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EXPRESSION OF MDR-1 AND GSTΠ GENE IN HUMAN SARCOMAS.

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The poor response rate of sarcomas to chemotherapy led to this study of some chemoresistance mechanisms that could be involved in these tumors. MDR-1 and GSTπ gene expression were determined in 57 soft tissue and bone sarcomas. For MDR-1, Northern and Dot Blot analysis were done; moreover, 22 samples were studied by mRNA *in situ* by hybridization and immunohistochemistry. Only one sarcoma was found to overexpress MDR-1 mRNA and P-glycoprotein. GSTπ expression was evaluated only by Northern and Dot Blot analysis. Thirty-eight samples out of 41 analyzed had detectable levels of GSTπ mRNA, without any correlation with clinical data. Thus, in our experience, MDR-1 gene overexpression is unusual in sarcomas. However low expression levels, undetectable by the methods used, may be clinically significant. Therefore, for accurate evaluation a more sensitive method such as RT-PCR could be used.

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THE ANTI-TUMOR PROPERTIES OF NOVOBIOCIN IN SENSITIVE AND MULTI-DRUG RESISTANT B16 MELANOMA

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The antibiotic drug novobiocin affects multiple biochemical targets, the best known of which is topoisomerase II.
This study describes the anti-tumor properties of novobiocin in sensitive and multi-drug (MDR) resistant B16 melanoma cells. Novobiocin was found to inhibit the growth of B16 melanoma cells in vitro and in vivo and to induce differentiated characteristics in these cells. Combined treatment of B16 melanoma cells with novobiocin and the GTP-depleting differentiating agent tiazofurin resulted in a synergistic growth inhibitory effect in vitro and in vivo. A synergistic interaction of novobiocin and microtubule disrupting agent was found in drug sensitive B16 melanoma cells. Novobiocin also reduced colchicine resistance in MDR B16 melanoma cells, by interfering with drug efflux.
These findings may suggest that novobiocin should further be evaluated as an anti-tumor agent in melanoma.

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AMIODARONE AS MODULATOR OF DRUG-RESISTANCE OF DOXORUBICIN, VINCRISTINE AND MITOXANTRONE Van der Graaf, WTA, de Vries, EGE, Mulder, NH. Dpt of Medical Oncology, Univ. Hosp. Groningen, 9713 EZ Groningen, The Netherlands.
Key words: amiodarone, mitoxantrone, drug-resistance
Amiodarone (AM) is a modulator of multidrug-resistance (MDR) in vitro. Its efficacy in modulation mitoxantrone (M) cytotoxicity (CX) has been tested in vitro and the feasability of combining AM with MDR drugs was studied in vivo. In vitro 3 cell lines were tested in a CX assay. In a human MDR-neg SCLC cell line 10 μM AM did not change the CX of doxorubicin (DOX) and hardly of vincristine (VCR), but increased the CX of M 4.7x. In a MDR-pos P-glycoprotein (P-gp) neg DOX-resistant subline, AM increased the CX of M 1.7x, with no effect on DOX and hardly any effect on VCR. In a P-gp pos colon cancer cell line AM increased the CX of DOX: 1.6x, of VCR: 2.7x and of M 2.0x. In a phase 2 study the feasibility of high-dose oral AM (1400 mg/m2, d1-7, every 3 weeks) in combination with DOX (50 mg/m2) and Vindesine (3 mg/m2), both d7, was studied. The whole treatment was performed in an out-patient setting. Apart from transient bradycardia in 1 of 25 patients, no serious toxicity due to AM was observed. The serum levels of AM and its active metabolite, taken before the infusion with chemotherapy were 7.7. μM, corresponding with an in vitro effective MDR modulation concentration. One patient with a P-gp pos uterus sarcoma obtained PR, 5 pts SD. In conclusion, an effective MDR modulation concentration of AM can be reached in vivo. Whether AM is in vivo effective combined with M has to be determined.

