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EFFECT OF THE SYNTHETIC ALKYL-LYSOPHOSPHOLIPID BM 41 440 ON GYNAECOLOGICAL TUMORS

H.M. Runge, H.A. Neumann, W. Pahlke, W. Kleine

Several gynecological tumors were tested for their susceptibility to synthetic Alkyl-Lysophospholipids (ALP). The ALP's are new antimetabolic tumor agents that interfere with the phospholipid metabolism preferently of neoplastic cells. In addition ALP proved to activate normal macrophages increasing their tumorcidal capacity. 2 ovarian, 2 cervix uteri, and 2 endometrial carcinomas were plated in a methylcellulose monolayer colony assay. 2 x 10^5 cells were plated in 30% fetal calf serum and Iscove's modified Dulbecco's medium containing methylcellulose (0,9% w/v) as viscous support. ALP was added at concentrations ranging from 2-16 µg/ml. The drug effect was expressed as percent reduction of colony formation as a function of the drug concentration. In all tumors a reduction of colony formation to 30-20% of control was observed. 3 tumors (1 cervix, 1 endometrial, 1 ovarian carcinoma) were xenotransplanted into Balb-C nude mice. 10 animals/group were treated p.o. with 2,5 mg/kg and 25 mg/kg every second day for 3 weeks. Growth inhibition was assessed by controlling the tumor diameters. In the ovarian carcinoma a growth delay of 50% compared to the control animals was observed. In the group that was treated with 2,5 mg/kg, 6/10 animals were free of tumor, in the group treated with 25 mg/kg 4/10 animals were free of tumors. No therapeutic effect was observed in the cervix uteri and the endometrial adenocarcinoma.

Universitäts-Frauenklinik Freiburg
Hugstetterstraße 55, D-7800 Freiburg

NCy 20

THE FIRST SPECTROPHOTOMETRIC ASSAY FOR DETERMINATION OF O-ALKYL CLEAVAGE ENZYMES

J. Kötting, C. Unger and H. Eibl

Aim of research: (Alkyl)-lysophospholipids have antineoplastic activity. In the present discussion on the cytotoxic effects, the accumulation of ether lipids in tumour cells may be the result of an enzyme defect, the lack of the O-alkyl cleavage enzyme (E.C. 1.14.16.5). At present, two methods have been described for the determination of this important enzyme. One method depends on radiolabelled substrates (J.F. Soodsma et al., J. Biol. Chem. 247, 3923, 1972), the other on the presence of phosphate (C. Unger et al., Cancer Res. 45, 616, 1985). We describe the first spectrophotometric assay for this enzyme.

Methods: The assay couples the oxidation of the cofactor of the O-alkyl cleavage enzyme with the NAD/NADH redox system.

Results: The spectrophotometric determination of the O-alkyl cleavage enzyme allows the measurement of specific activities down to 1 nMol/min/mg protein. Furthermore, the assay does not depend on structural limitations as demonstrated for a large variety of different substrates, e.g. glycerol ethers, (ether)-lysophospholipids, glycerol thioethers and (thioether)-lysophospholipids.

Conclusions: This new spectrophotometric assay is an ideal tool to analyze the structural requirements which determine the substrate properties of the O-alkyl cleavage enzyme. We can present a comprehensive overview about substrates which are helpful to develop an enzyme concept which results in specific toxicity for tumour cells.

Max-Planck-Institut für biophysikalische Chemie
Am Faßberg, D-3400 Göttingen

NCy 21

ALKYLPHOSPHOCHOLINES AS ANTICANCER AGENTS

M.R. Berger, D. Schmähl, C. Muschiol, C. Unger, H.J. Eibl

Three alkylphosphocholines were administered by gavage 5 x/week over 5 weeks to SD-rats bearing overt mammary lesions. Hexadecylphosphocholine (HPC) and Octadecylphosphocholine (OPC) were administered at six logarithmically spaced dosages ranging from 7 to 115 µmol/kg, tetradecylphosphocholine was administered at four doses ranging from 51 to 172 µmol/kg. Therapeutic effectiveness was evaluated by measuring tumor volumes of treated and control animals (T/C %). Toxicity was expressed by the percentage of animals which died during the treatment period. 15 rats were used per dosage group. The following results in terms of T/C% - % mortality were obtained at week 6. TPC 51 µmol/kg: 92-20; 77 µmol/kg:43-13; 115 µmol/kg: 72-13; 172 µmol/kg: 42-13. HPC 7 µmol/kg: 74-10; 15 µmol/kg: 28-10, 34 µmol/kg: 39-7; 51 µmol/kg: 13-0; 77 µmol/kg: 2-40, 115 µmol/kg: not determined - 93. OPC 7 µmol/kg: 74-20; 15 µmol/kg: 23-0, 34 µmol/kg: 6-0, 51 µmol/kg: 2-33; 77 µmol/kg: 1-80; 115 µmol/kg: not determined - 100. Controls: 100-15. The toxicity observed was inversely correlated with the length of the alkyl chain. Both HPC and OPC were highly effective in inhibiting the growth of established mammary carcinomas. Since recent in vitro findings on four types of leukemic cells showed marginal to high activity, highly specific anticancer efficacy might be responsible for the extinction of MNU-induced mammary carcinoma.

Institut für Toxikologie und Chemotherapie, DKFZ. Im Neuenheimer Feld 280, D-6900 Heidelberg

NCy 22

HEXADECYLPHOSPHOCHOLINE, A NEW ANTINEOPLASTIC AGENT: CYTOTOXIC PROPERTIES IN LEUKAEMIC CELLS

H. Eibl, C. Unger, E.A.M. Fleer, D.-J. Kim, M.R. Berger and G.A. Nagel

Aim of research: (Alkyl)-lysophospholipids (ALP) are potent antitumour agents (H.U. Weltzien and P.G. Munder, in: (U.K. Mangold and F. Paltauf, eds.) Ether Lipids, Academic Press, New York, 277, 1983). In our search for ALP with minimum structural requirements we have prepared hexadecylphosphocholine (HPC), since a long chain alkyl residue and phosphocholine are important.

Methods: Cytotoxic effects of HPC were studied against different leukaemic cells by inhibition of (3H)-thymidine incorporation and with the trypan blue dye exclusion test. Additionally, the metabolic fate of hexadecylphospho(3H-methyl)choline was followed in Raji cells.

Results: HPC is toxic against tumour cells and the glycerol residue is not necessary to generate toxicity. HPC inhibits the in vitro incorporation of tritiated thymidine into HL 60 and Raji human leukaemic cells. Complete cell destruction, as the trypan blue dye exclusion test demonstrated, was achieved with concentrations of 10 µg/ml for HL 60 cells and 32 µg/ml for the Raji cells after 72 hours of incubation. In addition, we analyzed the metabolic products formed during incubation with hexadecylphospho(3H-methyl)choline. We showed for the very first time that a direct transfer of the phosphocholine residue from HPC to diacylglycerol represents the toxification step. Hexadecanol, generated during incubation, is most probably responsible for cell death. First results with murine tumour models indicate that HPC is powerful in the treatment of mammary carcinomas of the rat.

Conclusions: HPC represents a prodrug principle. The toxine, most probably hexadecanol, leads to complete cell destruction of the leukaemic cells.

Max-Planck-Institut für biophysikalische Chemie,
Am Faßberg, D-3400 Göttingen