Bone Marrow Dosimetry in Rats Using Direct Tissue Counting After Injection of Radioiodinated Intact Monoclonal Antibodies or F(ab')$_2$ Fragments

Franz Buchegger, Yves Chalandon, André Pèlegrin, Norman Hardman, and Jean-Pierre Mach

Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland and Ciba-Geigy AG, Section of Biotechnology, Basel, Switzerland

Normal rats were injected intravenously with ^{131}I- and ^{125}I-labeled intact murine and chimeric mouse-human monoclonal antibodies directed against carcinoembryonic antigen or with the corresponding F(ab')$_2$ fragments. At different times after injection, individual animals were killed and radioactivity of blood and major organs, including bones and bone marrow, was determined. Ratios comparing radioactivity concentration in different tissues with that of bone marrow were calculated and found to remain stable during several effective half-lives of the antibodies. Mean bone marrow radioactivity was 35% (range, 29%–40%) of that of blood and 126% (range, 108%–147%) of that of liver after injection of intact Mabs or F(ab')$_2$ fragments. In nude rats bearing human colon carcinoma xenografts producing carcinoembryonic antigen, relative bone marrow radioactivity was slightly lower than that in normal rats.

J Nucl Med 1991; 32:1414–1421

Bone marrow is one of the most radiosensitive organs. When cancer patients receive intravenous injections of radiolabeled monoclonal antibodies (Mabs) for the purpose of radioimmunotherapy (*1–5*), high radiation doses are delivered to bone marrow, since blood circulation is intense in it. Such radiation doses are even increased when radiolabeled Mabs accumulate preferentially in bone marrow as in the case of Mabs which either react with bone marrow cells (*6*) or are denatured by radiolabeling procedures. In any case, bone marrow radiation toxicity represents the major limiting factor in dose escalating therapeutic protocols using radiolabeled antibodies (*1–5,7*). Clinically, bone marrow dosimetry after injection of radiolabeled antibodies is difficult to perform in patients and leads to highly variable results depending on the method of calculation used (*2–5*).

Received July 10, 1990; revision accepted Dec. 21, 1990.

For reprints contact: Franz Buchegger, MD, Institute of Biochemistry, University of Lausanne, CH-1066 Epalinges, Switzerland.

In the present study, we have measured bone marrow radioactivity using direct counting of tissues from normal rats and from tumor-bearing nude rats after intravenous injection of radiolabeled intact Mabs or F(ab')$_2$ fragments directed against carcinoembryonic antigen (CEA). Radiation doses to bone marrow and different organs were calculated from time course radioactivity distribution studies in normal rats. The results were also compared with dosimetric studies performed in tumor-bearing nude mice in the course of successful radioimmunotherapy (*8, 9*). A good correlation was found for all organs analyzed in the two species. In mice, however, radiation doses to bone marrow could not be measured. A good correlation was also obtained with experiments performed on dogs, where bone marrow radioactivity was measured 24 hr after injection of ^{125}I-labeled irrelevant Mabs (*6*).

These experiments should be helpful for clinical dosimetric calculations. Data obtained in one patient with colon carcinoma metastasis in the liver, who was injected with therapeutic doses of ^{131}I intact anti-CEA antibodies, suggest that this may indeed be the case.

MATERIALS AND METHODS

Monoclonal Antibodies

Intact antibodies and F(ab')$_2$ fragments against two specific determinants of CEA have been used: Mab 35 [(*10*), directed against epitope Gold 2 (*11*)] and two chimeric human-mouse molecules derived from mouse Mab CE25-B7 [(*8,12*), directed against epitope Gold 4, (*11*)]. These Mabs had been selected according to criteria required for diagnostic or therapeutic injection in patients (*13–15*), including high binding rate to purified CEA and *no* binding to: (a) cross-reacting antigens NCA-55 and NCA-95, (b) biliary glycoprotein, and (c) fresh human granulocytes (*16*).

Preparation of Intact Mabs and F(ab')$_2$ Fragments

The intact Mab 35 (mouse IgG$_1$ isotype) and one human mouse chimeric Mab (human IgG$_2$ isotype, derived from Mab CE25-B7) were purified from mouse ascites by ammonium sulfate precipitation and ion-exchange chromatography (*16*). Chimeric Mab CGP 44290 (*12*), (human IgG$_4$ isotype, derived from

CE25-B7) was purified from culture medium by the manufacturer (CIBA-GEIGY, Basel, Switzerland). $F(ab')_2$ fragments of Mab 35 and of both chimeric Mab were obtained from purified antibodies by digestion with pepsin (Sigma, St. Louis, MO; pepsin 2-4% (w/w), pH 4, 6–22 hr at 37°C) and fractionation on Sephadex G-150 (Pharmacia, Uppsala, Sweden) and ion-exchange chromatography (*16*). $F(ab')_2$ fragments of chimeric Mab of IgG_4 subclass were found to be unstable in nude mice experiments and were successfully replaced by the $F(ab')_2$ of chimeric Mab of IgG_2 subclass.

Labeling of Mabs and $F(ab')_2$ Fragments and Injection into Rats

Antibodies (intact or $F(ab')_2$) were labeled by the chloramine T method with ^{131}I or ^{125}I to a specific activity of 0.8–0.9 $\mu Ci/\mu g$. Binding of radiolabeled intact Mabs and $F(ab')_2$ to purified CEA insolubilized on CNBr-Sepharose (Pharmacia), determined as described (*16*), ranged from 78% to 92%. Nonspecific binding to irrelevant proteins on CNBr-Sepharose was below 2%. Five to 15 μCi of ^{125}I and ^{131}I labeled Mabs, either intact or $F(ab')_2$, were mixed together and each fraction was adjusted to 20 μg protein with unlabeled, but otherwise identical protein and injected intravenously into rats.

