

Effects of Experimental Chemoendocrine Therapy With a Combination of a Pure Antiestrogen and 5-Fluorouracil on Human Breast Cancer Cells Implanted in Nude Mice

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Abstract: The antitumor effects of an experimental chemoendocrine therapy combining a new pure antiestrogen ICI 182780 and 5-fluorouracil (5-FU) were studied on MCF-7 human breast cancer cells implanted in nude mice. ICI 182780 had a dose-dependent antitumor activity, which was potentiated by the concomitant use of 5-FU. When compared with the control group, the estrogen receptor (ER) level in the ICI 182780 group was lower and that in the combination group was markedly lower. Cell cycle analysis by flow cytometry (FCM) resulted in a lower percentage of S-phase cells (%S) in the treated mice. No significant difference was observed in the 5-FU concentrations in tumor cells, while the 5-FU content in RNA was significantly higher in the combination group. The changes in free thymidylate synthetase (TS) concentration indicated TS synthesis after the administration of 5-FU to be more greatly suppressed in the combination group than in the 5-FU group. These results suggest that ICI 182780 and 5-FU exert their combination effect mainly on ER-positive cells, and that the suppression of TS synthesis in tumor cells and the potentiation of the 5-FU-induced metabolic dysfunction of RNA are thus involved in the mode of action of this combination therapy.

Key Words: chemoendocrine therapy, pure antiestrogen, 5-fluorouracil, nude mouse, breast cancer

Introduction

Breast cancer is a hormone-dependent cancer which is generally known to respond to chemoendocrine therapy. Evidence has also been obtained that estrogen

receptor (ER)-positive breast cancer is sensitive to endocrine therapy while ER-negative cancer is not very sensitive.¹ Meanwhile, even apparently ER-positive breast cancer is intermingled with ER-negative cells, so that a combination of endocrine therapy with chemotherapy which has a different mode of action is required to improve the response rate. In addition, a possible reduction of adverse reactions of chemotherapy by concomitant endocrine therapy is of clinical significance.²

Among the various forms of endocrine therapy for breast cancer, antiestrogens, especially tamoxifen (TAM), have been the most widely used. TAM, although an antiestrogen, also has estrogenic activity³⁻⁵ and has recently been reported to be associated with the occurrence of endometrial cancer.^{6,7} Among the newly developed antiestrogens, ICI 182780, a pure antiestrogen free from estrogenic activity, is expected to be particularly useful in the treatment of breast cancer.^{8,9}

In this experiment, the antitumor effects of a combination of ICI 182780 and a widely used fluorinated pyrimidine, 5-fluorouracil (5-FU) were studied, and the mode of action of this chemoendocrine therapy on human breast cancer implanted in nude mice was evaluated.

Materials and Methods

The animals used were BABL/c nu/nu female nude mice aged 7–8 weeks and weighing 19–22 g, obtained from Nippon Crea, Tokyo, Japan. The cell line used was MCF-7, a nude mouse transplantable human breast cancer. It was a hormone-dependent, human breast cancer cell line originally established by Soule et al.¹⁰ in 1970 from cancerous pleural effusion of a postmenopausal breast cancer patient who was 69 years old, donated 3 years ago by Dr T. Kubota, Department of Surgery, Keio University Medical School, and since then has been serially cultivated by the authors.

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The tumor was cut into fragments measuring about 8mm³ which were implanted into the dorsal subcutaneous tissue of the mice by a trocar. For the tumor to grow in the exponential growth phase, immediately after the implantation 5mg/kg estradiol dipropionate and 250mg/kg progesterone caproate were intramuscularly injected into the mice.¹¹ The nude mice bearing tumors weighing 40–90mg were then assigned, 14–16 days after implantation, to receive either one of the trial medications. The tumor weight was estimated by the formula [long axis × (short axis)² × 1/2], which has been reported to correlate with the actual tumor weight.¹²

The drugs used were pure antiestrogen ICI 182780 and 5-FU. ICI 182780 was formulated as a 50mg/ml solution in castor oil and 5-FU was dissolved in distilled water by an ultrasonic cleaner to make a 12mg/ml concentration. The nude mice in the ICI 182780 group were administered a single dose of 0.5, 1, 3, or 5mg subcutaneously on the first day of treatment. Those in the 5-FU group were administered intraperitoneally a 60mg/kg dose of 5-FU three times at 4-day intervals on and after the first day of treatment. The combination group received 1mg of ICI 182780 and 60mg/kg of 5-FU in the same manner as that for the individual monotherapy groups.

