CRITICAL CONSIDERATIONS ON THE TREATMENT OF CANCER: EFFECT OF THE ADMINISTRATION OF ANTICANCER AGENTS BEFORE, DURING, OR AFTER SURGICAL OPERATION (Plates IX and X)

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Synopsis

Based on experimental studies, administration of anticancer agents before, during, or after a surgical operation was found to prevent metastatic recurrence of tumor to some extent. Clinical application of anticancer agents in surgical operation is described in detail.

For the treatment of cancer many surgeons make efforts to completely eradicate cancer, including the metastatic lymph nodes. In the present state, unfortunately, recurrence is not infrequent even after radical operation, in spite of the recent progress in surgical technique and anesthesia. There is still the disappointing result of postoperative 5-year-survival rate of cancer of breast with metastasis to the axillary lymph node of only 48.0% (Atlan and Biblen), although the mammary cancer is usually more easily detected and completely removed than other kinds of cancer.

According to Kazitani,9) only 7 out of 55 patients with gastric cancer survived over 5 years following such a superradical operation in which the tail of the pancreas, spleen, and regional lymph-nodes along the celiac and splenic artery were removed in addition to total gastrectomy. The autopsy of patients with gastric cancer revealed that the residual cancer was encountered in as frequent as 38% (Ranson14) and 39% (Hamaguchi6) after a radical operation in which the removal of all cancer tissues had presumably been made. Those results make us even doubt the true significance of the present surgical treatment of cancer. Hence, it is currently stressed that the satisfactory results can never be obtained by the present surgical treatment alone.

At present there is no perfect answer to the questions of how cancer recurrence
can be eliminated, although various factors are anticipated in their cause, and if there are any effective procedures for the prevention of cancer recurrence.

Among various types of recurrence of cancer, surgeons are interested in local recurrence of cancer following operation, because the local recurrence may be at times secondary considered to the operative procedure itself. The local recurrence involves the following features:

1) Recurrence due to an incomplete extirpation of cancer tissues (residual cancer).
2) Dissemination of cancer cells in the local area (disseminated cancer recurrence).
3) Metastatic recurrence in regional lymph nodes (local metastatic cancer recurrence).
4) Intracanalicular implantation in the intestine or other organs (local implanted cancer recurrence).
5) Hematogenous metastatic recurrence, especially in damaged tissues (local hematogenous metastatic cancer recurrence).

Even though many of these local recurrences may be eliminated by further improvement of the surgical technique, it is questionable if all metastatic or disseminated recurrences could be completely prevented by surgical procedure alone.

We have been investigating anti-cancer chemotherapy combined with radical operative procedure for the past several years and we present in this paper the results of its basic experiments and the effectiveness of a combined therapy in the prevention of cancer recurrence.

I. Significance of Anticancer Agents for Treating Cancer Patients

No one currently throws doubt on the usefulness of chemotherapy for infectious diseases. The answer to the question of whether anticancer chemotherapy is also effective for cancer patients or not may be obtained if following experimental and clinical data are taken into consideration.

For the past several years, it has been proved from many animal experiments that cancer cells appear in the vascular canals after inoculation into the extravascular space. Recently, Kurata found that various strains of rat ascites hepatoma cells appeared in lungs and intracardiac blood after intraperitoneal inoculation of tumor cells. The earliest appearance of tumor cells was on the first day, while the latest was about one week later. This result seems to indicate the difference of not only the biological property of tumor cells but also the emigration of tumor cells into vascular canals. This emigration rate was faster than was suspected. This behavior of tumor cells is analogous to septicemia induced by bacterium. It is uncertain, however, whether human cancer cells behave similarly as bacteria.

Recently, Engel succeeded in detecting cancer cells from the drainage veins in cancer of intestine, stomach, mammary gland, and lungs as much as in 58% of 107 patients. In addition, he found cancer cells even in peripheral vein (cubital vein) of
cancer patients. Following his work, a number of similar evidences have been presented by various workers (Fischer and Turunbell, Packard, Cole, and Sontwick, Moore, and Tazaki). Thus, some of human cancer is by no means a localized disease, but spreads all over the body even in the early stage as in infectious diseases. This also suggests that surgical treatment alone never provides a complete cure of cancer.

The evidence of the acceleration of exfoliation of tumor cells associated with operative manipulation has also been presented. For instance, the recent work of Kamimura revealed that the exfoliation of cancer cells in the abdominal cavity occurred as frequently as in 60% of cases following surgical removal of stomach cancer. These exfoliated tumor cells were probably one of the causes of the local recurrence of cancer. It is out of the question to remove these exfoliated tumor cells completely by surgical treatment alone.

