Proc. Natl. Acad. Sci. USA
Vol. 74, No. 5, pp. 1983–1987, May 1977
Biophysics

Solid tumor models for assessment of different treatment modalities: Therapeutic strategy for sequential chemotherapy with radiotherapy*

(combined chemotherapy and radiotherapy/sequential therapy/clinical relevance)

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Communicated by Jesse W. Beams, January 21, 1977

ABSTRACT **A therapeutic strategy for combined radiotherapy and chemotherapy of experimental solid tumors has been devised. More effective utilization of combined chemotherapy and radiotherapy may be realized clinically if comparable information is obtained in man. The overall treatment efficiency of successive courses of treatment has been determined by a method that defines tumor response quantitatively over an entire spectrum of tumor responses. The findings of this study have shown that an individual tumor that responds well to the first course of therapy will respond well to the second and third courses of combined modality therapy. Various solid tumors in different animal species have demonstrated variability of response to treatment, analogous to the many types of response found clinically.**

The use of different treatment methods such as radiotherapy and chemotherapy, or combined chemotherapy, indicates that further improvement in management of solid tumors may be realized if the temporal relationship between tumor and patient response is better understood. One of the more promising approaches to the improvement of cancer treatment is the optimal sequencing of different therapeutic modalities to produce maximal effects on the tumor with minimal effects on the host. Many of the questions related to solid tumors in man can only be answered at this time by use of solid tumor models in animals. Such well-defined and rapidly analyzable tumor models can yield quantitative information concerning the time sequence of toxicity to therapy and the kinetics of recovery of host and tumor.

Previous reports from these studies (1–6) have demonstrated that recovery from the effects of a large dose of 5-fluorouracil (5-FUra) in rats occurs 10–11 days after treatment. The maximal rate of tumor volume change occurs 12 days after 5-FUra (4). It has also been demonstrated that the rate of proliferation in the tumor is at a maximum 11–12 days after 5-FUra (3). Studies on the solid tumor model hepatoma 3924A indicated that neither radiation alone [375-1500 roentgens (0.10–0.38 C/kg)] nor 5-FUra alone (50–250 mg/kg) would control tumor growth. However, these results suggested that radiation in combination with 5-FUra treatment could in principle transform the management of a chemotherapeutically resistant solid tumor from an untreatable situation to a treatable situation by the combined modality treatment every 11 days until the tumor is eradicated (2).

The present report describes the effectiveness of a therapeutic strategy for the sequential use of chemotherapy with radiotherapy. In addition, a method has been developed to compare the treatment efficiency of successive courses of treatment so that successive effects can be distinguished from integrated ones.

Abbreviations: 5-FUra, 5-fluorouracil; OTE, overall treatment efficiency.

* This is paper no. 5 of a series. Paper no. 4 is ref. 2.

MATERIALS AND METHODS

The analytic methods developed for the evaluation of the effectiveness of single and combined modality therapy on tumor growth curves categorized tumor response as follows: class I, regression (tumor volume is less than initial tumor volume somewhere in the 11-day interval); class II, pseudoregression (tumor volume is always greater than initial tumor volume but, at some point, shows a definite diminution from a previously attained maximum); and class III, slowdown (tumor volume never decreases, but the growth rate slows down) (4). These analytical methods have been extended so that the efficiency of response can be expressed as a continuous spectrum. The response varies uniformly across the boundaries of the three classes of response.

Female ACI rats were inoculated subcutaneously in the back with 3924A hepatoma cells by Harold Morris in Washington, DC, and shipped to this laboratory. The rats were maintained under standard laboratory conditions including commercial laboratory rat chow (Charles River Laboratories, Wilmington, MA) supplied ad lib. and a 12-hr lighting schedule, the dark period beginning at 8:00 p.m.

5-FUra (Roche Laboratories, Hoffmann-LaRoche, Inc., Nutley, NJ) prepared in sterile saline was given by intraperitoneal injection between 8:00 and 8:30 a.m. Control animals were injected with saline alone.

