HOST RESISTANCE AGAINST TUMOR AND ITS SYNERGIC EFFECT WITH CANCER CHEMOTHERAPY

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Synopsis

The treatment of animals with non-neoplastic or neoplastic tissue before or after tumor transplantation inhibited the growth of transplanted tumor cells. This phenomenon was most clearly demonstrated when a sub-effective dose of chemotherapeutic agent was administered. In animals bearing a spontaneous tumor, the growth of auto-transplanted tumor was inhibited as compared with that of the same tumor transplanted in isologous animals. These findings suggested that the acquired resistance of the host against tumor inhibits the growth of a newly transplanted tumor.

INTRODUCTION

Up to the present, various studies have been made on the host resistance against transplanted tumor.1, 4, 8, 10, 12, 13, 15 It is well known that a pretreatment with isologous non-neoplastic tissue, isologous or homologous tumor tissue, or even with B.C.G. can induce resistance in the host against the growth of a transplanted tumor,1, 5, 6, 7 but attempts to extend these findings to the treatment or prevention of neoplastic diseases have not succeeded as yet.

On the other hand, it is almost impossible to destroy spontaneous tumors completely even with a maximum tolerated dose of chemotherapeutic agents. It seemed that one of the more favorable means in cancer chemotherapy would be to find some conditions which may work synergically with chemotherapeutic agents.

The author has been studying host resistance9 against transplantation of Yoshida sarcoma16 in Donryu rats17 and found some conditions which were synergic with chemotherapeutic agents to show a strong therapeutic effect. The most interesting was the fact that even if such a resistance was seemingly unapparent, the conditions worked synergically with chemotherapeutic agents to effect cure.10

Whether animals and human beings have natural resistance to the growth of a spontaneous tumor or not is an interesting problem. As one of the experiments concerned with this problem, the following experiment was carried out.

A part of a spontaneous tumor was resected and back-transplanted in the same animal. The growth of such an auto-transplanted tumor was markedly inhibited, as compared with that of the same tumor transplanted in an isologous animal.11

MATERIALS AND METHODS

Animals The Donryu and Wistar rats of both sexes, weighing 80~120 g, and ddN mice of either sex, weighing 20~22 g, were used. Female C3H/He mice, weighing 22~25 g, were also used in some experiments. All the animals used were supplied from

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the Central Laboratory for Experimental Animals, Tokyo. There were no differences in experimental results between male and female animals.

**Tumors** Ascites form of Yoshida sarcoma, serially transplanted in Donryu rats for about 2 years, was used. Spindle-cell sarcoma was induced by injection of 10 mg of 3-methylcholanthrene*2 dissolved in 0.2 ml of olive oil in subcutaneous tissue of Donryu and Wistar rats, and serially transplanted in these strains. The Ehrlich ascites tumor had been serially transplanted in ddN mice in the National Cancer Center.

For the transplantation of Yoshida sarcoma, five million cells were transplanted intraperitoneally in a Donryu rat, the abdomen was opened aseptically on the 4th day, and the ascites was collected by washing out with physiological saline. Five million Yoshida sarcoma cells suspended in the physiological saline were transplanted intraperitoneally in a Donryu rat.

Ehrlich tumor was transplanted similarly in ddN mice, the ascites was collected on the 7th day, and five million tumor cells thus collected were transplanted in the same strain mouse.

HeLa cells grown on 199 medium added with 20% of bovine serum were used.

Rat and mouse tissues were obtained under ether anesthesia and pieces measuring 4 × 4 mm were used. Blood was collected by heart puncture and immediately injected intraperitoneally, 0.2 ml per rat.

For the autologous transplantation of spontaneous tumor in Donryu rats and C3H mice, a part of the tumor was resected under ether anesthesia and 0.3 g of it was immediately grafted subcutaneously, as far away as possible from the primary tumor. As a control, the same tumor tissue was transplanted in intact animals of the same strain, at the same site and under the same conditions as in tumor-bearing animals.

In another experimental group, the spontaneous tumor was completely removed, 0.3 g of it was transplanted subcutaneously in the same animal, and only the animals with no recurrence of the primary tumor were counted as the effective number of animals.