Although early vascular emigration and dissemination of cancer cells are proved both in experimental and human tumors, a question is aroused whether or not all emigrating cancer cells are always lodged in the various remote organs, resulting in metastatic formation. Engel and Moore and Sandberg reported that the frequency of appearance of cancer cells in blood vessels could not always be correlated with metastatic formation, whereas contrary results were presented by Spiggs and by Balders. Spiggs said that cancer cells were found in blood vessels in 7 out of 100 patients, and all those 7 cases had a poor prognosis. The opinion of the latter workers seems conceivable from the following experimental data.

Experiment was performed by Sato using mouse ascites hepatoma MH-134. He examined the interrelation between the frequency of appearance of cancer cells in blood and tumor growth in lungs of the host mice after inoculation. As indicated in Table I, a clear correlation was noted in the two; the more frequently tumor cells were found in blood stream, the more frequently metastatic formation was found in the lungs of the mice. In addition, it is usual to find cancer cells in peripheral blood of the late-stage cancer patients with widespread metastasis more easily than in early cancer patients. Therefore, the appearance of cancer in blood vessels should be the sign of a poor prognosis.

II. Effect of Anticancer Agents for the Emigration of Cancer Cells

As mentioned above, the usefulness of anticancer chemotherapy is probably similar to the antibacterial chemotherapy. The cytological effect of anticancer agents on tumor cells has been well investigated, but relatively little is known of their effect on metastatic formation, especially on the emigration of tumor cells. Therefore, Satou examined anticancer drugs to see if they could inhibit the metastatic formation of tumor cells.
EXPERIMENT A

Method: Rat ascites hepatoma cells (AH-13, $\times 10^7$) were inoculated into the muscle of the right thigh of rats. In the control group, venous blood (2.0 ml) was aspirated from vena cava inferior 10 days later, nuclear cells were collected from the blood by the hematocrit method, and smeared on five glass slides. For microscopic examination, Wright-Giemsa staining was applied. When even one tumor cell was detected on a slide, the slide was evaluated as positive. The frequency of appearance of tumor cell was indicated by the number of positives among the tested slides. In other groups, the preparation and identification of tumor cells were just the same as those of the control group but nitrogen mustard N-oxide (HN$_2$-O, 0.5 mg/rat) was injected daily for 4 days before aspiration of the venous blood on the 10th day.

Result: As indicated in Table II, migration of tumor cells into local vein was proved to be markedly inhibited by the injection of HN$_2$-O. This result was presumed to indicate that anticancer agents caused not only noxious effect on tumor cells, but also inhibited tumor metastasis.

EXPERIMENT B

In the previous experiment, the inhibitory effect of anticancer agents on migration of tumor cells was demonstrated. One of the reasons why anticancer agents display such an effect was clarified by the following experiment.

Yamada carried out systematic studies on tumor metastasis and intercellular adhesiveness, using rat ascites hepatoma island. The first step of tumor metastasis was thought to depend on the separation of tumor cells from the primary tumor. Therefore, intercellular adhesiveness of tumor cells was examined. For the study of this problem, rat ascites hepatoma islands seemed to be suitable, because the islands were composed of cell aggregates without interstitial tissue, forming epithelial arrangement (Photos 5 and 6).

Table I. Metastatic Spread of Tumor in the Lung of Mice bearing either Tumor-positive or -negative Blood (MH-134)$^{15}$

<table>
<thead>
<tr>
<th>Days after</th>
<th>Tumor cell in blood</th>
<th>Tumor growth in the Lung</th>
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<td></td>
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<td>nodules</td>
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<td>10</td>
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<td>20</td>
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<td>1/18</td>
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<td>10</td>
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<td>20</td>
<td>—</td>
<td>0/23</td>
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<tr>
<td>30</td>
<td>—</td>
<td>0/2</td>
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</table>

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a) Screening for the agents effecting separation of intercellular bonding of hepatoma islands:

As indicated in Photos 6 and 7, AH-601 strain of rat ascites hepatoma cells was

Table II. Effect of Nitromin (HN₂-O) on Emigration of Tumor cells into Local Vein
            (Rat ascites hepatoma AH-13)¹⁷)

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Frequency of tumor-positive specimen* every 10 days</th>
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<tr>
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<td>Control group</td>
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<td>1</td>
<td>7/8</td>
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<td>5/5</td>
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<td>3</td>
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<td>2/4</td>
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<td>9</td>
<td>1/5</td>
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<tr>
<td>10</td>
<td>0/5</td>
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Blood (2.0 ml) was aspirated from local vein 10 days after inoculation of tumor cells (10⁷) into right thigh of rats and examined by hematocrit method. The numerator is the number of positive specimens and denominator, the total number of specimens examined.

