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Differential metabolic fate of octadecyl-methyl-glycero-3-phosphocholine (ET18-OMe) in tumoral cells, normal cells and isolated and perfused liver.

Cytotoxicity of Ether Lipids (EL) *in vitro* is high and tumor specific but little effect was found against tumors *in vivo* . Since one possible reason for this lack of activity in the whole animal could be a high degree of metabolic degradation, we have studied the metabolic route of 1-octadecyl-2-methyl-glycero-3-phosphocholine (ET18-OMe) in tumor and normal cells *in vitro* and in a more complex system, for example the isolated and perfused rat liver.

ET18-OMe labeled with (^{3}H) in the position 9-10 of stearyl alcohol was incubated at 37°C for different periods of time with rat serum and eritrocytes, with HL60 (sensitive to EL effect) and K562 (resistant) leukemic cells, HT29 adenocarcinoma cells and in primary culture of hepatocytes. In other experiments labeled ET18-OMe was added to the perfusion fluid in a isolated and perfused liver system. ET18-OMe and its metabolites after lipid extraction and TLC separation were identified and quantified.

We found that in tumor cells 98% of ET18-OMe remained undegraded after 24 h incubation. Plasma and erythrocytes from rats metabolized not more than 4-5 % of the original compound.

In primary cultures of hepatocytes, incubated for 6 and 24 hours with ET18-OMe, radioactivity was incorporated in different compounds; three were products of direct metabolisation by phospoholipase C and D (1-O-alkyl-2-methyl-glycerol and 1-O-alkyl-2-O-methyl-phosphatidic acid respectively) and alkylhydrolase (stearyl alcohol); however other phospholipids, like phosphatidilcholine(PC) and phosphatidyl ethanolamine (PE), and in fatty acids (F.A) were also labeled. In isolated and perfused rat liver after 3 hours perfusion about 15% of total radioactivity was found in products of metabolisation by phospholipases C and D and alkylhydrolase.

We conclude that EL are substrates of phospholipase C and D, which are ubiquitary in the body, while tumor cells lack of this capacity.

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NEW IN VITRO APPROACH FOR INDIVIDUALIZATION OF TREATMENT IN CHILDREN WITH ACUTE LEUKEMIA (AL).

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To provide species-specific therapy of AL sensitivity of fresh tumor cells from 20 children (13-ALL, 6-ANNL, 1-CML in blast crisis) to the panel of cytostatic agents (ADR,ARA-C,VP-16,VCR, PRd,MTX) and cytokines (alpha and gamma IFN, TNF and new hybrid protein TNF-T<tymosin-alpha>) and their combinations was studied in MTT-assay.LC-50 can be variable ten-hundred times even when AL is of the same immunophenotype,cytogenetics, especially for PRd and MTX. Cytotoxic activity of both TNF and TNF-T was equal and without lineage dose-dependence, their combination with ARA-C was more effective then ARA-C alone, and in one case of TNF-T+ gamma-IFN combination even more effective then ARA-C. In 3 ALL patients when ARA-C was combined with gamma-IFN the LC-50 was 2-3 times less then for ARA-C alone. Cells survived treatment were incubated to receive the model of relapse and to explore it (immunophenotype, cytogenetic, sensitivity in MTT-assay). This will be compared with biology of the blasts in relapse if occur. Consolidation phase in newly diagnosed AL and relapse protocols will be based on this MTT-assay data.

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ANTITUMORAL ACTIVITY OF THE NEW ANTIESTROGEN RU 58668 ON THE HUMAN MAMMARY CELL LINE MCF-7.

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RU 58668 is a pure antiestrogen with potent antiproliferative activities in vitro on human mammary cell lines which express the estrogen receptor, it also induced the regression of MCF-7 tumors implanted in nude mice. To better investigate the antitumoral properties of this product we extented our study using cell cycle analysis of MCF-7 cells, invasiness in embryonic chick of very highly tumorigenic MCF-7 (MCF-7vht) and histological analysis of MCF-7 xenografted in nude mice. RU 58668 effect on MCF-7 cell cycle for 48 hr resulted in a large decrease of 32% cells in S-phase with a corresponding increase in G0/G1-phase cells. Effect on invasiveness of MCF-7vht resulted in a decrease of the presence of epithelial cells in chick embryonic heart. At last tumor from nude mice treated with RU 58668 demonstrated important stromal composition. Angiogenic activity was also observed. Thus, in addition with the in vivo studies these results argue that this antiestrogen is a potential candidate for the treatment of human estrogene sensitive mammary carcinoma.

