7.019

THERAPEUTICAL EFFECT OF ADRIAMYCIN- AND IODODOXORUBICIN-TREATED LAK CELLS IN MICE BEARING LUNG METASTASES.

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Many attempts have been made to deliver a cytotoxic agent to the tumor site in order to increase its antitumor activity and reduce side effects. We explored the possibility of using cytotoxic cells as carriers of two antineoplastic drugs, Adriamycin and its derivate 4'-Deoxy-4'-Iododoxorubicin. Drug uptake and release by lymphokine activated killer (LAK) murine cells was evaluated at various intervals following incubation with either ^{14}C labelled or unlabelled drug; ^{14}C incorporation or fluorescence emission of this drug by means of FACS analysis was then evaluated. In vitro experiments demonstrated that LAK cells were able to internalize both drugs and release them in an active form. Adoptive immunotherapy experiments performed by transferring LAK cells loaded with drugs into recipient mice bearing lung metastases showed that Adriamycin-treated LAK cells had little protective effect while Iododoxorubicin-treated cells brought about a significant reduction in the number of lung metastases.

7.020

DEVELOPMENT AND CHARACTERIZATION OF A NEW BISPECIFIC MAb FOR RIS AND RIT OF CEA-EXPRESSING CARCINOMAS.

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A panel of MAbs specific for DTPA-radioisotope complexes had been generated.After accurate characterization, clone 1LF5 was selected to generate a hybrid-hybridoma secerning a bispecific MAb able to selectively target DTPA-radiosotope complexes on human tumour cells and therefore made resistant to geneticin. CEA-specific IgG secerning hybridoma F023C5 was selected as fusion partner and turned resistant to methotrexate.After PEG-mediated fusion was selected and estabished a hybrid-hybridoma cell line secerning MAb with double specificity for DTPA and CEA. The bispecific MAb has been purified and characterized for use in radioimmunoscintigraphy and radioimmunoteraphy of CEA-expressing carcinomas.

7.021

SYNERGISTIC ENHANCEMENT BY TUMOR NECROSIS FACTOR OF "IN VITRO" CYTOTOXICITY FROM CHEMOTHERAPEUTIC DRUGS TARGETED AT DNA TOPOISOMERASE I AND II.

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Recombinant human tumor necrosis factor (rHuTNF) is a cytokine, with some antitumor activities, released by stimulated monocytes-macrophages. "In vivo" and "in vitro" cytotoxicity studies testing the effectiveness of rHuTNF alone or in combination with chemotherapeutic agents have been carried out. We have evaluated the direct cytotoxic effect of rHuTNF on four human epithelial ovarian cancer cell lines "in vitro" (A2780, A2774, SW626, PA1), alone or in combination with VP16, Doxo, Epidoxo, mAMSA, Ellipticine, Topoisomerase II-targeted drugs, or in combination with Camptothecin, a Topoisomerase I-targeted drug, or in combination with CDDP, a not Topoisomerase interactive drug. Our results suggest that rHuTNF is directly cytotoxic and it is also able to induce a potentiation of the cytotoxicity of Topoisomerase-targeted drugs but it is unable to potentiate CDDP-cytotoxicity. These data represent a reasonable basis for combining rHuTNF with Topoisomerase I and II inhibitors within phase I studies. We suggest that rHuTNF may be a useful adjuvant to these drugs which have well-known antitumor activity.

7.022

BEHAVIOUR OF A RUTHENIUM(III) COMPLEX IN COMPARISON TO CISPLATIN IN MICE WITH SOLID METASTASIZING TUMORS.

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Ruthenium complexes appear to be the most promising tool for investigating new anticancer drugs within transition metals of platinum group. They match many favourable properties such as tumor tissue accumulation and chemical reactivities that *cis*-dichlorodiammine platinum(II) (cisplatin) does not exibit in biologic conditions. The present investigation shows the main differences of antitumor activity observed with a new ruthenium(III) complex characterized by the presence of dimethylsulphoxide (DMSO) and nitrogen-containing heterocycle (Im) ligands, namely [*trans*-RuDMSOImCl$_4$]. [*trans*-RuDMSOImCl$_4$] reduces the growth of Lewis lung carcinoma and of MCa mammary carcinoma of CBA mice with peaks of activity statistically not different from those of cisplatin but, unlike cisplatin, it significantly prolongs the survival time of the same animals as well. The effects on host survival are unrelated to the effects on primary tumor growth, are higher with the schedule of administration on days 1,5,9,13 and seem attributable to a selective antimetastatic effect, more pronounced than that on primary tumor growth. The antitumor effects of the ruthenium complex depend on the dosage used less than for cisplatin and are significantly pronounced also after rather low non-toxic dosages. These data stress the importance of screening new compounds of ruthenium origin for cancer chemotherapy.

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7.023

MEASUREMENT OF CELL GROWTH IN CYTOTOXICITY ASSAY USING A SOLUBLE TETRAZOLIUM FORMAZAN DERIVATIVE

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A semi-automated microculture cytotoxicity assay using MTT has already been described by our laboratory.In this assay, the amount of reduced MTT measured with the ELISA plate reader is proportional to the number of living cells.However, the reduced product had to be solubilized in DMSO which is a safety hazard for the laboratory personnel ; moreover, DMSO has a deleterious effect on laboratory plasticware and its use increases the inherent error of the assay.We have tested a new tetrazolium salt ,XTT, of which reduced form is soluble in cell culture. We report the optimization of the assay using human cell lines with respect to XTT concentration and volume ,incubation period,the amount of phenazine methosulfate used as a color enhancer.A linear relationship was obtained between the optical density at 450nm and the cell concentration .The assay was used both for anchorage dependent and for cells growing in suspension.The assaywas reproducible with a C.V. of less than 10%. This new assay offers many advantages since it is simple,reproducible and it avoids the DMSO solubilisation step.

7.024

TREATMENT OF OSTEOSARCOMA AND ADENOCARCINOMA CELLS IN VITRO WITH METHOTREXATE ANTIBODY CONJUGATES

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Chemotherapy has increased the five year survival rate from 25% to 65% in the last decade but its toxicity still limits its use in cancer therapy.In order to increse its efficiency,methotrexate was coupled to a sarcoma specific monoclonal antibody and to anti carcinoembryonic antigen. Since methotrexate (MTX) is usually coupled to macromolecular carriers through its carboxyl end with some loss of pharmacological activity, new derivatives of MTX were synthesized on the peptide synthetizer using the Fmoc method.Their free amino group was activated with glutaraldehyde and the activated drug was purified and conjugated to the antibody. This chemical bond is stable at physiological pH but easily lysed in the lysosomes after internalisation. These conjugates were tested in vitro respectively on human osteosarcoma cells and on colon cancer cells. we found that these conjugates inhibited dihydrofolate reductase in vitro;as a free drug,the new derivative was not toxic but after conjugation the drug was as toxic as free MTX. The lack of cytotoxicity of the free drug and the pharmacological activity of the conjugate suggest that this system could be used for a combined prodrug and targetting effect in cancer therapy.