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RELATION BETWEEN CURABILITY AND TUMOR VOLUME IN A MURINE CARCINOMA AND SARCOMA

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Although it has long been known clinically that an inverse relationship exists between tumor volume and curability by irradiation (ALLEN & FREED 1956, VON ESSEN 1960, COHEN 1966), equations relating these two parameters never have been formulated over a wide range of tumor volumes. Similarly, reports on tumor volume and curability in animals have not quantified this relationship in solid tumors ranging in size from subclinical foci to detectable ones (NICE 1957, SIKOV & LOFSTROM 1960, SUIT et coll. 1960, 1965, WILSON 1961, REINHOLD & DEBREE 1968).

The importance of knowing sterilizing radiation doses for tumors not only at clinically detectable levels but also at subclinical stages is underscored by the fact that a greater number of patient deaths result from treatment failures of undetected regional or hematogenous metastases than from treatment failures of primary tumors (SUIT 1970, SCHABEL 1976).

The relationship between the curability and tumor volume over a wide range of tumor volumes including microscopic foci as well as palpable tumors was investigated and the result is now reported. TCD_{50} (radiation dose to control 50 per cent of the tumors) values were calculated for a murine sarcoma and carcinoma irradiated and assayed in vivo either as pulmonary colonies ranging from 3×10^{-5} to 1×10^{-2} mm^3 or as palpable subcutaneous tumors of about 300 mm^3.

Supported by USPHS Grant CA 13300-03 from the National Cancer Institute. Submitted for publication 26 January 1977.

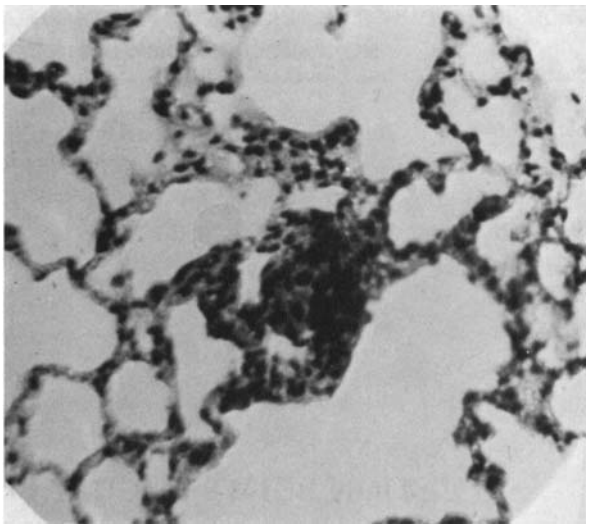

Fig. 1. High-magnification view of tumor nest in lung of mouse killed 7 days after injection of C3HBA mammary adenocarcinoma. Based on such serial sections, tumor volume calculations were found to be in good agreement with extrapolated portion of tumor growth curves (SHAEFFER et coll. 1974, 1975 a).

Materials and Methods

Details regarding the mice, tumors, growth rates, cell suspension preparations, irradiation techniques, and lung colony assays have been described elsewhere (EL-MAHDI et coll. 1974 a, SHAEFFER et coll. 1973 a, 1973 b, 1975 a, 1976). The two tumors used were the C3HBA mammary adenocarcinoma and the Dunn osteogenic sarcoma. Both are transplantable tumors of spontaneous origin, the former grown in 8 to 10-week-old female C3H/HeJ mice (Jackson Labs, Bar Harbor, Me, USA) and the latter in 8 to 10-week-old female C3H/HeDub mice (Flow Research Animals, Dublin, Va, USA).

For pulmonary colony production, intravenous injections of single cell suspensions prepared from subcutaneously grown tumors were used. Tumor inocula were adjusted to produce nominally 100 colonies per mouse in untreated animals. Mice that were anesthetized with i.p. 0.065–0.070 mg sodium pentobarbital per g body weight were given single ^{60}Co anterior thoracic treatments at either 1, 7 or 14 days after tumor cell injection. Animals were killed 30 days after tumor injection for lung colony assays.

For production of palpable tumors, the right hind limbs were injected subcutaneously with tumor cell suspensions. Tumors had an average volume of about 300 mm^3 11 days following the injection of about 1×10^5 viable cells from the C3HBA suspension and 16 days after the injection of 2×10^5 viable cells from the Dunn tumor suspension. Single ^{60}Co doses to the tumor-bearing limbs were given to anesthetized mice.

