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Chemotherapy and surgery of spontaneous tumors of mice

S.R. Humphreys, N. Mantel, A. Goldin

Drug Evaluation Branch, Cancer Chemotherapy National Service Center and Biometry Branch, National Cancer Institute, Bethesda, Maryland, USA

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Abstract

Daily treatment with 3′5′dichloromethotrexate, 6-mercaptopurine or Cytoxan retarded the growth of spontaneous mammary tumors in C_3H/HeN mice. However, with possibly a few exceptions, no increases in survival time of the mice resulted.

Surgical excision of the spontaneous tumors plus weekly treatment with Cytoxan did not result in any increases in survival time of the mice. The combination, nevertheless, appeared to be more effective than surgery alone or Cytoxan alone in controlling tumor growth. It increased the incidence of cases in which no tumor growth, whether excised tumor regrowth or new tumor formation, was evident at the time of death. In addition, there was a decrease in the number of mice that died with multiple tumors. However, with the combination of surgery and chemotherapy, new tumors arose despite the continuation of therapy.

The data appear to lend support to the concept that reduction in tumor mass may augment therapeutic response.

Résumé

Un traitement quotidien par le 3′5′dichlorométhotrexate, la 6-mercaptopurine ou le cyclo-phosphamide retarde la croissance de tumeurs mammaires spontantées de souris C_3H/HeN. Cependant, sauf de rares exceptions, la survie des souris n'est pas prolongée.

Une exérse chirurgicale des tumeurs spontanées, associée à un traitement hebdomadaire par le cyclophosphamide, n'augmente pas la survie. Néanmoins, la combinaison semble étre plus eficace que la chirurgie seule ou que le cyclophosphamide seul pour contrôler la croissance des tumeurs. Elle augmente l'incidencede cas dans lesquels on ne peut mettre en évidence aucune croissance tumorale – que ce soit sous forme d'une récidive locale aprés exérése ou formation d'une nouvelle tumeur – au moment de la mart. De plus, il y a un moins grand nombre de souris qui meurent avec des tumeurs multiples. Cependant, avec l'association de la chirurgie et de la chimiothérapie, de nouvelles tumeurs apparaissent malgre la poursuite du traitement.

Ces données semblent appuyer l'hypothese que la reduction de la masse tumorale augmente l'efficacit de la chimiothtrapie.

Zusammenfassung

Die tägliche Behandlung mit 3′5′ Dichloromethortexat, mit 6-Mercaptopurin oder mit Cytoxan verlangsamte das Wachstum von spontanen Brusttumoren in C_3H/HeN-Mäusen. Trotzdem wurde, von einigen möglichen Fällen abgesehen, keine Verlángerung der Lebenspanne der Mäuse erzielt.

Die chirurgische Entfernung der Spontantumoren kombiniert mit wöchentlicher Anwendung von Cytoxan führte zu keiner Verlängerung der Lebenspanne der Mäuse. Trotzdem schien diese Behandlungsmethode das Tumorwachstum besser zu kontrollieren als chirurgische Behandlung oder Cytoxananwendung allein. Die Anzahl der Fälle, bei denen nach dem Tode kein Geschwulstgewebe, sei es ein Rezidiv oder ein Neugewächs, zu finden war, schien grösser zu sein. Ausserdem nahm die Anzahl der Fälle, die nach dem Tode multiple Tumoren aufwiesen, ab. Trotzdem entstanden neue Geschwulste, selbst bei ununterbrochener Behandlung, wenn die Kombination van Chirurgie and Chemotherapie angewandt wurde.

Diese Resultate scheinen die Hypothese zu bestäligen, dass die Verminderung der Tumormasse die Wirksamkeit der Chimiotherapie erhöht.

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1. Introduction

The transplantable tumor has been the principal tool in the experimental evaluation of anticancer agents. Primarily this has been justified by convenience and the need for standardized methodologies. Further, some correlation has been noted between the chemo-therapeutic responses of human tumors and transplantable mouse tumors, e.g. acute lymphoblastic leukemia of children and the transplantable mouse leukemia L-1210 [1,2].