Assessment of the Antitumor Effects

The long and short axes of each tumor were measured by a sliding caliper twice a week over the period from day 1 to day 21 to estimate the tumor weight and the mean tumor weight for each group. The mean tumor weight on Day X (W) was divided by the initial mean weight (W_0) and the obtained values (the relative mean tumor weight ratio: W/W_0) were plotted against the time (days) after the start of the treatment so as to produce a tumor growth curve for each group. The ratio of W/W_0 of each treated group to W/W_0 of the control group was also calculated (T/C). For the ER measurements and cell cycle analysis by flow cytometry (FCM), the tumors were resected on day 21, cut in half to remove any necrotized tissue, and then frozen until use.

ER Measurements

ERs in the frozen tissues were determined by the dextran-coated charcoal (DCC) method and the results were analyzed by Scatchard plots to determine the number of binding sites (B_{max}).

Cell Cycle Analysis by FCM

The frozen tissues were minced, mixed with 0.1% ribonuclease and 0.1% polyoxyethylene(10)octylphenyl ether, and filtered with a 40- μ m nylon mesh. The filtrate was mixed with the same volume of propidium iodine solution (to make the final concentration of 50 μ g/ml). A DNA histogram was prepared using a flow cytometer

(FACScan, Becton Dickinson, San Jose, CA, USA) and the percentage of cells in S phase (%S) and G phases (%G₀G₁) were determined by the software Sum of Broaded Rectangles (SOBR). The tumor 5-FU and thymidylate synthetase (TS) concentrations, and 5-FU contents in tumor cell RNA were determined in the 5-FU monotherapy and the combination groups. Nude mice bearing MCF-7 intramuscularly received 5mg/kg estradiol dipropionate and 250mg progesterone caproate immediately and 14 days after implantation. At 28 days after implantation, treatment with 5-FU alone or in combination with ICI 182780 was started in the same manner as mentioned before. The tumors were resected at either 1 to 12h after the last administration of 5-FU, cut in half and frozen within 5min after the necrotized tissue was removed. The tumors resected at 1h after the last administration of 5-FU were used for the tissue 5-FU assay, those taken at 1 and 12h after the last administration of 5-FU for the TS assay, and those taken at 12h after the last administration of 5-FU for 5-FU contents in RNA.

Tumor 5-FU Assay

The tumor tissue specimens were mixed with silica gel and cold acetonitrile, homogenized, and centrifuged. The obtained supernatant was concentrated to dryness, reconstituted with ethanol, passed through a silica gel column to be adsorbed by silica gel, eluted by acetone, then concentrated to dryness. The resulting solid was reconstituted with a solvent and subjected to high-performance liquid chromatography (HPLC).¹³

Tumor TS Assay

In accordance with the method reported by Spears et al.¹⁴ the tumor tissues were homogenized and centrifuged. Sufficient amounts of ³H-labeled fluorodeoxyuridine monophosphate (³H]FdUMP) and methylenetetrahydrofolate (CH₂FH₄) were added to the resulting cytosol fraction to form a ternary complex. Free [³H]FdUMP was removed by DDC for TS and radioactivity was determined by a liquid scintillation counter. Buffer, pH 8.0, was added to the cytosol fraction to cut the TS-FdUMP bond and, as in the free TS measurement, sufficient amounts of [³H]FdUMP and CH₂FH₄ were added, and radioactivity was counted by scintillation counter to determine the total TS. The TS inhibition rate (TSIR) was calculated by the equation [(total TS — free TS)/(total TS) × 100%].