Normal Rats and Tumor Model in Nude Rats

For biodistribution studies, normal female rats (OFA-SD, IOPS, from Iffa Credo, l'Arbresle, France) 9–11 wk of age, weighing between 240 and 308 g (mean 257 g) were used. For comparison, biodistribution was measured in female nude rats (LEW/Mol rnu/rnu), Mollegaard, Skensved, Denmark) aged 7–10 wk and weighing between 126 and 160 g (mean 140 g) bearing human CEA producing colon carcinoma. Nude rats were pretreated with 500 rad whole-body external irradiation (for prevention of human xenograft rejection) 2 days before receiving the human colon carcinoma transplants. Human colon carcinoma T380 (*17*) was transplanted into the right flank and human colon carcinoma LS174T (ATCC, Rockville, MD) into the left flank of the rats. Fifteen to 20 days after tumor transplantation, animals were injected intravenously with radiolabeled intact Mabs or $F(ab')_2$ fragments.

Biodistribution of Radiolabeled Intact Mabs and Fragments

At different times after injection (see Tables 1 and 2), individual rats were killed. About 2 ml of blood were taken and normal organs including six bones per animal (femurs, tibias and humeri) were dissected and radioactivity was determined per gram of tissue. Then, both ends of the six bones were clipped off and bone marrow chased out using metal wires of different diameters. Bone marrow was collected on carefully weighed, small pieces of filter paper excluding any bone particles. Fifty to 100 mg of bone marrow were collected from each animal in three different fractions (fraction 1: left femur and tibia bone marrow; fraction 2: right femur and tibia bone marrow; fraction 3: bone marrow from both humeri).

Eight tumor-bearing nude rats were similarly injected with either intact antibodies or $F(ab')_2$ fragments and analyzed for tissular radioactivity distribution at times indicated in the Results section.

Dosimetry

Calculation of radiation doses was based on concentrations of radioactivity measured in bone marrow and other tissues at different times after injection of radiolabeled Mabs in normal rats assuming uniform distribution of antibody in all organs as anticipated from the "Medical Internal Radiation Dose" (MIRD) calculations (*18*). The injected diagnostic doses of ^{125}I- and ^{131}I-labeled antibodies or fragments were extrapolated to therapeutic doses of ^{131}I radioactivity, allowing comparison with previously reported dosimetry results in mice.

The integral activity in $\mu Ci \times hr$ was calculated per gram of normal organs and blood. Tissue absorbed β-radiations were then calculated according to (*19*):

$$D_\beta = 2.13 \times \mu Ci/g \times hr \times E_\beta \text{ rad}$$

[E_β of $^{131}I = 0.19$ g/(μCixhr)]

Additional γ-radiation was assumed to be equally distributed in whole animals. Calculations including the six major γ-radiation energies emitted by ^{131}I indicated that whole-body γ-radiation represents approximately 10% of the whole-body β-radiation for a 30-g mouse and about 16% for 240–308-g rats (*19*). Similarly, for an average 65 kg patient treated with intact ^{131}I-labeled Mabs, γ-radiation delivered to the central parts of the body was calculated to represent 84% of the whole-body β-radiation (*19.*).

RESULTS

Tissue Distribution of Intact Mabs and Fragments in Normal and Tumor-Bearing Nude Rats

Nine OFA rats were injected with two radiolabeled intact anti-CEA Mabs (^{125}I-chimeric Mab of human IgG_4 subclass and ^{131}I-Mab 35 of mouse IgG_1). Individual animals were killed at different times after injection and tissue radioactivity distribution was measured and expressed in percent of injected dose (% ID) after correcting for physical half-life of the iodine isotopes (Table 1). The decrease of tissue radioactivity after injection of both intact antibodies revealed two phases in most organs. In blood, we observed an initial half-life (lasting for approximately 1 day) of 26 and 15 hr, followed by slower half-life of 194 and 100 hr for Mab 35 and chimeric Mab, respectively.

For bone marrow, the figures shown in Table 1 represent the mean bone marrow radioactivity concentrations of individual rats. Half-lives of intact antibodies in bone marrow were similar to those observed in blood with initial half-lives of 24 and 20 hr followed by slower half-lives of 149 and 88 hr for Mab 35 and chimeric Mab, respectively. Comparing bone marrow from different bones (data not shown), revealed that bone marrow of left and right tibia and femur contained very similar radioactivity amounts, with a mean difference of 0% and 3% for each of the two intact antibodies. When bone marrow from both humeri was compared with that of femurs and tibias, however, the mean radioactivity concentration in humeri was 24% and 23% lower for intact Mab 35 and for chimeric Mab, respectively. This was statistically significant with $p = 0.03$ and $p < 0.007$ for Mab 5 and chimeric Mab, respectively, using two factors-analysis of variance.

Muscles behaved differently from other organs with maximum radioactivity concentration reached at 24 hr after injection only, followed by a slow decrease.

TABLE 1
Time Course Tissue Distributions Measured in Normal Rats Injected with Two Intact Mabs Labeled with Two Iodine Isotopes

