EFFECT OF PACLITAXEL, EPIRUBICIN AND TAMOXIFEN ON LABELLING INDEX IN CULTURED EHRLICH ASCITES TUMOR CELLS

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SUMMARY:
The effect of Paclitaxel (PAC), Epirubicin (EPR) and Tamoxifen (TAM) on 3H-thymidine labelling index (3H-TdR LI) of Ehrlich ascites tumor cells (EAT) was investigated in cultured. In the present study, an estrogen receptor positive ER(+) hyperdiploid cell lines were studied. We used optimum doses of PAC, EPR and TAM (12μg/ml, 12μg/ml and 2μg/ml, respectively). Cells were treated with these doses for 0, 4, 8, 16 and 32 hours. At the end of these periods, both control and treated cells were labelled for 5μCi/ml 3H-thymidine for 30 minutes. The results showed that inhibition of DNA synthesis in cultured EAT cells were increased in the combined treatment of two drugs when compared to the treatment of a single drug (p<0.01). In the treatment of three drugs, however, this effect reached a maximum (p<0.001). As a result, PAC+EPR+TAM treatment’s had a maximum synergistic effect at 4 hours treatment.

Key words: EAT cells, in vitro, DNA synthesis, Paclitaxel, Epirubicin, Tamoxifen

INTRODUCTION:
Ehrlich ascites tumor cells (EAT), very convenient in cancer research, arised as spontaneous mammary gland carcinomas in senile female mice (Stewart et al., 1959). Paclitaxel (PAC, Taxol) is a natural product derived from the western yew tree, shown promise for efficacy in treatment of breast cancer. It has a unique action and broad spectrum of antineoplastic activity. Over the last decade a wide variety of tumors have been found to respond to PAC, including ovarian cancer and breast cancer. PAC acts by promoting the assembly of microtubules from tubulin dimers and stabilizing microtubules by preventing depolymerization. This inhibits the normal functioning of microtubules during mitosis, as well as during interphase. In addition, PAC induces the formation of abnormal arrays or “bundles” of microtubules throughout the cell cycle, and of multiple asters of microtubules during mitosis (Rowinsky et al., 1992). Epirubicin (EPR, Farmorubicin) is the epimer of the anthracycline antibiotic doxorubicin, with inversion of the 4’-hydroxyl group on the sugar moiety, and has been used alone or in combination with other cytotoxic agents in the treatment of a variety of malignancies. EPR is a cell cycle phase non-specific anthracycline, with maximal cytotoxic effects in the S and G2 phases. In vitro studies showed that EPR possesses cytotoxicity at least equivalent to that of doxorubicin against a variety of animal and human tumor cell lines. Findings of various studies reveal that EPR
forms a complex with DNA, by intercalation between DNA strands (Pigram et al., 1972). This intercalation inhibits DNA replication and transcription (Tewey et al., 1984). Cell culture studies exhibit that EPR enters the cells rapidly, is localised in nuclei and inhibits nucleic acid synthesis and cell division (Ozcan et al., 1997). In the treatment of tumors, surgery, radiotherapy, chemotherapy and endocrine therapy are sometimes employed solely, or in a combined of two or more of them. Of the hormonal therapies available, Tamoxifen (TAM, Nolvadex) is a semi-synthesitical estrogen antagonist used in the management of pre and post menopausal breast cancer. This drug binds to intracellular estrogen receptors, and prevents endogenous estrogen from binding to their own receptors. TAM is a competitive inhibitor of estradiol binding to the ER, which can cause G1 arrest in sensitive cell lines (Osborne et al., 1983). Prolonged TAM exposure blocks ER (+) cell lines in G0-G1 of the cell cycle, while it has no obvious effects on ER (-) cell lines (Mc Guire, 1978). Since there have been few studies about the combined effects of PAC, EPR and TAM on labelling index (LI) of rapidly proliferating cells. We determine the in vitro cytotoxic activity of PAC, EPR and TAM and their combined effect of cytotoxicity in cultured EAT cells.

