Temporary Interruption of Regional Blood Flow Combined with Local Hyperthermia for Cancer Chemotherapy

Tsuneo Baba, Ken Aoki, Yoshiro Kidera, Mihoko Kimura, Takashi Kanematsu, and Noritaka T. Kimura

Laboratory of Cell Research, Cancer Research Institute, Faculty of Medicine, Kyushu University, Fukuoka, Japan 812

SUMMARY

A striking chemotherapeutically curative effect on tumor was obtained by means of temporary interruption of regional blood flow combined with local hyperthermia. By analyzing various basic conditions required for this system using Ehrlich tumor implanted in the hind limbs of mice, the following were found to be essentially indispensable to obtain satisfactory chemotherapeutic effects: (a) a time interval of 1 to 3 min after systemic i.v. administration of drug to the mouse, (b) use of a tourniquet on the tumor-bearing mouse limb to stop blood flow, and (c) warming at 37-41°C (d) for a period of at least 30 to 60 min.

Among the chemotherapeutic drugs tested in the present study, Carbazilquinone (NSC 134679) was the most effective because it revealed the strongest antitumor effect despite its relative innocuousness to nontumorous adjacent normal tissues. Applying the present method, a large syngeneic mouse sarcoma transplanted to the limb 7 days before the experiment also completely regressed in 6 of 9 mice.

INTRODUCTION

In cancer chemotherapy the discovery of a drug that selectively attacks only cancer cells but not normal cells is of primary importance. Improvements in the method or mode of drug administration may also be considered essential in order to make the drug display the selectivity.

It was previously reported in our in vitro study (10) that, among the various drugs tested, Carbazilquinone showed the strongest tumoricidal action under the condition of the decrease of extra- and intracellular pH due to the glycolysis of cancer cells themselves. It was also reported in another paper (1) that a large V2 carcinoma in the rabbit leg completely regressed by the "regional perfusion of the drug with circulation-stop method."

In recent attempts to establish the optimal conditions as to time length, temperature, dose of drug, etc., we have developed a more simple in vivo experimental system using mice.

The experimental system essentially consisted of i.v. injection of drug followed by temporary interruption of blood flow of the tumor-bearing mouse hind limb and warming of the limb for a certain period of time. Because about 50 to 60 mice could be tested simultaneously by this system, analytical studies on various basic conditions could be performed with a fairly high efficiency.

MATERIALS AND METHODS

Mouse. Over 1000 2- to 4-month-old female highly inbred DDD mice propagated in and supplied from the Animal Center of Kyushu University were used in the present study.

Tumor Cells. Eight- to 10-day-old Ehrlich ascites tumor cells that had been maintained in DDD mice by weekly passage in our laboratory were mainly used. The Ehrlich tumor cells suspended in phosphate-buffered saline (pH 7.2; components/liter: 100 mg CaCl2, 200 mg KCl, 200 mg KH2PO4, 100 mg MgCl2·6H2O, 8000 mg NaCl, and 150 mg Na2HPO4·2H2O) were counted using a Coulter counter (Model ZB-1), and 105 tumor cells were inoculated i.m. into the right hind limb of each mouse weighing 20 to 25 g. A syngeneic mouse tumor, i.e., a methylcholanthrene sarcoma, was also used instead of an allogeneic tumor system for the specific purpose of testing the effectiveness of the present therapy under much more difficult therapeutic conditions. This sarcoma has so far been maintained s.c. in mice of the same strain by serial transplantation for about 50 generations. For the present experiment, 1 trocar unit of sarcoma tissue was transplanted i.m. to the right hind limb of each DDD mouse weighing 25 to 28 g.

As a rule, 4 to 5 groups of 10 to 12 mice each were used in each experiment. In the case of the Ehrlich tumor-bearing mice, all treatments were initiated at 24 hr after tumor transplantation. In the case of the methylcholanthrene sarcoma, on the other hand, the treatments were started after allowing 7 days for tumor growth in the mouse hind limb.

