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.