NCy 15

OVERADDITIVE TOXICITY OF COMBINATIONS OF METHYLATING AGENTS WITH 2-CHLOROETHYLNITROSOUREAS

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Some human tumor cell lines are able to prevent chloroethylnitrosourea-induced DNA-interstrand crosslinks by removing O^6-chloroethyl DNA monoadducts before crosslinks can form. The same cell lines can repair O^6-methyl-guanine adducts by guanine-O^6-methyl-transferase and were designated as Mer^+ (methylation repair$^+$). Methylating agents like MNNG or MNU are able to inactivate this repair system followed by an increase in chloroethylnitrosourea-induced DNA interstrand crosslinks and in vitro cytotoxicity in Mer^+ tumor cell lines. With regard to the potential benefit of such combinations for the treatment of human tumors knowledge of whole animal and organ toxicity is indispensible. We determined the combination toxicity index of MNU plus BCNU according to a method proposed by Skipper. The sums of the decimal fractions of the LD_{10}s of both agents were opposed to the observed mortality. The LD_{10}(sum) was calculated by probit analysis. Altogether 14 different combinations of both agents were investigated. The results revealed a combination toxicity index of 0.32, the lowest value reported so far for the combination of 2 cytostatic drugs. The main targets of toxicity were the intestinal tract and the bone marrow. The results suggest that a clinical application of such combinations should be performed with the respective precautions.

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NCy 16

EVALUATION OF A SOFT-AGAR COLONY FORMING ASSAY IN GYNECOLOGIC TUMORS AND BREAST CANCER

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The culture of human tumor cells in a soft-agar colony-forming assay ("human tumor stem cell assay") has been suggested as an in vitro method to predict the clinical response to chemotherapeutic agents. We performed the assay using tumor samples from 74 patients. The primary tumors were as follows: breast (42), ovary (19), cervix (2), Fallopian tubes (1), endometrium (1), and vulva (1). Tumor samples were mechanically and enzymatically disaggregated and filtered through meshes with pore sizes down to 60 µm. We were unable to prepare one pure single cell suspension. Cells were plated in a bilayer soft agar system containing one of several cytotoxic drugs. Cell groups with a minimum diameter of 100 µm were scored after 18 to 21 days. In vitro sensitivity was defined as a 50% or more decrease in the number of colonies. Only cell suspensions that were cytologically positive or suspect were able to grow in soft agar. Of the 74 samples processed, only 39 gave a cell yield sufficient for plating. According to our criteria of evaluability, only 14% (10/74) of samples were considered evaluable. Patients' treatment was independent of in vitro results. Prospective correlations of in vitro results with clinical outcome were feasible in only 3 of the 10 evaluable samples and were correct in 2. We draw the following conclusions: (1) due to the low rate of evaluable samples, the assay has only limited value at our institution, (2) major improvments are necessary before the assay can be considered a useful tool in clinical gynecologic oncology.

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NCy 17

SPECIFIC ALTERATIONS OF PROTEIN PATTERNS IN ADRIAMYCIN-RESISTANT SARCOMA 180 CELLS

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Different protein classes (membran proteins, cytosol proteins, intermediate proteins) in adriamycin-resistant and sensitive S 180 cells were analysed by one-and two-dimensional gel electrophoresis. The object of this study was to identify resistance specific marker proteins. Adriamycin-resistant S 180 cells reveal a specific fluorescence with the P170 antibody. However, the distribution of the fluorescence in the adriamycin resistant cells did not indicate a membrane localization of the antigen in comparison with colchicine-resistant CHO-cells. Consequently, electrophoretic analyses do not reveal gross differences within the proteins of isolated membranes from adriamycin-resistant cells, whereas a control experiment with the original CHO cells well reproduces the postulated alteration. Resistant S 180 cells show a 30 kd protein species within both the soluble cytoplasmic and the Triton-insoluble membrane proteins. Labelling studies with galactose mannose and fucose result in differences mainly in fucose labelled proteins. One- and two-dimensional electrophoretic separation of Triton-soluble membrane proteins show protein species of 180 and 200 kd which are increased and 250 kd which is expressed exclusively in resistant cells. The net incorporation of fucose and the reactivity of the cells with fucose specific lectins are increased in resistant cells and seem additionally to be associated with the proliferative state of the cells.

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NCy 18

EFFECTS OF THE SYNTHETIC ALKYL-LYSOPHOSPHOLIPID BM 41 440 ON CLONOGENEITY OF HUMAN TUMOR CELLS FROM VARIOUS HISTOLOGICAL TYPES.

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Alkyl-lysophospholipids (ALP) are synthetic analogues of natural lysophosphatidylcholine and represent a new class of cytotoxic agents. ALP interfere with the membran phospholipid turnover and cause damage of the cell membrane A number of in vitro experiments with experimental cell lines showed cytotoxic effects. In this study the effect of the ALP BM 41 440 on clonogenic tumor cells from human spontaneous tumors was tested. Tumor samples were cultured immediately after resection or were taken from samples grown in nude mice. A single cell suspension of tumor cells was plated in Iscove's modified Dulbecco's medium, 30 % fetal calf serum and methylcellulose. Cultures were incubated at 37°C in 7,5 % CO_2. ALP was added at concentrations of 1, 2, 4, 8 and 16 ug/ml. The tumor samples revealed individual dose response curves. 8 out of 22 tumor probes showed a reduction of colony formation to less than 20% of control growth at a concentration of 16 ug/ml (2 colorectal tumors, 2 malignant melanomas, 1 ovarian carcinoma, 1 squamous cell carcinoma, 1 adenocarcinoma, 1 small cell carcinoma of the lung). In two cases no reduction of colony formation was observed. The other tumors yielded a colony reduction between 20 and 60% of the controls. We conclude that the ALP BM 41 440 acts cytotoxic on human clonogenic tumor cells in vitro. This new class of tumor agents deserves further investigations.

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