Local tumor irradiation was carried out with a 250-kV, 30-mA General Electric Maxitron 250 using 0.25 mm of Cu and 1.0 mm of Al as filters. Prior to irradiation the animals were anesthetized with ether and placed in a lead-shielded box through which the tumor protruded. The midpoint of the tumor was approximately 6 cm from the x-ray tube target and received the calculated dose while the animal body received 0.5% of the dose delivered to the irradiated tumor. A Plexiglas cover was placed over the animal and the target cone was lowered to prevent tumor displacement. The 5-FUra was given 12 hr after local tumor irradiation on each of the three 11-day treatment courses to take advantage of the partial synchrony of the cells by local tumor irradiation at this time (5).

Four groups of 15 animals per group were used in this experiment. The rats in group A received 1500 roentgens of local tumor irradiation, group B received 5-FUra at 100 mg/kg, and group C received 1500 roentgens of local tumor irradiation followed by 5-FUra, 100 mg/kg, 12 hr later. Group E were controls. The controls were anesthetized with ether (as the irradiated groups A and C) and were given 1 ml of saline intraperitoneally to simulate the 5-FUra injections in groups B and C.

Tumor volumes (mm^3) were calculated (0.5 × length × width × height) from measurements made daily before and after treatment during the period of major changes in tumor

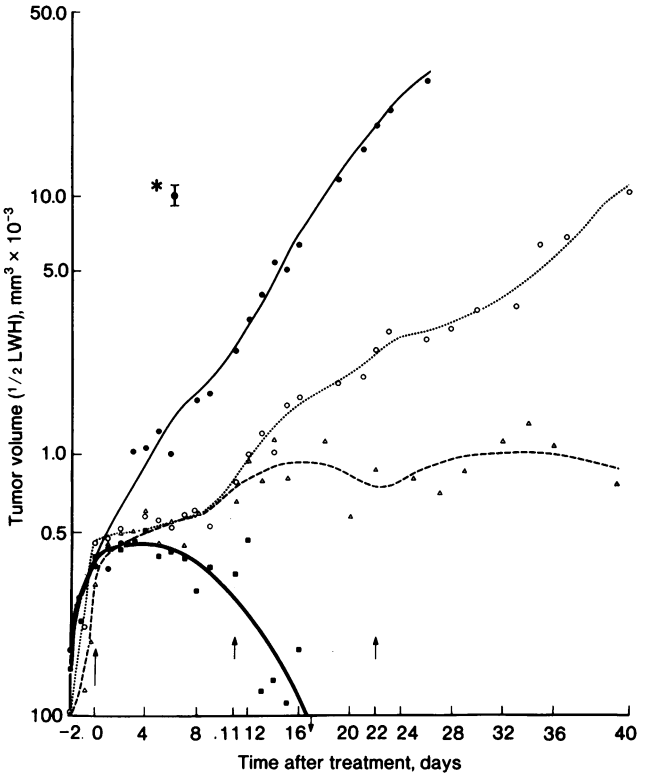

FIG. 1. Representative growth curves for one control (●, E-4) and three treated tumors (○, C-2; ■, C-4; △, C-12). The different symbols represent actual tumor measurements. The lines are computer-fitted growth curves for each tumor. Vertical arrows indicate treatment days. * Representative tumor volume error, representing the accuracy with which the caliper measurement of tumor dimension can be made.

growth rates (1). Measurements were made three times weekly during the remainder of the experiment.

RESULTS

Previous studies of this series have demonstrated variability of response to treatment within the same treatment group. The divergence of these differences in response was accentuated in this multiple course study of combined modality therapy given over longer periods of time (Fig. 1). Combined modality therapy obviously did not control growth in tumor C-2. The tumor volume remained essentially unchanged over the entire period of therapy in tumor C-12. The tumor volume in C-4 showed a rapid decrease at the end of the 11-day interval after the first course of combined modality therapy. The second course of therapy, 11 days after the first, resulted in eradication of the tumor. In some tumors, rapid reductions in tumor volume were delayed until after the third course of therapy, 22 days after the initial treatment.

The mean tumor volume curve for the group of 15 animals treated successively with radiation and 5-FUra was similar to that of C-12 (Fig. 1). The standard error of the mean increased with time after therapy because of the increasing divergence of the tumor curves as illustrated by the growth curves for C-2 and C-4. The mean (±SEM) for the treated group was 452 ± 52 mm^3 on day 7, 462 ± 129 mm^3 on day 16, and 636 ± 234 mm^3 on day 28. Eleven of the 15 tumors showed regression: 7 regressed completely whereas 4 regressed and regrew.

