A Mitomycin C-resistant Jensen Rat Sarcoma: Isolation and Transplantation Studies*

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SUMMARY

A transplantable rat tumor, the Jensen sarcoma, has been selected for resistance to the action of mitomycin C. Initial treatment consisted of 0.15 mg/kg. After seven passages through rats, the treatment dose was raised to 0.5 mg/kg. Under normal circumstances, this dose of antibiotic completely inhibits tumor growth. The stability of this resistance was proved by passage of the tumor through more than twenty generations in untreated rats.

The antibiotic-resistant tumor was also found to have the following characteristics: (a) growth as a firm mass with less necrosis and central liquefaction than is ordinarily observed in the sensitive sarcoma; (b) a greater tendency to persist for longer periods of time in the rat host before showing drastic signs of regression; (c) a slow growth rate in cortisone-treated Swiss mice; (d) poor transplantability to neonatal mice; and (e) cross-resistance to nitrogen mustard in tests with the cortisone-treated Swiss mouse.

As an aid in experiments designed to study the effect of heterologous transplantation on tumor response to drug treatment, a mitomycin C-resistant Jensen rat sarcoma was isolated. It is the purpose of this paper to describe the isolation of the tumor and its growth characteristics.

Methods and Procedures

Female rats (Sprague-Dawley) weighing 80–120 gm. were used for transplantation and maintenance of the mitomycin C-sensitive Jensen rat sarcoma (to be designated JRS). The tumor was routinely passaged every 9–11 days by means of subcutaneous inoculations of 1.0 ml. of a 50 per cent minced tumor suspension prepared in physiological saline fortified with streptomycin sulfate (2 mg/ml) and penicillin G (500 units/ml).

Mitomycin C was prepared every 2 weeks as a stock solution in saline (0.86 per cent) in a concentration of 0.3 mg/ml. During this time the stock solution was kept refrigerated. Dilutions of the stock were made with saline. Rats were treated daily with single intraperitoneal injections starting 24 hours after tumor transplantation. Treatment was then continued for 9–10 days.

RESULTS

At the start of these experiments, rats bearing the sensitive tumor (JRS) were treated with 0.15 mg/kg of mitomycin C to select a “resistant” tumor passage. After four passages in rats treated with 0.15 mg/kg mitomycin C, experiments revealed that the tumor had become resistant to 1.0

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mg/kg dose levels. In an experiment performed at this time, “resistant” tumors (to be designated hereafter as JRS/MC) grew in three out of six animals treated with 1.0 mg/kg. The three tumors attained an average diameter of 4.56 cm. Under ordinary circumstances, this dose of mitomycin C (1.0 mg/kg) causes complete destruction of JRS tumor transplants.

After the seventh passage of tumor in rats treated with mitomycin C at 0.15 mg/kg, the JRS/MC tumor was passed to rats treated with 0.5 mg/kg. The tumor is now maintained routinely in such antibiotic-treated rats.

During the first 2-week period after tumor transplantation, resistant tumors grow in mitomycin C-treated rats as firm, well vascularized masses which show very little central necrosis. This is in contrast to the growth of sensitive tumors which are less firm on palpation, whitish in appearance, and display a greater degree of gross necrosis with central liquefaction.

Several attempts were made to increase the treatment dose level to 1.0 mg/kg. One such attempt was made after the tumor had been passed for ten generations in animals treated with 0.5 mg/kg of antibiotic; these tumors were successfully transferred for eleven generations, but no tumor takes were obtained on the 12th passage, and the line was lost.

On the tenth passage in mitomycin C-treated rats, the JRS/MC tumor was transplanted into rats which were not subsequently treated with antibiotic. This was done to establish the stability of resistance. Data presented in Chart 1 illustrate that the JRS/MC tumor after fourteen passages in rats not treated with antibiotic retained its resistance to the drug.