MATERIALS and METHODS:

In the present study, an estrogen reseptor positive hyperdiploid cell lines have been studied. A line of Ehrlich ascites carcinoma was supplied from the Pathology Institute of Cologne University, in Germany, and was maintained in our laboratory by weekly intraperitoneal transplantation of 6.10^6 cells/mouse. EAT cells are grown in Minimum Essential Medium (MEM, Gibco BRL) containing 10% foetal bovine serum (FBS, Gibco BRL) as suspension culture. Test cells were divided into one control and seven test groups. Tumor cells, cultured in 24-well tissue culture plates were exposed to PAC, EPR, TAM, PAC+EPR, PAC+TAM, EPR+TAM and PAC+EPR+TAM. Microplate wells were covered with 3.10^4 cells/cc and incubated 5% and 95% air. ER levels were studied by the methods of Lippman, Huff, Raynaud and et al. ER activity as demonstrated by the dextran-coated charcoal technique has been closely correlated with the clinical ability of TAM to inhibit tumor growth. We used optimum doses of PAC, EPR and TAM (12 μ/cc, 12 μ/cc and 2μ/cc, respectively). Cells were treated with these doses for 0, 4, 8, 16 and 32 hours. At the end of these periods, both control and treated cells were labelled for 5 microCi/cc^3H-thymidine (Amersham, England, sp. Act. 185 Bq/mmol) for 30 minutes. Smear cells were prepared on glass-slides. Slides were rinsed with 2% perchloric acid twice at 4^0C for 30 minutes to remove dissolved radioactive material. They were then coated with gel emulsion (Ilford, England, K.2) kept at 4^0C for 3 days for exposure and then developed with D19b. After slides were stained with Giemsa, LI values were calculated, by counting at least 2500 cells from each slide. Multiple comparisons and the differences between the percentage distribution of S phase of the various treatment groups were compared by the student-t test (n=150).
RESULTS:

It was observed that depending on the drug treatments, inhibition of DNA synthesis in cultured EAT cells were increased, being of statistically significant (p<0.01) but combined treatment of PAC+EPR and PAC+EPR+TAM gave more successful results relative to the other groups (p<0.001). When the differences of $^3$H-TdR LI levels between different treatment groups and different times were analyzed, significant differences were found between treatments groups and between times (Table I). Table I indicated Pulse Thymidine Labelling Index values of treatment groups on the basis of hours. From the value of 4 hours, we saw that all the drugs had a rapid effect (Figure 1). In subsequent hours, this effect seemed to continue. The inhibition of DNA synthesis was higher in the combined treatment of two drugs when compared to the treatment of a single drug. This revealed that a synergistic effect was resulted in combined treatment of two drugs. However, in the treatment of three drugs, this effect reached a maximum. In addition, PAC+EPR+TAM treatment’s results had a maximal synergistic effect at 4 hours treatment. As a result, concomitant treatment combined with endocrine therapy has given improved results compared with treatment alone.

DISCUSSION:

Initial studies of PAC as a single agent, conducted at MD Anderson Cancer in Texas (Holmes et al., 1993) and Memorial Sloan-Kettering Cancer Center in New York had shown that PAC was effective against metastatic breast cancer (Norton et al., 1992). Preliminary studies suggested that drug sequence had an effect on the efficacy of the combination of PAC plus cisplatin. In vitro studies had shown that when PAC was followed by cisplatin, this sequence produced maximal cytotoxicity in L1210 leukemia cells (Holmes et al., 1991). Anthracyclines including EPR, resulted in maximal cell death in the S and G2 phases of the cell cycle, but cytotoxic effects might occur in the G1 and M phases at higher drug concentrations (Plosker et al., 1993). Maximal lethal effects of EPR were demonstrated in the S and G2 phases of the cell cycle in murine and human tumor cell lines (Hill et al., 1982). For many years, animal studies have been performed to study the effects of TAM on rodent mammary tumors (Gullino et al., 1975). Treatment with TAM significantly reduced the incidence of contralateral breast cancer (Cuzick et al., 1985). In TAM-treated patients, TAM was shown to reduce LI in human breast tumors in vivo (Clarke et al., 1993). Laboratory studies had demonstrated that chemotherapeutic cell killing was inhibited in the presence of TAM (Osborne et al., 1994). In the present study, adding TAM to PAC plus EPR treatment had a maximal synergistic effect. Thus, the results of our study seem to be concordant with the above mentioned studies suggesting that combinations of drugs are superior to single agents. In conclusions, the data suggest that TAM treatment may modify the effect of cytotoxic combine chemotherapy in cultured ER-positive EAT cells. The growth, mitotic index, continuous labelling index, cytotoxic and cell cycle effects must be examined and compared in ER-positive and negative breast cancer cell lines.
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<table>
<thead>
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<th>Treatment group</th>
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<td></td>
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<tr>
<td>Control</td>
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</tr>
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<tr>
<td>Paclitaxel+Epirubicin+Tamoxifen</td>
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Table I. Pulse Thymidine Labelling Index values of various treatments groups in cultured EAT cells. Statistical significance 1: p<0.5, 2: p<0.01, 3: p<0.001

Figure 1. Pulse Thymidine Labelling Index values of various treatments groups in cultured EAT cells.