The individual tumor growth curves were analyzed by using techniques previously described (6). Briefly, in each 11-day treatment period the data for each tumor are fitted to a function of the form $\ln V/V_0 = a_0 + a_1 t + \ldots + a_n t^n$ in which V_o is the volume at the time of treatment. The response of the tumor during each treatment period is then categorized as being in one of the three classes defined in *Materials and Methods*.

Table 1. Percentages of tumors showing responses, by class of tumor response

Treatment	Treatment course	% Tumors responding		
		Class I	Class II	Class III
Group A, x-ray	1	60	13	27
	2	71	0	29
	3	55 (64)*	0	45 (36)*
Group B, 5-FUra	1	0	0	100
	2	0	0	100
	3	0	0	100
Group C, x-ray + 5-FUra	1	70	10	20
	2	82	0	18
	3	57	0	43

* Percent if the two animals in class I that died from anesthesia are included.

One way of characterizing the response of the experimental tumors is simply to report what percentage of the tumors show a response in each of the three categories during each treatment period (Table 1). The most striking fact is that the percentage of tumors that respond in a given way to a given treatment does not seem to change significantly when that treatment is repeated. In other words, in this model the tumors exhibited a persistence of response and, once a particular type of response was seen during one treatment period, that same type of response was seen during subsequent periods as well.

Some other points should be made about Table 1. In group A (radiotherapy only), there appeared to be a decrease in the percentage of tumors exhibiting class I responses in the third treatment interval. One animal died during anesthesia during the second and third intervals. These animals had exhibited class I responses in previous intervals. Had they survived and continued this response pattern, the percentages for the third interval for group A would have been equivalent to what was seen in earlier intervals.

The persistence of response in group B (chemotherapy only) is related to the fact that only a class III (slowdown) response can be produced by 5-FUra alone (1). Animal toxicity precludes giving enough 5-FUra to elicit a greater response in this chemotherapeutically resistant solid tumor.

There was a marginally significant increase in treatment effectiveness in group C (radiotherapy plus chemotherapy) over group A.

If it is to be concluded from the data that the persistence of response is actually a feature of the system, it is clear that it is necessary to find a better way of characterizing the response of a given tumor to a given treatment than simply assigning that response to a class. While such a method can give an overall view of the responses, it clearly fails to characterize the more detailed features that might be the most helpful in evaluation of the sequential utilization of combined modality therapy. It would not be possible to say that two responses of a tumor were significantly different for a tumor that on the first course of treatment had its volume reduced just below V_0 but on the second course of treatment had a minimal volume just greater than V_0. Yet, in this classification scheme, one would call the first class I and the second class II and conclude that the tumor had responded differently to each treatment.

A more detailed description of tumor response was realized by introducing the concept of treatment efficiency for each

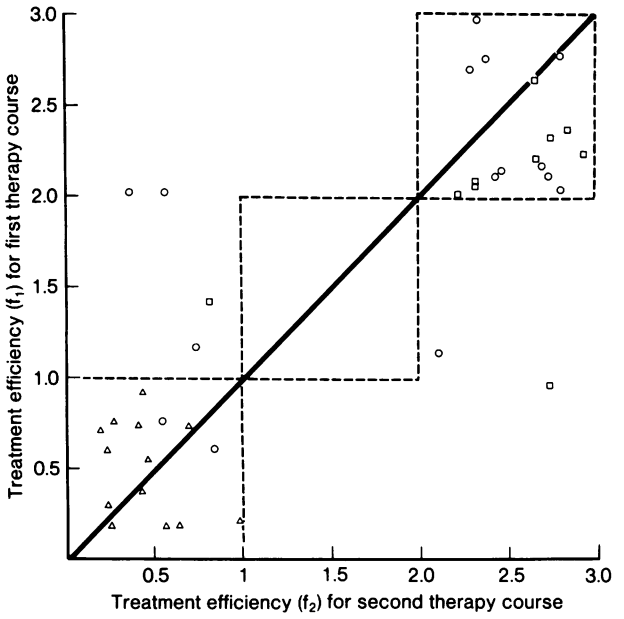

FIG. 2. OTE of the first treatment course in relation to the second treatment course 11 days later. O, radiotherapy; △, chemotherapy; □, combined therapy.