Test Drugs. Carbazilquinone (NSC 134679, Esquinone; Sanky Co., Tokyo, Japan), nitrogen mustard-N-oxide (Nitromin; Yoshitomi Pharmaceutical Ind., Ltd., Osaka, Japan), Mitomycin C (Kyowa Hakko Co., Ltd., Tokyo, Japan), Adriamycin (Kyowa Hakko Co.), Thio-TEPA (Sumitomo Chemical Co., Osaka, Japan), cyclophosphamide (Endoxan; Shionogi Co., Ltd., Osaka, Japan) and Chromomycin A3 (NSC 58514; Toyomycin; Takeda Chemical Ind., Ltd., Osaka, Japan) were used as antitumor drugs.

As a rule, each drug was dissolved or diluted just before use in sterilized 0.1 M phosphate buffer solution, pH 7.2. The drug solution was prepared to contain a dose of one-decade,

1 Supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan, and by the Princess Takamatsu Cancer Research Fund.

Received December 5, 1975; accepted March 8, 1976.
Blood Flow-interrupting Hyperthermic Chemotherapy

Table 1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Time length of warming (min)</th>
<th>Local warming temperature (°C)</th>
<th>Tumor growth*</th>
<th>Tumor wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Drug</td>
<td>Tourniquet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>CQ</td>
<td>+</td>
<td>60</td>
<td>37</td>
</tr>
<tr>
<td>B</td>
<td>CQ</td>
<td>-</td>
<td>60</td>
<td>37</td>
</tr>
<tr>
<td>C</td>
<td>PB</td>
<td>+</td>
<td>60</td>
<td>37</td>
</tr>
<tr>
<td>D</td>
<td>PB</td>
<td>-</td>
<td>60</td>
<td>37</td>
</tr>
</tbody>
</table>

* Number of tumor bearers/number of mice surviving 4 weeks after treatment.

Method of Interruption of Regional Blood Flow. Mice of all groups were anesthetized by an i.p. injection of sodium pentobarbital (47 mg/kg) and then the test drug was injected slowly into their tail veins. Since a lower speed of injection was found to be an important factor in the present study, we usually adhered to the speed of 0.1 ml/15 sec at every injection. Unless otherwise stated, 1 min after the conclusion of injection, a rubber tourniquet was fastened so tightly on the tumor-bearing limb at the level of the right inguinal ligament that the blood flow of the femoral vasculature of the limb could be temporarily interrupted for 30-60 min.

Warming of the Tumor-bearing Limb. Immediately after the tourniqueting, mice were settled on a specially designed roof-shaped wooden table that was set in a water bath (Taiyo Incubator M-1), and their tumor-bearing limbs were immersed and warmed at various temperatures for a certain period of time (Fig. 1). As soon as the warming was finished, the rubber tourniquet was cut off and blood flow of the limb was allowed to recommence. In the experiment using Ehrlich tumor, the above treatments were performed only once. In the case of methylcholanthrene sarcoma, the treatments were performed once a week for 3 weeks.

Measurement. After the treatments, general conditions of the animals were inspected daily and each mouse was weighed every week.

The size of tumors was calculated weekly by measuring the tumor-bearing limb in 2 orthogonal diameters. All the mice were killed at 4 weeks after the initial treatment, and each tumor mass was enucleated, weighed, and photographed. Both the treated and the control hind limbs of mice were histologically examined in some of the experiments.

RESULTS

In several preliminary experiments that had been performed by almost the same system as the present one, Carbazilquinone had been found to be the best drug for treatment, with respect to its antitumor effect, general toxicity, and relative harmlessness to the regional normal tissue elements. Therefore, using Carbazilquinone as the test drug, we first examined and made analyses on various basic conditions involved in the present method of temporary interruption of regional blood flow combined with local hyperthermia.

Importance of Temporary Interruption of Regional Blood Flow

For determination of whether or not the interruption of blood flow is essentially important for the present system, 4 groups of mice bearing Ehrlich tumor in their right hind limbs were put to the test. Groups A and B were given i.v. injections of Carbazilquinone, and Groups C and D received phosphate buffer solution alone. One min after the injection of the drug, a tourniquet was fastened at the inguinal region on mice of Groups A and C but not on those of Groups B and D. Then all tumor-bearing limbs were immediately warmed at 37° for 60 min in the water bath described above. As shown in Table 1, there was a striking difference in the number of the tumor-free survivors among the groups. In Group A, receiving both Carbazilquinone injection and tourniqueting, 9 of 10 mice showed no tumor growth at all at the end of 4 weeks. On the contrary, in Group B, receiving the same treatments as those of Group A except for tourniqueting, practically no antitumor effect could be observed. All mice of both Groups C and D given injections of 0.1 M phosphate buffer solution alone showed tumor growths, but no difference ascribable to the tourniqueting was found between the groups.