	Time after injection (in hr)								
	1	4	8	12	24	48	72	96	144
Intact Mab 35									
Bone marrow	1.23*	1.73	1.32	1.53	0.76	0.53	0.34	0.35	0.31
Blood	3.79	3.77	3.32	2.94	2.13	1.50	1.33	1.10	1.07
Kidney	0.87	1.01	1.00	0.86	0.62	0.33	0.31	0.30	0.29
Lung	1.90	1.69	1.45	1.43	0.85	0.53	0.50	0.47	0.51
Liver	1.15	0.98	0.99	1.08	0.60	0.34	0.31	0.30	0.32
Heart	0.65	0.93	1.20	0.77	0.55	0.36	0.33	0.30	0.29
Spleen	0.80	0.66	0.57	0.61	0.38	0.25	0.24	0.19	0.22
Bone	0.23	0.35	0.33	0.28	0.19	0.12	0.11	0.09	0.11
Muscle	0.05	0.08	0.09	0.11	0.14	0.09	0.09	0.10	0.10
Intact chimeric Mab									
Bone marrow	1.40*	1.49	1.11	1.17	0.50	0.31	0.18	0.20	0.13
Blood	3.45	2.97	2.31	2.02	1.16	0.77	0.65	0.61	0.39
Kidney	1.58	1.52	1.34	1.22	0.65	0.34	0.29	0.28	0.19
Lung	1.95	1.58	1.41	1.28	0.67	0.36	0.32	0.34	0.23
Liver	0.99	0.80	0.72	0.69	0.32	0.18	0.15	0.16	0.11
Heart	0.67	0.88	1.01	0.67	0.38	0.21	0.19	0.20	0.12
Spleen	1.53	0.70	0.76	0.56	0.33	0.16	0.14	0.13	0.10
Bone	0.25	0.32	0.29	0.25	0.14	0.07	0.06	0.07	0.05
Muscle	0.06	0.09	0.08	0.11	0.11	0.07	0.06	0.07	0.05

* Percent injected radioactivity (after correction for physical half-life) per g of tissue in rats dissected at different times after injection of either intact Mab 35 or intact chimeric human-mouse Mab.

Eight other OFA rats were injected with radiolabeled $F(ab')_2$ fragments of the two anti-CEA Mabs. $F(ab')_2$ fragments of chimeric Mab of human IgG_2 subclass were labeled with ^{125}I and the fragments of murine IgG_1 Mab 35 with ^{131}I. For the two antibody fragments, both in blood and bone marrow, mean initial half-lives of 6 hr (range, 5.7–6.5 hr) followed by half-lives of 12 hr (range, 11.7–12.3 hr) were observed (results calculated from data of Table 2).

The data in Table 2 represent the mean bone marrow radioactivity concentrations of individual rats. Comparison of radioactivity concentration in bone marrow from different bones (data not shown) revealed that bone marrow of left and right tibia and femur contained very similar amounts of radioactivity with mean differences of 0% and 3% for each of the two Mab $F(ab')_2$. Mean humeral bone marrow radioactivity, however, was 14% and 17% lower for $F(ab')_2$ fragments of Mab 35 and of chimeric Mab, respectively, as compared to that of femurs and tibias. Statistical comparison using two factors-analysis of variance indicated that this was significant with $p < 0.007$ and $p < 0.01$ for Mab 35 and chimeric Mab, respectively.

Four nude rats, bearing two human colon carcinoma xenografts each, were injected with the same mixture of intact antibodies as that given to normal rats. Two rats were killed and analyzed for tissue radioactivity distribution at Day 1 and the two others at Day 3 after injection (Fig. 1A-B). Intact chimeric Mab again had a shorter half-life than Mab 35 in normal organs and tumor. Tumor-to-normal tissue ratios were very similar for both antibodies, except that the ratio comparing tumor-to-kidney was slightly higher for murine Mab 35.

Four other nude rats bearing the same two human tumor xenografts were injected with the same $F(ab')_2$ fragments as the normal rats. Two animals were dissected after 8 hr and 2 others after 24 hr. Radioactivity distribution expressed in % ID/g of tissue of two of these animals bearing colon tumor T380 transplants of 0.3 and 0.47 g and LS174T tumors of 0.8 and 1.5 g are shown in Figure 1C-D. In the two other rats, T380 tumors were larger (1.9 and 2 g) and % ID/g of T380 tumor were lower (between 2.5% and 3.8%, results not shown), as observed earlier for large tumors in nude mice (*9*). The radioactivity concentrations in normal tissues, however, were similar to those obtained in nude rats bearing smaller tumors.

In general, the percent injected radioactivity per gram tissue in all normal organs of tumor bearing nude rats were higher than those observed in normal rats. This is probably due to the smaller body size of these animals (mean weight of nude rats was 140 g versus 257 g for normal rats).

Organ-to-Bone Marrow Ratios in Normal and Tumor-Bearing Nude Rats

Organ-to-bone marrow ratios for all dissected normal rats were calculated and found to remain stable (except when comparing muscle and heart with bone marrow)

TABLE 2
Time Course of Tissue Distributions Measured in Normal Rats Injected with Two Mab F (ab')$_2$ Fragments Labeled with Two Iodine Isotopes

	Time after injection (in hr)							
	0.25	1	2	4	8	12	24	48
Mab 35 F(ab')$_2$ fragments								
Bone marrow	1.27*	0.91	1.06	0.80	0.47	0.60	0.31	0.06
Blood	4.40	4.07	3.48	3.12	1.88	1.73	0.85	0.20
Kidney	1.41	1.68	1.39	1.55	0.90	0.81	0.51	0.15
Lung	1.88	1.47	1.60	1.19	0.86	0.95	0.44	0.13
Liver	1.02	0.85	0.84	0.79	0.50	0.50	0.24	0.07
Heart	0.70	0.78	0.80	0.82	0.50	0.53	0.26	0.05
Spleen	0.77	0.70	0.60	0.59	0.39	0.42	0.21	0.07
Bone	0.24	0.20	0.22	0.23	0.14	0.19	0.10	0.03
Muscle	0.05	0.05	0.08	0.08	0.08	0.08	0.08	0.02
Chimeric Mab F(ab')$_2$ fragments								
Bone marrow	1.48*	1.05	1.11	0.85	0.54	0.56	0.23	0.06
Blood	4.04	3.50	2.96	2.62	1.50	1.34	0.46	0.15
Kidney	2.35	2.79	1.89	2.03	0.82	0.82	0.44	0.12
Lung	1.82	1.42	1.50	1.12	0.98	0.92	0.41	0.11
Liver	1.17	0.87	0.81	0.72	0.44	0.44	0.16	0.05
Heart	0.68	0.79	0.78	0.80	0.50	0.50	0.20	0.05
Spleen	1.27	1.23	0.87	0.88	0.57	0.55	0.19	0.06
Bone	0.25	0.21	0.21	0.21	0.14	0.18	0.08	0.02
Muscle	0.05	0.07	0.08	0.08	0.08	0.08	0.06	0.02

