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SYNERGISTIC INHIBITION BY LONIDAMINE AND VERAPAMIL OF P-GLYCOPROTEIN-MEDIATED MULTIDRUG RESISTANCE IN DIFFERENT HUMAN CANCER LINES: A PRECLINICAL MODEL

Valenti A.M., Niero A., and Marangolo M. Medical Oncology Unit and Istituto Oncologico Romagnolo for Cancer Research. City Hospital - Ravenna - Italy.

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Resistance to cancer chemotherapy is a major obstacle to successful treatment of advanced or recurrent human tumors. In recent years, a comparable experimental phenomenon, termed multidrug resistance (MDR) has been extensively studied. The pharmacologic basis of MDR appears to be decreased intracellular drug accumulation due to enhanced efflux. This process is mediated by a glycoprotein p170 which functions as an energy-dependent multidrug transporter. A number of compounds reverse MDR in vitro; it has been demonstrated that Lonidamine (LND) and particularly Verapamil (VPM) are able to overcome drug resistance in clinical setting. The conditioning treatment with high-dose VPM is considered as a high risk regimen for cardiotoxicity. Aim of this preclinical study is to identify a possible synergistic effect of LND and VPM, which have different sites of action in the p170 molecule, in order to reduce the dose of VPM. Two human tumor cell lines (MCF 7 and LOVO), both resistant to ADM, have been employed; escalating doses of ADM (0.1-0.3-0.6-0.8-1 ug/ml), of VPM (0.1-0.5-1 ug/ml) and fixed dose (50 ug/ml) of LND have been tested. Clonogenic test (IC50) is employed for ADM alone, ADM and VPM in different combinations with or without LND. The reduced drug efflux achieved by the conditioning treatments is demonstrated by Fluoro-Cytometry. The preliminary results seem to demonstrate that VPM dose may be reduced by 30%, with the same overcoming drug resistance power, when employed with LND.

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THE MECHANISM OF ACTION OF THE ANTINEOPLASTIC DRUG LONIDAMINE - NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY STUDIES

O Kaplan, H Ben-Horin, G Navon
Department Surgery B, Tel-Aviv Medical Center and School of Chemistry, Tel-Aviv University, Israel.

Lonidamine is a relatively new antineoplastic agent, and it was previously suggested that its main targets in the cell are the mitochondria and plasma membranes. Multinuclear magnetic resonance spectroscopy (MRS) studies enabled continuous in-vivo monitoring of Lonidamine effects and its cytotoxicity in perfused MCF-7 human breast cancer cells. Phosphorous (31P) MRS demonstrated a pronounced decrease in intracellular pH, followed by a depletion of ATP and phosphomonoesters. Carbon (13C) MRS experiments showed that lactate content in the cells increased, while its content in the perfusate decreased, indicating that Lonidamune may be an inhibitor of lactate transport. It seems therefore, that the main effect of Lonidamine is direct acidification of the intracellular milleu. MRS may provide useful information on drug mechanisms, which is hardly attainable by conventional biochemical techniques.

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INFLUENCE OF TEMPERATURE ON ACTION AND PHARMACOKINETICS OF IFOSFAMIDE
A PRECLINICAL STUDY

Th. Wagner, A. Wiencke, M. Mentzel
G. Wiedemann, and Ch. Weiss
Depts. of Internal Medicine and Physiology,
Medical University of Lübeck, Germany

Nude mice with transplanted human MX1 tumors were i.v. injected with either 32, 65, 125, or 250 mg/kg b.w. ifosfamide (IFO). Subsequently, the mice were immersed in a temperature controlled water bath of either 37, 39, or 41°C for 1 hr. Core temperature was continously controlled in the rectum by micro thermo couples.The percentage of tumor free survival (TFS) at day 60 was determined, and the concentrations of IFO and its activated form 4-OH-IFO in blood and tumor tissue were followed up during the immersion in the waterbath.
A clear drug and thermodose dependent therapeutic effect was observed.
At 37°C 250 mg/kg b.w. IFO led to 100% TFS. At 41°C only half the dose of the drug attained the same therapeutic effect. The half life times and the AUC for IFO in blood were not significantly different at 37°C and at 41°C. The calculated (apparent) concentration of IFO and 4-OH-IFO in the tumors within the 60 min. at 41°C exceeded more than 2-fold that at 37°C.