These results strongly suggest that the temporary occlusion after injection of the drug is of specific importance for obtaining an antitumor effect in the present chemotherapy system.

Effect of Local Warming on the Chemotherapy

While several preliminary experiments had been carried out without any special attention to temperature, it came to our attention that the results of these experiments were much influenced by the room temperature at which an
experiment was carried out. Therefore, we tried to find out how much the warming of the part isolated from the general circulation influenced the chemotherapeutic efficiency of the antitumor drug. After i.v. injection of Carbazilquinone, the right hind limbs of the mice with Ehrlich tumor growths were tourniqueted to stop the blood flow, and the limbs thus tourniquet were immersed in water bath at various temperatures. Five groups of mice were used in this experiment. As shown in Table 2, with Group A, which received drug injection followed by the occlusion for 60 min combined with warming at 37°C, a similarly remarkable antitumor effect was obtained as in the former experiments, and tumors of 10 of 12 mice surviving until the end of the 4th week had completely vanished.

By contrast, Group B, which received the same treatments as Group A in terms of both drug and tourniqueting but was kept at room temperature (20°C) for 60 min without warming, showed a markedly diminished antitumor effect of the treatments, and none of the mice had complete regression of the tumor.

Furthermore, as seen with Group C, when the tumor-bearing limbs were cooled at 0°C in an iced water bath, no effect on tumor was brought about, despite the fact that the mice were otherwise given the same treatments as in Group A. With Group D, left at room temperature after drug injection without tourniqueting, no antitumor effect of the drug was observed when compared with control Group E.

Thus it became clear that antitumor effects in the present experimental system depended strongly on how high a temperature was applied to the tumor-bearing limbs during the treatments.

**Time Length of the Interruption of Regional Blood Flow**

Although all the above experiments were carried out under the time length of 60 min, we did not exactly know whether this 60-min warming was really the minimum and adequate condition. It is of course desirable from any practical point of view to make the time length of the occlusion as short as possible. Thus, for the purpose of determining the optimal time length, a further experiment was carried out with 5 groups of mice using Carbazilquinone again as the test drug. Group A, for which the regional blood flow had been stopped for 60 min at 37°C, again revealed a similarly remarkable antitumor effect as in the former experiments, and tumors in 9 of 11 mice surviving 4 weeks were completely regressed. In contrast with Group A, tumors were found in all of the 9 surviving mice in Group B, for which the regional blood flow had been stopped for only 30 min at 37°C. The size of the tumors of this group, however, was significantly decreased as compared with those of control Group E. Group C, for which the warming temperature was raised to 41°C and the interruption of blood flow was for 60 min, showed such a significant tumor regression that all the tumors of 6 mice surviving until the end of the 4th week had completely vanished. However, the normal tissues in the treated limbs of the mice of this group were so severely damaged that one-half of the mice died within 10 days. With Group D, in which the animals received the temporary occlusive treatment for 30 min at 41°C, again a remarkable antitumor effect was obtained. Actually, no tumors were found in 8 of the 11 mice that had survived 4 weeks. All 11 mice of the control Group E showed tumor growths as large as in the control groups of the former experiments.

The results indicate that the suitable time length for the occlusion depends on the warming temperature and that 60 min in the case of the warming at 37°C or 30 min in the case of the warming at 41°C is probably the most expedient for the present experimental system.

