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RESPONSE OF HUMAN BREAST CANCER CELLS TO A COMBINATION OF CYTOTOXIC DRUGS AND PHARMACOLOGICAL CONCENTRATIONS OF OESTROGENS.

R Clarke, H W van den Berg & R F Murphy
The Queen's University of Belfast, Depts of Biochemistry and Therapeutics, Belfast, N Ireland, UK

We have previously reported that 10^{-6}M 17β oestradiol (E2) increased the cytotoxicity of methotrexate in hormone responsive MCF-7 cells whilst reducing its cytotoxicity towards hormone unresponsive MDA-MB-436 cells (Br J Cancer 1985; 51: 365-369). We have subsequently investigated the ability of 10^{-6}M E2 to modulate the effects of vincristine (VCR) melphalan (MEL) and adriamycin (ADR) in these cell lines. DNA synthesis was determined by the incorporation of $[^3H]$ - deoxyuridine into acid precipitable material and membrane fluidity by the steady-state polarization of fluoresence of the probe diphenylhexatriene.

10^{-6}M E2 alone produced a variable stimulation of cell proliferation in MCF-7 cells. However, the inhibitory effects of MEL & VCR on DNA synthesis and cell proliferation were consistently potentiated. In MDA-MB-436 cells E2 alone did not influence cell proliferation and failed to modulate the cytotoxicity of these drugs. Thus the potentiation of the effects of MEL & VCR may be related to the mitogenic potential of E2 although perturbation of other biochemical pathways not currently identified may also be involved. 10^{-6}M E2 failed to potentiate the cytotoxicity of ADR but reduced the fluidity of the cellular membranes. Since ADR may act on the plasma membrane (Tritton & Yee, Science 1982; 217: 248-250) alterations in membrane fluidity may obscure any increased cytotoxicity resulting from the mitogenic effects of E2. Thus, the ability of E2 to modulate the effect of cytotoxic drugs may be influenced by the oestrogen receptor content of the cell and the choice of drug.

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HORMONAL MANIPULATION OF MCF-7 BREAST CANCER CELLS: IMPLICATIONS FOR CHEMOTHERAPY.

M. Bontenbal, P. Sonneveld*, J.A. Foekens and J.G.M. Klijn. Dr Daniel den Hoed Cancer Center, P.O. Box 5201, Rotterdam, The Netherlands and *The Radiobiological Institute TNO, Rijswijk, The Netherlands.

The efficacy of chemotherapy in disseminated breast carcinoma has reached a plateau phase. Therefore we investigate whether selective hormonal manipulation of MCF-7 human breast cancer cells in vitro can influence the effect of chemotherapy with Adriamycin. The Adriamycin induced kill of MCF-7 cells was investigated as regards the incubation duration (1-72h) and drug-concentration (0.03-30μM). Adriamycin induced cell death was maximal following long-term (72h) incubation with low dose (0.03μM) Adriamycin. At short-term exposures (1 and 24h) the Adriamycin induced cell kill was correlated with the intracellular drug-concentrations as measured by high pressure liquid chromatography (H.P.L.C.). Lase activated flow cytometry was used to determine the proliferation kinetics of MCF-7 cells as a result of the estrogen administration to the incubation medium. When cells were grown in steroid hormone-deprived culture medium an 88% arrest of these cells in G_0G_1-phase was observed. Subsequent administration of 30 pM Estradiol revealed a significant increase of cells in S-G_2M-phase, which was maximal (51%) at 24h after pulse stimulation, compared to 12% in the control group. Estrogen receptors (ER) were demonstrated with a monoclonal antibody directed against the ER-receptor. This study supports the opinion that one day interval between the administration of Estradiol and cytostatic drugs, as used in some clinical trials, might be sufficient.

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THERAPEUTIC SYNERGISM HORMONES-ANTINEOPLASTIC DRUGS ON HUMAN BREAST CANCER CELL LINES EVALUATED BY MONOCLONAL ANTIBODY (MoAb) AGAINST α-DNA POLYMERASE.

R.E.Favoni, A.Nicolin, P.Del Monte, L. Miglietta and A.Alama
Inst.Scientifico Tumori, Dept.of Pharmacology, Genoa, ITALY.

Sequential antihormonal and hormonal kinetic modulation of hormone-responsive human tumors may be exploited to potentiate the cytotoxic effect of chemotherapy. We have studied doxorubicin (Dx) cytotoxicity on two human breast cancer cell lines exhibiting different estrogen receptors (ER) and androgen receptors (AR) phenotype: MCF-7(ER+) and Evsa-T(ER-/AR+). In order to increase cytotoxic Dx effect we induced a proliferative arrest by an antihormonal drug (4-hydroxytamoxifen on MCF-7 and cyproterone acetate on Evsa-T) followed by kinetic recruitment mediated by an hormonal treatment (17-β-estradiol on MCF-7 and 5-α-dihydrotestosterone on Evsa-T). Drugs activity was monitored, evaluating tumor cell growth fraction (GF), by MoAb to α-DNA polymerase, and percentage of intranuclear enzyme in proliferating cells was estimated by an immunoperoxidase method. Our results show: 25% and 70% GF inhibition after antihormonal treatment; 30% and 15% GF increase after hormonal drug administration in MCF-7 and Evsa-T respectively vs.controls. In MCF-7 cell line Dx treatment of synchronized cells induced a significant increase in cytotoxic effect (40%) vs.unsynchronized cells, in contrast with the unresponsiveness of Evsa-T line. In conclusion, growth stimulatory action of 17-β-estradiol improves the cytotoxic activity of Dx on ER+ MCF-7 cell line while synergistic hormone-Dx activity has not been found in ER-/AR+ Evsa-T cell line.

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SENSITIVITY TO CYTOTOXIC DRUGS OF HUMAN BREAST CANCER CELLS RESISTANT TO SPECIFIC HORMONES.

Hannelore Braunsberg, N.G. Coldham, St. Mary's Hospital Medical School, London W2. U.K.

Sequential hormonal and cytotoxic therapies are used widely for the treatment of advanced breast cancer. Prior hormonal therapy may or may not affect a tumour's subsequent sensitivity to cytotoxic agents, but although attempts have been made to resolve this question this is difficult by means of clinical trials. We have attempted to produce hormone-resistant sub-populations of human breast cancer cells (MCF-7) in order to study their sensitivities and that of hormone responsive cells to methotrexate (MTX) and vincristine (VCR), two agents with different modes of action. Cells grown and passaged with 2 μM tamoxifen (TAM) 4μM dexamethasone (DEX) 20μ M oestradiol (E_2) or 1μM medroxyprogesterone acetate (MPA) for 6 to 22^2 months remained weakly responsive to the hormone used. Experiments carried out so far suggest that cells resistant to the growth-inhibitory effects of MPA are as sensitive to MTX and VCR as "wild type". Other sublines are under study.

The prolonged survival of some hormone-sensitive cells in the presence of high concentrations of growth-inhibitory hormones, as demonstrated in these experiments, may or may not be indicative of the in vivo behaviour of tumour cells. However, it seems reasonable to include in chemotherapeutic regimens hormonal drugs which can be shown to have no adverse effects on the actions of cytotoxic agents. In vitro studies such as these can provide information which may be applicable to chemo-hormonal combinations.