Modulation of Cancer Chemotherapy by Green Tea

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ABSTRACT
Biochemical modulation has played an important role in the development of cancer chemotherapy. We have directed our attention to the intake of common beverages and investigated the effects of green tea and tea components on the antitumor activity of doxorubicin. We carried out the combined treatment of doxorubicin and green tea on Ehrlich ascites carcinoma tumor-bearing mice. The oral administration of green tea enhanced 2.5-fold the inhibitory effects of doxorubicin on tumor growth. The doxorubicin concentration in the tumor was increased by the combination of green tea with doxorubicin. In contrast, the increase in doxorubicin concentration was not observed in normal tissues after green tea combination. Furthermore, the enhancement of antitumor activity of doxorubicin induced by green tea was observed in M5076 ovarian sarcoma, which has low sensitivity to doxorubicin. These results suggest that drinking green tea can encourage cancer chemotherapy and may improve the quality of life of clinical patients.

INTRODUCTION
In cancer chemotherapy, biochemical modulation has been studied extensively (1–4), and the enhancements of the activity of antitumor drugs, e.g., 5-fluorouracil (3, 4), are confirmed in clinical treatments. However, when the modulators induce the enhancement of antitumor effects, the examination of its side effects are not enough, and there are some cases of patients dying as the result of biochemical modulation. The development of a new modulator, which enhances antitumor activity and reduces the side effects of antitumor agents, has been needed. In addition, the use of modulators increases the number of medications and adds to the patient’s burden. Simultaneously, the study to improve the quality of life of patients is also necessary. If the intake of food or beverage as modulator enhances antitumor activity (biochemical modulation), then the improved antitumor activity by this type of biochemical modulation will reduce the patient’s burden.

We have directed our attention to Japanese green tea (Camellia sinensis), one of the common beverages that has been popular in Japan and China for a long time. Some green tea components have been reported to have useful effects (5–9), and the interest in these evaluations is high. Green tea inhibits carcinogenesis and is known to be effective for the chemoprevention of cancer (8, 9). However, the effects of green tea on cancer chemotherapy have never been investigated previously. Frequently, drinking green tea, coffee, or black tea is restricted for clinical patients. These restrictions may hinder the mental stability of the patients and/or occasionally have a bad influence on the therapy. If green tea has a positive action on cancer chemotherapy, we could expect that cancer therapy would become more effective and that the patient’s mental condition would become better by drinking green tea as usual.

To examine the effects of the oral administration of green tea on cancer chemotherapy, we have performed animal experiments, designed to consider the effects of drinking green tea during clinical treatment. In this study, we investigated the combination of green tea with DOX, an antitumor antibiotic widely used in clinical therapy (10–12), on tumor-bearing mice. The effects of theanine and caffeine, which are known to enhance the activity of antitumor agents (13–16), as green tea components (5), were examined as well.

MATERIALS AND METHODS
Chemicals. DOX injection, 10 mg/vial (Adriacin®), was purchased from Kyowa Fermentation, Inc. (Tokyo, Japan). Theanine was purchased from Tokyo Kasei Co., Ltd. (Tokyo, Japan). Caffeine was purchased from Wako Pure Chemical Industries, Ltd. Green tea powder was a product of Shizuoka (Shizuoka, Japan). The drugs were dissolved in sterile isotonic saline. The other chemicals used in this study were of the highest purity available.

Animals. Male CDF, and BDF, strain mice, 5 weeks of age and 20–25 g, were obtained from Japan SLC, Inc. (Hamamatsu, Japan). The animals were housed in a room maintained at 25 ± 1°C with 55 ± 5% relative humidity and were given free access to regular chow pellets (MF; Oriental Co., Ltd., Tokyo, Japan) and water.

Tumors. Ehrlich ascites carcinoma (1 × 10⁶ cells/animal) were i.p. transplanted into the CDF₁ mice. The ascites were collected on the 7th day after transplantation. M5076 ovarian sarcoma was kindly provided by Dr. T. Tashiro (Japanese Foundation for Cancer Research, Tokyo, Japan).