Questions may nevertheless be raised as to the appropriateness of the use of transplantable tumors. It has been shown by Woolley *et al.* [3] and Scholler *et al.* [4] that spontaneous tumors are more resistant to chemotherapy than are similar transplanted tumors and that, although transplanted tumors may be affected or eradicated by chemotherapy, the corresponding autochthonous tumor may be unresponsive. In recent studies Chirigos *et al.* [5] and Glynn *et al.* [6] in investigations on the chemotherapy of Moloney virus-induced leukemia also observed a relative resistance of viral-induced autochthonous tumors as compared to corresponding transplantable tumors. Hirschberg, in a comprehensive survey of patterns of response of animal tumors to anticancer agents, concluded that the available information indicates that spontaneous and induced mouse tumors are less readily inhibited by chemotherapeutic agents than are transplanted neoplasms [7].

A number of investigations have indicated that the effectiveness of chemotherapy may decrease as the amount of tumor in the animal is increased. In the experimental chemotherapy of transplanted tumors, Goldin *et al.* [8] have reported on the marked refractoriness to treatment of the advanced tumor albeit they eventually achieved success against advanced mouse leukemia, L-1210, through use of appropriate drugs [9]. Goldin *et al.* [10] and Skipper *et al.* [11] found that the effectiveness of therapy with a folic acid antagonist diminished as the number of cells in a leukemic inoculum was increased. Surgical excision of established transplantable tumors increased the effectiveness of chemotherapy [12–15]. Similarly, surgery and adjuvant chemotherapy was reported to increase the percentage of 'cures' of spontaneous mammary mouse carcinoma, as compared with surgery or therapy alone [16–18]. Whether the refractoriness to treatment of the autochthonous tumor is essentially similar to that of the advanced transplanted tumor is then a possibility.

Our experience in two investigations into the treatment of spontaneous mouse tumors with chemical agents, surgery, or both are presented in this report.

2. Methods

2.1. Experiment 1

In this experiment, conducted over a period of 7 months, female C_3H/HeN mice bearing spontaneous tumors [19] [1] were employed. They were obtained from the NIH breeding colonies following detection of the tumors in the course of routine handling. [2] Age data for the mice were not available, though such mice are not ordinarily kept beyond 9 months as breeders.

On receipt, mice varied markedly in their body weight, general physical appearance, and in the size of their tumors; some mice had multiple tumors. According to the order of receipt from the breeding colony, mice were assigned to one of four treatment groups: untreated controls; daily subcutaneous injections of either 50 mg/kg 3′5′dichloromethotrexate (DCM) dissolved in 2% $NaHCO_3$, [3] or 60 mg/kg 6-Mercaptopurine (6-MP) dissolved in dilute NaOH and neutralized with dilute HCl, or 50 mg/kg Cytoxan dissolved in N-saline. Statistical randomization procedures were employed in determining the order of assignment. Mice were caged individually. Treatment was started on the day of receipt and was continued until eventual death of the mice. Tumors were palpated and mice weighed weekly, though initially more frequent palpation measurements were made.

2.2. Experiment 2

This experiment was directed towards evaluating the efficacy of treatment of spontaneous tumors in female C_3H/HeN mice by surgery, or by treatment with Cytoxan, 180 mg/kg injected subcutaneously at weekly intervals, or by combined surgical-chemotherapeutic procedures.

The great variability among mice in the first experiment suggested the need for standardized procedures to achieve uniformity. To this end we maintained in our own laboratory 200 C_3H/HeN female mice, obtained in weekly groups of fifty each from the breeding colony, and initially 4 weeks old. The female mice were caged together with C_3H male mice and permitted to breed; they were not, however, permitted to nurse their offsprings. Mice were examined at weekly intervals for palpable tumors, the first such tumors being observed in the eighth month (32nd week) following the average time of birth of the mice. By the twelfth month more than half of the mice (109/200) had developed palpable tumors and had been admitted to the experiment.

[1] Although pathology was not done on the current tissues this strain has been reported to have a high incidence of mammary tumors [19].

[2] The NIH Animal Production Unit kindly provided tumorous mice from its breeding colony.

[3] The drugs employed in these experiments were obtained from the Cancer Chemotherapy National Service Center.