* Percent injected radioactivity (after correction for physical half-life) per g of tissue at different times after injection of F(ab')$_2$ fragments from either Mab 35 or chimeric human-mouse Mab.

with no trend to either increase or decrease over the whole period of observation. This was confirmed when mean organ to bone marrow ratios and their standard deviations were calculated for each Mab in the intact or fragment form. As shown in Tables 3 and 4, the standard deviations of these ratios calculated for four to nine animals were small for comparing radioactivity in blood, kidney, lung, liver, heart, spleen, and bone with that in bone marrow.

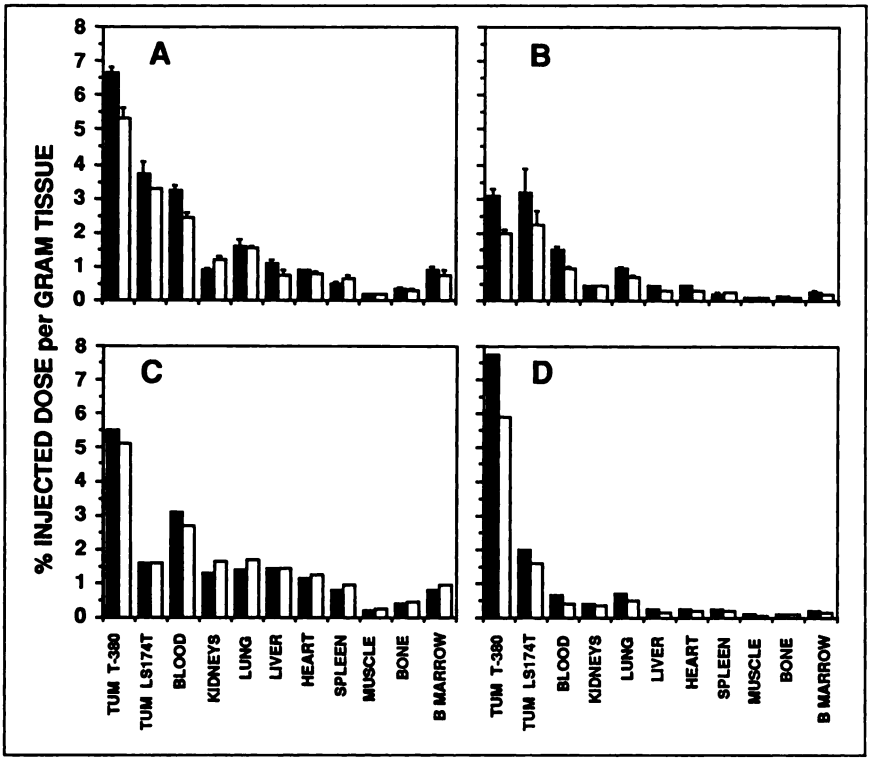

FIGURE 1. Tissue distributions of radioiodinated intact Mabs and F(ab')$_2$ fragments in tumor-bearing nude rats. Panels A and B show the %ID for intact antibodies, Mab 35 (black bars) and chimeric Mab (open bars) from groups of two rats dissected 1 day (A) and 3 days (B) after injection (half the range is indicated by vertical lines). Panels C and D show the % ID for F(ab')$_2$ fragments of Mab 35 (black bars) and chimeric Mab (open bars) from individual rats dissected 8 (C) and 24 hr (D) after injection. The different tissues shown are, from left to right, human colon carcinomas T380 and LS174T, blood, kidney, lung, liver, heart, spleen, muscle, bone, and bone marrow. Tumor weights (in grams) were for tumor T380: 0.3 and 0.47 (A), 0.38 and 0.38 (B), 0.78 (C), and 0.66 (D); and for tumor LS174T: 0.9 and 1.1 (A), 0.7 and 1.4 (B), 1.49 (C), and 1.87 (D).

TABLE 3
Organ-to-Bone Marrow Ratios Calculated from Biodistribution Studies in Normal Rats and Tumor-Bearing Nude Rats After Injection of Radiolabeled Intact Antibodies

	Mabs 35		Chimeric Mabs	
	Normal rats	Tumor-Bearing nude rats	Normal rats	Tumor-Bearing nude rats
Blood/BM	2.85 ± 0.60*	4.43 ± 0.95†	2.52 ± 0.59*	4.02 ± 0.91†
Kidney/BM	0.75 ± 0.14	1.27 ± 0.28	1.25 ± 0.20	1.86 ± 0.34
Lung/BM	1.23 ± 0.26	2.55 ± 0.87	1.39 ± 0.29	2.86 ± 0.86
Liver/BM	0.80 ± 0.14	1.42 ± 0.24	0.68 ± 0.11	1.20 ± 0.24
Heart/BM	0.73 ± 0.18	1.24 ± 0.27	0.78 ± 0.20	1.34 ± 0.29
Spleen/BM	0.53 ± 0.13	0.66 ± 0.09	0.68 ± 0.19	1.05 ± 0.18
Bone/BM	0.24 ± 0.05	0.40 ± 0.04	0.27 ± 0.07	0.46 ± 0.06
Muscle/BM	0.16 ± 0.11	0.28 ± 0.09	0.20 ± 0.14	0.38 ± 0.14

* Organ-to-bone marrow radioactivity ratios (mean ± s.d.) calculated from nine normal rats after injection of intact Mabs 35 and intact chimeric Mabs.

