WALKER CARCINOSARCOMA 256 IN STUDY OF ANTICANCER AGENTS. I. METHOD FOR SIMULTANEOUS ASSESSMENT OF THERAPEUTIC VALUE AND TOXICITY

V. M. Rosenoer, B. C. V. Mitchley, F. J. C. Roe, and T. A. Connors

SUMMARY

Described is the screening system with the Walker tumor in rats currently used at the Chester Beatty Research Institute. The biologic criterion of activity is defined in terms of the chemotherapeutic index measured in a combined therapeutic-toxicity assay.

INTRODUCTION

Drug screening refers to the initial testing of compounds and other substances for some specific pharmacologic activity; the immediate goal is to select compounds which show promise for more intensive study, and to indicate for testing other compounds structurally or pharmacologically related to the promising ones. The main function of a screening procedure is to distinguish between potentially active and inactive substances (1). The more economically this is done, in terms of animals and time, the greater is the proportion of the available facilities which can be devoted to more intensive study of promising compounds.

In the procedure adopted at the Chester Beatty Research Institute in the study of certain types of anticancer agents, particularly the alkylating agents, the biologic criterion of activity is defined in terms of the therapeutic index (TI) measured in combined therapeutic-toxicity assays in groups of rats with Walker carcinosarcoma 256.

MATERIALS AND METHODS

Walker Carcinosarcoma 256. This tumor arose in a pregnant albino rat in 1928 in the laboratories of Dr. George Walker, Johns Hopkins University School of Medicine. Histologically a mammary adenocarcinoma, it was transplanted successfully into the Doure strain of rats and later into other strains. Earle (2) reported that the adenomatous structure disappeared early in the transplant history, and though the stromal elements varied from generation to generation, the stock strain in his laboratory appeared free from any signs of sarcomatous elements.

A Walker tumor line has been maintained continuously at the Chester Beatty Research Institute since 1944. It has been serially transplanted in 6- to 8-week-old randombred, male, albino, Chester Beatty rats at 6- to 8-day intervals by implanting carefully selected 4- to 6-cu mm fragments subcutaneously into the right flank with a No. 8 trocar. The tumor has grown reproducibly, killing the host in 14-16 days when the tumor is about 8 by 5 by 5 cm and weighs about 60 gm.

Histologically the tumor is pleomorphic but usually contains a prominent spindle cell element. The tumor grows so rapidly that it soon outstrips its blood supply and central necrosis is usual from the 7th day onward. However these central necrotic areas still contain viable tumor cells (4).

The Walker tumor line maintained in this Institute has remained reasonably stable in its sensitivity to different classes of cancer chemotherapeutic agents. For example, it is now as sensitive to certain alkylating agents, and as insensitive to certain antimetabolites, as it was 10 years ago. However no tumor, regardless of how long it has been serially transplanted, should be regarded as completely stable. Koller (5) has shown that the modal chromosome number of this Walker tumor line has fluctuated during the past 15 years.

Preliminary Toxicity Studies. If adequate information is available on the toxicity of the compounds to be studied, it provides the basis for selecting doses for the subsequent combined therapeutic-toxicity test. Otherwise, preliminary toxicity studies are done in 8 normal rats at 4 widely separated and loga-

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rhythmically spaced doses—usually 5, 25, 125, and 625 mg/kg—given intraperitoneally (i.p.) as a single
dose or daily doses depending on the proposed treatment schedule. The rats are observed daily for 14
days and the results are recorded as death, weight loss, or no apparent effect. Two untreated rats housed
in the same cage serve as controls. The results of this test have proved sufficiently reliable and informative
for selecting the dose range for a combined therapeutic-toxicity test (Table 1).

Combined Therapeutic-Toxicity Test. This test is done in a group of 24 rats bearing the Walker
tumor. Usually 6 logarithmically spaced doses are given i.p., or occasionally by other routes, to 18
rats (3 rats per dose) as a single dose or daily doses starting 24 hours after tumor implant. The other 6
animals are the untreated controls. In most studies a factor of 2 has separated doses and the actual
doses have been selected as indicated in Table 1. The animals are killed 10–14 days after implantation
and the tumors are removed by careful dissection, blotted dry, and weighed to the nearest 0.5 gm. Careful
clinical records are kept and a thorough postmortem examination is done on each animal.