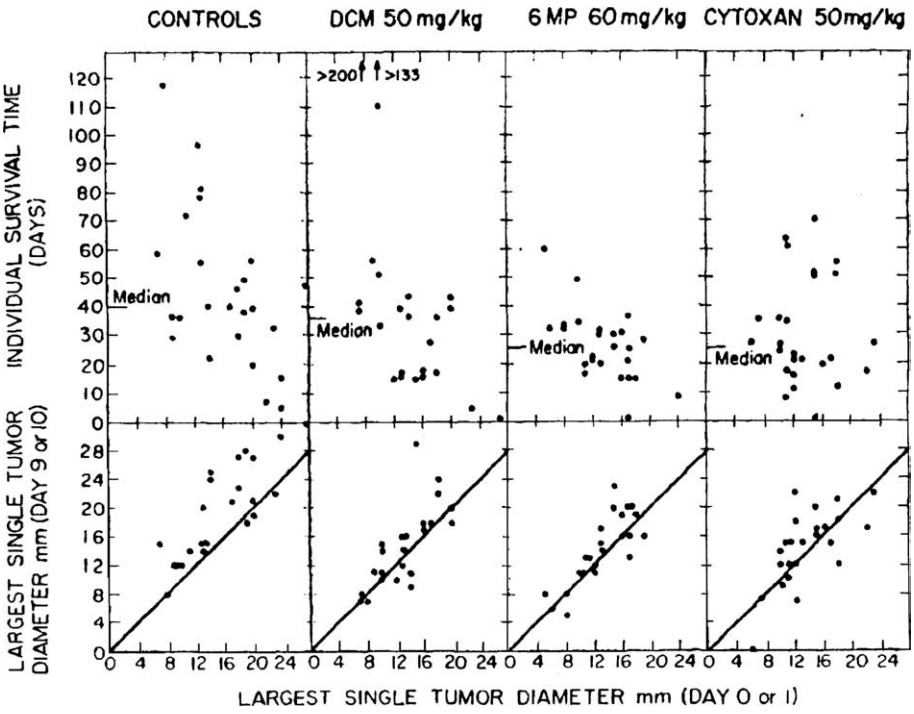

Fig. 1. Efficacy of various drug treatments for reducing tumor size and prolonging survival time of C_3H/HeN mice bearing spontaneous mammary tumors. The panels show the individual survival times and the maximum single tumor diameter on day 9 or 10 as a function of the maximum single tumor diameter when the mouse was entered into the study. The diagonal lines in the lower panels indicate where a point would fall if there was no change in tumor diameter over the early treatment period.

The mice found with palpable tumors each week were assigned at random to one of the four treatment groups: untreated controls; surgery only; cytoxan only; surgery followed the next day and at weekly intervals thereafter by cytoxan. Simple surgical removal, by techniques previously described [15], of grossly visible tumor tissue was performed under nembutal anesthesia and the wound closed with clips. Tumorous mice admitted to the experiment were caged individually and observed daily for general appearance, weekly for weight changes, growth or regrowth of old tumors or appearance of new tumors.

3. Results

3.1. Experiment 1

The various panels of Fig. 1 show the significant results, individually for each mouse, of this experiment. The upper control panel shows that untreated mice survived for as little as 5 days or for as much as 117 days (median 40 days) following admission to the experiment. This extreme variation in survival time could be related to the great differences in initial tumor sizes, the diameter of the largest tumor in a mouse varying from 7 to 28 mm. [4] The control data do indicate that with increasing initial tumor size the survival time of mice following admission into the study decreases. But even for a fixed initial tumor size there remains considerable variation in survival times.

The corresponding three drug treatment panels also show considerable variability in survival times and do not indicate any of the three drugs to be efficacious in prolonging the life of tumorous mice (medians: DCM, 36 days; 6-MP, 26 days; Cytoxan, 26 days); if anything, survival time has been clearly reduced for the 6-MP and Cytoxan groups. Suggestive of some possible efficacy for the DCM is the occurrence in that group of two cases of quite prolonged survival time.