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COMPARISON OF S 9788, A NEW MODULATING AGENT, TO VERAPAMIL AND CYCLOSPORIN IN REVERSING MULTIDRUG RESISTANCE (MDR)

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S 9788, a new MDR reversal agent acting on the Pgp catalysed drug efflux, has been compared to verapamil (VRP) and cyclosporin (CSA), in reversing MDR both in vitro and in vivo. In vitro, a large panel of rodent and human cell lines of various origins (lung, kidney, colon, hematological malignancies...), expressing either induced or intrinsic resistance was tested.
Reversion by S 9788 was shown to depend on both the MDR expression of the cell line and the antitumoral agent (adriamycin ADM, vincristine VCR). Reversion factors (RF = IC50 (cytotoxic agent) / IC50 (cytotoxic agent + modulator)) were determined for each modulator and compared. The RF ratios (RF S 9788 / RF VRP or CSA) ranged from 0.3 to 238 (median : 1,6 in 30 cell lines tested) for VRP, and from 0.6 to 119 (median : 1 in 12 cell lines tested) for CSA.
In vivo, S 9788 (50-100 mg/kg) administered with VCR (0.25 mg/kg) daily for 4 days, completely reversed the resistance in the murine leukemia model P388 / VCR (increase in T/C = 61%). In the same conditions, VRP only partially restored the sensitivity to VCR (increase in T/C = 10%).
Based on these results, phase I studies of S 9788 combined to ADM or VCR have been initiated.

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FLOWCYTOMETRIC DETECTION OF P-GLYCOPROTEIN IN HEMATOLOGIC DISORDERS

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Peripheral blood and bone marrow samples of patients with haematologic disorders, presenting at the University Hospital of Antwerp between June 1992 and January 1993, were screened for expression of P-glycoprotein (P-gp). A flow cytometric assay using the monoclonal antibody (MoAb) MRK-16 (kindly provided by Dr. Tsuruo) was used. Briefly, samples (10^6 cells) were incubated with 10 μg of rabbit IgG to saturate aspecific binding sites. After washing the samples were incubated with 1μg of the P-gp specific MoAb MRK-16. The cells were labeled with 25 μg of rabbit-anti-mouse-F(ab')$_2$ fragments conjugated with fluoroisothiocyanate. Samples were analysed on a FACScan instrument.
422 samples of 196 patients were screened. Arbitrarily samples were termed mdr-positive when ≥ 2% of the cells showed a fluorescence intensity over one log decade. 35 (8.3%) samples from 26 (13.2%) patients were found to be mdr-positive. Levels of positivity were generally low (between 2% and 5%). No positive cells were found in benign or preneoplastic disease. No correlation between stage of the disease and mdr-positivity was found.
The present data indicate that expression of the multidrug transporter P-gp at detectable levels can be identified in hematologic tumors. The expression level however is low in comparison to data of other studies in hematologic cancer.

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5'-DEOXY-5-FLUOROURIDINE ACTS AS AN MULTIDRUG RESISTANCE REVERSAL AGENT.

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Resistance to anticancer agents of colorectal cancer can, among other, be related with overexpression of a membrane glycoprotein (P-gp 170) that is associated with multidrug resistance (MDR). Fluoropyrimidines such as 5'-deoxy-5-fluorouridine (dFUrd) are amongst the few treatment options. They execute their cytotoxic activity in a P-gp 170 independent way. In the present study, the cytotoxic activity of dFUrd against cell lines with different P-gp 170 expression was assessed with the MTT assay. A rat colon carcinoma cell line with low MDR expression ($CC531^{mdr+/-}$), an MDR variant ($CC531^{mdr++}$), a revertant type ($CC531^{mdr-}$, lacks P-gp 170 expression) and a human ovarian cancer cell line (A2780) with its MDR variant (2780^{AD}) were tested. Sensitivity patterns for dFUrd in combination with Daunorubicine (DNR) have also been determined. Differences in DNR-uptake after growth under dFUrd pressure were determined by flowcytometry. dFUrd demonstrated highest activity in cells with highest P-gp 170 expression. The Resistance Factor $ID_{50}2780^{AD}/ID_{50}A2780$ calculated was 0.02. In CC531 cell lines a similar pattern was found: $ID_{50}CC531^{mdr++}$: 19±2μM, $ID_{50}CC531^{mdr-}$:51±3μM, $ID_{50}CC531^{mdr+/-}$:17±3μM. It is concluded that dFUrd activity is not hindered at all by increased expression of P-gp 170. The results also show that dFUrd improves the DNR-uptake in MDR positive cells which is related with increased cytotoxicity.