Animal Experiments. Ehrlich ascites carcinoma (5 × 10⁵ cells/animal) were transplanted onto the backs of CDF₁ mice. DOX (2.0 mg/kg/day for 4 days) was i.p. administered on the 10th, 12th, 14th, and 16th days after transplantation. Green tea powder (1.0 g/kg/day for 4 days) and theanine and caffeine...
Green Tea Enhances Cancer Chemotherapy

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor Weight Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>DOX</td>
<td>37%</td>
</tr>
<tr>
<td>DOX + Green Tea</td>
<td>55%</td>
</tr>
<tr>
<td>DOX + Theanine</td>
<td>75%</td>
</tr>
<tr>
<td>DOX + Caffeine</td>
<td>80%</td>
</tr>
</tbody>
</table>

Fig. 1: Effects of green tea and tea components on the changes in tumor weight (Ehrlich) induced by DOX. Each column is the mean of eight mice expressed as percentage of control level: bars, SD. Significant differences from the level of the DOX-alone group are indicated by: a) $P < 0.001$; b) $P < 0.01$; and c) $P < 0.05$.

Fig. 2: Effects of test drug on the DOX concentration in the tumors of mice. Each point is the mean of eight mice; bars, SD. Significant differences from the level of the DOX-alone group are indicated by: a) $P < 0.001$.

(100 mg/kg/day for 4 days) was p.o. administered on the 11th, 13th, 15th, and 17th days, or i.p. injections (10 mg/kg/day for 4 days) of tea components were performed on the same day. The mice were killed on the 18th day, and the solid tumors and tissues were rapidly removed and weighed.

The tissue samples were homogenized in 10 volumes (w/v) of 10 mM phosphate buffer (pH 7.8); then DOX was extracted by chloroform:methanol (4:1, v/v). The DOX concentration was determined with fluorescence spectrophotometer (excitation wavelength, 470 nm; emission wavelength, 585 nm).

M5076 ovarian sarcoma (1 x 10⁶ cells/animal) was transplanted onto the backs of BDF₁ mice. DOX (2.0 mg/kg/day for 4 days) was i.p. administered on the 14th, 16th, 18th, and 20th days after the transplantation. Green tea powder (100 mg/kg/day for 4 days) and theanine (10 mg/kg/day for 4 days) were p.o. administered on the 15th, 17th, 19th, and 21st day. The mice were killed on the 22nd day, and solid tumors were rapidly removed and weighed.

RESULTS

Effects of Green Tea and Tea Components on the Antitumor Activity of DOX in Ehrlich Ascites Carcinoma Tumor-bearing Mice. Tumor weights after treatment are shown in Fig. 1. The injection of DOX alone reduced the tumor weight by 25% compared with the control level (0.675 ± 0.108 g). The combined treatment of DOX and green tea significantly reduced the tumor weight to 37% of control level (significant difference from the DOX alone group, $P < 0.001$). Oral administration of theanine and caffeine enhanced the antitumor activity of DOX by 2.1-fold ($P < 0.01$) and 1.9-fold, respectively.

The DOX concentrations in the tumors are shown in Fig. 2. Green tea combined with DOX increased the DOX concentration in the tumor by 1.7-fold compared to the DOX-alone group ($P < 0.001$). Similarly, oral administration of theanine and caffeine increased the DOX concentration by 2.2-fold ($P < 0.001$).

DOX did not increase the DOX concentration in the heart and liver of tumor-bearing mice. In particular, the combination of theanine and DOX reduced the DOX concentrations in the heart ($P < 0.05$).

**Effects of Green Tea and Theanine on the Antitumor Activity of DOX in M5076 Ovarian Sarcoma Tumor-bearing Mice.** Tumor weights are shown in Fig. 3. The injection of DOX alone did not reduce the tumor weight compared with the control level, whereas green tea and theanine combined with DOX significantly reduced the tumor weight to 55% ($P < 0.05$) and 37% ($P < 0.01$) of control level.

DISCUSSION

Oral administration of green tea was combined with DOX treatment on Ehrlich solid tumor-bearing mice. Tumor growth was inhibited by DOX, and the administration of DOX alone decreased the tumor weight by 25% compared with the control level, whereas the addition of green tea remarkably reduced the tumor weight to 37% of the control level and significantly enhanced by 2.5-fold the DOX inhibitory effect on tumor growth. This result indicates that drinking green tea causes the enhanced activity of antitumor agents. This effect of green tea is new evidence and provides the possibility that green tea acts as a biochemical modulator. We may expect that green tea promotes cancer chemotherapy. Similarly, the enhancement of the antitumor activity of DOX was observed by the i.p. and oral administration of theanine or caffeine with DOX. Thus, theanine and caffeine were indicated to be effective as biochemical modulators, and it is suggested that the effect of green tea depends on these components.