The bottom panels of Fig. 1 show the effect of drug treatment on tumor size. To take into account the great variation in initial tumor sizes, the panels show the diameter of the largest tumor 9 or 10 days after beginning of treatment plotted against the initial or day 1 largest single tumor diamcter. [5] The diagonal lines shown on these lower panels help judge changes in tumor size over the interval. Tumor growth is indicated by points falling above the diagonal line, stasis by points falling on or near the line, regression by

[4] Volumewise this could reflect on the order of a 100-fold difference in tumor sizes.

[5] This day, 9 or 10, was selected for comparison purposes as a compromise. With too early a day there would be inadequate opportunity for treatment to affect tumor size; with too late a day, selection effects, because of death of animals, could have become important.

points falling below the line. Comparing the lower panels, it can be seen that untreated controls typically showed tumor growth while the treated groups showed lesser amounts of growth and perhaps also some degree of stasis or regression. But such antitumor effects of therapy were not accompanied by increases in survival time, as already noted.

3.2. Experiment 2

Fig. 2 and Table 1 present the outcome of the second experiment.

For each animal in each experimental group, Fig. 2 shows the survival time following tumor appearance and initiation of treatment. Whether death was with none, one, or multiple tumors is indicated. Where tumors were excised surgically and subsequently recurred, it is indicated by the symbol R.

From Fig. 2 it is possible to gauge the role of animal age at time of tumor appearance, and the control panel data do suggest that tumors in a older mice do take longer to kill than do tumors in younger mice. It may be noted from the figure that tumors did not appear until week 32.

Table 1 summarizes the data of Fig. 2, with results combined for all mice in an experimental group irrespective of week of tumor appearance, From the Table the following can be seen:

1. Mice on Cytoxan only had the shortest survival time, median survival times being substantially equal for the three remaining groups. The decrease in survival for the mice treated with Cytoxan alone appears to be attributable to increased drug toxicity in the presence of a large tumor mass, since longer survival times were observed when the same treatment was given to mice which had undergone surgical removal of the tumors. The wide variation in individual survival time for all groups should also be noted.
2. Late deaths, after day 50, without tumors occurred 9 times in the group receiving surgery plus Cytoxan but not at all in the group receiving surgery only. With Cytoxan alone only one mouse died late without tumor.
3. Excised tumors recurred in 20 of 28 mice on surgery only, but in only three of 28 mice which, in addition, received Cytoxan.
4. The occurrence of new tumors was reduced in mice on Cytoxan, whether or not accompanied by surgery.