The volumes of the palpable subcutaneous tumors were calculated using the formula $V = 0.524\ d_1\ d_2\ d_3$, where d_1, d_2 and d_3 are the tumor diameters in 3 orthogonal planes measured with calipers. The growth curves for the pulmonary colonies

Table 1

Mean pulmonary colonies ± standard error in mice injected intravenously with C3HBA adenocarcinoma and given thoracic ^{60}Co irradiation at various times

Dose (Gy)	Age of tumor at ^{60}Co irradiation		
	1 day	7 days	14 days
0 (control)	74.0 ± 11.4	110.6 ± 7.2	58.9 ± 5.2
3.50	55.4 ± 12.2	76.3 ± 14.7	—
7.00	28.9 ± 8.4	—	51.9 ± 6.6
10.00	11.6 ± 3.2*	47.9 ± 8.9	46.6 ± 4.9
14.00	3.9 ± 2.5	22.8 ± 4.9	33.9 ± 2.6
21.00	0.33 ± 0.21	6.3 ± 3.5	6.9 ± 1.8

* Treated with a dose of 10.50 Gy.

Table 2

Mean pulmonary colonies ± standard error in mice injected intravenously with Dunn osteosarcoma and given thoracic ^{60}Co irradiation at various times

Dose (Gy)	Age of tumor at ^{60}Co irradiation		
	1 day	7 days	14 days
0 (control)	66.9 ± 17.3	181.3 ± 25.8	78.0 ± 15.5
3.50	48.7 ± 6.8	151.2 ± 25.0	62.4 ± 11.0
7.00	17.5 ± 6.5	108.1 ± 24.2	47.4 ± 7.5
10.00	6.8 ± 3.4	74.9 ± 13.5	45.1 ± 7.9
14.00	3.0 ± 1.9	33.1 ± 4.7	29.3 ± 4.7
17.50	1.0 ± 0.7	28.4 ± 8.0	16.8 ± 4.1

of both tumors have been published previously (SHAEFFER et coll. 1974, 1975 a). Tumor volumes for 14-day lung colonies were measured using a dissecting microscope with a calibrated reticle. The volumes of the 7-day lung colonies were initially estimated from the extrapolated portion of the growth curves, but have subsequently been calculated by measuring tumor diameters in serial histologic sections of the lungs (Fig. 1). The volumes of 1-day lung colonies have been estimated from the growth curves.

Results

A total of 131 mice were injected intravenously with C3HBA adenocarcinoma cells and irradiated thoracically or sham-irradiated at either 1, 7 or 14 days post injection. The average numbers of lung colonies in these mice at 30 days post injection

Table 3

Local tumor control of subcutaneous tumors irradiated with single ^{60}Co doses. Figures within parentheses represent percentages

Dose (Gy)	C3HBA adenocarcinoma	Dunn osteosarcoma
0	0/17 (0)	0/18 (0)
40.00	—	1/13 (8)
50.00	7/15 (47)	2/14 (14)
55.00	9/15 (60)	—
60.00	12/16 (75)	9/15 (60)
70.00	—	14/15 (93)

Table 4

Volumes and TCD_{50} values for tumorr irradiated with single ^{60}Co doses

Tumor			
Type	Age	Volume (mm^3)	TCD_{50} (Gy)
C3HBA	1 day old	3.1×10^{-5}	5.50
Dunn	pulmonary	5×10^{-5}	4.70
C3HBA	7 day old	3.3×10^{-4}	7.30
Dunn	pulmonary	1×10^{-3}	8.50
C3HBA	14 day old	1.2×10^{-2}	14.50
Dunn	pulmonary	1×10^{-2}	10.40
C3HBA	palpable	3.1×10^{2}	51.15
Dunn	subcutaneous	2.7×10^{2}	57.80

are presented in Table 1. The mean lung colony numbers in the untreated control groups differ because each experiment (1, 7 or 14 days) was done separately. Results of similar experiments using 235 mice injected intravenously with Dunn osteosarcoma cells are summarized in Table 2.

The rates of local tumor control for subcutaneous tumors of both the mammary carcinoma and osteosarcoma are given in Table 3. These values are for a 30-day observation period to be consistent with the 30-day observation period for the lung colony assay.

Tumor volumes and TCD_{50} values are summarized in Table 4. TCD_{50} values were calculated from the experimental data in Tables 1 to 3 using least squares regression analysis. The power function $TCD_{50} = 23.57\ TV^{0.15}$ was the best empirical fit for these data (Fig. 2).

