

Different Chemo- and Endocrino- Sensitivity of MCF-7 Cells with or without Estradiol Supplement *in Vitro*

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Abstract. The sensitivity of MCF-7 cells to tamoxifen (TAM) and mitomycin C (MMC) was assessed in rapidly and slowly growing cells with or without estradiol supplementation, respectively. The growth of MCF-7 was inhibited by MMC in a concentration-dependent manner with or without estradiol (E_2) supplementation. Preincubation with MMC suppressed subsequent E_2 stimulated growth of MCF-7. TAM inhibited the growth of MCF-7 supplemented with E_2 and preincubation with TAM prevented subsequent E_2 stimulated growth of MCF-7. However, TAM did not inhibit the growth of MCF-7 cells in E_2 free medium. These results suggested that MMC may be more effective than TAM on breast cancer cells in the dormant or slow-growth phase.

The recurrent mode of breast cancer is different from the other solid carcinomas, and breast cancer often recurs even 10 years after radical mastectomy (1). Early Breast Cancer Trialists' Collaborative Group (EBCTCG) reported that chemotherapy and endocrine treatment were beneficial in patients with early stage breast cancer (1,2).

To clarify the behavior and the sensitivity of breast cancer cells during the subclinical phase of the cancer, we have investigated the chemo- and endocrino-sensitivity of breast cancer cells in rapid and slow growing phases in order to design appropriate chemotherapy and endocrine treatment regimens for the long subclinical stage after mastectomy.

Materials and Methods

Tumour cell line. MCF-7 cells were kindly supplied by Dr.Y. Nomura, Kyushu Cancer Center.

Agents. Commercially available mitomycin C (MMC) was purchased from Kyowa Hakko Kogyo, Co. Ltd., Tokyo. Tamoxifen (TAM) was kindly sup-

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plied by I. C. I., Pharma Ltd., Osaka. Estradiol (E_2) was purchased from Sigma Chemical Co. Ltd. St. Louis, MO.

Chemosensitivity assay of monolayer cultures with MTT endpoint. The 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) endpoint was used on monolayer cultures according to the method of Mosmann (3) with some modifications as reported elsewhere. The treatment schedule is shown in Figure 1. MCF-7 cells at $2 \times 10^4/400 \mu\text{l}$ /well were plated into 24-well plates (Sumitomo Bakelite Co., Ltd. Japan) and preincubated for 24 h with complete culture medium consisting of Eagle's minimum essential medium and 10% fetal calf serum treated with dextran-coated charcoal with E_2 at a concentration of 10^{-8} M for Group 2, or without E_2 for Groups 1 and 3. MMC at final concentrations of 0.1, 1.0 or 10 $\mu\text{g/ml}$ was added for 2 h at the initial incubation. TAM at final concentrations of 10^{-8} , 10^{-7} or 10^{-6} M was added to the medium. The plates were incubated at 37°C in a humidified atmosphere containing 95% air and 5% CO_2 until evaluation on day-6 for Groups 1 and 2, and on day-11 for Group 3. The medium change was carried out on day 1-3 with the same medium, while on day-6 the medium change was carried out with complete culture medium containing E_2 added at 10^{-8} M for Group 3.

MTT (Sigma) dissolved at 4 mg/ml phosphate-buffered saline (PBS) and sodium succinate dissolved at 0.1 M PBS were filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA) and these solutions were added to each well at 50 μl /well. Following a 4 h incubation at 37°C , 400 μl of dimethyl sulfoxide per well were added to dissolve the formazan salt and the plates were shaken for a few min. Two hundred microliters were transferred from each well of 24-well plates to 96-well plates, which were read on a plate reader, model EAR 340 (SLT-Labinstruments, Austria) at 540 and 630 nm. All drug concentrations were tested in quadruplicate. The inhibition rate (IR) was calculated as $\text{IR} = (1 - \text{mean absorbency of the treated cultures} / \text{mean absorbency of control cultures}) \times 100$.

Results

The effect of E_2 on the growth of MCF-7 is shown in Figure 2. The growth of MCF-7 was enhanced by the addition of E_2 at a concentration of 10^{-8} M.

The antitumour activity of TAM on MCF-7 cells incubated with or without E_2 is shown in Figure 3. No antitumour activity of TAM was observed on MCF-7 cells in E_2 -free medium. The growth of MCF-7 was somewhat stimulated under both conditions. TAM suppressed the E_2 stimulated growth of MCF-7 cells in a concentration-dependent manner. Preincubation with TAM suppressed subsequent E_2 stimulated growth.

