FACTORS RELATED TO RESPONSE TO ANTICANCER DRUGS IN BREAST CANCER

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Abstract

In this study, we examined the correlation of in vitro sensitivity to anti-cancer drugs, histological tumor response and alteration of apoptosis in breast cancer. In vitro sensitivity was examined by the method of succinate dehydrogenase inhibition test. Histological changes and alteration of apoptosis in tumor cells were investigated by staining with hematoxylin and eosin and immunohistochemical staining, respectively. Patients with locally advanced breast cancer were treated with adriamycin (ADM). Using resected specimens, in vitro sensitivity and apoptosis were examined before and after treatment. After three courses of treatments with ADM, in vitro sensitivity in tumor cells to anticancer drugs including ADM significantly decreased. On the other hand, apoptotic index altered independent of in vitro sensitivity.

It is concluded that in vitro sensitivity is useful in predicting tumor response to treatment with anticancer drugs in breast cancer. The alteration of apoptosis in tumor cells induced by treatment with an anticancer drug is independent of the in vitro sensitivity to the drug.

Introduction

Breast cancer is advocated as the general disease; therefore, treatment with...
anticancer drugs takes an important role in the management of the breast cancer. The study of the factors relating with the tumor response to the anti-cancer drugs following the definite indication in the clinical treatment is demanded to attain the beneficial quality of life in the patients suffering from the advanced or recurred tumors which show biological diversity.

Recent studies reported that apoptosis was induced by anti-cancer drugs in tumor cells (Micheau, Hammann et al., 1999, Fuda, Susin et al., 1998). On the other hand, we performed the treatment in advanced or recurred breast cancer based on the in vitro sensitivity.

In this report, we present the correlation between the in vitro sensitivity and the pathological response, the alteration of the in vitro sensitivity before and after treatment with anticancer agents, and changes in apoptosis of tumor cells after the treatment.

Materials and Methods

Among the patients with locally advanced breast cancer, we examined 7 patients who received preoperative chemotherapy and were evaluated for in vitro sensitivity and pathological findings before and after the chemotherapy.

(1) In vitro sensitivity

The examination of in vitro sensitivity was done in accordance with succinate dehydrogenase inhibition test (SDI method) utilizing 3-(4, 5-dimethyl-2-thazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) assay (Iwata and Kanematsu 1999). For culture, tumor tissues obtained from a patient were mechanically minced, and cells were suspended in ASF301 (Ajinomoto Co., Ltd., Tokyo). The number of cells was adjusted to $1 \times 10^4$ /ml. The subjects were divided into control and examined cells. Cells were then incubated at $37^\circ$ C in a humidified 5% CO2 atmosphere. The exposure concentration of anti-cancer drugs was adjusted at 10-fold the peak plasma concentration in intravenous administration (adriamycin [ADM]; 4µg/ml, cis-diaminedichloroplatinum [CDDP]; 20 µg/ml, 5-fluorouracil [5-FU]; 100µg/ml, etoposide [VP-16]; 100µg/ml, methotrexate [MTX]; 4µg/ml,). After 72 hours of incubation followed by centrifugation, the
Figure 1: Histological findings in tumor tissue before and after the initiation of treatment with ADM. From Iwata et al (Annals of Cancer Research and Therapy, Vol. 11, 2003). ©2003 by PJD Publications Ltd., Westbury, NY 11590-0966.
supernatant was removed and 0.1 mol/l sodium succinate solution and 0.4% MTT (Sigma Chemical Co., St Louis) solution were added to control and examined cells. Then incubation was continued for 3 hours. The supernatant was removed, and formazan was dissolved in 2N KOH solution containing 50% dimethyl sulfoxide. After leaving the plates covered in the dark at room temperature for overnight, SD activity was taken as the absorbance of formazan measured at 565 nm. The sensitivity in tumor cells was presented as the ratio of the percentage of SD in treated cells to that in control cells. The in vitro sensitivity was examined prior to and after the chemotherapy.

(2) Treatment with anti-cancer agents

In the treatment, ADM was administrated at the dose of 20mg/m². Treatment was repeated 3 times with each interval of 10-14 days. The post-therapeutic in vitro sensitivity was examined at 4 weeks after the last therapy.

(3) Histological examination

The resected specimens were fixed in 10% formalin and utilized for examination of histological response and the change in apoptosis of tumor cells due to the treatment. In the examination in histological tumor response, tumor cells were stained with hematoxylin and eosin and counted the number in every 10 fields at 400 folds. The histological tumor response was presented as the ratio of the percentage of number in treated cells to that in non-treated cells. The change in apoptosis of tumor cells was examined by the method of immunohistological staining (TUNEL) (Gavrieli, Sherman et al., 1992). The apoptotic index (AI) was presented as the ratio of the percentage of the apoptotic cells in tumor tissue in every 10 fields at 400 folds. In the individual patient, the apoptotic index in the tumor tissue which was obtained prior to and after the treatment was compared.

Results

Typical histological changes coming from the treatments with ADM in the two patients are shown in Figure 1. In vitro chemosensitivity to ADM in each patient was 40% and 80%, respectively. After the three courses of treatment, SD ratio in ADM was elevated in all patients. Moreover, SD ratios in the other agents which were not utilized
**Figure 2:** (LEFT) Changes in *in vitro* sensitivity of tumor cells to anti-cancer drugs at the peritreatment with ADM. Case 1 - before the treatment and after the treatment; Case 2 - before the treatment and after the treatment.

**Figure 3:** (RIGHT) Correlation between *in vitro* sensitivity, remnant cancer cells and apoptotic index at the peritreatment with ADM.
in the treatment showed elevations with statistical significance (Figure 2). In individual patients, SD ratio in ADM prior to the therapy had a close correlation with the remnant tumor cells after the treatment. However, the in vitro sensitivity prior to the therapy did not show any correlation to the change in AI (Figure 3).

Discussion

Mitotic index (Akahashi-Tanaka, Tsuda et al., 1996), S-phase fraction (Game, Meyer et al., 1995), and p53 (Ellege, Gray et al., 1995, Jacquemier, Penault-Llorca et al., 1994) have been reported as the factors that affect the sensitivity of breast cancer cells to anti-cancer drugs. Recent studies have shown that anti-cancer drugs induce apoptosis in cancer cells (Micheau, Hammann et al., 1999, Fulda, Susin et al., 1998, Mizutani, Yoshida et al., 1998). The over-expression of mutant p53 interfered with p53-independent programmed cell death (Li, Sutphin et al., 1998). On the other hand, wild-type p53 increased MDR1 (Li, Zhu et al., 1997) resulting in low-sensitivity of cancer cells to ADM. In this study, the in vitro sensitivity of cancer cells to ADM did not seem to be eligible as a factor that predicts the alteration in apoptosis in cancer cells by treatment with ADM. This may support the clinical report that expression of bcl-2, which is a factor preventing apoptosis, did not predict response to treatment with anti-cancer drugs including ADM (van Slooten, Clahsen et al., 1996).

In this study, the in vitro sensitivity to ADM closely correlated with the remnant cells after the treatment. Further, the remnant cells showed low sensitivity to other anticancer drugs, as a matter of course to ADM. This may suggest that the selection of drugs combined with ADM is important. The drugs combined with ADM in clinical usage should be required to act synergistically to increase apoptosis in tumor cells, even if the drugs do not show a high in vitro sensitivity to cancer cells.

References


