Enhancement of the Cancer Chemotherapeutic Effect by Anticancer Agents in the Form of Fat Emulsion

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Takahashi, T., Kono, K. and Yamaguchi, T. Enhancement of the Cancer Chemotherapeutic Effect by Anticancer Agents in the Form of Fat Emulsion. Tohoku J. exp. Med., 1977, 123 (3), 235-246 — Utilizing the lipid-absorbing ability of lymphatic capillaries, anticancer agents were given in the form of fat emulsion in order to deliver them to regional lymph nodes. The emulsion, in which the drug solution is contained as the innermost phase, yielded high drug concentration in the lymphatic system. Intratumoral injection of emulsified anticancer agent resulted in significantly prolonged retention of the drug within the tumor tissue. Therapeutic experiments of the emulsion also disclosed remarkable tumor reduction and cure rate as compared with aqueous solution of drugs. Oral administration of emulsified 5-Fluorouracil (5-FU) was also attempted for stomach cancer. With 5-FU, the maximum concentration of drug in thoracic lymph and stomach was greater when administered as an emulsion than as an aqueous solution, and a high concentration persisted longer. As a clinical trial of the emulsion method, eight patients with inoperable malignant growth were injected locally with emulsified anticancer agents and 121 patients were given 5-FU emulsion orally. From the clinical and histological findings, it was thought that the emulsion enhanced the chemotherapeutic effect of the anticancer agent on lymph node metastasis.

For increasing the efficiency in cancer chemotherapy, it is important to supply anticancer agents in sufficiently high concentration to both primary growth and metastasis with minimal side effects. In our previous studies we demonstrated that anticancer agents, when administered in the form of fat emulsion, accumulated in regional lymph nodes and were retained there in high concentration over an extended period (Takahashi et al. 1973, 1976).

The present paper deals with the results of experimental and clinical studies of emulsified anticancer agents.

MATERIALS AND METHODS

Preparation of emulsified anticancer agents. Various types of emulsions which contained anticancer agents were prepared by ultrasonification. The detailed procedures for the preparation of emulsified anticancer agents have already been described (Takahashi et al. 1973). Water in oil (W/O) emulsions were made up of 65% sesame oil, 30% aqueous.

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solution of anticancer agent and 5% emulsifier (Span 80). Oil in water emulsions (O/W) were prepared with 30% sesame oil with or without anticancer agents, 65% aqueous solution with or without anticancer agent and 5% emulsifier (Tween 80, HCO-60). Multiple water-oil-water (W/O/W) emulsions consisted of 65% distilled water, 19.5% sesame oil, 9% aqueous solution of anticancer agent and 6.5% emulsifier (Pluronic F-68, Span 80).

RESULTS

Drug concentration in regional lymph nodes

Donryu rats were divided into five groups and injected with 30 μCi of 3H-5-fluorouracil (3H-5FU) as follows: Rats of group 1 were injected into the testis with W/O 3H-5FU emulsion, group 2 with O/W 3H-5FU emulsion, group 3 with W/O/W 3H-5FU emulsion and group 4 (W) with aqueous solution of 3H-5FU. Rats of group 5 were injected intravenously with an aqueous solution of 3H-5FU. Five animals of each group were sacrificed at intervals of 0.5, 1, 3, 6, 12 and 24 hr after injection and the regional lymph nodes were removed. Radioactivity was measured with a Packard liquid scintillation counter.

The results are shown in Fig. 1. In group 1 (W/O) and group 3 (W/O/W), maximum values of drug were reached in 3 hr, and were 2.7 and 7.9 times, respectively as high as the maximum value after injection of the aqueous solution, and 3.1 and 9.1 times as high as after intravenous injection. Group 2 (O/W)
also showed the highest value at 3 hr and this level was 1.5 times higher than the maximum observed after intravenous injection.

**Drug concentration in tumor tissue**

In this series of experiment, 180 rats of the Donryu strain were used. Rats were transplanted acites hepatocarcinoma AH-66 subcutaneously in the back, and the following treatments were begun 10 days after implantation, by which time the tumor had grown to about 15 to 20 mm in diameter: 1) a W/O emulsion containing 1 mg bleomycin was injected directly into the tumor; 2) the same dose of the anticancer agent in the form of aqueous solution was also topically administered, or 3) a comparable dose of bleomycin as aqueous solution was given by intravenous injection. Ten animals were sacrificed 10 min, 30 min, 1 hr, 5 hr and 10 hr after injection. To measure the drug concentration in the tumors, each tumor was totally resected and the same amount of saline was added to it. Then the tumors were homogenized and centrifuged for 10 min in a cold room to produce a supernatant. The method of bleomycin assay was a modification of the zone inhibition technique of Clarkson (Clarkson et al. 1965), in which agar plates with subtilis PCI 219 as test organism were employed. Table 1 shows the mean levels of drug concentration of each ten tumor tissues at the times mentioned above following administration of 1 mg of bleomycin by the three different methods. A large amount of the drug was present in the tumors 10 min after direct injection of the emulsion and of aqueous solution, whereas at the same time interval the concentration of bleomycin in the tumor after intravenous injection was only at a trace level. At 30 min after direct administration, the bleomycin level in the tumor still remained as high as 3.13 $\mu$g/g in animals which received the emulsion, whereas it was less than 0.1 $\mu$g/g in those given the aqueous solution. On the other hand, the drug was no longer demonstrable in rats given the drug by the intravenous route. The drug level in the tumor was 2.04 $\mu$g/g 1 hr after injection of the emulsion. In this group of rats, trace amounts were still demonstrable in the tumor even 10 hr after administration.