Table III. Dissociation of Hepatoma Islands into free Hepatoma Cells after Treatment with Various Agents (in vitro) (AH-601)²⁴)

1. Trypsin (pH 7.1) 1–5 mg/ml #
2. Hyaluronidase 5000 U/ml −
3. Hyaluronidase-EDTA 5000 U + 0.2% 1 ml −
4. EDTA (disodium ethylenediaminetetraacetate) 0.04–0.5% −
5. NaCl solution of various osmotic pressure Aq. dest. 10% −
6. Buffer solution of various pH pH 1–8 + (pH 8)
7. Nitrogen mustard N-oxide 0.001–1 mg/ml −
8. Carboxymethylcellulose 0.1–2.0% −
9. Surface-active agents
   1) Span 20 1% −
   2) Span 60 #
   3) Span 85 #
   4) Tween 20 #
   5) Tween 40 #
   6) Tween 60 #
   7) Tween 80 #
   8) Tween 85 #
10. Steroidal hormones
    1) Progesterone (E–P Hormone) 10% −
    2) Esterone (Ovahormone) 0.2 mg, 1 ml ±
    3) Androsterone (Durabolin) 10 mg, 1 ml −
    4) Testosterone (Enarmon) 0.5 mg, 1 ml −
    5) Adrenocortical hormone (Interenin) 0.25 mg, 1 ml ±
collected 5 days after inoculation into the intraperitoneal cavity of rats. The tumor cells were washed several times with physiologic saline and prepared into 20% tumor cell suspension in physiologic saline. Then, the frequency percentage of the free tumor cells and islands (composed of more than 3 cells) was estimated as a control. The tumor suspension was divided into test tubes into which various kinds of agent (Table III) were added. All these tubes were incubated at 37° for 1 hour and then shaken in an agitation device. After this procedure, the frequency percentage of free tumor cells and islands was again calculated. If the free cells increased after the above procedure, separating effect on the intercellular bond of tumor islands was deemed positive and its grade was indicated as −, +, ++, and +++.

As indicated in Table III, a marked separating effect on the intercellular bond of tumor cells was found in trypsin, Tween 80, and Tween 20 among the various agents tested but not in HN2-O. This result indicates that HN2-O does not have any direct influence on the intercellular bonding of tumor cells in vitro.

b) The separating effect of Tween 80 ("Tween 80 effect") on the intercellular bonding of tumor island cells (AH-601), with special reference to the growth of tumor cells:

Tumor suspension was prepared each day following intraperitoneal inoculation, as in the previous experiment and the separating effect of Tween 80 (1%) on those different-aged tumor cells was tested in vitro. As indicated in Fig. 2, the "Tween 80 effect" was found to vary with time after inoculation; it increased and reached a peak on the 4th or 5th day and decreased thereafter to return to the starting point. This result seemed to indicate that the separating effect of Tween 80 on hepatoma islands was variable with the age of tumor cells used.

\[\text{Fig. 1 Increased percentage of single cells isolated from island of ascites hepatoma (AH-601) treated with Tween 80 in vitro}^{24}\]

c) "Tween 80 effect" on rat ascites hepatoma island (AH-601) influenced by anticancer agents:

Tumor-bearing rats were injected with additional 0.5 mg of HN2-O every day until
5th day after intraperitoneal inoculation of tumor cells (the total dose varied in each group of rats). On the 5th day, all the rats were sacrificed and tumor cells were collected. The 5-day-old tumor cells were tested in vitro. The "Tween 80 effect" was evaluated as in the previous experiment. A similar test was carried out using triethylenetriphosphoramide and Mitomycin-C. As the control, 5-day-old tumor cells without injection of anticancer agents were used.

The results are demonstrated in Fig. 2. The "Tween 80 effect" on tumor islands decreased in proportion to the dose of the previously injected anticancer agent. In other words, the injection of anticancer agents made the intercellular bond of hepatoma islands more intimate. The above results seem to indicate why the anticancer agents have an inhibitory effect on the migration of tumor cells, as demonstrated in Experiment A.

