emits high-energy beta rays as well as gamma rays (*15*). Activity determination with Cerenkov counting was within 6% of the activity determined using gamma ray spectroscopy.

Survival of GM-CFCs

The survival of GM-CFCs was determined using the procedures described by Metcalf (*11*). The animals were killed with an overdose of ether, cleaned with 70% ethanol and transferred to a laminar flow hood. The muscle tissue was removed from the femur with sterilized instruments, and the femur shaft was separated from the tibia as described by Metcalf (*11*). The epiphysis of the proximal end was cut off with scissors. A 21-gauge needle was carefully inserted through the cartilaginous sheet at the distal end of the femur shaft, and a 3-ml culture medium was gently injected into the shaft under aseptic conditions to purge the marrow into a

50-ml tube. The culture medium contains 13.37 g Dulbecco's modified Eagle's medium HG powder (GIBCO, Grand Island, NY), 980 ml distilled/deionized water, 4 ml L-asparagine (20 μg/ml; GIBCO), 2 ml DEAE dextran (75 μg/ml; Sigma Chemical Co., St. Louis, MO), 10 ml penstrep (5000 units/ml penicillin and 5000 μg/ml streptomycin; GIBCO), 3.7 g $NaHCO_3$ (GIBCO) and 2% horse serum (GIBCO) (*12*). After aspirating the medium through the shaft several times, we performed three additional 1-ml flushes with fresh medium to ensure complete removal of the bone marrow. The resulting cell suspension was placed on ice. The cells from all the animals within a group were pooled, centrifuged at 200 g for 5 min, the supernatant decanted and the cells resuspended in 5 ml of culture medium. The cells were gently pipetted with a sterile 10-ml pipette to produce a dispersed cell suspension. The mononucleated cells were separated from these cells using density gradient centrifugation. The 5-ml cell suspension was layered on top of 3.5 ml Histopaque-1077 (Sigma Chemical Co.) in a 15-ml centrifuge tube and centrifuged at 400 g for 30 min at 4°C. The mononucleated cell layer was removed carefully with a 3-ml syringe, transferred to a 15-ml centrifuge tube and centrifuged at 200 g for 5 min at room temperature. The cells were washed three times with 15 ml of culture medium and resuspended in 2 ml of double-strength culture medium. The viability of the cells was tested using Trypan blue exclusion. More than 95% of the cells were viable. A 10-μl aliquot of the cell suspension was transferred to a Coulter counting vial containing 20 ml Isotone II diluent (Coulter Electronics, Inc., Hialeah, FL), three drops of Zap-O-Globin (Coulter Electronics) were added, and the cells were then counted with a Coulter Model ZM cell counter.

After preparing the mononucleated cells as described earlier, the surviving fraction of the GM-CFCs compared with unirradiated controls was determined using cell culture techniques (*12*). A double-strength culture medium with 60% horse serum was prepared and maintained in a 37°C dry bath. A 0.6% Bacto agar solution (dissolved in water and autoclaved) also was similarly maintained. Appropriate dilutions of the cells were prepared for culturing by mixing the double-strength culture medium with the required amount of cell suspension to a final volume of 2 ml. To this solution was added 2 ml of the 0.6% agar solution. One-milliliter aliquots of the final cell suspension were transferred to wells in six well-culture plates containing 1200 units of granulocyte-macrophage colony stimulating factor dissolved in 20 μl of culture medium. The plates were allowed to stand at room temperature until the agar gelled firmly (10–15 min) and transferred to an incubator at 37°C with 100% humidity, 5% CO_2 and 95% air, for 7 days to allow for colony formation. The resulting GM-CFC colonies were scored with an Olympus dissection microscope at 40× magnification, and the survival fraction compared with unirradiated controls was determined.