<table>
<thead>
<tr>
<th>Preparation for injection</th>
<th>Time after injection</th>
<th>10 min (Mean±S.E.)</th>
<th>30 min (Mean±S.E.)</th>
<th>1 hr (Mean±S.E.)</th>
<th>3 hr</th>
<th>5 hr</th>
<th>10 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulsion, into tumor</td>
<td></td>
<td>7.80±1.47</td>
<td>3.13±0.48</td>
<td>2.04±0.15</td>
<td>$&lt;0.1^*$</td>
<td>$&lt;0.1^*$</td>
<td>$&lt;0.1^*$</td>
</tr>
<tr>
<td>Aqueous solution, into tumor</td>
<td></td>
<td>5.70±1.74</td>
<td>$&lt;0.1^*$</td>
<td>$\circ$</td>
<td>$\circ$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aqueous solution, intravenous</td>
<td></td>
<td>$&lt;0.1^*$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</table>

* Trace amounts of bleomycin.
In order to assess the effect of emulsified anticancer agent on the tumor and its metastasis, 0.5 mg of bleomycin was administered in three different ways.

As the model of primary and metastatic growth, Donryu rats bearing a left axillary tumor and a metastasis were used in this series. 10⁶ cells of ascites hepatoma AH–66 were inoculated in the left axillary region of the rats. Ten days after inoculation, by which time the tumor had grown to about 15 mm in diameter and metastasized to the axillary lymph node, the rats were divided into four groups according to the way of treatment, as follows; those given intratumoral and peritumoral injection (local injection) of W/O bleomycin emulsion, those given intratumoral and peritumoral injection (local injection) of aqueous solution of bleomycin, those given intravenous injection of aqueous solution of bleomycin, and untreated control. Tumor growth was evaluated by the average increase or decrease in the size of the tumor in comparison with the pretreatment value for each group. Axillary lymph node metastasis as examined by autopsy 20 days after injection.

Fig. 2 illustrates serial changes in the tumor size. A rapid reduction of the tumor occurred in animals given the emulsion; the mean diameter of the tumor decreased by 35% at 7 days after injection and 50% at 14 days. In contrast, the tumor size in the group receiving bleomycin in water by local injection increased by 15% at 7 days after administration and decreased by 5% at 14 days. No reduction of the tumor was found in untreated controls or in animals receiving the bleomycin solution by intravenous injection. Axillary lymph node metastasis was seen in 5 of 20 rats given local injection of bleomycin in emulsion, 7 of 20 rats receiving local injection of bleomycin in aqueous solution, 15 to 20 rats receiving intravenous
injection of bleomycin, and 14 of 20 untreated controls.

_Drug concentration in oral administration of 5-Fluorouracil emulsion_

Oil in water (O/W) emulsion containing 44 mg/ml of 5-FU and Donryu rats weighing about 200 g were used in this series.

_Drug concentrations in thoracic lymph_

Rats were given 100 mg/kg of 5-FU emulsion or 5-FU aqueous solution orally. A polyethylene tube was intubated into the thoracic duct just below the diaphragm to collect the thoracic lymph continuously. The concentration of 5-FU in thoracic lymph was determined by bioassay.

Fig. 3 shows the time course of the average 5-FU level in the thoracic lymph in each group of 10 rats. In the rats given 5-FU emulsion, the peak level of the drug concentration was 26.1 µg/ml at 1 hr after administration, then the drug concentration in the lymph decreased gradually. On the other hand, in the rats given 5-FU aqueous solution the peak level was 8.9 µg/ml, and decreased rapidly. The difference was statistically significant (p<0.01).

![Fig. 3. Drug concentration of thoracic lymph of rats after oral administration of 100 mg/kg of 5-FU emulsion (●—●) or 5-FU aqueous solution (○—○).](image)

_Drug concentration in blood and organs_

Rats were sacrificed at various intervals to examine the drug concentration in blood and organs. The maximum drug concentration in blood of rats given 5-FU aqueous solution was higher than that of rats given 5-FU emulsion, but the difference was not significant.