**Time Interval between Drug Injection and Tourniqueting**

We also tried to find out what time interval between the drug injection and tourniqueting should be adopted to obtain the highest chemotherapeutic efficiency in the present system. The experiment was repeated several times by using Carbazilquinone at a dose of 1 mg/kg or 0.5 mg/kg. All the experiments of this series were carried out under the warming at 37°C for 60 min. Four groups of mice were treated by varying time intervals of 1, 3, 10, and 30 min, respect-

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Drug</th>
<th>Time length of warming (min)</th>
<th>Local warming temperature (°C)</th>
<th>Tumor growtha</th>
<th>Tumor wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CQb</td>
<td>+</td>
<td>60</td>
<td>37</td>
<td>2/12</td>
</tr>
<tr>
<td>B</td>
<td>CQ</td>
<td>+</td>
<td>60</td>
<td>20</td>
<td>12/12</td>
</tr>
<tr>
<td>C</td>
<td>CQ</td>
<td>+</td>
<td>60</td>
<td>0</td>
<td>12/11</td>
</tr>
<tr>
<td>D</td>
<td>CQ</td>
<td>-</td>
<td></td>
<td>12/12</td>
<td>1.70 ± 0.252</td>
</tr>
<tr>
<td>E</td>
<td>PB</td>
<td>-</td>
<td></td>
<td>12/12</td>
<td>2.44 ± 0.236</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of tumor bearers/number of mice surviving 4 weeks after treatment.
<sup>b</sup> CQ, Carbazilquinone, 1 mg/kg (1/5 LD<sub>50</sub>); PB, phosphate buffer solution (pH 7.2).
<sup>c</sup> p < 0.001, Fisher's direct probability test.
<sup>d</sup> Mean ± S.E.
<sup>e</sup> p < 0.01, t test.
<sup>f</sup> p < 0.005, t test.
tively. A control group was always added to each experimental group for comparison. According to the results obtained in several repeated experiments, the time interval of 1 to 3 min was concluded to be the most effective; the 10-min interval was also found to be effective, but the 30-min interval was of little effect.

Effects of the Treatments on Normal Tissues

In the present experimental system, regardless of the kind of drugs used, marked edematous swelling of the limbs appeared within 1 or 2 days and lasted for about 1 to 2 weeks whenever the limbs were treated by the temporary occlusion under warming. In the case of the best results obtained by Carbazilquinone, grossly the treated limbs were somewhat atrophic without any other remarkable changes in appearance at autopsy. The function of the hind limbs was relatively well preserved, namely, in the majority of mice little or no ankylosis was observed at the knee, ankle, and phalangeal joints and they could walk in an almost normal gait by using the treated limbs that no longer bore viable tumors. A small number of mice, however, suffered from moderate degree of ankylosis at the joints. Histologically, the most remarkable change observed was some degree of muscle atrophy, but no remarkable changes were detected in the skin and s.c. tissues as well as in the vascular systems.

Results Obtained Using Various Other Antitumor Drugs

Using Carbazilquinone as the antitumor drug, several basic conditions involved in the present system were analyzed as mentioned above, and it was revealed that the drug could display a fairly high efficiency for tumor chemotherapy under certain specified conditions. Then we extended our test to other commercially available antitumor drugs to compare their therapeutic effects with those of Carbazilquinone.

Nitrogen Mustard-N-oxide and Mitomycin C. Mice were treated with 1/5 LD50 of each drug, with tourniqueting at 1 min after the drug injection and with 60 min warming at 37°. As shown in Table 3, in Group A nitrogen mustard-N-oxide revealed as strong an antitumor effect as that of Carbazilquinone, with which Group C was treated. In both groups there was no tumor growth in all at 4 weeks after the treatments. With Group B, treated with Mitomycin C, the drug showed a slightly lower antitumor activity than that of Carbazilquinone, and 3 of 11 mice proved to be positive in tumor growth in their hind limbs at 4 weeks after the treatment, although there was a significant difference compared with the control.

Although nitrogen mustard-N-oxide was found to be as active as Carbazilquinone in its antitumor effect, the side effects of the former on regional normal tissues proved to be much more severe than those of the latter. A high degree of edematous swelling was observed in the treated limbs, and a marked skin erosion was frequently detected at the toes. Severe atrophy of the limbs was always accompanied by the notable ankylosis of several joints. In the most severe cases, total necrosis or mummification of the treated limbs was observed.

In histological examination, vascular wall damage, necrosis and degeneration of muscle, and skin erosion were frequently observed. In particular, hyaline degeneration of the walls of arteries and arterioles associated with marked periarteritis was demonstrated in the case of nitrogen mustard-N-oxide. Changes in normal tissues of the tumor-bearing limbs induced by the treatment with Mitomycin C were almost the same as those induced by Carbazilquinone both grossly and histologically, namely, a relatively good function was preserved in the treated limbs in many animals, although there was some degree of atrophy.