A comparison of percentage “takes” and average sizes of the JRS/MC and JRS tumors at the end of 9–11 days of growth revealed no significant differences (Table 1). However, in terms of host mortality rate and the onset of tumor regression in surviving animals, differences were noted between the two tumors (Chart 2). The general impression to be gained from a comparison of the data presented in Chart 2 is: (a) the JRS and JRS/MC tumors have approximately the same growth rate during the first 2-week period after transplantation. Thereafter, the resistant tumor does not necessarily continue to increase in size. (b) JRS tumor-bearing rats die sooner than JRS/MC tumor-bearing animals. Data for this representative experiment show that 50 per cent of the

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td>A COMPARISON OF A MITOMYCIN C-RESISTANT (JRS/MC) AND SENSITIVE (JRS) JENSEN SARCOMA GROWING SUBCUTANEOUSLY IN RATS*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PASSAGE</th>
<th>JRS/MC†</th>
<th>JRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>9/9</td>
<td>7/14</td>
</tr>
<tr>
<td>6</td>
<td>15/18</td>
<td>15/18</td>
</tr>
<tr>
<td>9</td>
<td>14/18</td>
<td>15/15</td>
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<tr>
<td>12</td>
<td>10/18</td>
<td>15/15</td>
</tr>
<tr>
<td>15</td>
<td>15/17</td>
<td>17/19</td>
</tr>
</tbody>
</table>

* Female Sprague-Dawley rats weighing 80–120 gm.
† Mitomycin C-resistant tumor passaged in untreated rats.
‡ Final tumor sizes determined after 9–11 days of growth.
JRS tumor-bearing rats were dead by the 20th day, whereas only 25 per cent of the JRS/MC tumor-bearers had died during this same time period. (c) In rats which survive for 27–28 days, JRS tumors regress sooner than JRS/MC tumors. Experimental data in Chart 2 show that JRS tumors began to regress on the 17th day after transplantation in those rats which survived for 27 days, whereas regression of the resistant tumors began after the 24th day. Although this represents a lag in regression time of only approximately 7 days, it has been observed rather consistently and hence represents an important difference.

Growth in cortisone-treated mice.—As an outcome of our interest in heterotransplantation (12), experiments were designed to study the growth of the resistant and sensitive tumors in cortisone-conditioned mice. When transplanting tumors to mice, females (Swiss-Webster) weighing 18–20 gm. were given inoculations intramuscularly of 0.5 ml. of a 50 per cent tumor suspension; the mice were then conditioned with a single, subcutaneous dose of 3 mg. cortisone acetate (Cortone acetate, Merck). Mice were maintained for 10–14 days, at which time tumors were measured, and, in most cases, the mice were sacrificed and tumors removed and weighed. In those cases when tumors were sectioned and examined, the resistant tumor showed no remarkable changes in histology.

One of the characteristics noted for the JRS/MC tumor was its slow growth on heterotransplantation to cortisone-treated mice. A difference in growth between the JRS and JRS/MC tumor was first noted on the fourth passage of the tumor in rats treated with antibiotic at 0.15 mg/kg. Data are presented in Chart 3 to illustrate the striking difference in growth pattern of the two tumors.

In addition to showing differences in growth rate, Chart 3 presents data which demonstrate the response of JRS tumor to antibiotic therapy and the lack of response of the resistant tumor to similar treatments when tests are made in the conditioned heterologous host. Chart 4 shows that after passage of the JRS/MC tumor in rats not treated with antibiotic the tumor retained its pattern of slow growth in the cortisone-treated mouse.

Growth of the resistant tumor in newborn mice.—In view of the slow growth observed in cortisone-treated mice, experiments were designed to study the growth of transplants in neonatal mice. The suspensions of tumor cells used in these transplantation studies were prepared by first mincing the tumor with scalpels and then gently homogenizing the tissue in 9 volumes of cold physiological saline; cells were then washed and finally suspended in physiological saline fortified with penicillin G (500 units/ml) and streptomycin sulfate (2 mg/ml). The cell concentration was adjusted so that the required number of cells could be inoculated subcutaneously in volumes ranging from 0.05 to 0.1 ml. Newborns were first examined for tumor 7 days after inoculation, and final tumor measurements were made on the 11th–12th days.

