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RETINOIC ACID RECEPTORS (RAR) AS TARGETS IN RETINOID RESPONSIVE TUMORS - PRECLINICAL INVESTIGATION IN HUMAN OVARIAN CANCER CELL LINES (OCCL)

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The growth inhibitory effects of all-trans and 13-cis retinoic acid (RA) as well as the synthetic retinoids TTNPB, TTNPB-ethylester and TTNN (J.E. Eliason; Hoffmann-La Roche) were investigated in 7 epithelial OCCL (HOC-7, HEY, H134, SK-OV-3, CAOV-3, OVCAR-3, TR-170) and 1 ovarian teratocarcinoma cell line PA-1. 6 OCCL were inhibited by RA and the synthetic retinoids in a dose dependent manner. No response was observed for PA-1. As RA and retinoids exert their action on the cells via nuclear receptors the expression of RAR-α, -β and -γ mRNA was examined by PCR following reverse transcription without/with exposition to RA. All cell lines expressed RAR-α and -γ mRNA; 6 cell lines additionally RAR-β mRNA, among them PA-1. Our data revealed no direct association between the presence of RAR subtype transcripts and the response to retinoids in OCCL.

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PRECLINICAL EVIDENCE FOR THE ANTI-CANCER EFFECT INDUCED BY ZILASCORB(²H) - A REVERSIBLE PROTEIN SYNTHESIS INHIBITOR WITH MINOR TOXIC EFFECTS

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Zilascorb(²H) is a deuterated analog of benzylidene-ascorbate [5,6-benzylidene-d_1-ascorbate]. The presence of deuterium increased the degree of protein synthesis inhibition and cell inactivating effect induced by the compound. Zilascorb(²H) treatment reversibly inhibited protein synthesis in a number of malignant or transformed cell lines (NHIK 3025, A549, PANC-1, B16), while no such effect was observed in normal or primary cells (V79, 3T3, primary oral fibroblasts), inducing a potential **oncostatic** effect. Zilascorb(²H) did not affect cellular uptake of amino acids into cells, however, the compound did inhibit translation of exogenous mRNA in a reticulocyte lysate. The LD_{50} of zilascorb(²H) in mice is 2200 mg/kg, indicating that this compound induces little systemic toxcicity - a find confirmed by toxicological testing in rats and dogs. The antitumor effect induced by zilascorb(²H) is demonstrated in a number of human tumor xenografts. A significant effect on tumor volume growth curves, and degree of necrosis as evaluated by histological examination is shown for SK-OV and OVCAR ovarian carcinoma, EE melanoma, and PANC-1 pancreatic carcinoma grown in nude mice.

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Arterial chemoembolisation of rat tissue-isolated tumours with microcapsules containing cisplatin and SR423

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The use of chemotherapy is limited by its secondary effects. One possible approach to increase the therapeutic gain of drug treatment is to use arterial chemoembolisation. This approach has the advantage of killing some cells by mechanical obstruction of tumour vessels and increasing the amount of drug trapped within a tumour (internal reservoir of drug) leading to reduced systemic exposure. Using an existing isolated rat tumour system currently being used for physiological studies at the CRC Gray laboratory, we compared the effect of chemoembolisation with microcapsules loaded either with cisplatin or SR4233. Using a bioreductive drug, such as SR4233, has the additional advantage of producing a hypoxic tumour environment by embolization of the tumour vasculature, thereby activating the drug within the tumour. The tissue-isolated tumour used was the rat carcinosarcoma P22 transplanted in the fat pad supplied by the epigastric artery. Intra-arterial injection of microcapsules, loaded with 7 mg per rat of either SR4233 or cisplatin, unloaded microcapsules, or unencapsulated drug was carried out on tumours after 3-4 weeks of growth. Microcapsules containing cisplatin and SR4233 were prepared by the coacervation method, previously described (Hecquet et al. 1984). The microcapsules are 50 - 150 mm in diameter and about 70% in drug content. In all cases the injection of loaded microcapsules induced a regrowth delay from one to several months. The unloaded microcapsules, on the other hand, did induce a regrowth delay of maximum one month and the unencapsulated drug produced death of the animals or major toxicity. Pharmacokinetic data will be presented. In conclusion, arterial chemoembolisation dramatically increases the therapeutic gain of the treatement with both cisplatin and SR4233. *Work supported by Cancer Research Campaign UK.*

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REDUCTION OF DOXORUBICIN-INDUCED CARDIOTOXICITY IN THE RAT BY THE USE OF A N-(2-HYDROXYPROPYL) METHACRYLAMIDE DOSE DELIVERY SYSTEM.