† Organ-to-bone marrow radioactivity ratios (mean ± s.d.) calculated from four tumor-bearing nude rats after injection of intact Mabs 35 and intact chimeric Mabs.

The mean coefficient of variation of the above ratios was 20%, ranging from 10% to 31%.

Muscle is clearly an exception to the rule of a stable ratio between tissue and bone marrow. As already stated, muscle radioactivity increased during 4 to 24 hr after injection of intact antibodies or $F(ab')_2$ and then progressively decreased (Table 1 and 2).

Heart muscle represents another exception, similar to skeletal muscle, but radioactivity in heart increased more rapidly. It reached a maximum at 4–8 hr (Table 1 and 2) and then decreased like in the other organs.

Comparison of organ-to-bone marrow ratios obtained in tumor-bearing nude rats after injection of intact antibodies (Table 3) shows a good correlation with normal rats (correlation factor $r^2 = 0.96$ and 0.97 for Mab 35 and chimeric Mab, respectively). In tumor-bearing nude rats, however, all ratios comparing organ radioactivity with that of bone marrow are higher (Table 3), indicating that bone marrow in tumor-bearing nude rats took up relatively less radioactivity than other organs.

Similarly, organ-to-bone marrow ratios after injection of $F(ab')_2$ fragments in nude rats correlate well with normal rats (Table 4; correlation factors $r^2 = 0.91$ and 0.89 for fragments of Mab 35 and chimeric Mab, respectively). Again, however, most ratios in tumor-bearing nude rats were higher than those observed in normal rats (Table 4), except for ratios comparing blood and kidney with bone marrow, which are similar in both groups of rats.

Since bone marrow of irradiated nude rats might be less relevant for comparison with human bone marrow, nude rat results are not included in the following dosimetry study.

TABLE 4
Organ-to-Bone Marrow Ratios Calculated from Biodistribution Studies in Normal Rats and Tumor-Bearing Nude Rats After Injection of Radiolabeled Mab $F(ab')_2$ Fragments

	Mab 35 $F(ab')_2$ fragments		Chimeric Mab $F(ab')_2$ fragments	
	Normal rats	Tumor-Bearing nude rats	Normal rats	Tumor-Bearing nude rats
Blood/BM	3.49 ± 0.59*	3.33 ± 0.27†	2.72 ± 0.40*	2.81 ± 0.19†
Kidney/BM	1.68 ± 0.40	1.76 ± 0.16	1.96 ± 0.41	1.99 ± 0.22
Lung/BM	1.62 ± 0.19	2.52 ± 0.60	1.54 ± 0.29	2.46 ± 0.52
Liver/BM	0.92 ± 0.13	1.44 ± 0.24	0.80 ± 0.08	1.20 ± 0.24
Heart/BM	0.86 ± 0.16	1.20 ± 0.17	0.81 ± 0.17	1.33 ± 0.18
Spleen/BM	0.75 ± 0.16	1.11 ± 0.18	0.99 ± 0.14	1.14 ± 0.07
Bone/BM	0.28 ± 0.07	0.47 ± 0.05	0.26 ± 0.08	0.55 ± 0.12
Muscle/BM	0.14 ± 0.10	0.29 ± 0.11	0.14 ± 0.09	0.28 ± 0.10

* Organ-to-bone marrow radioactivity ratios (mean ± s.d.) calculated from eight normal rats after injection of $F(ab')_2$ fragments of Mab 35 and of chimeric Mab.

† Organ-to-bone marrow radioactivity ratios (mean ± s.d.) calculated from four tumor-bearing nude rats after injection of $F(ab')_2$ fragments of Mab 35 and of chimeric Mab.

Calculation of Tissue Radiation Doses in Normal Rats in Comparison with Results Obtained in Tumor-Bearing Nude Mice

In order to compare the dosimetric results in normal rats with those obtained in previous radioimmunotherapy experiments in tumor-bearing nude rats (*8,9*), we selected theoretical injected amounts of ^{131}I-labeled Mabs and fragments which would deliver the same radiation dose to the blood of rats as was delivered to that of nude mice. These amounts were 3.65 mCi of ^{131}I for intact Mabs (pool of Mab 35 and chimeric Mab) and 10 mCi of ^{131}I for F(ab')$_2$ (pool of F(ab')$_2$ of Mab 35 and chimeric Mab). Calculated radiation doses compared with the previously reported results in tumor-bearing nude mice are shown in Table 5.

The amount of injected radioactivity selected to deliver identical radiation doses to blood delivered also very similar radiation doses to other normal organs including kidney, lung, liver, heart, spleen, bone, and muscle in the two types of animals. Statistical comparisons of radiation doses calculated for the normal organs of rats and mice exhibit a very high degree of correlation ($r^2 = 0.993$ and 0.978), the slope of the regression line being close to one (0.992 and 1.056) for intact Mabs and F(ab')$_2$, respectively. A similar comparison of the present rat tissue dosimetry data with those from another therapeutic experiment in mice (*8*), in which a single dose of 2200 μCi of ^{131}I Mab F(ab')$_2$ had been injected, gives also a high degree of correlation with $r^2 = 0.987$ and a slope of the regression line of 0.96.

The high correlation of calculated radiation doses between eight organs of normal rats and of tumor-bearing nude mice indicates that the radiation doses to bone marrow are very similar between the two types of animals. The calculated radiation doses to bone marrow of 1180 and 800 rads after injection of rats with ^{131}I intact Mab or F(ab')$_2$, respectively, can thus be considered as realistic for mice treated with 0.56 and 2.43 mCi of ^{131}I intact Mabs and F(ab')$_2$, respectively.

