810 POSTER

Expression of the multidrug resistance-associated protein (MRP) and chemoresistance of human non-small-cell lung cancer cells

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Purpose: Human non-small-cell lung cancer (NSCLC) is considered a chemotherapy-refractory malignancy. Using 11 unselected NSCLC cell lines, expression and drug-transporting activity of the multidrug resistance-associated protein (MRP), mediating a multidrug resistance (MDR) phenotype, as well as its correlation with chemoresistance were analysed.

Methods: MRP mRNA and the corresponding protein were detected by RT-PCR and immunoblot, respectively. Southern hybridisation was used to analyse MRP gene amplification. Functional activity of MRP was determined by drug accumulation studies using ^{3}[H]daunomycin and calcein as MRP substrates and probenecid, genistein, benzbromarone, N-ethylmaleimide and verapamil as MRP-modulators. Chemosensitivity was evaluated by an MTT-based survival assay.

Results: The MRP gene is intrinsically expressed at markedly varying intensity in NSCLC cells. Two cell lines expressed MRP at levels comparable to those detected in drug-selected control cell lines (GLC4/ADR, HL-60/AR), however, without MRP gene amplification. Functional analysis revealed a transporting activity of MRP, correlating significantly with the gene expression data. Moreover, a significant correlation between MRP expression and chemoresistance against daunomycin, doxorubicin, etoposide and vinblastin, but not cisplatin was detected.

Conclusion: Our data suggest that MRP may be involved in the intrinsic MDR phenotype of NSCLC cells.

811 POSTER

Influence of melatonin on mutagenicity and anti-tumor effect of cyclophosphamide and nitrosomethylurea in mice

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Purpose: We examined the ability of pineal hormone melatonin (MLT) to modulate the mutagenicity of N-nitrosomethylurea (NMU) and cyclophosphamide (CP) in the tests for chromosome aberrations (ChA) in bone marrow cells and sperm head anomalies (SHA) in mice.

Methods: Animals were killed 24 h (for ChA) or 17 days (for SHA) after single injections of MLT (5 mg/kg, s.c.), NMU (50 mg/kg, i.p.), CP (200 mg/kg, i.p.) or co-administration of MLT and NMU or CP.

Results: MLT reduced the level of ChA (%) from 15.9 (NMU) and 13.7 (CP) to 4.5 and 4.3, respectively ($p < 0.05$). Similarly, SHA frequency (%) was reduced from 18.6 (NMU) and 17.7 (CP) to 9.9 and 6.1. Exposure to MLT (20 mg/l, in drinking water at night) alone or in combination with NMU (50 mg/kg, i.p. ×1) or CP (200 mg/kg, i.p. ×1) failed influence s.c. transplanted carcinoma Ehrlich size in comparison to relevant controls. However, MLT significantly increased the life-span of CP-treated mice.

Conclusion: MLT has antimutagenic effect and potentiates anti-tumor action of CP.

812 POSTER

Inhibitory effect of radiosensitizer AK-2123 on experimental hepatic metastases and CA^{2+} active transport

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The purpose of the work is identification of new active agents for treatment colorectal cancer hepatometastases.

The effect of radiosensitizer AK-2123 (nitro-triazole) on hepatometastases and on Ca^{2+} active transport was studied.

The metastases were induced by intrasplenic injection of colon adenocarcinoma cells in singenic mice. The average number of colonies in control and treated groups was estimated on day 22. The active transport of calcium ions by the (Ca^{2+}-Mg^{2+})-dependent ATP-ase of sarcoplasmic reticulum was evaluated pH-metrically.

The average number of metastases in control and treated (10 mg/kg) groups was 15.8 ± 2.1 and 3.8 ± 1.5 respectively. The part of treated animals was free of metastases. 100% of inhibition of Ca^{2+} active transport was observed.

The AK-2123 radiosensitizer exhibits significant antimetastatic effect which is suggested to be related to the inhibition of active calcium transport.

813 PUBLICATION

In vivo resistance of murine leukemia P388 towards platinum complexes

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Numerous attempts have been done to synthesize novel platinum drugs with improved therapeutic features. We synthesized previously the platinum complex with diaminonitroxyl radical-PtII(DAPO)Ox, which displayed the more antitumor activity in tumor-bearing mice and less overall and specific toxicity than CDDP. The purpose of this work was to examine comparatively development of P388 leukemia resistance to these drugs. The resistance was induced by successive transplantations of tumor cells from mice treated by each drug, with the stepwise increase of doses. There were obvious differences in duration of resistance development to CDDP and PtII(DAPO)Ox – at 5th and 11th generations respectively. Resistant substrains exhibited the mutual cross resistance to CDDP and Pt(DAPO)Ox. Tumorogenicity of resistant and parent strains was similar. Resistance was not disappeared during 16 generations in the absence of own drug. The resistant substrains retained the high sensitivity to ADR, DAU, VCR. They had the cross resistance to ThioTEPa. One substrain (P388/DAPO) maintained the high sensitivity to MTX whereas the other showed the cross resistance to this drug. P388/DAPO, but non P388/CDDP, had a collateral sensitivity to etoposide. Thus, the introduction of nitroxyl radical in platinum complex results in the delay of resistance development and certain changes of chemosensitivity.