The minimum effective dose (MED) is the dose which inhibits the growth of the tumor to 10% of the
control tumor weight, estimated by linear interpolation on the log dose-tumor weight curve at doses
spanning the required value. The LD50 is estimated by the Spearman–Karber method (3) from Table 2.
This method has been adopted since the dose-toxicity slope for most of the compounds tested is very
steep. An LD90/LD10 ratio greater than 2 is exceptional and the 3 patterns of survival shown in Table 2
apply to most of the compounds.

The therapeutic index (TI) is LD50/MED. When testing chemically related compounds it has been
possible to predict the TI from the results obtained with previously tested members of the series. In these
cases as few as 3 and as many as 10 logarithmically spaced doses have been required to span the MED-
LD50 dose range and, when appropriate, the test is modified to meet these requirements.

Tests are rejected if the mean control tumor weight is not between 20 and 60 gm, if the standard
error of the mean control tumor weight exceeds 4 gm, or if the MED or the LD50 cannot be estimated in a
single test. This occurs infrequently with the system described but even in these cases sufficient infor-
mation is provided for a second, more adequate assay.

Solvent Control. Past experience indicates that simple solvents and suspending media, e.g.,
water and arachis oil, given i.p. have no effect on the growth of the Walker tumor. Therefore a group of
animals given solvent alone is not included in the screening test unless unusual solvents are used. The
possibility of drug-solvent synergism cannot be excluded by a test of this type and must be studied sepa-
rately in the more extensive investigations reserved for compounds of interest.

Host Body Weight. During the therapeutic test, clinical records are kept and postmortem examina-
tions are done on all animals. Each animal is weighed at the beginning and at the end of the test and
body weight changes are noted, excluding the tumor weight. The observations are valuable in the subse-
quent study of interesting compounds.

RESULTS

The detailed results of typical tests with chlorambucil, aniline mustard, and naphthylamine mustard
are shown in Charts 1–3. The results of repetitive testing of these compounds are given in Table 3 to
illustrate the degree of stability of the system.

DISCUSSION

The test system described was introduced in 1962. It replaced an earlier, more empirical system
designed to select those materials which, at the maximum tolerated dose, produced an antitumor effect
equal to or greater than a prescribed minimum. In this earlier system carcinostatic activity was expressed
as the ratio of the average tumor weight in control animals (C) to the average tumor weight in treated ani-
mals (T) after a defined period of treatment and observation. Although the responses were quantitative,
they were interpreted in an all-or-none manner: the compounds were either rejected or accepted. The
screen was not designed to compare the activity of compounds quantitatively, and it was not possible to
compare quantitatively the results from this test system with results from other similar tests.
Walker Carcinosarcoma 256 in Study of Anticancer Agents. I. Therapeutic Value and Toxicity

This type of test has been of great value in detecting anticancer activity, and the activity of several clinically useful alkylating agents was first revealed in the empirical Walker tumor test. However in recent years justifying the clinical trial of new drugs has become increasingly difficult. Superiority over existing drugs must be clearly demonstrable. It has long been realized that a relative tumor inhibition-toxicity index would provide a more rational and informative basis for comparing potential antitumor agents than a tumor-inhibition index alone (6). The present Walker tumor system has been designed to obtain an estimate of the chemotherapeutic index of each compound tested, enabling the quantitative comparison of the therapeutic value of new agents with that of known drugs.

The stability of the system in terms of tumor growth and of the estimates of the MED, LD50, and TI of drugs is shown by the results with chlorambucil, aniline mustard, and naphthylamine mustard. Five independent estimates of the TI of chlorambucil gave a mean of 8.9 with a range of 6.6 to 12.4, while 5 estimates of the TI of aniline mustard gave a mean of 10.5 with a range of 5.1 to 15.3 and 3 estimates of the TI of naphthylamine mustard gave a mean of 9.2 and a range of 5.7 to 11.3. The data now available are insufficient for full statistical analysis but it is stressed that TIs differing by less than a factor of 3 should not be regarded as significantly different unless confirmed by further experiments.