The higher dose delivered to bone marrow by intact antibodies as compared with fragments is in agreement with the more severe toxicity observed in mice treated with intact Mabs (*9*). The high bone marrow radiation doses were well tolerated by the mice and only 1 out of 18 animals showed a decrease of peripheral white blood cells below 1000 cells per mm^3 (*9*).

TABLE 5
Radiation Doses Absorbed by Tissues (in rads) Estimated from Biodistribution Studies

	^{131}I-intact Mabs		^{131}I-Mab F(ab')$_2$	
	In rats	In mice	In rats	In mice
	^{131}I injected (mCi)			
	3.65	0.56	10.00	2.43
BM	1180*	nd	800*	nd
Blood	3100	3120†	2200	2220†
Kidney	1120	1150	1360	1450
Lung	1500	1580	1220	1400
Liver	940	1120	680	580
Heart	930	890	710	730
Spleen	740	790	690	510
Bone	390	500	290	310
Muscle	320	300	190	190
Whole animal	nd	660	nd	440
Tumor (in mice)	nd	9420	nd	9170

* Bone marrow and normal tissue radiation doses (in rads) calculated from radioactivity distributions in rats.

† Normal tissue and tumor radiation doses (in rads) calculated from radioactivity distributions in tumor-bearing nude mice as described (*9*).

DISCUSSION

The lack of reliable radioactivity measurements in the bone marrow of cancer patients after injection of radiolabeled antibodies limits the monitoring and prediction of side effects. Clinical estimations of radiation doses delivered to the bone marrow vary widely between different centers: at the lower extreme, some radiotherapists assume that bone marrow radiation dose is similar to whole-body dose. This assumption is based on results from an experimental study in mice, where bone marrow dose has been reported to be 33 and 11 times lower than that of blood after injecting iodinated irrelevant Mab and anti-Thy 1.1 Mab, respectively (*20*). In another study, bone (not bone marrow) radiation dose was calculated to be 8.3 and 6.7 times lower than that of blood for iodine- and yttrium-labeled antibodies, respectively (*21*). At the other extreme, some investigators assume that the radiation dose delivered by radiolabeled antibodies to bone marrow is approximately the same as that to blood (*2*).

Results from our studies in mice and rats indicate that the difference between whole-body and blood radiation doses can be as high as six-fold (Table 5). The measured bone marrow radioactivity in rats (Tables 3 and 4) and the resulting calculated bone marrow radiation doses (Table 5) were between 2.5 to 3.5 times lower than blood and about two times higher than whole-body doses, both after injection of intact Mabs or F(ab')$_2$ fragments.

Our present kinetic tissue distribution data in rats indicate that the ratios comparing radioactivity in bone marrow with that in several organs remain stable during observation periods ranging from 1 hr to 144 hr after injection of intact antibodies or from 15 min to 48 hr after injection of fragments. This may be important for further dosimetry studies. For instance, the mean bone marrow radioactivity at different times was 37.4% of that of blood (range, 35.1%–39.7%) after injection of intact antibodies and 32.7% (range, 28.7%–36.8%) after injection of F(ab')$_2$ fragments.

In individual rats, a significant difference was observed when radioactivity of bone marrow from humeri was compared with that of femurs and tibias, suggesting that variable amounts of ^{131}I-labeled antibodies can be retained in bone marrow located in different bones. It has not been possible to obtain representative bone marrow samples

from other bones of rats. In patients, after injection of iodinated anti-CEA Mabs without crossreactivity with granulocyte glycoproteins, external scintigraphy showed no evidence of enhanced accumulation of radioactivity in bone or bone marrow (*14,15,22*).

The relatively low radioactivity concentration observed in the bone marrow of tumor-bearing nude rats as compared to normal rats is surprising. Initially, rather an increased bone marrow radioactivity was expected in animals bearing large CEA-producing tumors due to accumulation of antigen-antibody complexes in the reticuloendothelial system, including bone marrow. One possible explanation for the relatively low bone marrow radioactivity in tumor-bearing nude rats might be that T cells and other white blood cells bearing Fc receptors are absent or reduced in nude rats, due to genetic immunodeficiency and to the 500-rad whole-body irradiation given to facilitate tumor grafting.

We have compared our experimental biodistribution results in rats with those obtained in four dogs that were all killed 24 hr after injection of intact ^{125}I-labeled irrelevant Mab and ^{131}I-anti-Ia (HLA-DR) Mab (*6*). In dogs, bone marrow radioactivity of irrelevant Mab at 24 hr after injection was 30% of that of blood, while in our normal rats the mean value for both intact antibodies in all nine rats (killed at different times) was 37.4% (±8%) of that in blood (Table 3).

Statistical comparison of the mean organ-to-bone marrow ratios calculated from the nine rats injected with intact antibodies (Table 3) with the data obtained in dogs (*6*) gives high correlation factors with $r^2 = 0.95$ and 0.85 for Mab 35 and chimeric Mab, respectively. This suggests that the rat data might be extrapolated to higher vertebrates and humans.

In calculating radiation doses to different organs, it was assumed that both intact Mabs and F(ab')$_2$ fragments were equally distributed in each organ and that ^{131}I β-radiation produced a homogeneous irradiation due to crossfire effect. Such assumptions might not always be valid, as it has been shown for tumor tissue, where antibody distribution and energy of β-particles of a given isotope can produce quite different irradiation patterns (*23,24*).

When comparing rat dosimetry data with those of tumor-bearing nude mice that have been treated with radiolabeled antibodies (*8,9*), we have extrapolated radiation doses for nude mice bone marrow that appear rather high (1180 rads for 560 μCi of ^{131}I intact Mab and 800 rads for 2430 μCi of ^{131}I-F(ab')$_2$, Table 5). Two arguments might explain the good tolerance of the mice to the calculated high radiation doses. First, radiation delivered to bone marrow by ^{131}I intact antibodies or fragments is highly fractionated and represents a low dose rate (maximally 25 to 45 rad per hr immediately after injection of intact Mabs and F(ab')$_2$, respectively), thus allowing DNA repair to occur (*23*). Second, a relatively high proportion of bone marrow cells lies close to trabecular bone structures or solid shafts (*25*) that contain low radioactivity concentrations. Thus, these bone marrow cells may be exposed to only one-half of the calculated radiation doses [similar to the decreased radiation exposure expected for cells at the periphery of tumor nodules (*26*)].