Optimal Day for GM-CFC Survival Assay

The animals must be killed on the day postinjection when GM-CFC survival is at a minimum. To determine this optimal day, we intravenously injected 10 groups of four animals with an equal amount of radiochemical (30 kBq/g mouse weight) on 1, 2, 3, 5, 7, 9, 12, 14, 17 and 20 days before the kill date. An additional two untouched groups were maintained as controls. All groups were killed on same day and assayed for GM-CFC survival. The survival fraction compared with controls was plotted as a function of time postinjection.

Calibration of the Biological Dosimeter

An essential component of our studies was calibrating the biological dosimeter (GM-CFC survival). This was accomplished using our custom-designed low-dose-rate ^{137}Cs irradiator, which facilitates delivery of exponentially decreasing dose rates that are similar to the dose rates delivered to the mouse femur by internal ^{90}Y-citrate (*16*). Mice were caged in groups of four and the cages were placed on shelves within the irradiator cabinet at different distances from the ^{137}Cs source. The initial dose rate (r_o) to the top cage was set to 8.0 cGy/hr, and the cages below received initial dose rates of 2.8, 1.3 and 0.68 cGy/hr, respectively. The dose rate to each cage was decreased exponentially in time with a dose rate decrease half-time of 62 hr, the time required for the dose rate to decrease by one half. This half-time was set equal to the effective half-time of the ^{90}Y in the femur because the dose rate to the femoral marrow is essentially dictated by the femur activity [cross-dose from other tissues is negligible (*17*)]. The dose rates and total doses to each cage were monitored during the irradiation period using MOSFET dosimeter probes customized for low-dose-rate measurements (Thomson & Nielsen Electronics, Ltd., Ottawa, Ontario, Canada). The animals were killed on the optimal day and the GM-CFC survival ascertained.

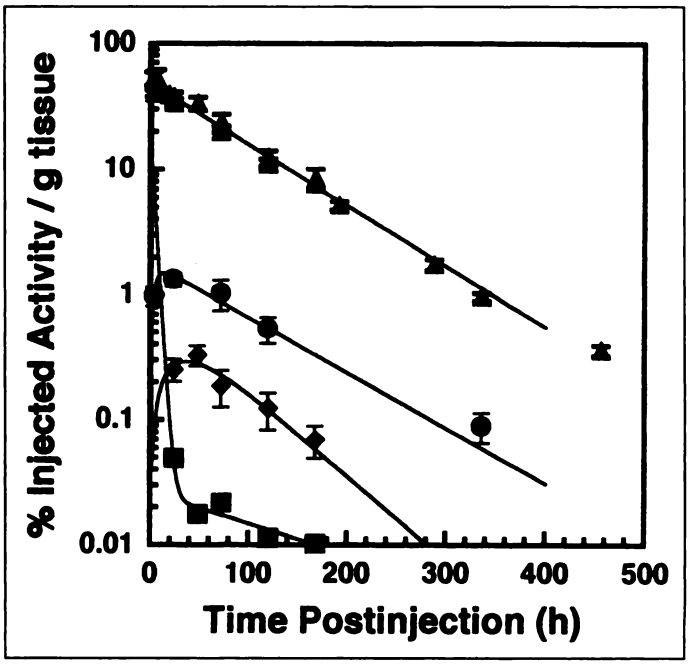

FIGURE 2. Effective uptake and clearance of radioactivity in various tissues after intravenous administration of ^{90}Y-citrate. The tissues represented are: femoral bone (▲), femoral marrow (●), muscle surrounding femur (◆) and blood (■). These data represent the average of three independent experiments. Standard deviations of the mean are indicated accordingly.

GM-CFC Survival as a Function of Femoral Activity

The relationship between extrapolated initial activity (activity extrapolated to t = 0) in the femur (A_o) and survival of femoral bone marrow GM-CFC was determined for ^{90}Y-citrate. Six groups of four mice were injected with a fixed 200-μl volume containing different activities of the radiochemical. The animals were killed on the optimal day (see the results section) and assayed for GM-CFC survival. The femoral bones, having been purged of marrow for the survival assay, were dried, weighed and assayed for activity content. The extrapolated initial femur activity was obtained by correcting these activities to the time of injection using the effective half-time of the radiochemical in the femurs obtained in biokinetics experiments.