**Experiment C**

Previously, Mizota and Satou\(^1\) succeeded in transplanting Yoshida sarcoma cells in the stomach of rats. In the present experiment, the exfoliating tendency of Yoshida sarcoma cells inoculated into stomach was investigated. The method is illustrated in Fig. 3. In three groups of rats bearing Yoshida sarcoma in the stomach, HN\(_2\)-O (0.5 mg) was injected intraperitoneally, and 8, 24, 48 hours before removal of the sarcoma from the stomach. In another group, 0.5 mg HN\(_2\)-O was injected daily for 4 days and these 4 groups were compared with the control group without HN\(_2\)-O injection. The exfoliating tendency was estimated by calculating the number of exfoliated Yoshida sarcoma cells. The results failed to indicate any clear difference between
the test and control groups. However, HN\textsubscript{2}-O never accelerated the exfoliating tendency of Yoshida sarcoma cells inoculated in stomach. This experiment is still being continued (Fig. 4).
All these experimental results indicated the necessity of the preoperative administration of anticancer agents and the reason is as follows: The surgical procedure itself gives the risk of pushing out the tumor cells into local vascular canals (hematogenous and lymphogenous), as cited previously. In addition, resistance of the host reduced by surgical procedure itself may cause a spreading of tumor cells which remain unremoved. Such postoperative spreading of tumor cells may depend on the decrease of intercellular adhesiveness of tumor cells. The present experiment revealed the possibility that the migration of tumor cells could be prevented by preoperative administration of anticancer agents, if the dose of the agents administered could be so chosen as to be sufficient to exhibit the effect, but not enough to reduce the host's resistance markedly.

In addition another experimental findings are presented proving that preoperative administration of anticancer agents was necessary.

Table IV. Inhibitive Effect of Nitromin upon a Small Number of Yoshida Sarcoma Cells

<table>
<thead>
<tr>
<th>Single dose (mg/rat)</th>
<th>No. of injection</th>
<th>Total dose (mg/rat)</th>
<th>No. of Yoshida sarcoma cells inoculated</th>
<th>10²</th>
<th>10³</th>
<th>10⁴</th>
<th>10⁵</th>
<th>10⁶</th>
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- No effect
+ Life prolongation effect positive
++ Life prolongation effect positive (more than 50% cured)
III. Anticancer Chemotherapy for a Small Number of Tumor Cells

When anticancer chemotherapy is combined with surgical operation, chemotherapeutic effect on a comparatively small number of tumor cells should be taken into consideration, because most of the tumor cells are usually removed by surgery and a small number of tumor cells would remain. In order to analyze this aspect, Satoh\textsuperscript{16}) carried out a comparative study of anticancer effect of HN\textsubscript{2}-O on a small number of Yoshida sarcoma cells (100\texttextup{\textasciitilde}1,000,000 cells), \textit{in vivo}. As shown in Table IV, the minimum dose of HN\textsubscript{2}-O which produced life prolongation effect was less in rats bearing smaller number than a large number of tumor cells. This result seems to indicate the reason why a small dose of anticancer agent is effective enough immediately after surgical operation.

IV. Effective Concentration of HN\textsubscript{2}-O in Circulating Blood

Before clinical application of anticancer agents, there are several problems to be investigated. Among them it is most important to know how long the anticancer agents keep their effectiveness in the circulating blood following administration through various routes. Tokuoka\textsuperscript{20}) investigated this point.

EXPERIMENT D

Dogs, 12\texttextup{\textasciitilde}20 kg in body weight, were administered HN\textsubscript{2}-O (10 mg/kg) through various routes, i.e. intravenous, intra-arterial, intraperitoneal, oral, and gastric
subserosal. HN$_2$-O in blood, urine, and chyle were measured by *in vitro-in vivo* tests (Satoh) at various intervals of time.

*In vitro-in vivo* test was carried out as follows: Yoshida sarcoma cells were mixed with the test sample of various dilutions and incubated for 30 minutes at 37° *in vitro*. These tumor cells were inoculated into the intraperitoneal cavity of rats. The maximum effective dilution of each sample was estimated by means of morphological changes such as appearance of abnormal mitotic figure and abnormal cytological features in Yoshida sarcoma cells.

This result is indicated in Fig. 5. Although all above methods caused no marked difference of HN$_2$-O concentration in blood, the longest effectiveness of HN$_2$-O in blood was obtained by intraperitoneal injection. Beside these finding, direct cytotoxic effect can be expected from HN$_2$-O on tumor cells which are disseminated into peritoneal cavity. Therefore, the intraperitoneal administration of anticancer agents is preferred in clinical use.