5-FU Assay in Tumor Cell RNA

Gas chromatography–mass spectrometry (GC-MS) was used.¹⁵ The tumor tissue specimens were homogenized in distilled water. Ice-cold trichloroacetic acid was added, mixed, and centrifuged to remove the supernatant. The precipitate was rinsed, hydrolyzed by potas-

sium hydroxide, and then perchloric acid was added. After centrifugation, the supernatant was neutralized by potassium hydroxide, and newly appearing precipitate was removed to obtain a mononucleotide solution. This solution was used to determine the concentrations of RNA and 5-FU by GC-MS, and the 5-FU content in RNA was also calculated.

Criteria for Antitumor Effects

The treatment was considered to be effective when the minimum T/C during the treatment was not higher than 0.42.¹² The significance of the intergroup difference in the mean tumor weight was tested by two-way repeated measures ANOVA (analysis of variance). The combination effect was considered to be synergetic when T/C in the combination group was smaller than the product of T/Cs in the two forms of monotherapy. The two-way repeated measures ANOVA was used to test the significance of intergroup differences in the nude mouse body weight change, and Student's *t*-test and the Mann-Whitney *U*-test were used for ER, %S, %G₀G₁, tumor 5-FU concentrations, TS concentrations, and 5-FU content in RNA. A difference with *P*-value less than 0.05 was considered to be significant.

Results

Antitumor Effects on MCF-7

ICI 182780 exhibited a dose-dependent antitumor activity which was statistically greater at 3 and 5 mg as compared with the control (Fig. 1). As the minimum T/C was as low as 0.35 (≤ 0.42), 5 mg was assessed to be effective (Table 1).

Although neither 1 mg of ICI 182780 alone nor 5-FU alone was significantly different from the control with

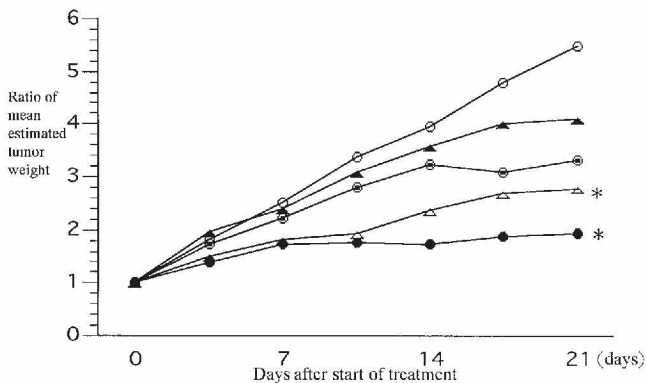


Fig. 1. Tumor response of MCF-7 to ICI 182780. Vertical axis, mean estimated tumor weight on a given day/that on day 0. Open circles, control (n = 14); closed triangles, 0.5 mg (n = 7); dotted circles, 1 mg (n = 11); open triangles, 3 mg (n = 10); closed circles, 5 mg (n = 14). **P* < 0.05 compared with control

regard to the antitumor activity, the difference between the combination and the control was significant (Fig. 2). The combination, however, failed to show a synergetic effect with a minimum T/C of 0.42 marginally exceeding the product of T/C of the two forms of monotherapy ($0.61 \times 0.68 = 0.41$) (Table 1).

Changes in the Body Weights of Nude Mice

The body weights of the nude mice decreased slightly in those treated with both 5 mg of ICI 182780 alone and with the combination. There was a significant difference between 1 mg and 5 mg of ICI 182780, but not between the control and any of the treatment groups (Table 2).

ER Values at the Time of Tumor Resection

The mean ER value at the time of tumor resection (B_{max}) was 605.5 fmol/mg protein in the control group,

Table 1. Tumor response to trial medication

Treatment	Minimum T/C
Control (n = 14)	1.00 (on day 21)
ICI 182780 0.5 mg (n = 7)	0.75 (on day 21)
ICI 182780 1 mg (n = 11)	0.61 (on day 21)
ICI 182780 3 mg (n = 10)	0.51 (on day 21)
ICI 182780 5 mg (n = 14)	0.35 (on day 21)
5-FU (n = 10)	0.68 (on day 21)
Combination (n = 10)	0.42 (on day 21)