RESULTS

Radionuclide Kinetics

The biokinetics of ^{90}Y in the femoral bone, femoral marrow, muscle surrounding the femur and blood are shown in Figure 2 as a function of time postinjection. The uptake of ^{90}Y by the

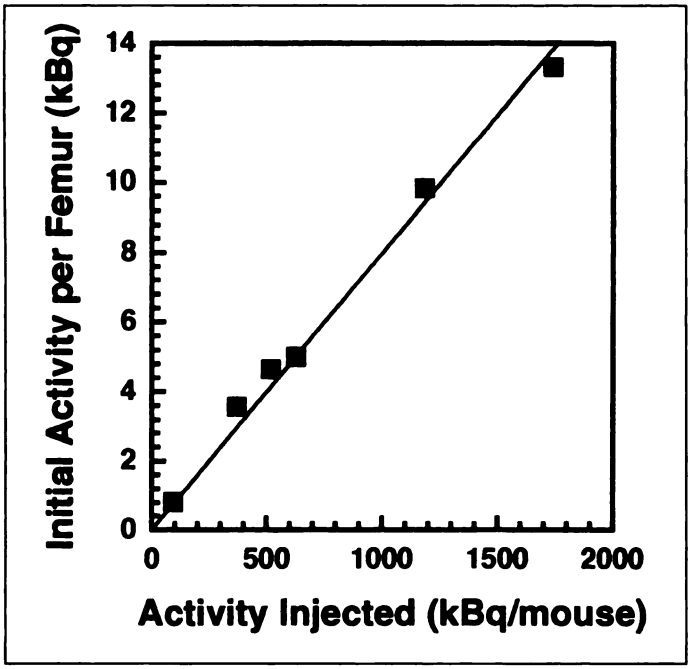

FIGURE 3. Uptake of radioactivity in the femoral bone as a function of injected activity after intravenous administration of ^{90}Y-citrate. The uptake is linearly proportional to the injected activity.

femoral bone was fast, and the bone activity cleared with an effective half-time of 62 hr. This effective half-time was close to the physical half-life of ^{90}Y (64 hr), indicating a long biological clearance half-time. The uptake of ^{90}Y in the muscle and femoral marrow was small, achieving maximum activities per gram that were only about 1% and 2% of the activity per gram in the femoral bone, respectively. The blood activity followed a two-component exponential clearance with the initial clearance being fast, followed by a slower second component. These data indicate that the absorbed dose received by the marrow was essentially due to the activity in the femoral bone. The contribution to the marrow dose from activity in the marrow and surrounding muscle was small.

The extrapolated initial activity A_o is plotted as a function of the injected activity A_{Inj} in Figure 3. The extrapolated initial activity in the femur was linearly dependent on the injected activity according to the relationship:

$$A_o = 8.00 \times 10^{-3} A_{Inj}. \quad \text{Eq. 1}$$

This indicates that 0.8% of the injected activity localizes in each femur of 5- to 6-wk-old female Swiss Webster mice.

Optimal Day

Figure 4 shows GM-CFC survival as a function of time after injection of a fixed activity of ^{90}Y. The survival fraction decreases rapidly, reaching a minimum on the seventh day postinjection. This minimum was subsequently followed by a resurgence in the GM-CFC population as demonstrated by the increasing survival fraction at longer times postinjection. These results indicate that the seventh day postinjection was the optimal day to kill the animals for the GM-CFC dose-response study.