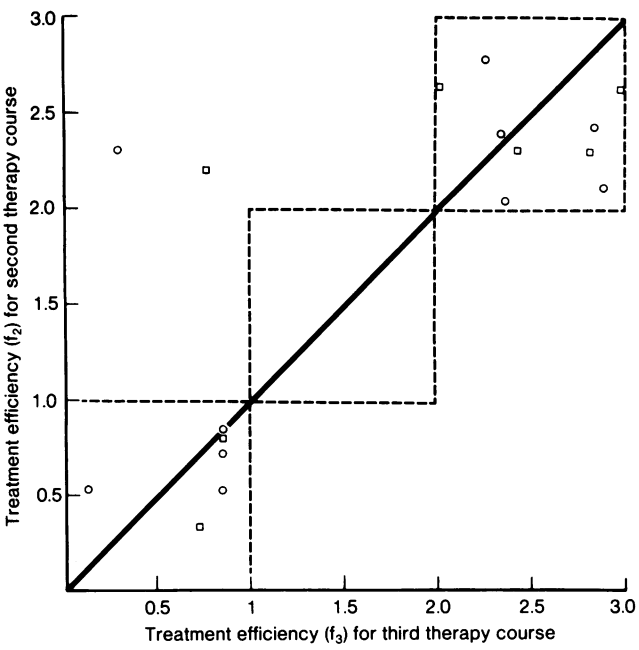

FIG. 3. OTE of the second treatment course in relation to the third treatment course given 11 days after the second treatment course and 22 days after the first treatment course. O, radiotherapy; □, combined therapy.

class. The basic idea here is to define a quantity that varies smoothly from 0 to 1, with the proviso that the quantity should be 0 for the least effective response for a given class and 1 for the most effective response.

For example, the treatment efficiency for a class I (regression) response could be defined by $\eta_I = 1 - (V_{min}/V_{max})$ in which V_{max} is the maximal volume attained by the tumor after treatment and V_{min} is the minimal volume. The most effective response for this class would be a complete regression of the tumor—i.e., a situation in which V_{min} became 0. In this case, $\eta_I = 1$. On the other hand, the least effective class I response would be one in which the volume after treatment remained near V_0 and the tumor eventually regrew. In this case, $V_{min} \approx V_{max} \approx V_0$ and $\eta_I = 0$. The concept of an efficiency takes the analysis one step beyond a simple classification. Saying a response is class I indicates that the tumor exhibited some regression. Stating the efficiency tells how much regression was seen.

In an analogous way, an efficiency appropriate to a class II response can be introduced as follows:

$$\eta_{II} = 1 - \frac{V_{min} - V_0}{V_{max} - V_0}$$

in which V_{min} and V_{max} are the minimal and maximal volumes attained by the tumor after treatment and V_0 is the volume at treatment. This index tells how much decrease in tumor volume is seen relative to V_0.

Finally, an efficiency for class III response is introduced as

$$\eta_{III} = 1 - \frac{b_{min}}{\langle b \rangle}$$

in which b_{min} is the growth rate at the point of minimal growth and $\langle b \rangle$ is the average growth rate of the controls at that point. Clearly, this quantity is a measure of how much the growth rate is reduced by the treatment.

An overall treatment efficiency (OTE) can be defined that allows the characterization, in one number, of the effect of a given treatment once these efficiencies have been defined, each one being calculated as appropriate for a given class of response. In this way, simple comparisons of successive treatments can be made by comparing the OTE of a given tumor in different treatment intervals.

The OTE for a given treatment interval is defined as

$$f_i = 3 - n + \eta.$$

In this equation, i represents the treatment interval. For our results, i will be 1, 2, or 3. "n" represents the class into which a particular response is placed and has the value 1 for class I (regression), 2 for class II, and so on. η is then the treatment efficiency for that class as defined in the preceding paragraphs.