**Chart 2.**—A comparison of the growth and regression rates of the mitomycin C-sensitive and -resistant Jensen rat sarcomas in female Sprague-Dawley rats.

Solid black circles represent the average diameter for all tumors measured at the specified time. Open circles refer to those tumors in rats which survived for the entire period of observation. Numbers in parentheses refer to the number of surviving animals.

Upper chart: Mitomycin C-sensitive Jensen sarcoma.
Lower chart: Mitomycin C-resistant Jensen sarcoma.
TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Swiss</th>
<th>DBA×C57BL/6</th>
<th>A×C57BL/6</th>
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<tbody>
<tr>
<td></td>
<td>JRS†</td>
<td>JRS/MC</td>
<td>JRS</td>
</tr>
<tr>
<td>No. newborns given implants</td>
<td>90</td>
<td>178</td>
<td>88</td>
</tr>
<tr>
<td>No. surviving newborns</td>
<td>74</td>
<td>152</td>
<td>14</td>
</tr>
<tr>
<td>No. tumor “takes”§ in surviving newborns</td>
<td>55</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>Per cent tumor “takes”§</td>
<td>47</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td>Per cent mortality</td>
<td>18</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>Av. diam. of tumors which grew (cm.)</td>
<td>1.09</td>
<td>1.14</td>
<td>1.02</td>
</tr>
</tbody>
</table>

* Tumor suspensions containing 1–7×10⁶ cells were inoculated into newborns less than 24 hours old. Final observations were made 11–12 days after transplantation.
† JRS: mitomycin C-sensitive tumor.
‡ JRS/MC: mitomycin C-resistant tumor.
§ Tumor “take” refers to measurable tumors growing subcutaneously.
subcutaneous or intraperitoneal tumors (although it is possible that microscopic foci of tumor cells remained from the original implant). The inability of F1 newborns derived from this cross to allow JRS/MC tumor implants to grow is unexplainable at this time.

Data in Table 2 also reveal that newborn random-bred Swiss mice, in contrast to hybrids, were less prone to die from inoculations of either JRS or JRS/MC tumor cell suspensions. Less than 20 per cent of the tumor-implanted, random-bred Swiss newborns died, whereas more than 50–60 per cent of the hybrid newborns succumbed. Deaths usually occurred within the first 7 days after tumor inoculation. During this period, it is unlikely that newborns would die of extensive invasion of vital organs by rapidly growing tumors. In fact, in the great majority of instances, gross inspection of the abdominal and thoracic cavities failed to reveal any extensive or untoward tumor formations. The cause of death is not known at this time.

Drug response.—Preliminary chemotherapy studies conducted in collaboration with Dr. Sugiuura revealed that the mitomycin C-resistant tumor was cross-resistant to alkylating agents (HN2, thioTEPA, and Cytoxan) and to the antimetabolite amethopterin. An investigation of the chemotherapeutic responses of the resistant tumor to a spectrum of chemical agents was therefore undertaken, and the results of these studies will be published elsewhere.

In addition to standard therapy studies on the rat, experiments were also attempted with the resistant tumor as it grew in the cortisone-conditioned mouse. Interest in this system was an outcome of earlier investigations on the response to therapy of a transplantable human tumor growing in conditioned mice (12). Data are presented for nitrogen mustard (Table 3) to illustrate cross-resistance of the tumor as it grew in cortisone-conditioned mice. Inhibitory effects were observed against the sensitive tumor, whereas no significant antitumor activity was observed with the resistant line.

### DISCUSSION

A mitomycin C-resistant Jensen rat sarcoma subline having an approximate three- to sixfold increase in drug resistance was isolated after four passages of the tumor in rats treated with 0.15 mg/kg of the antibiotic. This starting dose of mitomycin C represents a suboptimal therapeutically level for the Jensen rat sarcoma; normally, doses ranging from 0.25 to 1.0 mg/kg produce profound inhibitions in tumor growth (17). Other investigators have also isolated drug-resistant transplantable rat (6, 8, 9, 13) and mouse (16) tumors by initially using suboptimal treatment doses; however, this technic need not necessarily be used. Burchenal (1) and Clarke (2) were successful in isolating drug-resistant mouse neoplasms from groups of animals that had been treated with effective treatment levels of drug. In other instances, transplantable rat tumors have been observed to be resistant to drug action without any prior treatment of drug (18). The isolation of drug-resistant sublines of transplantable tumors can be complicated by the prior development of resistance to other chemicals (5).

