150

ANALYSIS OF NUCLEAR BINDING SITES FOR 1,25-DIHYDROXYCHOLECALCIFEROL IN HL 60 PROMYELOCYTIC LEUKEMIA CELLS: NUCLEAR TRANSLOCATION KINETICS OF CYTOSOLIC RECEPTOR

F.Jakob, A.Tresch, S. Hammer, F. Gieseler, K.Wilms
Medizinische Poliklinik, University of Würzburg, FRG

Estimation of receptors for 1,25-Dihydroxycholecalciferol (VDR) by classical methods (DCC-method, whole-cell-assay) proved to be difficult (proteolysis, unspecific binding). We now report a method to measure binding sites in isolated nuclei of HL 60 cells after in vitro incubation of whole cells with the 3H-ligand. Cells were incubated in RPMI medium made 10^{-8} Mol /l 3H-1,25-Dihydroxycholecalciferol (160 Cl/mmol) at 37°C for 0, 30', 1h, 2h, 3h, 4h time. Cells were washed in RPMI and resuspended in icecold STM-buffer 0,02 Mol/l DTT for 15 min. This suspension was made 1% v/v Triton X 100 and incubation continued for 15 min under constant gentle shaking. Nuclei were pelleted for scintillation counting (1800 rpm, 10 min). Control experiments were done with a 500-fold excess of unlabeled hormone.
Isolated nuclei, analysed as described above before incubation of whole cells, did not show significant amounts of VDR. Specific nuclear binding sites rose in a time-dependant manner reaching a maximum of appr. 1500 sites/nucleus after 3 h, slightly declining after 4h of incubation. The maximum number of binding sites/nucleus varied from appr. 1000 to 2000 binding sites/nucleus, while translocation kinetics were the same.
This method allows rapid, sensitive and highly specific analysis of VDR in a minimum number of 10^6 cells for single point analysis. VDR molecules - according to these data - do reside in the cytosol and are translocated into the nucleus upon ligand stimulation.
Purified samples from patients with leukemia/lymphoma can routinely be examined. Analysis of such biological samples may help to investigate the possibilities of induction of cell differentiation by ligand inducible transscription factors.

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151

SURAMIN-INDUCED GROWTH INHIBITION OF HUMAN BREAST CARCINOMA CELL LINES.

L.Miglietta,*F.Ravera,*P.Pirani, P.F.Conte and *R.E.Favoni.
Istituto Nazionale per la Ricerca sul Cancro, Dept.of Medical Oncology,*Dept. of Pharmacology - Genova, ITALY.

Suramin (Sur), a polyanionic naphtilurea used clinically for treatment of African Trypanosomiasis, has been shown to have an antagonistic activity for a variety of growth factors (GF) including PDGF, EGF, bFGF and TGF beta; moreover Sur has been demonstrated to have an antiproliferative effect on several human lymphoid and prostate carcinoma cell lines. It is also known that Sur can induce tumor regression in human kidney and surrenal carcinomas as well as lymphomas. In order to assess the effect of Sur on the proliferation of human breast cancer we have evaluated the optimal growth-inhibitory concentration of Sur as well as the reversibility of its effect on two estrogen-receptor positive (MCF-7 and T47D) and one estrogen-receptor negative (MDA-MB231) human breast cancer cell lines (HBCCL). Cells, plated in serum containing DMEM at 25,000/ml were exposed continously to Sur at 50, 100, 200, 400 ug/ml; viable cells were counted every 24 hrs up to four days. We found that Sur inhibited, in a dose-dependent manner, the growth of HBCCLs with a half-maximal effect of approximately 0.1 mM for MCF-7 and MDA-MB231 and 0.07 mM for T47D cell lines which corresponds to 50% of the clinically achievable dose. The inhibitory action of Sur did not appear to be due to cellular cytotoxicity since the removal of drug, after four days of treatment, resulted in the recovery of cell growth within two days. Studies evaluating the action of Sur on the mitogenicity of several GFs, particularly of insulin-like family, on HBCCLs are currently in progress in our laboratory. The goal of this investigation is to define a possible role of Sur in blocking the growth factors-induced proliferation of human breast tumor.

152

SURAMIN: PHARMACOKINETIC MONITORING, HAEMATOLOGICAL AND ANTITUMOR EFFECTS ON RATS BEARING TRANSPLANTABLE PANCREATIC TUMORS.

B.Setyono-Han, G.H.Bakker, H.A.Peters, A.M.Sieuwerts, A.M.M.Eggermont, J.A.Foekens and J.G.M.Klijn.
Dr.Daniel den Hoed Cancer Center, Division of Endocrine-Oncology. Rotterdam, The Netherlands.

Suramin, a polysulfonated naphtylurea is known to inhibit the binding of growth factors to their receptors and to antagonize the ability of these factors to stimulate growth of tumor cells. We have treated healthy rats as well as rats bearing the transplanted pancreatic tumor (EGFR+, IGF-1-R+, Somatostatin receptor+) with suramin (0.5, 2.5, and 5 mg/twice weekly s.c.). Rats were divided into groups of 18 rats each. Three rats from each group were sacrificed weekly. The plasma suramin concentration, haematological parameters, and the influence of suramin treatment on organ weights were examined. Suramin concentrations in the plasma measured by HPLC plateaued after about 5 weeks. Doses of 2.5- and 5-mg suramin/twice weekly resulted in significant tumor growth inhibition in the first 4 to 5 weeks post inoculation which became lower thereafter. The highest dose of suramin also resulted in reduced body weight; no significant effects could be detected on weights of various organs. However, kidney weight was exceptionally increased in some rats. The weight of mesenteric lymph-nodes was often reduced. The most pronounced suramin effect was on the spleen weight, which was already increased after two weeks of treatment. Suramin also caused higher white blood cell count and a slightly lower red blood cell count, when compared to those in healthy rats. Thrombocytopenia was also observed. Therefore, though significant tumor inhibition was found, the effects of suramin in treatment of experimental pancreatic carcinoma require further investigation.

153

IN VITRO AND IN VIVO EFFECTS OF SURAMIN ON RAT PROSTATE TESTOSTERONE 5α-REDUCTASE

E. di Salle, T. Zaccheo, G. Briatico and G. Ornati
Oncology Line, R&D, Farmitalia Carlo Erba, Erbamont Group, 20014 Nerviano (Mi), Italy

Suramin (S), an antihelminthic agent, has recently been shown to have clinical efficacy in the treatment of prostate cancer. Its postulated mechanism of action is via the binding to growth factors and inhibition of some cellular enzymes. We studied the possible influence of S on rat prostate 5α-reductase, the enzyme converting testosterone (T) to its active metabolite dihydrotestosterone (DHT). S was incubated with prostate particulate fraction and 1 μM [^{14}C]T. The compound inhibited the enzyme with an IC_{50} of 160 μg/ml, a concentration clinically achievable in humans. In castrated and T propionate (TP, 0.3 mg/kg s.c. for 10 consecutive days) supplemented rats, S was given at 100 mg/kg i.v., three days apart for four times. Ventral prostate and seminal vesicles weight increases induced by TP were not influenced by S, which completely antagonized the effect of TP on levator ani weight. Moreover S was found to reduce body weight gain by 38%, to increase both the absolute and relative weight of adrenals (30-58%), spleen (100-141%) and kidneys (16-41%) and to decrease thymus weight (41-30%). In conclusion in vivo S did not influence the T effects mediated by its 5α-reduced metabolite DHT, while in vitro it inhibited 5α-reductase activity.