757 POSTER

Breast cancer (BC) after curative chemotherapy (CT) in non-hodgkin's lymphoma (NHL): Drug resistance as the major cause of treatment failure

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Purpose: Patients with aggressive NHL are treated with curative intent by CHOP-based chemotherapy. However, these presumably cured pts carry a lifetime risk for the development of second solid tumors.

Methods: 18 pts presented with BC in complete remission (CR) after CHOP for NHL. The median age was 62 (49–70), all had high/intermediate grade NHL, treated with 6 × CHOP, and were in CR. BC tissue was stained by immunohistochemistry for drug resistance proteins (LRP, MRP, MDR).

Results: BC developed after a median of 26 mo (9–49) of NHL diagnosis; grade 2:9, grade 3:9 pts, 15 −ve; 3 weakly +ve for ER/PR, and stage IIIA/B: 12, IV:6. CT included CMF or CNFL. All progressed early in liver: 9, brain: 9, lung: 6, bone: 3, and received 2nd-line MMCNelbe or taxanes, with OS = 24 mo (10–45). 8/18 pts were +ve for all (LRP, MRP, MDR) and progressed; OS = 6.8 mo (4.5–11), while 10/18 were +ve in 2/3 (n = 8) and 1/3 (n = 2) drug resistance markers with an OS = 11.8 mo (6–26) [$p < 0.01$]. Time from NHL to BC development was 16 (14–27) mo in 1st (3/3 +ve) and 37 (26–56) mo in 2nd (1–2/3 +ve) group of pts [$P < 0.01$].

Conclusion: BC appearing shortly after CR in NHL is an aggressive variant with minimal response to CT due to induction of drug resistance by CHOP.

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A new approach to flow cytometric assay of ABC-transporters function in intact solid tumors for prediction of multidrug resistance and disease prognosis

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Expression of functional activity of ABC-transporters extruding cytostatics out of the cells is the marker of multidrug resistance (MDR) and poor disease prognosis. Flow cytometry (FC) is commonly used for assay of this index in terms of MDR-drug doxorubicin (Dox) intracellular accumulation before and after the ABC-transporters' inhibitor action. But the method does not allow accurate investigation of solid tumors because it requires obtaining cell suspension at the first step. This procedure results in damage of cell membranes and distorts Dox intracellular accumulation. Many efforts have been undertaken to make disaggregation of solid tumor samples less damaging.

Results: We have overcome this disadvantage of the method by simple change of procedure sequence: 1 – incubation of intact solid tumor specimen with Dox; 2 – disaggregation of the specimen for cell suspension. In this case any method of tissue disaggregation will not influence the results because the process of Dox intracellular accumulation is already finished. Taking into account that Dox penetrates in superficial layers only, gentle specimen shaking is used to obtain cell suspension with high proportion (nearly 100%) of Dox containing cells. Using this approach we have shown different level of Dox intracellular accumulation in various human solid tumors and increase of the index in some specimens preincubated with ABC-transporters inhibitors.

Conclusion: The new approach to FC firstly allows accurate intravital assay of ABC transporters' activity in intact solid tumor specimens. Supported by the Russian Foundation for Basic Researches.

759 POSTER

New antitumor agents: Cytostatic and cytogenetic activity of two modified steroidal alkylating agents

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Purpose: Comparative study of the antitumor and cytogenetic activity on *in vivo* and *in vitro* biological systems, treated by two modified steroidal derivatives of nitrogen mustard: 3β-hydroxy-5α,22α-spirostan-12-one-p-[N,N-bis(2chloroethyl)amino] phenylacetate (II) and 3β-hydroxy-12α-aza-C-homo-5α, 22α-spirostan-12-one-p-[N,N-bis(2-chloroethyl)amino] phenylacetate (II).