The antitumour activity of MMC on MCF-7 cells with or

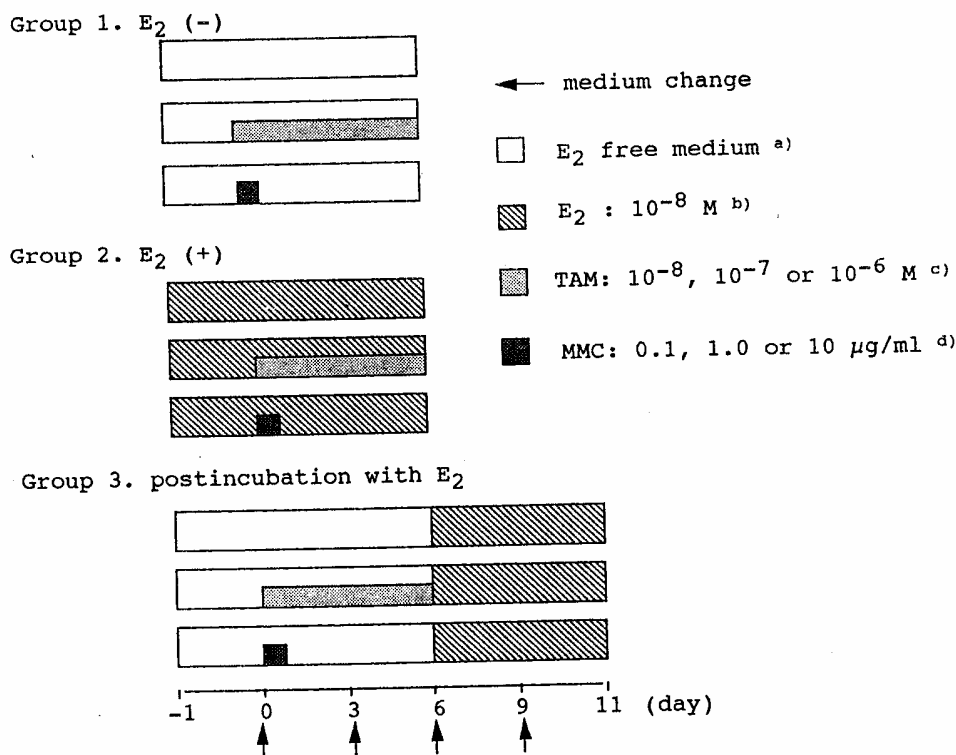


Figure 1. Schedule of treatment.

- a) Eagle's minimum essential medium with 10% fetal calf serum treated with dextran-coated charcoal.
 b) a) plus estradiol at a concentration of 10⁻⁸ M.
 c) Tamoxifen at concentrations of 10⁻⁸, 10⁻⁷ and 10⁻⁶ M.
 d) Mitomycin C at concentrations of 0.1, 1.0 and 10 µg/ml for 2 hours.

without E₂ supplement is shown in Figure 4. MMC inhibited the growth of MCF-7 with or without E₂ supplement, and this inhibition was also observed when MCF-7 was preincubated with MMC.

Discussion

It is well known that the survival of breast cancer patients declines even 10 years after operation⁽¹⁾. To explain this long subclinical state of breast cancer, we should consider the possibility that a majority of the tumour consists of long standing resting cells and we hypothesize that the rate of proliferation is almost equivalent to that of cell death. When recurrent tumours are observed after a long-term follow up, most of the remaining breast cancer cells are thought to be in a resting stage after surgery. The clinically "dormant" tumours may consist of either resting G₀ cells or the tumour may be at an equilibrium with cell proliferation and cell death.

The MCF-7 human breast cancer cell line was initially reported by Soule *et al*⁽⁴⁾ and has been used in many studies. This cell line was successfully transplanted into E₂ treated nude mice in 1983⁽⁵⁾, and this strain has been reported to be estrogen receptor (ER) positive *in vitro* and *in vivo*⁽⁴⁾. The growth of MCF-7 cells is accelerated by E₂ *in vitro*, whether E₂ is added

initially or at a late time of incubation. In our previous study, MCF-7 tumours were observed to be in a "dormant" state growing subcutaneously on the backs of the nude mice with a low level of endogenous E₂ which is less than 20 pg/ml without exogenously added E₂⁽⁶⁾. It was also demonstrated that proliferating cells were present in the MCF-7 tumours in the "dormant" stage, as detected by autoradiography using ³H-thymidine and immunohistochemical analysis with proliferating cell nuclear antigen as well as bromodeoxyuridine, and flow cytometry⁽⁶⁾.

The present study was conducted under the hypothesis that the rate of cell proliferation is almost equivalent to that of cell death in the "dormant" state, and that this "dormant" state would represent the long subclinical phase of breast cancer. MCF-7 cells could grow slowly in E₂-free medium even though the complete E₂-free state has a lower E₂ concentration than that in postmenopausal patients. Since this low-proliferating state is thought to be similar to the low growth fraction shown in subclinical tumours, this MCF-7 model in E₂-free medium was thought to be relevant to the subclinical state of tumour cells.