T/C, mean ratio of the estimated tumor weight to the baseline in the treated group/that in the control group
5-FU, 5-fluorouracil

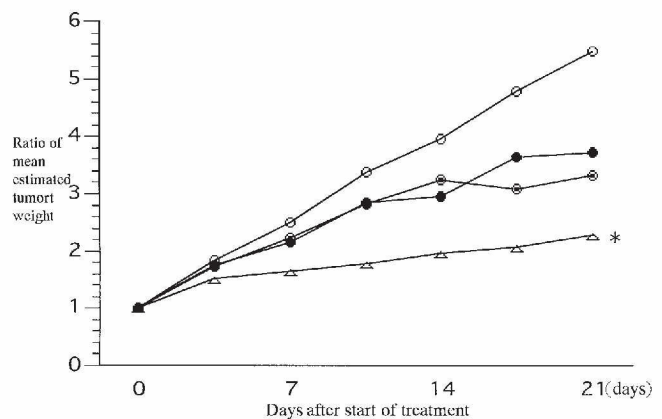


Fig. 2. Tumor response of MCF-7 to the combination of ICI 182780 and 5-fluorouracil. Vertical axis, mean estimated tumor weight on a given day/that on day 0. Open circles, control (n = 14); dotted circles, 1 mg ICI 182780 (n = 11); closed circles, 5-fluorouracil (n = 10); triangles, combination (n = 10). **P* < 0.05 compared with control

Table 2. Changes in body weights (BW) of the nude mice

Treatment	BW at baseline (g)	BW on day 21 of treatment (g)
Control (n = 7)	20.51 ± 1.43	21.40 ± 2.17
ICI 182780 1mg (n = 4)	22.00 ± 1.20	23.70 ± 1.42
ICI 182780 5mg (n = 6)	20.85 ± 1.13	20.78 ± 1.24
5-FU (n = 5)	20.38 ± 1.88	21.02 ± 3.16
Combination (n = 6)	23.03 ± 1.28	21.37 ± 2.27

*P < 0.05

Table 3. Estrogen receptor (ER) count on day 21 of treatment (by dextran-coated charcoal method)

Treatment	ER content (fmol/mg protein)
Control (n = 4)	605.5 ± 48.3
ICI 182780 1mg (n = 5)	198.5 ± 83.2
5-FU (n = 4)	418.1 ± 164.7
Combination (n = 4)	52.3 ± 76.8

*P < 0.05

while the mean ER decreased to 198.5, 418.1, and 52.3 fmol/mg protein after the administration of 1 mg of ICI 182780, 5-FU, and the combination, respectively. The fall of ER was significantly greater with 1 mg of ICI 182780 than with 5-FU, and with the combination than with either monotherapy (Table 3).

Cell Cycle Analysis by Flow Cytometry

The mean %S in the control group was 35.6%, while it was 14.2% for 1 mg of ICI 182780, 19.2% for 5-FU, and 19.8% for the combination therapy. The mean %G₀G₁ was 55.4% for the control, while the values in the treated groups were 82.8%, 76.4%, and 76.8%, respectively (Table 4). The treated groups showed a significant reduction in the %S and a significant increase in the %G₀G₁ as compared with the control group. ICI 182780 at 1 mg resulted in a greatest reduction of the %S and the greatest elevation of the %G₀G₁, with a significant difference in the %S from that of the combination therapy, and a significant difference in the %G₀G₁ as compared with 5-FU alone and the combination therapy.

5-FU and TS Concentrations in Tumors and 5-FU Contents in Tumor Cell RNA

The mean 5-FU concentration in the tumors was 302.0 ng/g in the 5-FU group, which was not significantly different from that of the combination group, 201.9 ng/g (Table 5). On the other hand, the mean 5-FU content in RNA was significantly higher in the combination group

(102.6 ng/mg RNA) than in the 5-FU group (65.2 ng/mg RNA) (Table 6).