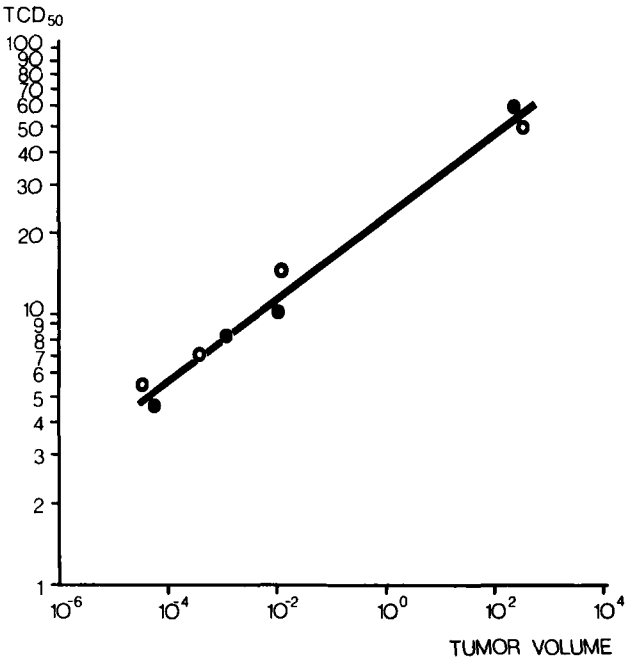

Fig. 2. TCD_{50} (Gy) of two murine tumors of various volumes irradiated and assayed in vivo as pulmonary colonies of palpable subcutaneous tumors on the hind limb. $TCD_{50} = 23.57\ TV^{0.15}$. ● = Dunn osteosarcoma. O = C3HBA adenocarcinoma.

Discussion

The relationship between tumor volume and radiation curability is one of the fundamental questions in radiation oncology which has yet to be answered adequately in mathematical terms. Basically, there are two different schools regarding this question. If tumors behave simply as aggregates of individual cells, then based on individual cell survival considerations the tumor cure rate (TCD_{50}) would be a logarithmic function of the tumor volume (TV), or $TCD_{50} = E + k \log TV$. The experimental data of SUIT et coll. (1965) agree with this theoretical equation derived from cell survival equations. One feature of this model, which is linear on a semilog plot, is that derived D_0 remains constant throughout the entire range of tumor volumes. Derived D_0 equals 1/2.3 multiplied by the change in TCD_{50} corresponding to a ten-fold change in tumor volume. The derived D_0 was about 3.00–3.25 Gy at all volumes in the experiments of SUIT et coll.

The second school relates tumor control to tumor volume by a power function of the general form $TCD_{50} = E \times TV^q$. This equation is linear on a log:log plot. Using clinical data from investigations by VON ESSEN (1960) on epidermoid carcinomas of the skin and lip, COHEN (1966) has calculated the equation $D = 3\,000\ L^{0.19}$ where D equals the tumor control dose and L the tumor diameter. With this power function model, derived D_0 varies with tumor volume. Derived D_0 values are lower with smaller tumors and become progressively larger as tumor volume increases. This concept has recently been confirmed by two other laboratories. Working with the EMT6

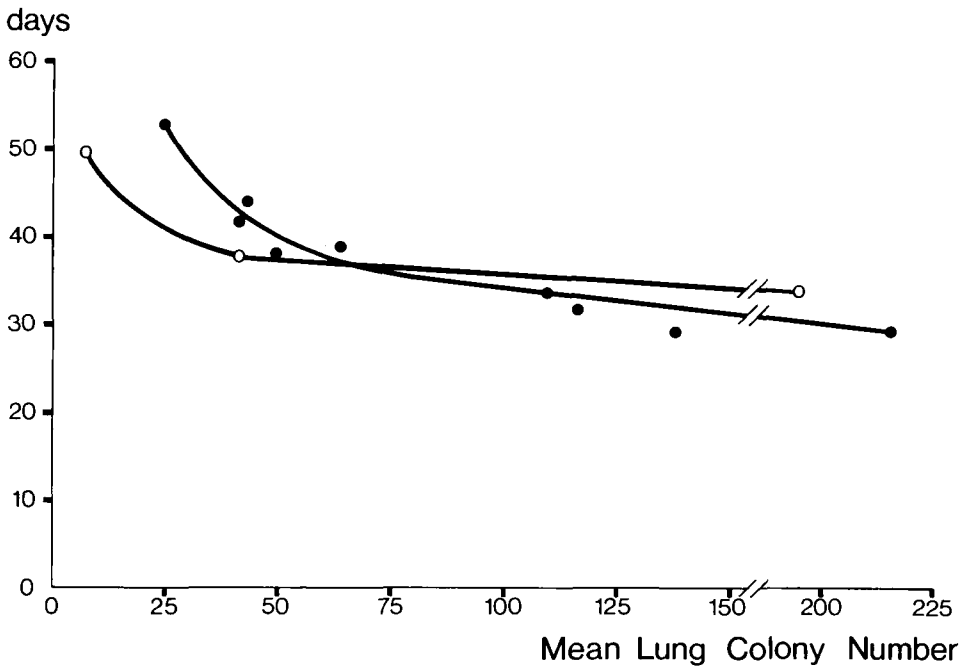

Fig. 3. Mean survival time versus lung colony number in 210 female C3H mice bearing C3HBA adenocarcinoma (○) and 213 female mice bearing Dunn osteosarcoma (●). These untreated groups of mice were given different intravenous tumor cell inocula.

tumor, Fu et coll. (1976) reported a lower D_0 for irradiated small lung nodules (0.5–2.0 mm diameter) than for larger flank tumors of 10 mm diameter. Similarly, Shipley et coll. (1976) reported lower D_0 values for 0.5 mm^3 Lewis lung tumors compared to the same tumors grown subcutaneously (500 mm^3).