As an example of clinical application of this bone marrow dosimetry based on extrapolation of sequential measurements of radioactivity in different blood samples, we shall analyze a patient who was included in our pilot radioimmunotherapy trial (*22*). The patient was a 49-yr-old female patient, weighing 65 kg, who had a large liver metastasis from a colon carcinoma and a circulating CEA level of 23 ng/ml. She was treated with two injections of 100 mCi of ^{131}I-labeled intact anti-CEA Mabs at 4-day interval.

There was no immediate side effect, but severe bone marrow depression developed between 29 and 37 days after the first injection. The patient recovered spontaneously from myelodepression and underwent a partial hepatectomy 2 mo later for removal of a single liver metastasis 8 cm in diameter. The patient is well and tumor-free 4 yr later without any sign of radiation toxicity.

Blood samples had been collected daily for the first 11 days and then every 2 days. Blood radioactivity decreased from 2 maximal values at 13 and 18 μCi per ml observed after the first and second injections, respectively, to 0.01 μCi per ml on day 25. Cumulative activity for blood was calculated as 1351 μCi × hrs corresponding to 546 rad of β-radiation (*19*). Based on direct measurement of radioactivity in normal rats, we assume that bone marrow radioactivity is 2.68 times lower than that of blood corresponding to 203 rad of β-radiation to bone marrow of this patient.

A great part of γ-radiation dose depends on whole-body radioactivity, since only a small amount of γ-radiation is absorbed locally within a single organ (*27*). Bone marrow γ-radiation dose shall therefore be calculated as percent of whole-body β-radiation, which in this patient was close to 90 rad, based on a whole-body effective half-life of 50 hr observed for the two injections of 100 mCi ^{131}I-labeled intact Mabs. Assuming that γ-radiation delivered to the central part of the body is 84% of whole-body β-radiation gives a bone marrow γ-radiation of 75 rad. Total bone marrow irradiation following the two injections of 100 mCi of ^{131}I anti-CEA Mabs would thus correspond 203 ± 75 rad = 278 rad (139 rad per 100 mCi), a dose consistent with the severe, but reversible bone marrow toxicity observed in this patient. Total dose-to-blood would in turn be 546 ± 75 rad = 621 rad, (311 rad per 100 mCi). Bone marrow dose thus represents 45% of blood dose.

Bigler et al. (*2*) reported similar blood radiation doses with a mean of 327 rad (±36 rad) per 100 mCi of injected ^{131}I-labeled anti-CEA Mabs in five of six patients presenting no human anti-mouse IgG antibodies. They assumed, however, that the dose to bone marrow was identical to that of blood, which, according to our experimental results,

is an overestimation. Siegel et al. (*29*), reported variable bone marrow radiation doses of 109 to 368 rad, (mean 226 rad), per 100 mCi of ^{131}I-labeled anti-CEA antibodies injected intravenously in eight patients, based on sacral scintigraphy dosimetry.

Slightly lower bone marrow radiation doses have been reported after intraperitoneal injection of different ^{131}I-labeled anti-tumor Mabs (*3,5*). Larson et al. (*3*) calculated that about 100 rad were delivered to the bone marrow per 100 mCi ^{131}I-Mab injected intraperitoneally, whereas Stewart et al. (*5*) assumed that bone marrow radiation dose is 25% of that of blood.

For ^{90}Y-labeled Mabs, bone marrow dose calculations, as reported here, might be misleading, since nonspecific accumulation appears to occur in the bone marrow with many antibody preparations labeled with this isotope. Severe bone marrow depression has indeed been observed in patients even if radiation doses calculated for bone marrow were relatively low (*5*).

Keeping in mind several reservations concerning proper selection of antibodies and isotopes, our rat bone marrow dosimetry based on direct measurements of radioactivity might be helpful for estimating bone marrow radiation doses in patients injected with radiolabeled Mabs and fragments.

ACKNOWLEDGMENTS

We thank Mrs. K. Fournier, M. Delessert-Pagani, and N. Monnet for excellent technical assistance. We are grateful to Dr. J. Lucas for providing purified intact IgG_4 chimeric Mab and to Dr. S. Betz-Corradin and S. Maillard for reviewing the manuscript. The clinical data from the patient reported in the discussion were obtained in collaboration with Drs. B. Delaloye and A. Bischof-Delaloye.