**Chemotherapeutic Agents** Mitomycin-C*2) and Nitromin*3) (nitrogen mustard N-oxide hydrochloride) were used. Their dosage and period of treatment will be given in the following section on experimental results.

**Results**

**Mitomycin-C** Fig. 1 shows the therapeutic results of Mitomycin-C given in doses of 1,000, 500, or 250 µg/kg, once a day for 3 days, starting 72 or 96 hrs. after intraperitoneal

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*2 The nomenclature of methylcholanthrene has been changed from 20-methyl- to 3-methyl cholangthrene in accordance with IUPAC nomenclature rule (A-23.1). The numbering and structure orientation of cholangthrene are given below:

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  H,C - CH₂
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transplantation of 5 million Yoshida sarcoma cells in Donryu rats. It was almost impossible to save the tumor-bearing animals even with a dose of 1,000 µg/kg if the treatment was started 96 hrs. or more after the transplantation, but if the treatment was started 72 hrs. after the transplantation, approximately 50% cure can be effected with 1,000 or 500 µg/kg, but not with 250 µg/kg of Mitomycin–C.

Synergic Effect of Host Resistance Isologous or heterologous tumor cells were transplanted in Donryu rats, 5 million Yoshida sarcoma cells per rat were transplanted intraperitoneally 3 weeks later, and injection of 250 µg/kg of Mitomycin–C or Nitromin per day was started 72 hrs. later, and continued for 3 days. These results are presented in Figs. 2 and 3.

When the sarcoma from Wistar rats was transplanted in Donryu rats, most of the transplantation failed, although a few took and the animals were transplanted intraperitoneally with Yoshida sarcoma and about 10% of the animals showed resistance to the second transplantation but the others died of tumor growth. When 250 µg/kg of Mitomycin–C was injected per day for three days 72 hrs. after the second transplantation of Yoshida sarcoma, a marked resistance to tumor growth was found in Donryu rats which had absorbed the sarcoma from Wistar rats (Fig. 2). The same result was obtained by the use of Nitromin.

The use of Ehrlich tumor cells or human HeLa cells, in place of sarcoma from Wistar rats, for the pretreatment also supplemented the therapeutic effect of 250 µg/kg of Mitomycin–C.
Fig. 2. Synergic effect of treatment with methylcholanthrene-induced sarcoma and Mitomycin-C or Nitromin on Yoshida sarcoma in Donryu rats

A- Synergism between pretreatment with Mitomycin-induced sarcoma and Mitomycin–C therapy (5 × 10⁶ Yoshida sarcoma cells inoculated intraperitoneally)

B- Synergism between pretreatment with Mitomycin-induced sarcoma and Nitromin therapy (5 × 10⁶ Yoshida sarcoma cells inoculated)
Effect of Tissue and Blood

After transplantation of isologous and heterologous normal tissues in Donryu rats, 5 million Yoshida sarcoma cells were intraperitoneally transplanted 3 weeks later. After 72 weeks, 250 µg/kg of Mitomycin-C per day was injected for 3 days. These results are shown in Figs. 4 and 5. The most powerful pretreatment which was synergic with the therapeutic effect of Mitomycin-C was blood from isologous animals, and this tendency was especially marked in the same strain of animals.

Effect of auto-transplantation and isologous host transplantation was also investigated. Tables I and II give results obtained on auto-transplantation of mammary tumor spontaneously produced in C3H/He mice and methylcholanthrene-induced sarcoma in Donryu rats. These results suggest that animals bearing spontaneous tumor possess some kind of a resistance to the growth of artificially transplanted tumor.
Fig. 4. Synergism of pretreatment with normal tissue and Mitomycin-C therapy (5×10⁶ Yoshida sarcoma cells transplanted intraperitoneally in Donryu rats)