This seemingly arbitrary definition of the OTE was chosen for a number of reasons. In the first place, it varies continuously from a value of 3 (for total regression of the tumor) to 0 (for controls). In the second place, it varies uniformly across the boundaries of the three main classes and assigns roughly an equal OTE to responses that differ only by a small amount but fall into different categories because of this difference. For example, we discussed above the case of two responses that were similar but overlapped the class I–class II boundary. The OTE for each of these responses can be calculated.

The first curve had a class I response with $V_{max} \approx V_{min} \lesssim V_0$ which gives $\eta_I = 0$. From this formula, we would have $f_1 = 3 - 1 + 0 = 2$. On the second course, $V_{max} \approx V_{min} \gtrsim V_0$, which gives a class II response with η_{ii}, 1. In this case, $f_2 = 3 - 2 + 1 = 2$. Thus, the OTE does indeed have nearly equal values for similar tumor responses, even when these responses straddle a class boundary. This makes it an appropriate one-number characterization of the response.

The main result suggested by Table 1, that responses of tumors tend to be the same in each treatment interval, can now be evaluated by means of the OTE. In Fig. 2 the OTE values for the tumors are plotted for treatment intervals 1 and 2; Fig. 3 gives the same results for intervals 2 and 3. These data support the hypothesis that has been advanced. The clustering of points inside the dotted squares shows that virtually all of the tumors will continue to exhibit the same response in each treatment

interval throughout the experiment. In the case of class I responses, this means that repeated treatments will eventually destroy the tumor if conditions can be extrapolated.

Once the persistence of response is established, one more question can be asked—Does the treatment become more effective or less effective as the repetitions increase? Any consistent trend for the OTE to increase or decrease from interval to interval would answer this question. For example, if the treatment became more effective with repetition, the lower right-hand side of the class I squares in Figs. 2 and 3 would contain a preponderance of the points; if the treatment became less effective, the points would tend to cluster in the upper left-hand corner. In Fig. 2, 12 tumors exhibited a higher OTE during the second interval than during the first, while only three had decreased OTE. This is an indication that the treatment was becoming somewhat more effective as it was repeated.

DISCUSSION

A therapeutic strategy analagous to clinical management of cancer patients has been devised to indicate how the optimal utilization of chemotherapy and radiotherapy may be realized clinically if comparable information is obtained in man. The two salient features of this strategy are (*i*) recovery of the animal and its hematopoietic system from the previous treatment course, and (*ii*) giving the treatment when the tumor has a maximal rate of proliferation after the previous treatment course.

Scheduling for optimal destruction of tumor cells must be within the constraints imposed by the vulnerable host tissues. The gastrointestinal epithelium and the bone marrow usually suffer the most life-threatening toxic reactions during cancer chemotherapy because of high rates of cell renewal normally occurring at these sites and the critical functions that these organs perform. Intermittent courses of therapy, which allow for recovery of essential function by critical organs, should be of greatest therapeutic benefit to the patient, but direct determinations in man of minimal intervals between treatments are not possible because of the risks involved (3).

It has been found in well-defined "split-dose" animal survival studies that rats recover rapidly and reach 100% survival levels when the second dose of 5-FUra is given 10–11 days after the first dose. All animals die when the second dose of 5-FUra is given 3–4 days after the first. This rapid recovery in animal survival at 10–11 days is also associated with a rapid return to normal values for total bone marrow DNA and peripheral leukocyte counts (3). The epithelium of the gastrointestinal tract recovers earlier than does the hematopoietic system. Thus, treatment of tumors in the rat with 5-FUra can be carried out every 10–11 days because hematopoietic tissue recovery occurs within this time. Studies on the recovery of the kinetics of tumor cell proliferation have shown that deoxyuridine incorporation into tumor cell DNA is markedly depressed for 2 days after treatment and returns to control levels by day 9; the maximal rate occurs at 11–12 days and returns to control value by day 21 (3). The maximal tumor volume change also occured 12 days after 5-FUra administratin (4).