REFERENCES

1. Leichner PK, Klein JL, Siegelman SS, Ettinger DS, Order SE. Dosimetry of ^{131}I-labeled anti-ferritin antibodies in hepatoma: specific activities in the tumor and liver. *Cancer Treat Rep* 1983;67:647–658.
2. Bigler RE, Zanzonica PB, Leonard R, et al. BM dosimetry for monoclonal antibody therapy. In: Schlafke AT, Watson EE, eds. *Proc Fourth Int Radiopharmaceutical Dosimetry Symposium 1985*. Oak Ridge: Oak Ridge Associated Universities; 1986:535–544.
3. Larson SM, Raubitschek A, Reynolds JC, et al. Comparison of bone marrow dosimetry and toxic effect of high dose ^{131}I-labeled monoclonal antibodies administered to man. *Nucl Med Biol* 1989;16:153–158.
4. Siegel JA, Pawlyk DA, Lee, RE, et al. Tumor, red marrow, and organ dosimetry for ^{131}I-labeled anti-carcinoembryonic antigen monoclonal antibody. *Cancer Res* 1990;50(suppl):1039s–1042s.
5. Stewart JS, Hird V, Sullivan M, Snook D, Epenetos AA. Intraperitoneal radioimmunotherapy for ovarian cancer. *Br J Obstet Gynaecol* 1989;96:529–536.
6. Appelbaum FR, Badger CC, Deeg HJ, Nelp WB, Storb R. Use of iodine-131-labeled anti-immune response-associated monoclonal antibody as preparative regimen prior to BM transplantation: initial dosimetry. *NCI Monogr* 1987;3:67–71.
7. Press OW, Eary JF, Badger CC, et al. Treatment of refractory non-Hodgkin's lymphoma with radiolabeled MB-1 (anti-CD37) antibody. *J Clin Oncol* 1989;7:1027–1038.
8. Buchegger F, Pfister C, Fournier K, et al. Ablation of human colon carcinoma in nude mice by 131-I-labeled monoclonal anti-carcinoembryonic antigen antibody F(ab')$_2$ fragments. *J Clin Invest* 1989;83:1449–1456
9. Buchegger F, Pèlegrin A, Delaloye B, Bischof-Delaloye A, Mach J-P. I-131-labeled F(ab')$_2$ fragments are more efficient and less toxic than intact anti-CEA antibodies in radioimmunotherapy of large human colon carcinoma grafted in nude mice. *J Nucl Med* 1990;31:1035–1044.
10. Haskell CM, Buchegger F, Schreyer M, Carrel S, Mach J-P. Monoclonal antibodies to carcinoembryonic antigen: ionic strength as a factor in the selection of antibodies for immunoscintigraphy. *Cancer Res* 1983;43:3857–3864.
11. Hammarstrom S, Shiveley JE, Paxton RJ, et al. Antigenic sites in carcinoembryonic antigen. *Cancer Res* 1989;49:4852–4858.
12. Hardman N, Gill LL, de Winter RFJ, et al. Generation of a recombinant human-mouse chimaeric monoclonal antibody directed against human carcinoembryonic antigen. *Int J Cancer* 1989;44:424–433
13. Mach J-P, Buchegger F, Forni M, et al. Use of radiolabelled monoclonal anti-CEA antibodies for the detection of human carcinomas by external photoscanning and tomoscintigraphy. *Immunology Today* 1981;2:239–249.
14. Delaloye B, Bischof-Delaloye A, Buchegger F, et al. Detection of colorectal carcinoma by emission-computerized tomography after injection of ^{123}I-labeled Fab or F(ab')$_2$ fragments from monoclonal anti-carcinoembryonic antigen antibodies. *J Clin Invest* 1986;77:301–311.
15. Bischof-Delaloye A, Delaloye B, Buchegger F, et al. Clinical value of immunoscintigraphy in colorectal carcinoma patients: a prospective study. *J Nucl Med* 1989;30:1646–1656.
16. Buchegger F, Vacca A, Carrel S, Schreyer M, Mach J-P. Radioimmunotherapy of human colon carcinoma by ^{131}I-labelled monoclonal anti-CEA antibodies in a nude mouse model. *Int J Cancer* 1988;41:127–134.
17. Martin KW, Halpern SE. Carcinoembryonic antigen production, secretion and kinetics in BALB/c mice and a nude mouse-human tumor model. *Cancer Res* 1984;44:5475–5481.
18. Loevinger R, Budinger TF, Watson EE. *MIRD primer for absorbed dose calculations*. New York: The Society of Nuclear Medicine; 1989.
19. Johns HE, Cunningham JR. The physics of radiology. In: Friedman M, ed. *Monograph in the Bannerstane division of American lectures in radiation therapy*, third edition. Springfield: Charles C. Thomas; 1978:564–574.
20. Badger CC, Krohn KA, Peterson AV, Shulman H, Bernstein ID. Experimental radioimmunotherapy of murine lymphoma with ^{131}I-labeled anti-Thy 1.1 monoclonal antibody. *Cancer Res* 1985;45:1536–1544.
21. Sharkey RM, Motta-Hennessy C, Pawlyk D, Siegel JA, Goldenberg DM. Biodistribution and radiation dose estimates for yttrium- and iodine-labeled monoclonal antibody IgG and fragments in nude mice bearing human colonic tumor xenografts. *Cancer Res* 1990;50:2330–2336.
22. Mach J-P, Bischof-Delaloye A, Curchod S, et al. Progress in diagnostic immunoscintigraphy and first approach to radioimmunotherapy of colon carcinoma. In: Srivastava S, ed. *Radiolabeled monoclonal antibodies for imaging and therapy*. New York: Plenum Publishing Corp; 1988:95–109.
23. Humm JL, Chin LM, Macklis RM. F(ab')$_2$ fragments versus intact antibody—an isodose comparison [Editorial]. *J Nucl Med* 1990;31:1045–1047.
24. Wessels BW. Current status of animal radioimmunotherapy. *Cancer Res* 1990;50(suppl):970s–973s.
25. Polig E, Jee WS. Bone structural parameters, dosimetry, and relative radiation risk in the beagle skeleton. *Radiat Res* 1989;120:83–101.
26. Klein JL, Nguyen TN, Laroque P, et al. Yttrium-90 and iodine-131 radioimmunoglobulin therapy of an experimental human hepatoma. *Cancer Res* 1989;49:6383–6389.
27. Humm JL. Dosimetric aspects of radiolabeled antibodies for tumor therapy. *J Nucl Med* 1986;27:1490–1497.
28. Siegel JA, Lee RE, Pawlyk DA, Horowitz JA, Sharkey RM, Goldenberg DM. Sacral scintigraphy for BM dosimetry in radioimmunotherapy. *Nucl Med Biol* 1989;16:553–559.