Fig. 4 sets forth the drug concentration in the stomach wall. In the rats given 5-FU in aqueous solution, the drug concentration in the stomach wall exhibited 188.5 µg/g in 30 min after administration and then decreased rapidly within 4 hr. On the other hand, in the rats receiving 5-FU emulsion, the maximum value was
Fig. 4. Drug concentration of stomach wall of rats after oral administration of 100 mg/kg of 5-FU in two forms. ● ●, 5-FU emulsion; ○ ○, 5-FU aqueous solution.

357.5 µg/g in 1 hr and this level was about 2 times as high as the maximum after administration of 5-FU aqueous solution. The drug concentration remained at a higher level for at least 4 hr. The difference in drug concentration between these two groups was statistically significant at 1 hr and 2 hr after administration of the drug. There was no significant difference in the concentration of drug in the liver and kidney between the two groups.

Clinical trials

Injection of emulsified anticancer agent. The above laboratory studies demonstrated that the effect of the anticancer agent was greater when it was injected into the tumor or lymph node area in the form of emulsion than of water solution. Encouraged by the well established fact that the purified sesame oil and the stabilizers employed in the preparation of the emulsion have no side effects in man (Janowits et al. 1953; Waldstein et al. 1954; Chusid and Diamond 1961), we conducted clinical trials of emulsified anticancer agents in patients with a variety of inoperable malignant tumors.

A total of 8 patients were treated by injecting emulsified anticancer agents into the tumor or its regional lymph node area. Emulsified bleomycin and mitomycin were used for the treatment of squamous cell carcinomas and adenocarcinomas, respectively. Patients with carcinoma of the skin, who were treated with emulsified bleomycin alone (3 cases) or in combination with surgical treatment (1 case), are still in good health. In both cases of postoperative recurrence and terminal stage of breast carcinoma, the tumor mass decreased to about 50% in size. However, both postoperative recurrence of carcinoma of the penis and carcinoma of the neck did not respond to bleomycin emulsion. The following is a brief report of demonstrable case.
A 67-year-old woman had a mass on the left cheek measuring $4.5 \times 4$ cm, which had grown for 3 months (Fig. 5). Biopsy examination revealed squamous cell carcinoma (Fig. 6). Intratumor bleomycin therapy was instituted with 5 mg bleomycin in W/O/W emulsion twice weekly. Three days after the first dose, spontaneous detachment of the necrotized central core of tumor occurred. With continuation of further local injection, the lesion further regressed. A biopsy of the margin of the ulcerated lesion after the 10th dose of bleomycin failed to show any tumor tissue. To complete the treatment, she was given three additional prophylactic doses into the adjoining areas; thus, she received a total of 13 doses or 65 mg of bleomycin. The ulcer was completely healed 3 months after the termination of medication, leaving only scars at the site (Fig. 7). The woman is well 4 years thereafter, showing no finding of metastasis or recurrence.

*Oral administration of emulsified 5-FU for stomach cancer.* A total of 121
patients with carcinoma of the stomach were given 500 mg of 5-FU preoperatively in the form of oil in water emulsion orally every day in three divided doses for 7 to 15 days. The age of the patients ranged from 26 to 82 years old, averaging 58.8. Ninety-six patients were subjected to gastrectomy with lymph node dissection, and the specimens were examined histologically.

Slight changes ascribable to 5-FU were indicated by swelling, vacuoles, pycnosis and frequent giant cells (Fig. 8). Moderate changes were indicated by destruction of structural patterns of cells and occasional degenerated flattened cells (Fig. 9). Marked changes of the cancer cells were characterized by extensive necrosis so that viable cells could not be found (Fig. 10), or those cells showed moth-eaten appearance due to destruction (Fig. 11). Since these changes were seen in
various degrees even on the same case, we graded the histological effect of 5-FU as follows: Grade 0, no histological changes; Grade 1, slight changes as above described; Grade 2, moderate changes; Grade 3, marked changes.

Lymph node metastasis in 62 of 96 cases treated with this method was graded histologically according to the above described criteria (Table 2). In the paragastric nodes, namely, superior or inferior gastric nodes including left and right paracardial nodes and supra- or subpyloric nodes, grade 2 and grade 3 were observed in 42% and 11%, respectively. In lymph nodes distal to the gastric wall, including lymph nodes along the common hepatic artery, left gastric artery, celiac axis and hepatic pedicle, grade 2 and grade 3 were observed in 23% and 25%, respectively.

Oral administration of 5-FU emulsion was effective not only for lymph node
Fig. 11. Photomicrograph of marked changes showing moth-eaten appearance.