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814 PUBLICATION

Antitumor effect oxygenic complexes of cobalt

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Purpose: The main objective in this work was to study the toxicity and antitumor activity of transition metal complexes (oxygen carriers); since it is supposed that these substances concrete as sources of superoxide and hydroxyl radicals that can destroy DNA. The 3 new cobalt complexes, containing jointly a molecular oxygen, amino acids and tetraazomacrocyclic ligands were examined.

Methods: These compounds: I. (cobalt, imidazole, lysine and molecular oxygen), II. (cobalt, histidine and molecular oxygen), III. (cobalt, tetraazomacrocyclic ligand and molecular oxygen) were synthesized by J. Bratuško and J. Stukalina, Institute of Physical Chemistry, Ukraine. The acute toxicity was determined in the intact mice. Ascite and solid tumor models were used: leukemia L 1210 (L 1210), Ehrlich ascite carcinoma (EAC) and Lewis lung carcinoma (LLC). Mice were injected intraperitoneally with compounds in various dose levels during 4–5 days. The antitumor effect of the compounds was measured as percentage tumor growth or metastases inhibition and percentage increase in life span.

Results: In acute toxicity test was determined that maximal tolerated doses were I and II-200 mg/kg, III-400 mg/kg. Treatment L 1210 and EAC failed to increase the life span, in spite of EAC marked inhibitory effect on ascite volume (40–90%). All compounds didn't inhibit the growth of primary tumor LLC, and only one of them (III) showed a significant reduction in the development of metastases LLC (80.6%).

Conclusion: One of the oxygenic complexes III (cobalt, tetraazomacrocyclic ligand and molecular oxygen) was found to have therapeutic effect on mouse Lewis lung carcinoma inhibiting the metastases growth.

815 PUBLICATION

8-Cl-cAMP induction of differentiation and apoptosis in Y-79 human retinoblastoma cell line

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Purpose: Treatment of retinoblastoma cells with certain agents induces a

partial differentiation to cell types resembling those of the mature retina. The effects of 8-Cl-cAMP on the Y-79 retinoblastoma cell line growth inhibition, differentiation and apoptosis were tested in vitro.

Methods: Y-79 cells were treated with increasing doses of 8-Cl-cAMP for growth inhibition. Apoptosis was evaluated by DNA laddering, acridine orange/ethidium bromide uptake and TUNEL assays.

Results: Y-79 cells treated with 8-Cl-cAMP produced short branching processes, whereas dibutyryl-cAMP induced differentiation toward a glial cell type. 8-Cl-cAMP treatment increased expression of the neuronal marker Neuron Specific Enolase, while dibutyryl-cAMP increased the glial marker Glial Acidic Fibrillary Protein. Y-79 cell proliferation was strongly inhibited by 8-Cl-cAMP at concentrations as low as 10–25 μM. 8-Cl-cAMP decreased expression of the RI regulatory subunit of cAMP-dependent protein kinase A, which is produced in abnormal quantities by Y-79 cells. Finally, 8-Cl-cAMP significantly increases the rate of apoptosis of Y-79 cells in a dose-dependent manner.

Conclusions: These data suggest that 8-Cl-cAMP can be a potential agent in the therapy of retinoblastoma.

816 PUBLICATION

Effect of β-interferon on vascular density, local metabolism and alkaline phosphatase activity in normoxia and hypoxia

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Purpose: While interferon's (IFN) are known to inhibit cellular proliferation rate, hypoxia is known to stimulate endothelial cell proliferation. In order to find out whether or not the angiogenic effect of hypoxia weakens the inhibitory effect of β-interferon experiments were performed in the early chick embryo.

Materials and Methods: For this study fertilised crossbred 'White-Plymouth-Rocks x Sussex' eggs were incubated in a commercial incubator in air (20.9% oxygen, normoxia), 10% oxygen (mild hypoxia) or 5% oxygen (severe hypoxia). After 48 hr of incubation, the egg shell was opened and 33000 IU interferon β were added locally 48 hr later, vascular density, local metabolism and activity of alkaline phophatase were determined in vivo.

Results: Vascular density was found to be significantly reduced in areas treated with β-interferon. This was observed in normoxia as well as in mild hypoxia. Simultaneously local metabolism decreased. Activity of alkaline phosphatase showed highest values in untreated eggs incubated in 10% oxygen. Treatment with interferon caused a significant reduction in AP activity. In severe hypoxia the antiangiogenic effect of β-interferon was abolished.

Conclusion: β-interferon decreased metabolic activity of tissue as well as vascular density as long as the local oxygen availability is above a critical level. At the same time the activity of AP is reduced. This may be due to an altered synthesis of extracellular matrix. Below a critical oxygen supply the inhibitory effect of interferon is overwhelmed by the angiogenic effect of hypoxia.