The screening system described is primarily designed to reject inactive compounds with a minimum of testing; only 8 normal, tumor-free and 18 tumor-bearing animals are used to test an inactive compound. Results are usually available 4 weeks after a compound is tested. Active anticancer agents are retained for further study; the priorities of further testing are determined by the novelty of chemical structure, and the magnitude of the TI and MED.

ACKNOWLEDGMENT

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REFERENCES


### TABLE 1
Selection of Doses for Therapeutic-Toxicity Tests

<table>
<thead>
<tr>
<th>RESULTS OF PRELIMINARY TOXICITY TEST IN 2 NORMAL RATS PER DOSE:</th>
<th>DOSES CHOSEN FOR THERAPEUTIC-TOXICITY TESTS (mg Ag, i.p.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOSES mg/kg, i.p. *--</td>
<td></td>
</tr>
<tr>
<td>625</td>
<td>125</td>
</tr>
<tr>
<td>0</td>
<td>1600</td>
</tr>
<tr>
<td>1 or +</td>
<td>1280</td>
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<tr>
<td>2</td>
<td>640</td>
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<tr>
<td>2 or +</td>
<td>320</td>
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<td>2</td>
<td>160</td>
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<tr>
<td>2 or +</td>
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</tr>
<tr>
<td>2</td>
<td>2.5</td>
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* Numbers are the rats that died; + = marked weight loss; 0 = no effect.

### TABLE 2
Estimation of LD50

<table>
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<tr>
<th>LOG DOSE</th>
<th>NUMBER OF ANIMALS SURVIVING</th>
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<tbody>
<tr>
<td>x</td>
<td>(\frac{3}{3})</td>
</tr>
<tr>
<td>x + d</td>
<td>(\frac{0}{3})</td>
</tr>
<tr>
<td>x + 2d</td>
<td>(\frac{0}{3})</td>
</tr>
</tbody>
</table>

Log \(LD50 = \log 80 + 0.833 \log 2 = 2.154\)

\(LD50 = 143 \text{ mg/kg}\).

### TABLE 3
Results of Repeated Tests With 3 Compounds

<table>
<thead>
<tr>
<th>ENTRY NO.</th>
<th>CB NUMBER</th>
<th>COMPOUND NAME*</th>
<th>MOLECULAR FORMULA</th>
<th>SOLVENT</th>
<th>DOSE SCHEDULE</th>
<th>MED (mg/kg)</th>
<th>LD50 (mg/kg)</th>
<th>TI</th>
</tr>
</thead>
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<tr>
<td>73581</td>
<td>1348</td>
<td>Chlorambucil; butyric acid, 4-[p-[bis(2-chloroethyl)]amino]phenyl]-</td>
<td>(\text{NO}_2\text{Cl}_9\text{C}<em>14\text{H}</em>{15})</td>
<td>Arachis oil</td>
<td>One dose i.p. Day 1</td>
<td>2.7</td>
<td>17.8</td>
<td>6.6</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>2.7</td>
<td>17.8</td>
<td>6.6</td>
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<td>1.8</td>
<td>22.4</td>
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<td>2.0</td>
<td>17.8</td>
<td>8.9</td>
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<tr>
<td>73582</td>
<td>1074</td>
<td>Aniline mustard; aniline, N,N-bis(2-chloroethyl)-</td>
<td>(\text{NCI}_9\text{C}<em>10\text{H}</em>{13})</td>
<td>Arachis oil</td>
<td>One dose i.p. Day 1</td>
<td>12.6</td>
<td>143</td>
<td>11.3</td>
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<td>12.7</td>
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<td>15.3</td>
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<td>17.8</td>
<td>90</td>
<td>5.1</td>
</tr>
<tr>
<td>73583</td>
<td>1048</td>
<td>Naphthylamine mustard; 2-naphthylamine, N,N-bis(2-chloroethyl)-</td>
<td>(\text{NCI}_9\text{C}<em>14\text{H}</em>{15})</td>
<td>Arachis oil</td>
<td>One dose i.p. Day 1</td>
<td>67</td>
<td>708</td>
<td>10.6</td>
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<td></td>
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<td>79</td>
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<td>125</td>
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* Source: Chester Beatty Research Institute.
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