We determined the DOX concentration in the tumor, as the targeted tissue, and in normal tissues. The DOX concentration in the tumor was significantly increased by the combination of green tea or tea components with DOX compared with the DOX alone group. This increment of DOX concentration in the tumor was regarded as the direct cause of the enhancement of antitumor activity, induced by green tea or tea components. In previous reports (13, 14), we have confirmed that theanine and caffeine increased the DOX concentration by 2.2-fold ($P < 0.001$) and 1.9-fold, respectively.
caffeine inhibit the DOX efflux from Ehrlich cells in vitro and then increase the DOX concentration in the tumor, thereby causing the enhancement of antitumor activity in vivo. The enhanced effect by green tea is considered mainly dependent on these components that increased DOX concentration in the tumor. However, it is possible that other components in green tea have efficacy on the antitumor effect of DOX as well.

In contrast, the increment in DOX concentration was not observed in normal tissues by green tea, theanine, or caffeine. Green tea and theanine decreased the DOX concentration in the heart. Thus, green tea was shown to enhance the antitumor activity of DOX due to specifically increasing the DOX concentration in the tumor only. On the other hand, DOX concentrations in normal tissues associated with side effects were reduced by the combination of green tea with DOX. These data suggest that green tea may reduce the side effects of DOX from the point of drug distribution.

Cardiac toxicity is very severe side effect of DOX and is reported to be caused by the elevation of lipid peroxide in the heart (12, 17, 18). Because green tea has some antioxidative components, e.g., catechins (5, 19–21), we could expect that green tea reduces the lipid peroxide level. Thus, we can expect that drinking green tea reduces the cardiac toxicity induced by DOX because of a decrease in the lipid peroxide level in the heart as well as the DOX concentration.

Many studies about biochemical modulation were not performed in the past in consideration of both the antitumor effects and side effects of a drug, whereas green tea described in this study can be an ideal biochemical modulator because it has beneficial effects on both the antitumor activity and side effects of antitumor agents. Because green tea is a common beverage, the combination of drinking green tea with chemotherapy is easy to try for humans in clinical treatment. We expect that cancer chemotherapy with the addition of green tea will induce a positive effect.

Furthermore, to investigate the broad usefulness of green tea, we examined these effects on M5076 ovarian sarcoma (22), which has low sensitivity to DOX. The inhibitory effect on M5076 tumor growth was not observed by the administration of DOX alone. In contrast, the combination of green tea, as well as theanine, with DOX significantly decreased the tumor weight to 55% of control level. Thus, these results demonstrate that green tea enhanced the antitumor activity of DOX against M5076 tumor, indicating that the effects of green tea as a modulator are not specific to Ehrlich ascites carcinoma. On the M5076 tumor, against which DOX was not effective, an antitumor effect of DOX was observed with the addition of green tea, without elevating the dose of DOX. Therefore, we may expect similar enhancement of antitumor effects by green tea against tumors of low sensitivity or drug-resistant tumors. These results suggest that green tea has a very important effect on cancer chemotherapy.

In this study, drinking green tea was demonstrated to enhance the antitumor activity and to reduce the side effects of DOX. We found that green tea has physiological effects as a biochemical modulator as well. For patients in clinical treatment with antitumor agents, it is easy to suggest drinking green tea with a meal. Green tea may have positive influences on cancer chemotherapy. We think that the intake of a favorite beverage favors a patient’s positive mental attitude and encourages the efficiency of the chemotherapeutic index, and that this efficacy is useful in improving the quality of life in cancer chemotherapy. This discovery appears important in the field of study of biochemical modulation.

The fact that green tea is useful not only for cancer chemoprevention but also for encouraging the efficacy of chemotherapy may be good news for clinical patients. The results of this study may be helpful to promote the eradication of cancer in the future. We hope that clinicians encourage their patients to drink green tea, according to these results, so that cancer therapy will be more successful.

**REFERENCES**

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