817 PUBLICATION

New dimethyltin (IV) compounds and their complexes with nitrogen containing ligands of antitumour activity

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Purpose: Organotin (IV) compounds and their complexes with various ligands have been investigated by many researchers and found to have a range of anti-tumour activity against certain types of tumour cell lines. In this study, a large number of new Me_2Sn (IV) compounds and their complexes with various nitrogen containing ligands were synthesized and their activities were examined.

Methods: The dimethyltin (IV) compounds, Me_2SnX_2, where $X_2 = C_2O_4$, $O_2(CO)_2CH_2$, $O_2(CO)_2(C_6H_{11})_2$, $O_2(CO)_2\{C(CH_3)_3\}_2$ and $O_2(CO)_2C\text{-}CH_2CH_2CH_2$ and their complexes of the general formula $Me_2SnX_2.L$ (or L_2), where L = nitrogenous ligand, have been synthesized and characterized physicochemically and spectroscopically. The cytotoxic activities of these compounds were evaluated *in vitro* using the MTT-assay against four tumour cell lines, one fluid suspension (P_{388}-leukemia) and three solid human cell lines (Hep-2, larynx; RD, embryonal rhabdomyosarcoma and HeLa, cervical carcinoma cells).

Results: Three of the above compounds exhibited an IC_{50} values similar to that for cisplatin against the three solid cell lines (Hep-2, RD & HeLa). Whereas, better cytotoxicity was achieved by these compounds against the four cell lines when compared with both the carboplatin and the oxaliplatin (the reference standards used in this study).

Conclusion: The present results are encouraging, yet, further studies are required to confirm them by using at least one of the known NCI-models like B16 melanoma or sarcoma 180.

818 PUBLICATION

Metphosphane – A potent anticancer agent

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Purpose: During the last several years the number of active new anticancer agents among derivatives of diethylenimidophosphoryl- and thiophosphoryl-amino-cyclohexylalcanoic acids were synthesized and examined. Continuing our research we prepared N,N'-tetramethyldiamido-N''-ethylenimidophosphate (metphosphane) with the aim to verify its antineoplastic activity.

Methods: Tumor models were used: lymphoidic leukemia L1210 (L1210), murine ascite tumor NK/Ly (NK/Ly), Ehrlich ascite carcinoma (EAC), Lewis lung carcinoma (LLC), carcinoma Jensene (CJ), sarcoma M-1 and 45 (S-M-1 and S-45), carcinoma Herene (CH), cholangioma PC-1 (ChPC-1), lymphosarcoma Pliss (PL), carcinosarcoma Walker 256 (W-256). Treatment of tumor-bearing animals with metphosphane was generally initiated on the 2–3 days following solid tumor implant or 24 h after ascite tumor inoculation and continued for a period 7–10 days. The antitumor effect was measured as percentage of tumor growth (TG) inhibition or percentage increase in life span (LS).

Results: The experimental data have shown that metphosphane did not increase the LS of mice with L-1210, EAC and LLC, but inhibited growth of ascite volume and reduced the tumor cell counts The mean white cell counts in the treated animals were significantly lower compare to the untreated animals. The TG of rats with wide spectrum of solid tumors treated with metphosphane was decreased 70–98%, and W-256 was inhibited 100%. Some preclinical examination of this agent is made too.

Conclusion: Metphosphane is active anticancer agent in experiment.

819 PUBLICATION

Influence of long-term storage of freshly explanted tumor cells on in vitro soft agar cloning

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The purpose of the present study was to investigate the viability, clonogenicity and chemosensitivity of freshly explanted tumor cells when stored in liquid nitrogen using a soft agar cloning system. Fresh tumor cells obtained by sterile standard procedures as part of routine clinical measures were cloned in the presence of clinically relevant concentrations of a variety of antitumor agents. Aliquots of the cells were cryopreserved in culture medium containing 10% DMSO by freezing at a rate of −1°C/min to −6°C/min down to −175°C and then stored in liquid nitrogen. After thawing and DMSO removal, the cells were cloned as described above. Viability, clonogenicity and chemosensitivity were investigated before and after various periods of N_2-storage. For longitudinal studies, 14 tumors were investigated and 10 clinical antitumor agents were used. After a freezing period of 2 hours the viability of the cells was reduced by 8.9% and clonogenicity was reduced by 22% in untreated controls. However, the chemosensitivity pattern was not changed. Experiments were repeated every 6 weeks and 528 determinations are available. Long-term storage of the cells up to 42 weeks had no further influence on viability, clonogenicity and chemosensitivity. 61 additional tumors were also investigated by independent investigators after various storage periods in liquid nitrogen. Again, no influence of N_2-storage on chemosensitivity and clonogenicity was observed. We conclude that storage of freshly explanted human tumor cells in liquid nitrogen is a simple and easy way to preserve samples for the preclinical evaluation of new antitumor agents.