Oxygen status may explain, at least in part, the difference between the logarithmic and power function curves. Suit et coll. rendered their tumors anoxic by application of clamp hypoxia, whereas the percentage of hypoxic cells would be expected to vary considerably in the tumors of the other investigators. Shipley et coll. estimate the hypoxic fraction as less than 0.5 per cent in the smaller lung foci, but about 36 per cent in the larger subcutaneous tumors.

That the same curve ($TCD_{50} = 23.57\ TV^{0.15}$) appears to fit the data for both the carcinoma and sarcoma may be related to similarities in these tumors of a long transplantation history, relatively low degree of immunogenicity (Miller & Fisher 1977), and comparable doubling times.

The choice of 30 days after intravenous tumor cell injection to kill the mice for the lung colony assay is based on the mean survival time of the tumor-bearing animals (Fig. 3). Longer observation periods with tumor-bearing mice are not possible without incurring considerable animal death. Animals which were tumor-free

('cured') at 30 days post tumor cell injection developed no recurrences when followed for over 340 days (SHAEFFER et coll. 1975 b).

Some of the TCD_{50} determinations are being repeated to get a better idea of their reproducibility. Compared to the values listed in Table 4, the mean $TCD_{50} \pm$ standard error of the mean for 3 replicate determinations each for the 1-, 7- or 14-day C3HBA lung colonies were 4.78 ± 0.89, 6.50 ± 0.99, and 13.08 ± 1.25 Gy, respectively. These current values are about 10 per cent lower than the original values and may reflect some change in the tumor itself as a consequence of serial transplantation over the course of the 2 years intervening the original and current experiments. Changes following serial transplantation in the in vivo radiation response of CD_2F_1 tumors as well as changes in the karyotype and sensitivity of tissue-culture derivatives of these tumors have been reported (EL-MAHDI et coll. 1974 b).

The empirical curve relating TCD_{50} to tumor volume is based on measurements using single doses on relatively unresponsive rodent tumors, and, as such, cannot be used to make direct clinical predictions whether human tumors of a known volume can be sterilized by fractionated radiation doses within the limiting constraints of the normal tissue tolerance. The curve is useful, however, in quantifying that tumor microfoci can be controlled by radiation doses that are substantially lower than those required to control clinically detectable tumors.

Acknowledgment

We gratefully acknowledge the assistance of Dr Michael Wilkins of Simon Fraser University in the analysis of the experimental data.

SUMMARY

Tumor control doses (TCD_{50}) using single ^{60}Co doses were determined on C3HBA adenocarcinoma and Dunn osteosarcoma ranging in size from microscopic pulmonary colonies (3×10^{-5} mm^3) to palpable subcutaneous tumors (300 mm^3) in the hind limb. The power function $TCD_{50} = 23.57\ TV^{0.15}$, with TCD_{50} in Gy and TV (tumor volume) in mm^3, fits the data for both tumors over the entire range of tumor volumes used.

ZUSAMMENFASSUNG

Die Tumor-Kontroll-Dosis (TCD_{50}) wurde bei Verwendung einer einzelnen ^{60}Co Dosis am C3HBA Adenokarzinom und Dunn Osteosarkom im Bereich der Grösse von mikroskopischen Lungen Kolonien (3×10^{-5} mm^3) bis zu palpablen subkutanen Tumoren (300 mm^3) im Hinterbein untersucht. Die Exponentialfunktion $TCD_{50} = 23{,}57\ TV^{0{\cdot}15}$, mit TCD_{50} in Gy und TV (Tumorvolumen) in mm^3, deckt die Daten für beide Tumoren über den gesamten Bereich des verwendeten Tumorvolumens.

RÉSUMÉ

Les doses de guérison de tumeurs (TCD_{50}) par des doses uniques de ^{60}Co ont été déterminées sur des adénocarcinomes C3HBA et des ostéosarcomes de Dunn dont la taille allait

des colonies pulmonaires microscopiques (3×10^{-5} mm^3) à des tumeurs sous-cutanées palpables (300 mm^3) dans la patte postérieure. La fonction exponentielle $TCD_{50} = 23{,}57\ TV^{0.15}$ avec TCD_{50} en Gy et TV (volume tumoral) en mm^3 est en accord avec les résultats obtenus sur ces deux types de tumeurs dans tout le domaine des volumes tumoraux étudié.

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