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IN VITRO PHARMACOLOGICAL MONITORING OF PHASE I - II TRIALS OF S 9788. A MULTIDRUG RESISTANCE (MDR) REVERSING AGENT

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S 9788, a novel triazinoaminopiperidine derivative, has already shown a major MDR reversing activity both in vitro and in vivo. As in vitro the pgp inhibition is dependent on reversing agent concentration and time exposure, we explored this issue on the human T leukemia cell line CCRF-CEM/VLB expressing the MDR phenotype. We had already shown that the S 9788 MDR reversing activity was increased when the cells were incubated for at least 24 hours in the presence of S 9788 alone (post-incubation), following a one hour incubation with both vinblastine (VLB) and S 9788. In this study, we evaluated the interest of various post-incubation times (over 6, 12, or 24 hours) with low doses of S 9788 (from 50 to 500 nM) following a one hour incubation with both S 9788 and doxorubicine (DOX) or VLB. The reversion factors [RF = IC50 (DOX or VLB) / IC50 (DOX or VLB + S 9788] were determined with or without post-incubation and their ratio (RF with post-incubation / RF without post-incubation) calculated below (in bold characters) :

One hour incubation	*Post incubation (S 9788 : 100 nM)*		
	6 h	12 h	24 h
DOX + S 9788	1.8	2.2	1.7

One hour incubation	*Post incubation (S 9788 : 125, 250 or 500 nM)*					
	12 h			24 h		
	125	250	500	125	250	500
VLB + S 9788	3.8	8.3	20	8.6	24.6	41

A six hours post-incubation after DOX treatment using at least 100 nM led to a two fold increase in the reversion factor. Longer post-incubation periods did not modify this effect. Conversely, longer post-incubation periods (12 or 24 hours) after VLB treatment led to a major increase (4x to 40 x) in the reversion factor. This effect was directly proportional to both duration of the post-incubation and concentration of S 9788. These results are the basis of phase I - II trial designs combining a loading dose of S 9788 before VLB or DOX administration, followed by a maintenance infusion with S 9788 alone.

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DAUNORUBICIN ACCUMULATION IS CORRELATED WITH TREATMENT OUTCOME IN ADULT ACUTE LEUKEMIAS

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To investigate whether multidrug resistance (MDR) could be involved in clinical resistance to chemotherapy in adult acute leukaemias, we have analyzed prospectively the expression of P-glycoprotein and the daunorubicin (Dnr) accumulation in bone marrow samples from 60 patients with primary acute leukaemias.
44 were studied at diagnosis and 6 after initial therapy (5 in first relapse, 1 in second relapse). There were 13 lymphoblastic and 57 myeloïd leukaemias. All the patients received chemotherapy which included MDR-related drugs. Fourty-four patients obtained a complete remission (CR) after one course of therapy, 16 were classified as failure (F). Expression of P-glycoprotein by using MRK16 monoclonal antibody and simultaneous determination of intracellular Dnr accumulation were performed by flow cytometry (Gheuens et al. Cytometry, 1991, 12, 636-644). No correlation was found between MRK16 labeling rate and Dnr accumulation. Mean of the MRK16 labeling rate was not significantly different in patients in CR as compared with pts in F (7.14 versus 8.9, p>0.4). For Dnr accumulation, a significant difference was observed between patients in CR (42.62± 26) as compared with patients in F (15.9±11) (p<0.001). These data suggest that Dnr accumulation is a reliable and useful parameter in designing therapy for patients with acute leukemias.

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FLUNARIZINE AND LONIDAMINE AS MODULATORS OF ANTICANCER DRUG ACTIVITY IN HUMAN COLON ADENOCARCINOMA CELLS.

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The potential of the calcium entry blocker, flunarizine (Flu), and of the energolytic derivative of indazole carboxylic acid, lonidamine (LND), to enhance the in vitro activity of cytotoxic agents was investigated in three human colon adenocarcinoma cell lines. The simultaneous exposure to doxorubicin (Dx) and Flu for 1 h selectively enhanced the anthracycline activity in a Dx-resistant cell line (LoVo/Dx) but not in the sensitive, parental cell line (LoVo). This potentiating effect was paralled by a 3-fold enhancement of intracellular Dx accumulation, as evaluated by fluorescence spectrophotometry, and by a significant increase in transmembrane potential, as assessed by flow cytometry. Again, a 24-h exposure to Flu considerably reduced the fraction of Dx-resistant cells able to immunoreact with a monoclonal antibody directed against an external epitope of the P-glycoprotein. As regards LND, a 24-h post-incubation with the agent induced an increase in mitomycin C (MMC) and BCNU activity in LoVo and HT29 cell lines. Flow cytometric analysis showed that such a potentiating effect was due to the stabilization by LND of the temporary MMC-or BCNU-induced accumulation of cells in S/G_2-M phases.