The total TS at 1h after the last administration of 5-FU was 30.8 pmol/g tissue in the 5-FU group and 30.5 pmol/g tissue in the combination group, with no significant difference in TSIR (94.1% and 97.2%, respectively). At 12h after the last administration of 5-FU, however, both the total and free TS (61.9 and 24.1 pmol/g tissue) in the 5-FU group were significantly higher than those in the combination group (51.3 and 17.5 pmol/g tissue, respectively) (Table 7).

Discussion

Breast cancer is a hormone-dependent tumor for which endocrine therapy is widely accepted as being effective. TAM is one of the most commonly used antiestrogens. However, TAM is reported to have partial estrogenic activity, and it has also been reported to produce estrogenic effects on some targets and antiestrogenic effects on others.³⁻⁵

ICI 182780, a new pure antiestrogen free from estrogenic activity, is a promising new therapy modality. One of the features of the drug is the lack of effect on gonadotropins.⁸ This means that the drug does not block cerebral ERs and therefore does not interfere with the hypothalamus-pituitary-ovary axis in breast cancer patients. In this sense, the drug is expected to be especially beneficial to premenopausal patients.⁸ Unlike TAM, in which the uterotrophic activity has been recently reported to be associated with a risk of endometrial cancer,^{6,7} ICI 182780 is devoid of uterotrophic activity and therefore free from this concern.

Meanwhile, even apparently ER-positive breast cancer heterogeneously consists of both ER-positive and ER-negative tumor cells. Antiestrogens are not so effective for ER-negative tumor cells.¹ Therefore, a combination of hormone therapy with chemotherapy, which has a different mode of action, can be expected to improve the response rate. There are a number of clinical reports using randomized controlled trials to compare chemotherapy alone and chemoendocrine therapy in recurrent breast cancer¹⁶⁻²⁹ and some studies comparing

Table 4. Cell cycle analysis on day 21 of treatment (by flow cytometry)

Treatment		%S	%G ₀ G ₁
Control	(n = 5)	35.6 ± 2.4	55.4 ± 2.3
ICI 182780 1 mg	(n = 4)	14.2 ± 3.3	82.8 ± 2.9
5-FU	(n = 5)	19.2 ± 4.3	76.4 ± 4.6
Combination	(n = 4)	19.8 ± 2.4	76.8 ± 2.6

%S, percentage of cells in S phase; % G₀G₁, percentage of cells in G phases

*P < 0.05

Table 5. 5-FU concentrations in the tumor^a

Treatment		5-FU concentration (ng/g)
5-FU	(n = 6)	302.0 ± 154.6
Combination	(n = 8)	201.9 ± 83.7

NS, not significant

^aTumors resected at 1 h after the last administration of 5-FU

Table 6. 5-FU contents in tumor cell RNA^a

Treatment		5-FU content in RNA (ng/mg RNA)
5-FU	(n = 6)	65.2 ± 18.0
Combination	(n = 5)	102.6 ± 34.6

*P < 0.05

^aTumors resected at 12 h after the last administration of 5-FU

Table 7. Concentrations of thymidylate synthetase (TS)

Treatment		TS total (pmol/g tissue)	TS free (pmol/g tissue)	TSIR (%)
5-FU at 1 h ^a	(n = 9)	30.8 ± 13.4	2.2 ± 2.6	94.1 ± 4.8
Combination at 1 h	(n = 11)	30.5 ± 7.1	0.9 ± 0.7	97.2 ± 2.2
5-FU at 12 h ^b	(n = 6)	61.9 ± 6.9	24.1 ± 2.3	61.0 ± 2.1
Combination at 12 h	(n = 5)	51.3 ± 8.1	17.5 ± 2.1	65.2 ± 7.2

TSIR, TS inhibition rate

*P < 0.05

^aTumors resected at 1 h after the last administration of 5-FU

^bTumors resected at 12 h after the last administration of 5-FU

endocrine therapy alone and chemoendocrine therapy.²⁰⁻²³ Most of these reports show higher response rates to the combination therapy. However, the expected combination effect of the chemoendocrine therapy has yet to be clinically demonstrated.