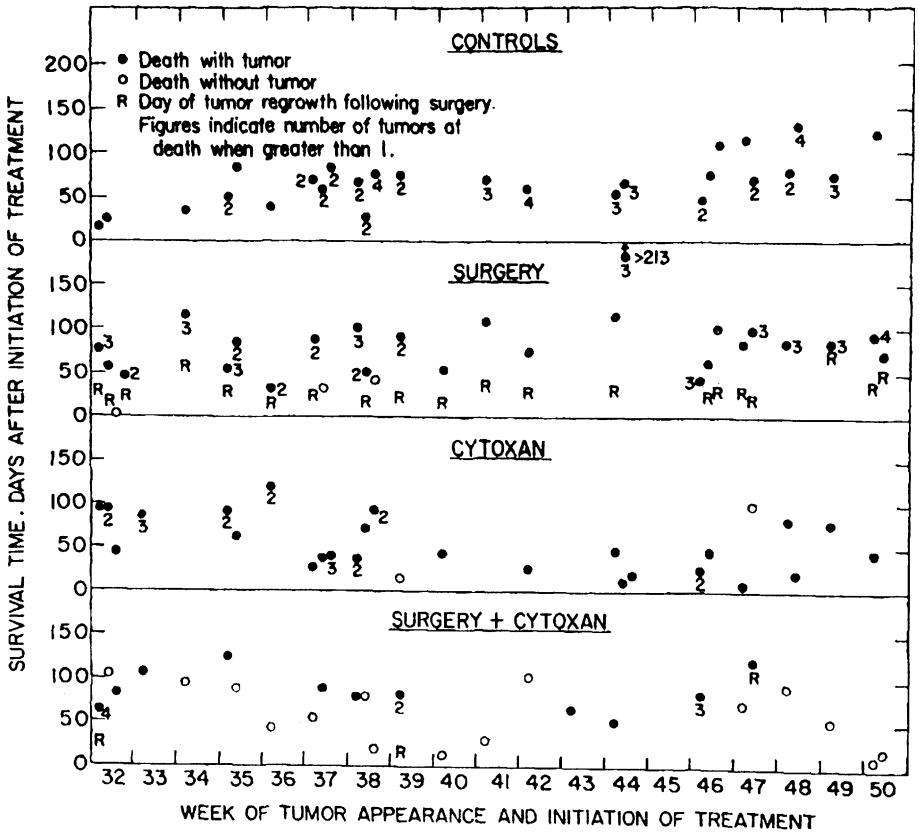

Fig. 2. Efficacy of drug treatment and of surgical removal, separately and in combination, in the therapy of spontaneous tumors arising in a defined cohort of C_3H/HeN mice. The figure shows for each mouse when its tumor appeared and its therapy was initiated and how long the mouse subsequently survived. The occurrence of new tumors subsequent to treatment initiation and the time of recurrence of excised tumors are indicated. New tumors have occurred when more than one tumor is indicated at time of death or if any tumors are indicated at death where the excised tumor is not shown to have regrown.

Table 1
Effect of surgery and Cytoxan, alone and in combination, in the treatment of spontaneous tumors in C_3H female mice

	Controls	Surgery only	Cytoxan only	Surgery + Cytoxan
No. of mice	26	28	27	28
Median	70	81	45	75
Survival time, days				
Range	18–131	0 to >213	9–119	1–124
Deaths without tumor after day 50, no.	0	0	1	9
Tumor recurrences, no.		20		3
Median day		29		29
Range		14–78		15–113
Deaths with multiple tumors, no.		16		3
	17		8	
Mice developing new tumors, no.		16		10
Average no. of new tumors				
All mice	1.0	1.1	0.4	0.5
Mice with new tumors only	1.6	2.0	1.2	1.4

4. Discussion

In studies with surgery and adjuvant chemotherapy in mice with spontaneous mammary carcinoma, Martin obtained a higher percentage of 'cures' for surgery plus Cytoxan than for surgery or Cytoxan alone [16,17]. Also surgery plus combination chemotherapy with thioguanine and mitomycin C or uracil mustard and mitomycin C prevented recurrence of surgically removed spontaneous mouse tumors in approximately 50 per cent of the animals. Surgery plus chemotherapy with only one of the drugs prevented recurrence in 20–30 per cent of the animals [18].

In the current experiments, 3'5'dichloro-methotrexate, 6-mercaptopurine and Cytoxan were effective in inhibiting tumor growth. The drugs, however, failed to increase the survival time of the animals.

Although therapy with the combination of Cytoxan and surgical enucleation of tumor did not result in tumor free survivors or in an increase in the survival time of the animals, the evidence does indicate that surgery plus Cytoxan was more effective in the control of tumor growth than the surgery employed or chemotherapy alone. With the combination of the surgery and weekly administration of Cytoxan there was a marked increase in the incidence of cases in which no tumor growth was evident at the time of death since in these animals the surgically removed tumors failed to regrow and new tumor growth was prevented. Surgery plus chemotherapy also resulted in a decrease in the number of mice that succumbed with multiple tumors.

The limitations to therapy with these drugs and with surgery plus chemotherapy would appear to be the toxicity of the drugs and the refractoriness to treatment of these spontaneous tumors. The continuous treatment in the current experiments increased the risk of deaths attributable to toxicity, and progressive weight loss in many of the mice suggests that this may have been an important contribution to death. Nevertheless, in neither drug-treated group did continuous Cytoxan administration wholly prevent growth of new tumors or regrowth of excised tumor. In fact new tumors appeared in mice where Cytoxan apparently prevented the regrowth of surgically excised tumors.

The current studies lend support to the concept that a decrease in tumor mass may improve the therapeutic effectiveness of drugs [10–12,14,20]. The reduced number of tumor cells may be more vulnerable to adjuvant chemotherapy and if immune mechanisms are implicated they may contribute to enhanced therapeutic effect against a reduced tumor mass [16,17]. Such attempts to improve therapy of human tumors have been reported by clinical investigators [13,21–28]. Further investigation of surgery and chemotherapy of spontaneous tumors is undoubtedly warranted.

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