The finding of the accelerated rate of tumor proliferation in the 5-FUra treated tumors compared to control tumors 11–12 days after 5-FUra has important clinical ramifications with regard to sequential combined modality therapy. The more rapidly proliferating a tumor becomes, the more sensitive it should be to cell cycle-specific chemotherapeutic agents such as 5-FUra. Because the maximal rate of proliferation occurs 11–12 days after 5-FUra administration, this should be the time for the maximal relative tumor sensitivity to 5-FUra after the previous course of 5-FUra. Increased thymidine labeling indices have been found 10, 12, and 15 days after treatment (compared to controls) of a plasmacytoma with cyclophosphamide (7). These findings in different experimental animal tumor models suggest that solid tumors in man might be more sensitive to the second and subsequent courses of chemotherapy if the therapy could be given clinically at the time the treated tumor is proliferating at a maximal rate after the previous treatment course.

Another way in which combined modality therapy may be more effectively utilized is to give the second form of therapy at a time when the maximal number of cells are in the most sensitive stages of the cell cycle as a result of partial synchrony by the first form of therapy. Previous studies have shown a 2- to 3-fold increase in numbers of tumor cells in the S phase of the cell cycle present after a single exposure to 5-FUra or radiation (5, 6). Maximal cell synchrony occurs 12 hr after a single exposure to radiation and 24 hr after 5-FUra. In these studies, 5-FUra was given 12 hr after radiation. In future studies, radiation would be given 24 hr after 5-FUra to take advantage of the later maximum in cell synchrony after 5-FUra compared to radiation.

The relationship between changes in tumor cellularity and changes in tumor volume is complex. Much additional information is needed to elucidate further the dynamic relationship between tumor cell kill after chemotherapy or radiotherapy and changes in tumor volume (6, 7). In addition to immediate changes in cell viability after radiotherapy or chemotherapy, more information is needed about the kinetic cellular changes and tumor histology over the entire period between courses of treatment. At present, changes in tumor volume provide an index for the net result of all of the complex internal cellular and histological changes that occur in solid tumors after treatment. In addition, it is one of the more clinically relevant measurements that can be made to assess therapeutic response. Tumor volume change is one of the most frequently used clinical methods for evaluating tumor response to different forms of therapy.

Previous studies of this series and other investigations using different solid tumor models in different animal species have all demonstrated the variability of response to different forms of treatment in experimental tumors that have been serially transplanted for many years in inbred hosts (1, 8, 9). This lack of a uniform response to treatment indicates that conventional methods of analysis such as mean values mask important experimental therapeutic results. For example, mean values mask the fact that tumor C-2 (Fig. 1) does not respond to therapy and the fact that tumor C-4 is eradicated after the second course of combined modality therapy. These findings also emphasize the differences in the response to therapy previously found after a single course of treatment in this animal tumor system as well as tumor systems in other animal species (1, 8). These differences in tumor response to treatment for the same tumor in different hosts is analagous to many clinical situations in which the same tumor type varies in therapeutic response from patient to patient. It is evident that a better understanding of these differences in experimental solid tumor models is needed to explain similar differences in therapeutic response in the same tumor type found in patients.

One of the important findings in this study with regard to clinical relevance is that relating to the type of response individual tumors demonstrate over successive courses of therapy. These findings have shown that a tumor that responds well to the first course of therapy will respond well to the second and third courses of therapy. The ability to predict which tumors

will respond to treatment has obvious clinical ramifications.

The suggestive evidence of increased efficiency of treatment with the second and third courses of therapy compared to the first course needs additional study to determine if this is a consistent finding. If this can be confirmed, it has obvious ramifications with regard to the sequential utilization of combined modality therapy. It also suggests that clinical management might be improved by the more effective sequencing of therapy. Combined chemotherapy and radiotherapy has the added advantage of permitting the chemotherapy to act prophylactically to prevent the metastatic spread of the cancer during the series of treatments designed to control the primary tumor. These results have also demonstrated the validity of the therapeutic strategy used in this study because successive combined doses of radiation and 5-FUra resulted in successively smaller tumor volumes with eventual tumor eradication in some tumors.

The assistance of Jean Wakefield, Mary Stuart, and Mark Evans is gratefully acknowledged. This investigation was supported in part by U.S. Public Health Service Research Grant 20516 from the National Cancer Institute.

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