- Control, physiological saline was injected
- Pretreated by liver of ddN mouse 3 weeks before the Yoshida sarcoma cell transplantation, and 250 μg/kg/day of Mitomycin-C was administered for 3 days from 72 hrs. after transplantation.
- Pretreated with liver of bovine 3 weeks before Yoshida sarcoma cell inoculation, and 250 μg/kg/day of Mitomycin-C was administered for 3 days from 72 hrs. after inoculation.
- Pretreated with spleen of a bovine 3 weeks before Yoshida sarcoma cell inoculation, and 250 μg/kg/day of Mitomycin-C was administered for 3 days from 72 hrs. after the inoculation.
Fig. 4. II Synergic effect of pretreatment with normal tissue and Mitomycin-C therapy (5 x 10^6 Yoshida sarcoma cells were transplanted intraperitoneally in Donryu rats)

- Control, pretreated with liver of Donryu rat 3 weeks before Yoshida sarcoma cell inoculation
- Pretreated with liver of a Donryu rat 3 weeks before Yoshida sarcoma cell inoculation and 250 μg/kg/day of Mitomycin-C was administered for 3 days from 72 hrs. after the inoculation
- Control, pretreated with spleen of Donryu rat in 3 weeks before Yoshida sarcoma cell inoculation
- Pretreated with spleen of a Donryu rat 3 weeks before Yoshida sarcoma cell inoculation, and 250 μg/kg/day of Mitomycin-C was administered for 3 days from 72 hrs. after the inoculation
- Pretreated with liver of a Wistar rat 3 weeks before Yoshida sarcoma inoculation
- Pretreated with liver of a Wistar rat 3 weeks before Yoshida sarcoma cell inoculation, and 250 μg/kg/day of Mitomycin-C was administered for 3 days from 72 hrs. after the inoculation
- Pretreated with spleen of a Wistar rat 3 weeks before Yoshida sarcoma cell transplantation
- Pretreated with spleen of a Wistar rat 3 weeks before Yoshida sarcoma cell inoculation, and 250 μg/kg/day of Mitomycin-C was administered for 3 days from 72 hrs. after the inoculation
Fig. 5. Synergism of pretreatment with blood of skin of isologous rats and Mitomycin-C therapy

- Control
- 250 $\mu$g/kg/day for 3 days
- 0.2 ml of Donryu rat blood/body
- 0.2 ml of Donryu rat blood and Mitomycin-C, 250 $\mu$g/kg/day, for 3 days
- 0.2 ml of Wistar rat blood/body
- 0.2 ml of Wistar rat blood/body and Mitomycin-C, 250 $\mu$g/kg/day, for 3 days

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- Control
- 250 $\mu$g/kg/day for 3 days
- Skin
- Skin and Mitomycin-C, 250 $\mu$g/kg/day, for 3 days

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3 weeks after pretreatment with blood of isologous rats, $5 \times 10^6$ Yoshida sarcoma cells were transplanted, followed 72 hrs. later with 250 $\mu$g/kg/day of Mitomycin-C for 3 days

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3 weeks after the graft of skin of isologous rats, $5 \times 10^6$ Yoshida sarcoma cells were transplanted and 250 $\mu$g/kg/day of Mitomycin-C was administered for 3 days, 72 hrs. after the inoculation

Table I. Autologous and Isologous Transplantation of Spontaneous Mammary Tumor (ca 2.5 $\times$ 4 g in weight)

<table>
<thead>
<tr>
<th>Pretreatment procedures</th>
<th>Challenge procedure</th>
<th>Total no. of animals used</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Tumor partially removed</td>
<td>Autologous transplantation</td>
<td>127</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Isologous transplantation</td>
<td>127</td>
<td>109</td>
</tr>
</tbody>
</table>

A: Increase in tumor weight  
B: Practically no increase in tumor weight  
C: Almost negative response
For the treatment of tumor as a systemic disease, chemotherapy and immunotherapy may be considered, especially the latter as a preventive measure. Therapy of tumor as a systemic disease requires an important prerequisite, which is a matter of fundamental difference between cancer cells and normal cells of the living body.

The use of chemotherapeutic agents like Mitomycin-C and Nitromin, if used in an early stage, has given good results in the case of transplanted tumor in experimental animals. In this case, if growth of a tumor has advanced beyond a certain stage, it becomes difficult to save the animals even by the use of a maximum tolerated dose of a chemotherapeutic agent. For example, the 50% lethal dose (LD50) of Mitomycin-C in rats is 2.9 mg/kg but when this antibiotic is used once a day consecutively, 1,000 μg/kg per day for four days is the limit.