Fig. 12. Left: Pyloric stenosis due to carcinoma of the stomach. Right: Improved stenosis by oral administration of 7,500 mg of 5-FU emulsion.

Table 2. Effect of 5-FU emulsion on histology of lymph node metastasis of human stomach cancer

<table>
<thead>
<tr>
<th></th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patagastric nodes</td>
<td>16(25.8)</td>
<td>13(20.9)</td>
<td>26(41.9)</td>
<td>7(11.2)</td>
</tr>
<tr>
<td>Distant nodes</td>
<td>6(13.6)</td>
<td>17(38.6)</td>
<td>10(22.7)</td>
<td>11(25.0)</td>
</tr>
</tbody>
</table>

metastasis but also for the stomach cancer itself. Pyloric stenosis due to carcinoma was occasionally improved by oral administration of 5-FU emulsion (Fig. 12). Table 3 summarizes the histological effect of 5-FU emulsion on stomach cancer.
TABLE 3. Effect of 5-FU emulsion on stomach cancer in histology

<table>
<thead>
<tr>
<th>Grade</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>5(11.3)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>15(29.5)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>20(45.5)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>6(13.6)</td>
</tr>
</tbody>
</table>

The sensitivity of well differentiated adenocarcinomas to 5-FU emulsion was higher than that of poorly differentiated adenocarcinomas and mucinous adenocarcinomas.

**DISCUSSION**

In order to attack lymph node metastasis which had not been completely eliminated by surgery, we have attempted to deliver anticancer agent in high concentration to the regional lymph nodes. Our previous studies have shown that anticancer agents injected into tissues in the form of fat emulsion are absorbed in a different way from that of aqueous solution of the agents (Takahashi et al. 1973). The emulsion is scarcely taken up by the blood vascular system but is retained within the tissue over a relatively extended period, being distributed slowly to the surrounding tissues. Eventually it is absorbed by lymph capillaries and conveyed to the regional lymph nodes. Utilizing this property of the emulsion, anticancer agents were given in the form of fat emulsion so that the agents can be transported to regional lymph nodes to give a higher concentration in the lymph nodes.

Among the various formulations for incorporating water soluble anticancer agents into emulsions, the water-in-oil (W/O) type and the water-in-oil-in-water (W/O/W) type proved to be the most effective. These emulsions, in which the drug solution is contained as the innermost phase, yield high drug concentration in the lymphatic system; namely, 2 to 7 times as high as those produced by local administration of aqueous solutions.

In rats bearing subcutaneous tumor AH–66, only a trace amount of bleomycin could be demonstrated in the tumor tissue 30 min after intratumor injection of the aqueous solution, whereas the concentration of the antitumor agent in the tumor was found to be considerably higher for up to 3 hr when the drug was locally injected in the form of emulsion. Although the quantity of the drug administered was quite small, it could be demonstrated in the tumor even 10 hr after injection when the drug was administered as an emulsion. These findings indicate that the retention of the drug within the tumor tissue is significantly prolonged when the drug was administered as an emulsion, but when locally administered in the form of aqueous solution, the drug rapidly dissipates from the tissue into the circulating blood. Intravenous administration of the same dose of aqueous solution gave rise
to only a slight accumulation of the drug in the tumor tissue which could be observed only immediately after injection, suggesting that by this route an extremely large dose would be required to achieve local drug concentrations comparable to those obtained by the injection of an emulsion.

The experiments conducted to assess the effect of bleomycin on AH-66 tumor and its metastasis in rats also disclosed a remarkable tumor reduction and cure rates with the emulsions as compared with aqueous solutions of drugs. Thus, it is likely that the drug concentration in the tumor tissue parallels the antitumor effect of the drug.

Oral administration of emulsified 5-FU was also attempted for stomach cancer. 5-FU was detected at higher levels in the stomach than in any other organs. A comparison of drug concentrations after administration of emulsion and aqueous solution of 5-FU indicated that the maximum value of 5-FU in the case of emulsion was higher than that of aqueous solution, and also high concentration of 5-FU persisted for a considerably long time when the emulsion was employed. This may be ascribed to the property of emulsion which permeates easily into the tissues and is retained there over a relatively long period. In thoracic lymph, the maximum level of drug attained with 5-FU emulsion was approximately 3 times that with the aqueous solution. The mechanism of absorption of 5-FU emulsion into the lymphatic system remains obscure. There is evidence that side effects of administration of the emulsion per se are virtually absent. This prompted us to conduct clinical trials of emulsions of anticancer agents. Carcinoma of the skin, in which emulsified bleomycin was locally injected, disappeared after 5 to 10 consecutive doses. In one of the carcinoma cases a marked therapeutic effect was also noted on metastatic regional lymph nodes.

Acknowledgment

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References