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Several approaches have been proposed to increase the therapeutic index of anthracyclines. A promising approach appears to be the use of a N-(2-hydroxylpropyl) methacrylamide (HPMA) co-polymer conjugate containing doxorubicin (DOX). This is stable in the circulation but DOX is liberated in cells. A rat model has been used to assess the reduction in both general acute and late cardiac toxicity of HPMA/DOX as compared with free DOX. Single iv doses of HPMA/DOX (4 mg/Kg DOX) produced no reduction in cardiac output (COP) or animal mortality after 12 weeks, whereas a similar dose of free DOX resulted in ~ 90% mortality from cardiac failure within 12 weeks. Higher iv doses of HPMA/DOX (8-12 mg/Kg DOX) only produced a 13% reduction in COP. Higher single ip doses of HPMA/DOX (24-36 mg/Kg DOX) were associated with animal mortality. Lower ip doses of the conjugate (12-18 mg/Kg DOX) were not generally associated with any significant late cardiotoxicity or general acute toxicity. This reduction in healthy tissue morbidity was not associated with a reduction in DOX cytotoxicity to tumour cells.

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THERMAL ENHANCEMENT OF TNF-α INDUCED LETHAL TOXICITY IN TUMOUR BEARING RATS

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Experimental studies strongly suggested synergistic anti-tumour acitvity of combined hyperthermia (HT) and TNF-α. However, some studies indicated an increased systemic toxicity of TNF by additional hyperthermia. In order to obtain safe starting dosages for a clinical phase I study, we investigated the toxicity of systemic rHu TNF-α (specific activity 6.7×10^6 U/mg protein, Knoll Amsterdam) combined with local HT (1 hr >42°C) of the tumour bearing hind leg, as compared with TNF alone at normal body temperature (NT; 37.5-38.5°C). When the tumour reached a diameter of about 1 cm, the animals recieved a single i.v. dose of TNF at either NT or HT. Both lethality and tumour cure were endpoints.

The LD_{50} (± SE) were 1135 ± 27 μg/kg at NT and 214 ± 26 μg/kg at HT, resulting in a thermal enhancement ratio of 5.3 (95% confidence limits 4.1-7.2). The TCD_{50} were 1202 μg/kg at NT and 188 μg/kg at HT, resulting in a thermal enhancement ratio of 6.4. We conclude that the systemic toxicity of TNF is increased by local hyperthermia. In view of the large variety in tumour sensitivity, the usefulness of this combined treatment modality has to be determined by clinical studies.

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IN VITRO ACTIVITY OF A CISPLATINUM CONJUGATED ANTI CA-125 ANTIBODY AGAINST HUMAN OVARIAN CANCER CELL LINES

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Immunoconjugates consisting of the murine monoclonal anti CA-125 antibody OC-125 (kindly provided by CENTOCOR Inc.) and Cisplatinum were synthesised using Diethylene-Triammine-Pentaacetic acid (DTPA) as linker substance as previously reported. The binding activity of the immunoconjugates against CA-125 was shown to be preserved despite the chemical modification of the antibody molecules by means of immuno-histo/ -cytochemical stainings using frozen sections of ovarian cancer tissue, amniotic epithelium, and the CA-125 positive ovarian cancer cell line NIH:OVCAR 3.

The antiproliferative activity of the immunoconjugates against the human ovarian cancer cell lines NIH:OVCAR 3 (CA-125 positive) and SKOV 3 (CA-125 negative) was tested with a kinetic microassay. 2×10^4 cells/well were seeded in 96 well microtitration plates and cultured for 48 h. Thereafter the immunoconjugates, pure antibody or Cisplatinum in various concentrations diluted in culture medium were added. After 30 minutes the media were removed, and the cells cultured for further 4 days in complete medium. Cells were fixed every 24 hours with 2% glutaric aldehyde and finally, after staining the nuclei with 0.02 % Crystalviolett and re-diluting the dye with 70 % Ethanol the optical density was measured with a 13 channel autoreader.

Despite the good binding properties of the immunoconjugates to CA-125 positive tissue sections and cells no antiproliferative effect of the immunconjugates on NIH:OVCAR 3 cells could be found. This rather unexpected effect may at least partially be due to the fact, that the target antigen CA-125 is shed in large amounts by the NIH:OVCAR 3 cells.