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DETECTION OF THE MULTIDRUG RESISTANCE GENE (MDR-1) EXPRESSION BY NON-RADIOACTIVE QUANTITATIVE POLYMERASE CHAIN REACTION

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A number of techniques including radioactive polymerase chain reaction (PCR) have been adapted to assess the expression of the multidrug resistance gene mdr-1. Although this PCR technique offers advantages over other methods in terms of sensitivity, speed and cell material required, its reliance on the use of radioactive isotopes limits its general applicability in a clinical setting. In view of the apparent importance of the multidrug resistance (MDR)-phenotype in clinical drug resistance we have adapted a non-radioactive PCR assay for quantification of the mdr-1 gene expression. We applied the technique to the detection of multidrug resistance in 66 patients with chronic myelogenous leukemia. In this series, 43 of the patients studied were in chronic phase, 5 patients were in accelerated phase, and 20 patients were in blast crisis. About one third of the patients in chronic phase showed no mdr-1 expression. Moderately increased mdr-1 expression levels (2-6 fold) were observed in about half of the patients in this group. During blast crisis, cases without mdr-1 expression were relatively rare. A significant proportion of these patients displayed rather high multidrug resistance levels (>6 fold). The data from the small group of patients in accelerated phase were not conclusive. We show that the non-radioactive technique reported may provide a convenient tool for the detection of the MDR-phenotype in clinical samples.

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STUDY OF MULTIDRUG RESISTANCE EVALUATED BY P-GLYCOPROTEIN STAINING AND FUNCTIONAL RELEASE OF RHODAMINE 123.

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Among the numerous mechanisms involved in multidrug resistance to chemotherapeutic drugs, overexpression of GP-170kd P-Glycoprotein (P-GP) is well documented. The aim of this study is to compare immunologic detection of P-GP with the functional release of Rhodamine 123 (R123) fluorescent dye on several human cancer cell lines. From these cell-lines, chemo and/or radio resistant subclones were selected and their chemo-resistance level (RL) was established from their viability index in presence of Doxorubicin (IC50). Cytocentrifuge preparation of these cell-lines were immunostained with the JSB-1 mAb followed by APAAP technique and analysed with an image digital analyser (Samba 200^R). For functional activity of P-GP, cells were incubated in the presence of R123 one hour at 37°c. The accumulation of R123 in the cells was monitored by flow cytometry after 60 min. The cells were then washed and resuspended in medium devoid of R123 and the evolution of fluorescence was recorded during 30 min. Antigenic and functional studies showed that cell lines displayed two phenotypes. Native cell lines and radiotherapy resistant subclones had a low IC50 and RL, were JSB1 negative and retained R123 during all the experiments, whereas, chemoresistant and radio-chemoresistant cell lines mutants had a high IC50 and RL, were JSB-1 positive and pumped actively the fluorescent dye out of the cells. The good correlations between the resistance level and the R123 index (r=0.82 p<0.001) and between the Quick Score obtained from the image analyser and the R123 index (r=0.71 p<0.001) indicates that the R123 staining of neoplastic cells is highly associated with the MDR phenotype .We conclude that the antigenic detection and functional analysis with R123 staining were complementary to diagnose the presence of a MDR activity and are useful in experimental and clinical approaches of multidrug resistance.

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EFFECT OF ADRIAMYCIN, VERAPAMIL AND TAMOXIFEN ON WEHI 164 MURINE FIBROSARCOMA CELLS AND A MULTIDRUG-RESISTANT SUBLINE DETERMINED BY PROTION NMR SPECTROSCOPY ON PCA-EXTRACTED METABOLITES

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The emergence of multidrug-resistant (MDR) tumor cells upon chemotherapeutic treatment of cancer is a major problem in cancer chemotherapy. ^{1}H-NMR spectroscopy of PCA-extracts of a MDR cell-line and the parental drug-sensitive cells before and after treatment with Adriamycin, demonstrated differences between the two cell-lines in the metabolic response to treatment. Upon 4 hrs of treatment with 30 µM Adriamycin, the intracellular level of cholinecontaining metabolites (phosphorylcholine and/or choline) showed a 50 % decrease in the drug-sensitive cells, but a 25 % increase in the resistant. After 4 hrs of treatment with both 30 µM Adriamycin and 30 µM Verapamil (a drug known to reverse MDR), the response of the resistant cells became very similar to that of the sensitive cells. Preliminary results indicate that treatment of the cells with 30 µM Adriamycin and 30 µM Tamoxifen for 4 hrs, gives a variation in the phosphorylcholine/choline and lactat levels that is similar to the experiment with Adriamycin and Verapamil, and that there is a marked increase in the levels of almost all detectable amino acids. These results show that there are metabolic differences between the two cell-lines in response to chemotherapeutic treatment, and that the reversal of drug-resistance by lipophilic drugs counteract these metabolic differences.