Adriamycin and Thio-TEPA. The results obtained using these 2 antitumor drugs as compared with those with Carbazilquinone are shown in Table 4. A slight but significant antitumor effect was observed in Group A tested for Adriamycin but no effect in Group B for Thio-TEPA. Again no tumor growth was seen in 8 of 9 mice of Group C for Carbazilquinone.

Cyclophosphamide and Chromomycin A3. A comparative
study was carried out in this experiment between the tourniquetted and the untourniquetted limbs bearing regional tumors (Table 5). Interestingly, in Groups A and B, both receiving cyclophosphamide, the antitumor effects obtained proved to be just the reverse of those obtained with the other drugs described. With the mice of Group B, receiving only the drug but no tourniqueting, a stronger inhibition of tumor growth was observed than with Group A, which received the identical treatments as Group B except for tourniqueting. As for Chromomycin A₃, a sign of slight effectiveness was obtained in Group C with tourniqueting but none in Group D without tourniqueting, as was revealed by comparison with the control Group E.

The toxic effects of these 4 drugs on normal tissues were relatively so mild that actual impairment of the function of the mouse limb was not found.

**Effectiveness of the Present Chemotherapy on a Large Syngeneic Mouse Sarcoma**

The purpose of this phase of experimentation was to find out how effective the above treatments were when tried on a therapeutically more difficult tumor system. In this experiment, mice bearing methylcholanthrene sarcomas grown for 7 days in their hind limbs were used. The 100% transplantability of the sarcoma in this strain of mouse has been proved in serial passages in our laboratory. The present chemotherapy system that had been developed as described above was applied, using Carbazilquinone as the test drug, on these tumor bearers once a week for 3 weeks. All surviving mice in each group were sacrificed at 5 weeks after transplantation.

As illustrated in Chart 1, Group A, which was treated with Carbazilquinone (1/₅ LD₉₀) injection followed by tourniqueting and warming at 37°C for 60 min, revealed such a marked chemotherapeutic result that the sarcoma was completely regressed in 6 of 9 mice, and in the remaining 3 mice only an atrophic tumor mass remained. A half-dose of Carbazilquinone (1/₁₀ LD₉₀) also revealed a significantly good antitumor effect, if combined with tourniqueting and warming (Group B). With Group C, which received no tourniqueting but otherwise the same treatments as those of Group A, only a slight inhibition of tumor growth was obtained when compared with control Group D; no complete regression of

### Table 4

**Effects of Adriamycin, Thio-TEPA, and Carbazilquinone on the growth of Ehrlich tumor in the mouse limb with temporary interruption of regional blood flow**

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Tourniquet</th>
<th>Time length of warming (min)</th>
<th>Local warming temperature (°C)</th>
<th>Tumor growth</th>
<th>Tumor wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Adr</td>
<td>+</td>
<td>60</td>
<td>37</td>
<td>9/9</td>
<td>1.93 ± 0.44₉₋¹, d</td>
</tr>
<tr>
<td>B</td>
<td>Thio-TEPA</td>
<td>+</td>
<td>60</td>
<td>37</td>
<td>9/9</td>
<td>2.30 ± 0.48₆</td>
</tr>
<tr>
<td>C</td>
<td>CQ</td>
<td>+</td>
<td>60</td>
<td>37</td>
<td>1/9</td>
<td>0.01 ± 0.00₅</td>
</tr>
<tr>
<td>D</td>
<td>PB</td>
<td>−</td>
<td></td>
<td></td>
<td>9/9</td>
<td>3.32 ± 0.39₆</td>
</tr>
</tbody>
</table>

a Number of tumor bearers/number of mice surviving 4 weeks after treatment.
b Adr., Adriamycin, 2.7 mg/kg (∆/₅ LD₉₀); Thio-TEPA, 3.4 mg/kg (∆/₅ LD₉₀); CQ, Carbazilquinone, 1 mg/kg (∆/₅ LD₉₀); PB, phosphate buffer solution (pH 7.2).
c Mean ± S.E.
d p < 0.05, f test.
e p < 0.001, Fisher’s direct probability test.
f p < 0.001, f test.