On the other hand, experimentally, Watanabe²⁴ reported that the chemoendocrine therapy combining TAM, the most widely used drug in clinical practice, and the fluorinated pyrimidine 5-FU, which is relatively widely used, exerted a synergistic effect on R-27 in vivo, while Kubota et al.²⁵ failed to demonstrate a synergistic effect of a combination of TAM and 1 M tegafur—4 M uracil (UFT) on Br-10. In vitro, Benz et al.^{26,27} reported a synergistic effect of the combination therapy on MCF-7. Sato²⁸ reported that dimethylbenzanthracene-induced rat mammary tumors responded to the combination therapy, while Yamamoto et al.²⁹ failed to demonstrate any synergistic effect. Although differences in the cell lines, dosages, and the types of administration tested

prevent us from making a simple comparison across these studies, there is still no agreement on the efficacy of this combination therapy. Under these circumstances, the authors investigated the antitumor effects and mode of action of the experimental chemoendocrine therapy combining pure antiestrogen and 5-FU.

A single administration of ICI 182780 at 0.5, 1, 3, and 5 mg showed a dose-dependent antitumor activity with a significant difference between 3 or 5 mg and the control. At 5 mg, the drug was assessed to be “effective” with a minimum T/C as low as 0.35. The toxic effect of the drug was considered to be minimal, as there were neither any deaths of the treated animals nor toxic symptoms other than a reduction of the mean body weight in the 5-mg group, which was not as significant as compared with the control.

Referring to the report of Kondo et al.³⁰ who reported the sensitivity of 5-FU on the tumor implanted in nude mice, the mice in the 5-FU group were administered

60 mg/kg of 5-FU three times at 4-day intervals on and after the first day of treatment. The 5-FU group failed to exert an antitumor effect with the minimum T/C of 0.68, and the effect was not significantly different from that of the control group. These findings agree with those of Koh³¹ who reported that MCF-7 did not respond to 5-FU *in vivo*.

In contrast to the minimum T/C of 0.61 for 1 mg ICI 182780 alone and 0.68 for 60 mg/kg 5-FU alone which were both assessed to be ineffective, the combination was assessed to be effective with a minimum T/C of 0.42, which was significantly different from that of the control but fell marginally short of the synergic effect. The combination therapy was associated with a reduction in the mean body weight, which was not significant as compared with the control. There were also no deaths seen in the treated mice. These results thus suggest the usefulness of this combination therapy.

A comparison of the ER values showed a significantly lower B_{\max} in the 1-mg ICI 182780 group than that of the control. This may be accounted for by the antitumor effect of ICI 182780 on ER-positive cells, as antiestrogen is expected to work through its effect on ER. The ER value in the 5-FU group decreased slightly but not significantly from that of the control. Judging from the observed mild antitumor effect of 5-FU and its mode of action, 5-FU seems to exert its effect directly on tumor cells regardless of the ER status. In contrast, the authors observed a marked reduction of the ER value in the combination group, which closely agreed with the findings of Watanabe who reported a marked reduction in the ER value in R-27 treated with a combination of TAM and 5-FU.²⁴ These findings as well as the increased antitumor response to the combination therapy thus suggest a potentiation of the effects on the ER-positive cells using this therapy.

The cell cycle analysis by FCM resulted in a decrease in the %S and an increase in the %G₀G₁ by ICI 182780. TAM was reported to reduce the ratio of the cells in the S phase and accumulate cells in the G₀G₁ phase.³² Like TAM, ICI 182780 also seems to slow down the cycle progression. On the other hand, 5-FU at a low dose is known to prolong the S phase and at a high dose to accumulate cells as in the G₁ phase.³³ Our findings of the decreased %S and the increased %G₀G₁ support Watanabe²⁴ who reported a reduction of the %S in R-27 treated with 5-FU.

An earlier report that DNA is synthesized in the S phase and the %S is inversely proportional to the tumor doubling time³⁴ is consistent with our findings that the %S was significantly lower in the groups which responded to the treatment than that of the control. The FCM used, however, failed to explain the combination effect from the results of the cell cycle analysis, as the greatest %S reduction was not related to the greatest

tumor response, which was seen in the combination group.