The chemosensitivity and endocrine sensitivity of the remaining breast cancer cells in the long subclinical stage is important for the design of appropriate adjuvant chemotherapy and en-

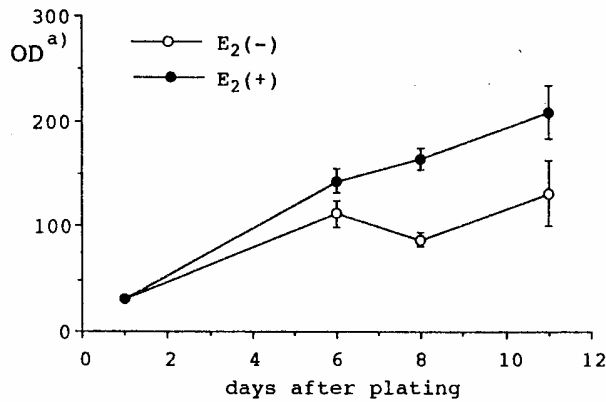


Figure 2. Growth curve of MCF-7 cells with or without estradiol. MCF-7 cells plated on 24 well plate at concentration of 2×10^8 /well with or without estradiol. Fetal calf serum was treated by dextran-coated charcoal and estradiol was or was not added at a concentration of 10^{-8} M. a) optical density at 540 and 630 nm.

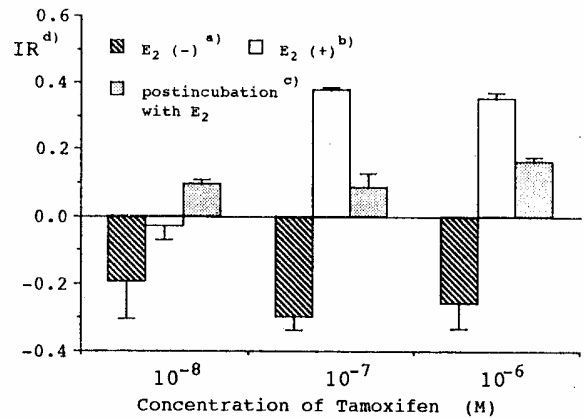


Figure 3. Treatment of tamoxifen with or without estradiol. a) Tamoxifen was applied without estradiol. b) Tamoxifen was applied with estradiol at a concentration of 10^{-8} M. c) Tamoxifen was applied without estradiol, and the postincubation was performed with estradiol at a concentration of 10^{-8} M. d) Inhibition rate compared to tamoxifen-free control.

doctrine treatment for post operative patients. In the present study, we have carried out chemo- and endocrino-therapy on MCF-7 cells with or without E₂.

E₂ at a concentration of 10^{-8} M represents the *in vivo* steady-state concentration and 10^{-7} and 10^{-8} M of TAM are reported to be clinically achievable concentrations. Although the binding ability of TAM to ER is reported to be approximately 1/100 E₂, it was apparent that the growth of MCF-7 was inhibited even by an equal molar concentration of TAM with E₂ in this study. This growth inhibition was also observed after the removal of TAM from the medium, suggesting that the competing activity of TAM remained for a few days after its removal from the medium. TAM, however, failed to inhibit the growth of MCF-7 cells in E₂-free medium, and rather enhanced it as a partial agonist. Moreover TAM inhibited the subsequent E₂-stimulated growth after preincubation with MCF-7 cells in E₂-free medium.

On the contrary, MMC was effective against MCF-7 cells with or without E₂ supplementation, suggesting that the antitumour activity of MMC on MCF-7 is not related to E₂ stimulation. The results presented here indicate that adjuvant chemotherapy, such as with MMC, may be more effective against slow growing tumour cells than endocrine treatment such as with TAM.

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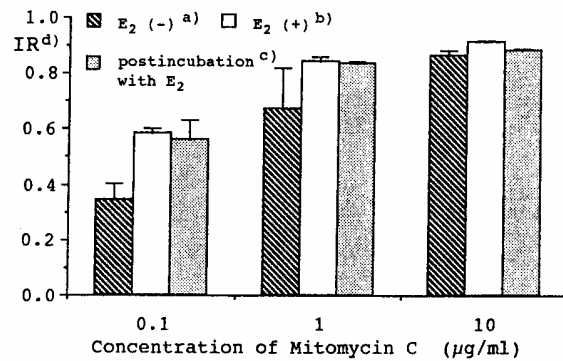


Figure 4. Treatment of mitomycin C with or without estradiol. a) Mitomycin C was applied without estradiol. b) Mitomycin C was applied with estradiol. c) Mitomycin C was applied without estradiol, and the postincubation was performed with estradiol. d) Inhibition rate compared to mitomycin C-free control.

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