GM-CFC Survival as a Function of Initial Femur Activity

The GM-CFC survival fraction as a function of extrapolated initial femur activity is shown in Figure 5. The data were least squares fitted to the following simple exponential relationship:

$$S = e^{-\frac{A_o}{A_{o,37}}}, \quad \text{Eq. 2}$$

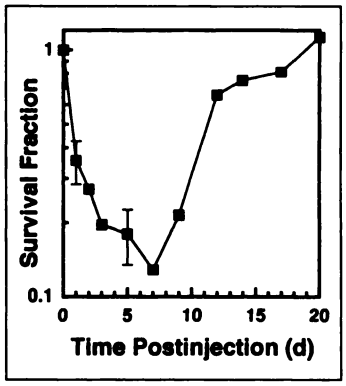

FIGURE 4. Survival of granulocyte-macrophage colony-forming cells (GM-CFCs) as a function of time postinjection of ^{90}Y-citrate. Data from two independent experiments are provided. The GM-CFC population experiences a rapid decline to the nadir on Day 7. This is the optimal day to assay GM-CFC survival for biological dosimetry.

where $A_{o,37}$ is the extrapolated initial femur activity required to obtain 37% survival. The fitted value of $A_{o,37}$ was 5.1 kBq per femur.

Calibration of the Biological Dosimeter

GM-CFC survival as a function of initial dose rate (cGy/hr) delivered by the ^{137}Cs irradiator is shown in Figure 6. The dose rate decrease half-time was 62 hr. A least squares fit of the data to

$$S = e^{-\frac{r_o}{r_{o,37}}}, \quad \text{Eq. 3}$$

where r_o is the initial dose rate (cGy/hr) and $r_{o,37}$ is the initial dose rate required to obtain 37% survival. A value of 1.9 cGy/hr was obtained for $r_{o,37}$.

Initial Dose Rate to the Femoral Marrow as a Function of Femur Activity

With knowledge of the GM-CFC survival as a function of extrapolated initial femur activity (Eq. 2) and the calibration curve (Eq. 3), one can obtain the relationship between initial absorbed dose rate r_o to the femoral marrow and the initial femur activity for ^{90}Y-citrate:

$$r_o = \frac{r_{o,37}}{A_{o,37}} A_o = 0.37\, A_o, \quad \text{Eq. 4}$$

where the initial femur activity A_o is in kBq per femur and the initial dose rate to the femur r_o is in cGy/hr. Because the femur activity was found to be proportional to the injected activity

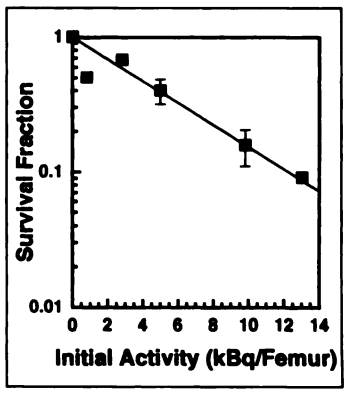

FIGURE 5. Survival of granulocyte-macrophage colony-forming cells (GM-CFCs) on the seventh day postinjection of ^{90}Y-citrate as a function of initial activity in the femur. The standard deviations of the mean for two independent experiments are shown.

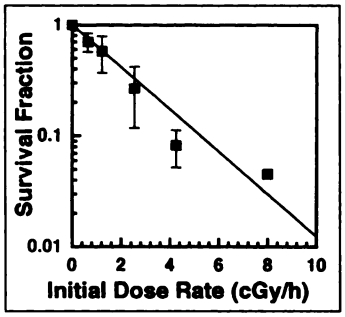

FIGURE 6. Survival of granulocyte-macrophage colony-forming cells (GM-CFCs) as a function of initial dose rate r_o when the mice were irradiated with exponentially decreasing dose rates of ^{137}Cs gamma rays. The dose rate decrease half-time was 62 hr. Animals were killed on the seventh day after the irradiation was initiated. Data are the average of two independent experiments, with the error bars representing the standard deviation of the mean.