It is, therefore, concluded from these experimental evidences that the administration of anticancer agents before, during, or after surgical operation is indispensable for preventing the recurrence of cancer.

V. Clinical Application

Although this method has been applied for all kinds of cancer, the emphasis is placed in the treatment of stomach cancer.

1) Preoperative Administration: A mixture of 25 mg of Nitromin–D* and 100 mg of Endoxan, dissolved in 500 ml of 5% glucose-Ringer solution, is intravenously infused twice, 24 and 48 hours before surgical operation. The combination of two different drugs is used to obtain a broad anticancer spectrum and the possibility of a synergistic effect of the two drugs. Broadening of the anticancer spectrum and synergistic effect of two or more drugs are still to be investigated and these points will be discussed in future.

2) Administration during Surgical Operation: A mixture of 25 mg of Nitromin–D* and 100 mg of Endoxan are dissolved in 50 ml of physiological saline. As much as 100 ml of the solution is sprinkled in the upper abdominal cavity, especially over the cancer of stomach and its neighborhood, during surgical operation. Before cutting out minor and major omenta, 3 ml of the solution is injected into the subserosal space at three points on each side of the stomach before resection of the tumor, as illustrated in Photos 1 and 2. This procedure is aimed mainly to kill the tumor cells which may

*3 Nitromin–D is composed of HN$_2$ N-oxide (Nitromin) and a dimer (2:3). This drug suppressed almost completely the cholinergic action of Nitromin on the extirpated intestine so far examined by the Magnus method. However, the effectiveness is almost the same as that of Nitromin on experimental tumor cells. Up to present, ill effect of this drug on clinical cases seemed to be less frequent than that of Nitromin.
be scattered microscopically in the stomach wall and partly to send anticancer drugs
directly into the regional lymph nodes.

After extirpation of stomach cancer, the remainder of the solution is sprinkled into
the abdominal cavity. In addition, a polyethylene tube of 1 mm in diameter is inserted
into the abdominal cavity and the tip of the tube is placed in site of the resected
stomach (Photo 3). The polyethylene tube is fixed to the abdominal wall before the
abdominal cavity is closed (Photo 4).

3) Postoperative Administration: Through the polyethylene tube, 100 mg of Endoxan
is administrated every day after the surgical operation. This postoperative instillation
of anticancer drugs may kill the tumor cells surviving in the abdominal cavity, on
the one hand, and may keep effective concentration of anticancer drugs in circulating
blood for longer period of time, on the other. The polyethylene tube is removed 7
to 10 days after the surgical operation. Meanwhile, blood transfusion (100~200 ml),
thiamine (10 mg), ascorbic acid (100 mg), Merthio-B (methionine+vitamin B), and
Cobalt-Greenpole (20 mg) are administrated intravenously every day.

4) Aftercare: The patient is recommended to visit the out-patient clinic once every
6 months for additional intravenous administration of Endoxan for the next two
years.

Up to the present, the above method was used in 42 cancer patients during the
past two years. The ill effect of chemotherapeutic agents was less frequently en-
countered than by the usual intravenous administration. Though it is our impression
that the present method of treatment of cancer is quite promising, our clinical ex-
perience of the method is still not enough to draw any final conclusions. It is hoped
that a more conclusive result of the method will be presented in the near future.

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**Explanation of Plate X**

Photo 5. Ascitic picture of AH-13 (acetic acid-Gentian Violet staining). This ascites hepatoma is composed of free cells.

Photo 6. Ascitic picture of AH-601 (acetic acid-Gentian Violet staining). This ascites hepatoma is composed mainly of islands.

Photo 7. Ascitic picture of AH-601 (phase-contrast microscopic view).

Photo 8. Ascites hepatoma cells (AH-13) circulating in the local vein (single cell).

Photo 9. Ascites hepatoma cells (AH-13) circulating in the local vein (pair cell).

Photos 10 and 11. Ascites hepatoma cell aggregates (AH-13) circulating in the local vein.
Each 3.0 ml of the solution of anticancer agent is injected into the subserosal space at three points of the stomach as illustrated.

The same procedure as above is performed on the back of the stomach.

A polyethylene tube of 1 mm in diameter is inserted into the abdominal cavity and the tip of the tube is placed in the site of the resected stomach.

The polyethylene tube is fixed to the abdominal wall and anticancer agent is injected postoperatively through this tube.