The maximum amount of Mitomycin-C that can be used in rats without loss of animals due to toxic death is 1,000 μg/kg/day for 3 days. When 5 million cells of Yoshida sarcoma are transplanted intraperitoneally in Donryu rats, consecutive injection of 1,000 μg/kg/day of Mitomycin-C for three days cannot save majority of the animals if the injection is started 96 hrs. after the transplantation. If the injection is started 72 hrs. after the transplantation, 50% of the animals can be cured completely.

In the present series of experiments, the treatment was started 72 hrs. after the intraperitoneal transplantation of 5 million Yoshida sarcoma cells in Donryu rats. At 72 hrs. after the transplantation, considerable infiltration was observed in the greater

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Table II. Isologous Transplantation with Methylcholanthrene-induced Sarcoma in Donryu Rats

<table>
<thead>
<tr>
<th>Treateda)</th>
<th>Control groupb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.8 (g)</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td>4</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
</tr>
<tr>
<td>5</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
</tr>
</tbody>
</table>

a) After the development of a sarcoma in Donryu rats by treatment with 3-methylcholanthrene, a piece (4 x 4 mm) of the sarcoma was removed and the pieces was back-transplanted in the same animal.

b) The same pieces of the sarcoma were transplanted in intact isologous rats.

DISCUSSION

For the treatment of tumor as a systemic disease, chemotherapy and immunotherapy may be considered, especially the latter as a preventive measure. Therapy of tumor as a systemic disease requires an important prerequisite, which is a matter of fundamental difference between cancer cells and normal cells of the living body.

The use of chemotherapeutic agents like Mitomycin-C and Nitromin, if used in an early stage, has given good results in the case of transplanted tumor in experimental animals. In this case, if growth of a tumor has advanced beyond a certain stage, it becomes difficult to save the animals even by the use of a maximum tolerated dose of a chemotherapeutic agent. For example, the 50% lethal dose (LD50) of Mitomycin-C in rats is 2.9 mg/kg but when this antibiotic is used once a day consecutively, 1,000 μg/kg per day for four days is the limit.

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In the present series of experiments, the treatment was started 72 hrs. after the intraperitoneal transplantation of 5 million Yoshida sarcoma cells in Donryu rats. At 72 hrs. after the transplantation, considerable infiltration was observed in the greater
omentum and in the subcutaneous tissue at the site of transplantation. Therefore, intraperitoneal injection of the chemotherapeutic agent was made once after 72 hrs., then after 96 hrs., and the animals were observed for 60 days thereafter without treatment.

The foregoing facts present various questions; whether the matter is a question of balance between the dosage of the chemotherapeutic agent used and the number of cancer cells, or whether the cure is effected by synergic effect with host resistance, and whether this host resistance becomes ineffective when the number of cancer cells increases and the cells infiltrate important organs. One the other hand, even a maximum tolerated dose of a chemotherapeutic agent is unable to cure animals with spontaneous tumor, although the size of the tumor may be reduced in some instances.

The present series of experiments have indicated that autologously transplanted spontaneous tumor shows much slower growth than the same tumor transplanted in a healthy animal of the same strain. This fact seems to be an example that there is a certain amount of resistance in the host animals even in the case of spontaneous tumor. If there is a resistance to cancer in animals, it should be possible to increase this resistance.

It was also revealed by the present experiments that a strong resistance to the growth of tumor cells is produced in animals transplanted with transplantable malignant tumor. Transplantable tumor, especially the ascites tumor, responds to the treatment with chemotherapeutic agents if used at an early stage. However, this is not the case in a spontaneous tumor. The reason for this fact seems to be the difference in the resistance of an animal to tumor cells and not to the essential difference in cancer cells between spontaneous and transplantable tumors. In other words, regression of a transplanted tumor is due not only to the effect of a chemotherapeutic agent alone but to the synergic effect of that with the host resistance.

It follows, therefore, that the future problem in the treatment of a spontaneous tumor is how to treat a resistance in the host which will work synergically with a chemotherapeutic agent.

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REFERENCES

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16) Yoshida, T., THIS JOURNAL, 40, 1 (1949).