(Fig. 3; Eq. 1), the following relationship can be obtained between initial absorbed dose rate to the femur (cGy/hr) and injected activity (kBq) of ^{90}Y-citrate:

$$r_o = 0.0030\ A_{Inj}. \qquad \text{Eq. 5}$$

Finally, the dose rate to the femur r(t) is given by Equation 6:

$$r(t) = 0.37\ A_o\ e^{-0.693t/62}, \qquad \text{Eq. 6}$$

where t is in hours, A_o in kBq/femur and r(t) in cGy/hr.

DISCUSSION

Internal radionuclides generally irradiate bone marrow chronically with exponentially decreasing dose rates that depend on the activity administered, biological clearance half-time of the radiochemical, physical half-life of the radionuclide and the properties of radiations emitted by the radionuclide (*3,4*). The biological response of bone marrow depends not only on total absorbed dose but also on the rate at which the dose is delivered and the length of the irradiation (*18*). Therefore, it is essential to determine the bone marrow dose rate pattern to predict biological response. Although computational approaches to bone marrow dosimetry exist, no experimental measurements have been made to verify the validity of such calculations. In our work, survival of GM-CFCs was developed as a biological dosimeter for bone marrow to determine the dose rate profile received by the marrow during irradiation by incorporated radionuclides.

Bone-seeking radionuclides are well suited for initial development of a biological dosimeter for bone marrow because their uptake in bone is high and they efficiently irradiate the marrow compartment. Yttrium-90, a choice radionuclide for radioimmunotherapy, is a bone seeker (*19*). As Figure 2 shows, the uptake of ^{90}Y in femoral bone is rapid and high compared with the marrow and surrounding muscle tissues. Given that the uptake in marrow and muscle are only about 2% and 1% of the bone uptake, respectively, the dose to the marrow is primarily a consequence of irradiation by the activity in the femoral bone. This absence of a significant cross-dose to the femoral marrow from activity in other tissues is a simplifying advantage in the initial development of biological dosimeters. Although the use of bone seekers and femoral marrow is advantageous in the initial stages of development, note that the cross-dose to the marrow as a whole from major organs such as the liver can be significant. Similar biological dosimetry approaches can be developed for these conditions after gaining a clear understanding in simple models such as the one described in this research.

Figure 3 shows the survival of GM-CFCs as a function of time postinjection of ^{90}Y-citrate. The nadir is observed on the seventh day postadministration, followed by a rapid recovery of the GM-CFC population that reaches control levels by Day 20. This time frame for the recovery is attributable to the onset of proliferation and is consistent with the observations of Wu and Lajtha (*20*). Given that the effective half-time of ^{90}Y in the femoral bone is 62 hr, more than 90% of the total dose is delivered to the marrow by the time the nadir is reached.

Because the dose rate patterns are similar for both the internal irradiation with ^{90}Y and the external irradiation with ^{137}Cs gamma rays (calibration), and the biological endpoint is the same in both cases (GM-CFC survival), the relationship between initial dose rate (cGy/hr) and the initial femur activity (kBq per femur) given by Equation 4 is valid for this radiochemical in 5- to 6-wk-old female Swiss Webster mice. A similar relationship must be established for each radiochemical of interest. In establishing these relationships, it is implicitly assumed that the relative biological effectiveness of ^{137}Cs 662-keV gamma rays is the same as for the energetic beta particles emitted by ^{90}Y, which have a mean energy of 935 keV. This assumption is not unreasonable considering that these low linear energy transfer radiations generally have the same biological effectiveness (*21,22*). It also should be recognized that even when the radionuclide distribution is highly nonuniform, the biological dosimeter gives a dose that is equivalent to the mean absorbed dose from ^{137}Cs that is required to produce the given effect. This dose may not be equal to the mean absorbed dose that one could calculate given a verified theoretical dosimetry model and exact distribution of the radioactivity in the femur. This, in fact, is the strength of biological dosimetry in that it always provides a quantity that can be reliably correlated with biological effect. On the other hand, the weakness of the biological dosimeter is that its calibration is radiochemical specific. In any case, these concerns are minimized by the use of radionuclides such as ^{90}Y, which emit energetic beta particles that can easily penetrate across the femurs and irradiate the marrow reasonably uniformly.