It is said that 5-FU may have two major modes of action. First, 5-FU is phosphorylated to FdUMP in the body, which formulates a ternary complex with methylenetetrahydrofolate and TS to inactivate TS and consequently inhibit the synthesis of DNA. Secondly, 5-FU is phosphorylated to fluorouridine triphosphate (FUTP), which is taken up by RNA instead of UTP and consequently interferes with the metabolism of RNA. Although much controversy remains as to which makes a greater contribution to the antitumor effect of the drug,^{14,35-37} the authors tried to elucidate the mode of action of the combination therapy from both possible modes of action of 5-FU, that is, the inhibition of the DNA synthesis and the metabolic dysfunction of RNA, by determining the 5-FU and TS concentrations in tumor cells and 5-FU contents in RNA for the 5-FU and the combination groups.

There was no significant difference in the 5-FU concentrations in tumor cells, although they were slightly higher in the 5-FU group (mean of 302.0 ng/g) than in the combination group (201.9 ng/g). Antiestrogen ICI 182780 is, therefore, unlikely to interfere with the uptake of 5-FU by tumor cells.

In contrast, the 5-FU content in RNA was significantly higher in the combination group than in the 5-FU group. The metabolic dysfunction of RNA was thus suggested to contribute to the increased tumor response of the combination. In addition to ICI 182780, 5-FU also slowed down cell cycle progression and accumulated cells in the G₀G₁ phase, as indicated by FCM. Ueki reported a decreased %S and increased %G₀G₁ of SC-115, an androgen-responsive mouse mammary tumor, at 24 h after administration of 5-FU, and a reincrease of %S at 1 week after administration.³⁸ The action of ICI 182780 to decrease %S and increase %G₀G₁ seems to last for some time, while that of 5-FU may disappear shortly after administration. 5-FU is said to work on RNA in the G₁ phase.³⁹ In our study, 5-FU was administered in the combination group, when the %G₀G₁ level was maintained at a high level by ICI 182780 and, as a result, the uptake of 5-FU by RNA may be facilitated.

There was no significant difference between 5-FU alone and the combination group with regard to the total TS, free TS, or TSIR at 1 h after the last administration of 5-FU. The TSIR approximated 100% in both groups. At 12 h after the last administration of 5-FU, the TSIR was of the order of 60% in both groups without any significant difference. The TSIR in both groups was as high as near 100% shortly after the administration of 5-FU and remained at a relatively high level of approximately 60% even at 12 h after the last administration of 5-FU. These findings suggest the possible contribution

of the inhibition of DNA synthesis by 5-FU to the anti-tumor effect in both groups. On the other hand, no difference in the TSIR between the groups suggests that a potentiation of the inhibition of DNA synthesis by 5-FU is not attributable to the increase in the antitumor response to the combination. The total TS and free TS in both groups at 12h after the last administration of 5-FU were higher than those at 1h after the last administration of 5-FU. These parameters were not significantly different between the groups at 1h after the last administration of 5-FU, but were significantly lower in the combination group than in the 5-FU group at 12h after the last administration of 5-FU. These findings thus suggested that the TS synthesis observed in the tumor cells in order to produce 5-FU induced a rapid decrease in free TS to be suppressed by the combination therapy. As the antitumor response is reported to correlate with the free TS concentration,⁴⁰ TS synthesis was more greatly suppressed by the combination therapy based on the higher antitumor response to the combination than to the 5-FU alone.

These results suggest the antitumor effect of ICI 182780 in ER-positive breast cancer cells, which was potentiated by the concomitant administration of 5-FU.

Conclusion

(1) ICI 182780 showed a dose-dependent activity against MCF-7, which was potentiated by the concomitant administration of 5-FU. (2) The observed marked reduction in the ER value in the combination therapy group of ICI 182780 and 5-FU suggests that the combination therapy may mainly work on ER-positive tumor cells. (3) ICI 182780 seems to slow down the cycle progression, as indicated by the decrease in the %S and the increase in the %G₀G₁. (4) In the combination group, the synthesis of TS after the administration of 5-FU was suppressed and the 5-FU-induced metabolic dysfunction of RNA was potentiated.

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