Methods: C57BL mice of both sexes were used for toxicity studies and antitumor evaluation. Lewis Lung Carcinoma (LLC) was used to detect the cytostatic effect and was maintained in solid form by rejection intramuscularly in the hind right leg of 2×10^7 cells/mouse. The antitumor activity against LLC was assessed from the inhibition of tumor growth by volume in cm^3 on day 14 after transplantation. Human lymphocyte cultures were used for sister chromatid exchange (SCE) assay, an additional method for evaluating the activity of the compounds.

Results: Drug treatments were given either as a schedule of a single dose (LD10/day 1) or as a schedule of intermitted doses (LD10/2 × 3) days. The compound II is the most effective in reducing antitumor effect, when the intermitted treatment schedule was used. Fourteen days after tumor inoculation the size of the implanted tumor was 2.04 cm^3 for the control group compared to 1.07 cm^3 for the group which received 2 mg/animal/day, producing 47.5% inhibition of the tumor growth. Compounds I and II were also active in inducing SCEs.

Conclusion: The results indicate that both substances demonstrate a good inhibitory effect against LLC and these results correlate positively with the *in vitro* effectiveness in SCE induction in normal human lymphocytes.

760 PUBLICATION

Collateral sensitivity of resistant leukemic cells to gemcitabine and alkylphosphocholines

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The aim of this study was to investigate the activities of gemcitabine (GEM) and the alkylphosphocholine derivative miltefosine (HPC) alone and in combination in leukemic cells (HL60, SKW-3, K562), and in two resistant sublines (HL60/dox and HL60/HPC). Cell proliferation was determined by MTT assay, and programmed cell death by gel electrophoresis of DNA isolated from treated cells. HPC and the ether lipid edelfosine were moderately active in HL60 cells (IC50: 7 and 3 μM; maximal Inhibition [maxIn]: 65 and 90% at 50 μM). Compared to the parental cell line, both agents were less active at low and more active at high concentrations in the HL60/dox subline (IC50: 22 and 6 μM; maxIn: 92 and 100% at 50 μM), but ineffective in the HL60/HPC subline. GEM was similarly active as edelfosine in HL60 cells (IC50: 1.5 μM; maxIn: 70% at 50 μM) and inactive in the subline HL60/HPC. In contrast, it was 100 fold more active in HL60/dox cells (IC50: <0.5 μM, maxIn: 100% at 0.5 μM). Both, HPC (>10 μM) and GEM (>5 μM) caused apoptosis in SKW3 and HL60 cells, and this effect was transferable by cytosolic extracts from treated cells to nuclei of untreated cells. Optimal growth inhibition was observed in primary resistant K562 cells when GEM was administered before HPC.

In conclusion, collateral sensitivity was observed for GEM in HL60/dox cells and the combination of GEM with APC appears promising.

761 PUBLICATION

Serotoninergic modulation of cell volume response to estramustine: An image-analysis study on perifused individual glioma cells

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A technique for microscopy with computerised detection of early morphological changes during continuous perifusion was used to monitor the geometry changes of cultured glioma cells (MG-251) when exposed to 40 mg/L estramustine phosphate (EMP) alone or in combination with granisetron (0.1 umol/L), ondansetron (0.1 umol/L), or serotonin (1 umol/L). When the cells were exposed to EMP, cell volume measured as projected cell area (PCA) rapidly increased. Serotonin and ondansetron, but not granisetron eradicated the acute EMP response (PCA). Serotonin but none of the 5-HT3 receptor antagonist protected against the cytotoxicity of EMP to the glioma cells as measured by a fluorometric microculture assay. The highly selective 5-HT3 receptor antagonist in the clinical situation has always been studied from their efficacy as antiemetics. Our results demonstrate hitherto unknown differences between selective 5-HT3 receptor antagonist on the cellular response to EMP and shows the necessity to also study the receptor antagonists from view-point of interference with the anti-tumour drug effects on malignant cells. The perifusion technique could be used to further study the effects of serotoninergic agonists and antagonists on cell volume regulation of cells exposed to anticancer drugs.