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A SLIGHT INHIBITION OF CELLULAR PROTEIN SYNTHESIS BY THE ONCOSTATIC AGENT ZILASCORB(²H) STRONGLY INHIBITS CELL CYCLE PROGRESSION

JE Melvik, TE Aastveit & JM Dornish
Dept. of Pharmacology, Pronova a.s, Gaustadalleén 21, N-0371 Oslo, Norway.

In vitro studies with the anticancer agent zilascorb(²H) [5,6-benzylidene-d_1-L-ascorbic acid] have demonstrated reversible inhibition of protein synthesis. This effect is, however, relatively low at clinically relevant doses. We have, therefore, compared the effect on protein synthesis with the effect on cell cycle progression in a human lung carcinoma cell line, A549. At a concentration of zilascorb(²H) (0.5mM) which induced only a slight inhibition of protein synthesis, a reversible block in cell cycle progression was seen. However, when higher zilascorb(²H) concentrations were given in the absence, but not in the presence of catalase, the cell cycle progression was additionally delayed after removal of zilascorb(²H). The additional delay in cell cycle progression may therefore likely be induced by a free radical mechanism. Our data demonstrate that even a slight inhibition of protein synthesis induces proportionately large effects on cell cycle progression. This supports the theory that the anticancer effect of zilascorb(²H) may be the result of primary effects on protein metabolism.

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EFFECT OF DECAPEPTYL ALONE OR IN COMBINATION WITH ANTIESTROGENS AND PROGESTINS ON THE GROWTH OF BREAST CANCER CELL LINES

L Marini, °G Robustelli della Cuna and G Sica
Institute of Histology and Embryology, UCSC, Rome; °Oncology Division, Fondazione Clinica del Lavoro, Pavia, Italy.

We studied the antiproliferative activity of Decapeptyl (DEC), an LH-RH analog, on various human breast cancer cell lines, both estrogen-sensitive and estrogen-insensitive. These cell lines have been grown in a medium supplemented with charcoal-treated fetal bovine serum (CH-FBS), in the presence or absence of estradiol, or unstripped serum (FBS) (not estrogen-depleted). DEC was ineffective when tested on estrogen-insensitive MDA-MB-231 cells. On the other hand, while ineffective when used alone (in presence of CH-FBS or FBS), DEC, at concentrations ranging from 10^{-11} to 10^{-6} M, significantly inhibited, in a dose dependent manner, the estradiol-stimulated growth of MCF-7 and CG-5 estrogen-sensitive cells. The inhibition reached 70% at the highest dose. Notably, DEC did not modify the antiproliferative action of Tamoxifen and Medroxyprogesterone acetate on the same cell lines. These data confirm the results obtained by other authors concerning the ability of DEC to inhibit cell proliferation only in the presence of sufficient quantities of estrogens. Moreover, our observations indicate that DEC is unable to affect cell growth in estrogen-insensitive mammary cells.

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INHIBITION OF HUMAN BREAST CANCER CELL GROWTH BY N-(4-HYDROXYPHENYL) RETINAMIDE AND 13-CIS-RETINOIC ACID ALONE AND IN COMBINATION WITH TAMOXIFEN

G Lama, C Angelucci, °F Recchia and G Sica
Institute of Histology and Embryology, UCSC, Rome; °Civil Hospital, Division of Medicine, Avezzano, Italy.

It has been known for many years that retinoids, the family of molecules comprising both the natural and synthetic analogs of retinol, are able to control both cellular differentiation and cellular proliferation. We investigated the ability of 13-cis-retinoic acid and N-(4-hydroxyphenyl) retinamide to affect the growth of estrogen-sensitive human breast cancer cells (CG-5) in vitro. All compounds were tested on cells grown in a medium supplemented with 5% fetal calf serum for 6 days. 13-cis-retinoic acid at concentrations ranging from 10^{-9} to 10^{-5} M inhibited cell proliferation in a dose-dependent fashion. Maximum reduction of cell number was 64% with respect to control. N-(4-hydroxyphenyl) retinamide induced a slight inhibition of cell growth at concentrations from 10^{-9} to 10^{-6} M, while it exhibited a strong cytotoxic effect at 10^{-5} M. Cotreatment of CG-5 cells with varying concentrations of 13-cis-retinoic acid and 10^{-7} M tamoxifen resulted in a growth inhibition which was higher than that induced by the antiestrogen alone only at the highest concentration of the retinoid. Nevertheless, the two drugs association did not enhance the growth inhibition produced by the retinoid alone. Our data are not in accordance with other reports concerning the effect of the combination of all-trans retinoic acid and other synthetic retinoids with tamoxifen. For this reason these findings warrant further investigations.