### Table 5

**Effects of cyclophosphamide and Chromomycin A₃ on the growth of Ehrlich tumor in the mouse limb with or without interruption of regional blood flow**

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Tourniquet</th>
<th>Time length of warming (min)</th>
<th>Local warming temperature (°C)</th>
<th>Tumor growth</th>
<th>Tumor wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CPA</td>
<td>+</td>
<td>60</td>
<td>37</td>
<td>9/9</td>
<td>1.16 ± 0.25₇₋¹, d</td>
</tr>
<tr>
<td>B</td>
<td>CPA</td>
<td>−</td>
<td>60</td>
<td>37</td>
<td>8/9</td>
<td>0.25 ± 0.06ₐ</td>
</tr>
<tr>
<td>C</td>
<td>Chr. A₃</td>
<td>+</td>
<td>60</td>
<td>37</td>
<td>9/9</td>
<td>1.19 ± 0.22ₚ</td>
</tr>
<tr>
<td>D</td>
<td>Chr. A₃</td>
<td>−</td>
<td>60</td>
<td>37</td>
<td>9/9</td>
<td>2.22 ± 0.28₃</td>
</tr>
<tr>
<td>E</td>
<td>PB</td>
<td>−</td>
<td></td>
<td></td>
<td>9/9</td>
<td>2.33 ± 0.22₅</td>
</tr>
</tbody>
</table>

a Number of tumor bearers/number of mice surviving 4 weeks after treatment.
b CPA, cyclophosphamide, 86.4 mg/kg (∆/₅ LD₉₀); Chr. A₃, Chromomycin A₃, 0.424 mg/kg (∆/₅ LD₉₀); PB, phosphate buffer solution (pH 7.2).
c Mean ± S.E.
d p < 0.005, f test.
e p < 0.001, f test.
A tumor was observed in any mouse. The size of each tumor striking regression of tumors in Group A is quite impressive. If compared with the size of the tumors of Group E, the fact that the peritumoral pH could not be affected by the acidic condition induced by cancer cell glycolysis. In a previous report, glucose solution as a vehicle for drug administration combined with some suitable modifications in the physical conditions of the recipient can display strong anticancer effects, in spite of the fact that no definite communication between the locus isostem and the systemic circulation. The possible contribution of this minor communication remaining after the isolation might not be ruled out when we consider the strong anticancer results obtained in our study, although we do not yet know its exact significance.

### DISCUSSION

It has been clearly demonstrated in the present study that drug administration combined with some suitable modifications in the physical conditions of the recipient can display strong anticancer effects, in spite of the fact that no definite effect was obtainable by the drug administration alone.

The present experiment has been carried out as a sequel to our previous studies (1, 10). The theory advanced in the previous papers had been based on the fact that the action of a drug such as Carbazilquinone could be potentiated in the acidic condition induced by cancer cell glycolysis. In a preliminary test of the present system, however, differing from the previous reports, glucose solution as a vehicle for Carbazilquinone was found to be not always superior to phosphate buffer solution as the medium for inducing the anticancer effect of the drug. This was presumably due to the fact that the peritumoral pH could not be affected by such a small dose of glucose solution injected into the general circulation system. Thus, we do not know exactly to what extent the cancer cell glycolysis is involved in the effectiveness of the present experimental system of chemotherapy, since outstanding anticancer effects could be demonstrated, as already mentioned, even by use of phosphate buffer solution as the vehicle.

As to the mechanism involved in the remarkable results obtained in the present system, several possibilities may be envisaged.

(a) As the blood circulation in the tumor region was interrupted by tourniqueting, the drug accumulated there may not have been inactivated by a specific organ such as the liver. Therefore, the drug concentration in blood at the tumor-bearing limb of the animal may have been kept much higher than that of the other parts of the body, even for a certain limited period of time. In other words, the rapid decrease in the drug concentration in blood that usually occurred in the systemic circulation may have been prevented by the tumor region isolated from the circulation. It is interesting to note in this connection that the anticancer action of cyclophosphamide was rather stronger in the uncurren Edward Group B than in the tourniqueted Group A (Table 5). Differing from other drugs, this drug has been considered to be activated at the liver.