### TABLE 3

**Effect of Nitrogen Mustard* on Jensen Rat Sarcoma Growing in Conditioned Swiss Mice†**

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>JRS‡</th>
<th>JRS/MC‡</th>
<th>JRS/MC‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mort.</td>
<td>Δ Body wt. (gm.)</td>
<td>Av. tumor wt. (gm.)</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>1</td>
<td>0/3</td>
<td>1/6</td>
<td>+0.5</td>
</tr>
<tr>
<td>2</td>
<td>0/7</td>
<td>0/7</td>
<td>+0.4</td>
</tr>
<tr>
<td>3</td>
<td>1/20</td>
<td>0/20</td>
<td>−1.8</td>
</tr>
</tbody>
</table>

* Dose—1.0 mg/kg/day x 7 days, I.P.
† Conditioning: Single subcutaneous injection of cortisone acetate (Cortone acetate, Merck) given on the day of tumor transplantation. Dose, 3 mg/mouse.
‡ Final observation: 9–10 days after tumor transplantation.
§ JRS: mitomycin C-sensitive Jensen sarcoma.
∥ JRS/MC: mitomycin C-resistant Jensen sarcoma.
tumor have not been significant, even though differences have been observed at the gross level. This failure to note any remarkable change of histological features in the resistant tumor is similar to that reported by Hirono (8) for nitrogen mustard N-oxide-resistant sublines of the Yoshida ascites sarcoma and the AH 13 ascites hepatoma. Although differences in growth rate during the 14-day period following transplantation have not been observed in the present study, data collected after the first 2-week period revealed that JRS/MC tumor-bearing rats died at a slower rate and the time of onset of tumor regression in surviving rats was somewhat delayed. Since the relationship of the sensitive and resistant Jensen tumors to the rat host is at best “homologous” rather than “isologous,” it is feasible to explain this observation on the basis that the tumor-host-relationship may now be changed as a consequence of the development of drug resistance in the tumor.

Transplantation data obtained from conditioned Swiss mice and newborns confirmed the fact that the JRS/MC tumor subline is fundamentally different from the JRS. Although changes in heterotransplantation of certain animal tumors have been observed to occur in the absence of attempts to produce drug resistance (14), it is still permissible in the present case to relate the observed changes to the actual development of drug resistance.

The JRS/MC tumor has been found to be cross-resistant to nitrogen mustard and other alkylating agents. This finding is similar to that reported by Oboshi (13); Kurita and co-workers (11) also have reported on the cross-resistance to alkylating agents of a mitomycin C-resistant Yoshida ascites sarcoma.

Other instances of cross-resistance in transplantable rat tumors have been demonstrated for a methylbis(β-chlorethyl)amine N-oxide-resistant tumor which displayed cross-resistance to other alkylating agents (7). Although it has been demonstrated that mitomycin C-resistant tumors can be cross-resistant to alkylating agents, the converse need not hold. Recently, Sakurai and co-workers (15) have shown that a nitrogen mustard-resistant Yoshida sarcoma was not cross-resistant to mitomycin C.

The development of drug resistance may be associated with a change in the capacity of a tumor to metabolize the compound to which it was formerly sensitive (3). In the present case metabolic studies on the mitomycin C-resistant tumor have revealed that, under conditions which support anaerobic glycolysis, whole homogenates of mitomycin C-resistant Jensen sarcoma do in fact have an increased capacity (approximately threefold) to metabolize the antibiotic even though the rate of anaerobic glycolysis by the resistant tumor is not significantly different from that of the sensitive tumor.

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