In this research, GM-CFC survival was used as a biological dosimeter to determine dose rate profiles in bone marrow. Because GM-CFCs reside within the bone marrow compartment, the dose received by the GM-CFCs in the femur is the same as the dose received by the femoral marrow. Although GM-CFC survival may or may not be truly representative of the hematopoietic stem cell survival, this assay can be used as a biological dosimeter. Although we chose survival of GM-CFCs for biological dosimetry, it is not unique in that there are a variety of other cells in the marrow that also could serve as dosimeters (*12,23,24*).

CONCLUSION

GM-CFC survival was used as a biological dosimeter to determine the dose rate pattern to murine femoral marrow delivered by intravenously administered ^{90}Y-citrate. The dosimeter was calibrated using a low-dose-rate ^{137}Cs irradiator to deliver exponentially decreasing dose rate patterns similar to those delivered by ^{90}Y-citrate. Such biological approaches to dosimetry can serve as valuable tools to determine experimentally the absorbed dose received by complex biological systems such as bone marrow. The absorbed doses thus determined can be used to verify and tailor theoretical bone marrow dosimetry models.

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An Alternative Method to Normalize Clinical FDG Studies

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An alternative method of determining the integrated input function, necessary in the quantitative [^{18}F]fluorodeoxyglucose (FDG) autoradiographic model, has been developed. Using erythrocytes as reference tissue, researchers require only one blood sample after injection of FDG to obtain the integrated input function. **Methods:** The amount of FDG-6-PO_4 in the erythrocytes is proportional to their exposure to FDG, that is, the integrated input function. Free FDG is removed by washing the erythrocytes twice. Inter- and intraindividual differences of the metabolic rate of erythrocytes are corrected for by an in vitro incubation with a known amount of FDG. **Results:** Validation of the proposed method was done by correlating the integrated input function, based on the glucose metabolism of the erythrocytes, to the integrated input function obtained by multiple venous blood samples. The new method provides the integrated input function with an accuracy better than ±8%. **Conclusion:** By using erythrocytes as a reference tissue, researchers can determine the integrated input function in the quantitative FDG autoradiographic model with an accuracy sufficient for clinical PET studies. The simplicity of the method also makes it suitable for FDG studies on small children. With two samples, the method can also be used for a simplified graphical Patlak analysis.

Key Words: erythrocytes; fluorine-18-fluorodeoxyglucose; integrated input function; normalization

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The problem of finding a simplified way of normalizing FDG-PET measurements has been addressed by different groups. The orthodox straightforward way is to use arterial blood samples to calculate the integrated FDG-input function. This, however, requires numerous samples and is a slightly traumatic experience for the patient. Phelps et al. (*1*) showed that the arterial samples could be replaced by venous samples. The blood activity in the aorta, measured with the PET scanner, has been used for the input function when the aorta is included in the field of view (*2*). Takikawa et al. (*3*) also developed a method using a population-based arterial blood curve.

A highly simplified method adopted by several groups is to only use the injected activity divided by body weight (SUV_{bw}) or body surface area (SUV_{bsa}). There are specific problems when using these indices (*4,5*), even after correcting for blood glucose concentration. A further discussion on these indices can be found in an editorial by Fischman AJ, et al. (*6*). When a reference tissue is used to normalize an FDG measurement, cerebellum seems to be the best choice (*7*). This, however, often requires an additional PET scan.

This article describes a new method based on the glucose metabolism of the erythrocytes, requiring only one blood sample taken in the middle of the PET scan to provide the integrated input function.

THEORY

Definitions of the symbols used in this article are listed in Table 1.

Consider an equation describing the metabolic rate (MR) of