(b) The anticancer action of the drugs used in the present study may have been temperature dependent by nature, as is well known in their ordinary chemical reactions. The anticancer results obtained in our system seem to have depended exclusively on the temperature applied to the tumor-bearing mouse limb. A complete explanation as to why the combination of a drug such as Carbazilquinone, interruption of the blood flow, and hyperthermia works so effectively may be still in question. Rapidity in chemical reaction of the drug, however, may have contributed much to the present good results.

(c) It must be considered, too, that the vascular permeability for drug may have been much affected and enhanced by the occlusion of the limb. If this was the case, every tumor cell in the locus in the treated animals might have come into contact with a highly concentrated dose of the drug. In this connection, we performed a few experiments using Evans blue dye instead of the drug under the same experimental system. Interestingly, the intensity of the coloration of the mouse limb by the dye clearly depended on the tourniqueting, temperature, and time length. The higher the temperature and the longer the time, the darker was the coloration in the tourniqueted limb of mouse. Without tourniqueting, however, no such phenomenon was observed.

Concerning the problem of the occlusion, a few comments should be added here. Although blood flow was completely interrupted by tourniqueting in almost all the vascular systems of the hind limb, it could not be interrupted in the collateral vascular system involving the bone marrow of the mouse limb. According to Creech and Kremetz (4), when a simple rubber tourniquet was used clinically for circulatory isolation of human extremities, there still remained some communication between the locus isolated and the systemic circulation. The possible contribution of this minor communication remaining after the isolation might not be ruled out when we consider the strong anticancer results obtained in our study, although we do not yet know its exact significance.
As for hyperthermia, for many years numerous attempts have been made by many investigators to elucidate the difference in thermosensitivity between cancer cells and normal cells.

In 1962, Crile (5) immersed the foot of a mouse implanted with a transplantable tumor in a water bath at various temperatures for various time lengths in about the same system as our present one but without either a chemotherapeutic drug or the occlusive procedure. He maintained that the inflammatory reaction caused by the prolonged exposure of certain cancers to temperatures between 42° and 50° selectively destroyed the tumor. Cavaliere et al. (6) carried out biochemical and clinical investigations and reported that the elevated temperature revealed profound and selective toxic effects on tumor cells. Bender and Schramm in 1968 (3), however, did not succeed in animal experiments to obtain a curative effect by hyperthermia alone or by additional treatment with Disulfiram and vitamin K₂. An in vitro study by Giovanella et al. (7,8) demonstrated a fairly distinct difference in thermosensitivity between cancer and normal cells. According to the recent report of Hahn et al. (9), when hyperthermia (43°) and Adriamycin were combined in the treatment of tumor-bearing mouse legs by immersing them in a hot water bath for a 1.8-hr period, a massive cell killing resulted.

Seen from our present results, however, it should be emphasized that neither hyperthermia alone nor the combination of hyperthermia with drug administration is sufficient to get ideal cancer chemotherapeutic effects. A striking improvement in the curative effect has been successfully achieved in our study by the combination of the temporary interruption of regional blood flow with the hyperthermic chemotherapy. Although producing the best result in our assay system, Carbazilquinone could never be considered as the ideal drug, since such normal tissues adjacent to the tumor as muscle and knee joint of the mice were more or less injured. When nitrogen mustard-N-oxide was administered at a dose equivalent in general toxicity to the Carbazilquinone dose, the damage of local tissues was too severe to use it practically, in spite of the fact that the tumor in the mouse limbs was completely regressed.

In order to get nearer to the treatment of clinical cancers, we are now applying the present system to an established autologous mouse sarcoma induced by a chemical carcinogen on the one hand and, on the other, testing the present theory on the chemotherapy of an experimental pulmonary metastasis of cancer with the aid of our newly devised technique of thoracotomy of rodents (2).

ACKNOWLEDGMENTS

We thank the late Dr. Waro Nakahara, former President of the National Cancer Center, for his continuing interest and encouragement. We appreciate Dr. Kiyoshi Muto's kind suggestions on the English translation. We also thank Dr. Mariko Kaku and Akiko Nakaji for their assistance.

REFERENCES

Temporary Interruption of Regional Blood Flow Combined with Local Hyperthermia for Cancer Chemotherapy

Tsuneo Baba, Ken Aoki, Yoshiro Kidera, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/36/7_Part_1/2146

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.