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THE SOMATOSTATIN (SST) ANALOGUE BIM23014 INHIBITS GROWTH OF THE SST RECEPTOR (SSTR) POSITIVE SMALL CELL LUNG CARCINOMA (SCLC) XENOGRAFT : SCLC-6

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SCLC is a neuroendocrine tumour with a poor pronostic. SSTR for SST-14 and its analogue BIM23014 were characterized in 3 human SCLC xenografts transplanted in nude mice.One major complex of 57kDa was identified by both ligands in all 3 tumours by cross-linking assay.. Two minor complexes were only detected by SST-14: 90kDa in all 3 tumours and 70kDa in 2 out of the 3 (SCLC-6, SCLC-75). Analyzed by Northern hydrization, SSTR-I expression was detected in all 3 tumours whereas SSTR-II expression was only detected in 2 out of 3 (SCLC-6, SCLC-75). No expression of SSTR-III. The antiproliferative effect of BIM23014 was evaluated on SCLC-6 growing in nude mice. BIM23014 alone (250µg t. i. d., 5 days) or concomitantly administered to cis-Platinum (1.5mg/kg/day, 2 days) inhibited the proliferation at day 10: BIM23014 57%, cis-Platinum 57% and BIM23014 + cis-Platinum : 78%. Our data suggest that BIM23014, alone or in combination with cis-Platinum could have a potential in the treatment of SSTR positive SCLCs.

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ANALOGUES OF CB 1954 AS SUBSTRATES FOR DT DIAPHORASE

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CB 1954 (5-(aziridin-1-yl)-2,4-dinitrobenzamide) is a monofunctional alkylating agent that upon bioactivation by the enzyme DT diaphorase forms the difunctional cytotoxic species 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide (4-NHOH). This activation results in a 10,000 fold increase in cytotoxicity in cells able to carry out this bioreduction. Although rat tumour cell lines are sensitive towards CB 1954 as would be predicted by their levels of DT diaphorase, human tumour cell lines with similar levels of DT diaphorase are insensitive to CB 1954 but show a similar sensitivity to 4-NHOH. This is because human DT diaphorase carries out this reduction at a slower rate than the rat form of the enzyme. Over 30, C1 substituted analogues, of CB 1954 have been examined for their rate of reduction by rat, human or mouse forms of DT diaphorase using HPLC and for their cytotoxicity towards human cell lines using colony forming assays. Of these, more than half are better substrates for the human form of the enzyme than is CB 1954 for the rat. Some compounds exhibit excellent selective cytotoxicity in tissue culture for high DT diaphorase containing cell lines but they do not approach the gross toxicity of CB 1954 in rat cells. It has now been demonstrated that the C1 substituent is more important than previously recognised, not only in determining the compound's ability to act as a substrate for the enzyme, but also in influencing the reactivity of the 4-hydroxylamino group. Quantitative structure activity relationship (QSAR) studies, particularly between C1 substituents and 4-hydroxylamine reactivity, have been used as an aid to the design and synthesis of more effective drug analogues.

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2',2',-DIFLUORODEOXYCYTIDINE AND INTERFERON-α AGAINST HUMAN RENAL CELL CARCINOMA IN NUDE MICE

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2',2'-Difluorodeoxycytidine (dFdC; Gemcitabine; GEM) has been shown to inhibit tumor cell growth by blocking DNA-polymerases and ribonucleotide reductases. Due to previous in-vitro data, where we observed a nearly equal cytotoxicity of GEM and vinblastin (VBL) against established cell lines from human renal cell carcinoma (rcc) (SN12C, ACHN, A498, Caki 1) at peak-plasma concentrations, an experimental study was employed to evaluate the potency of GEM against xenografts from human rcc alone or in combination with interferon-α. Methods: Cell suspensions of SN12C and ACHN cells ($5x10^6$) have been inoculated subcutanously (s.c.) in 3-4 week old balb/c nu/nu mice, and in additon by tail vein injection ($1x10^6$ vital cells) at first tumor appearance. Therapy was initiated at a s.c. tumorvolume of 100 mm^3 and lasted 16 weeks either with GEM (40 mg/kg Bw i.p.) and rhu-IFN-α2a ($8x10^6$ IU/kg Bw s.c.), VBL (0,6 mg/kg Bw i.p.) and IFN-α2a, or GEM alone. Results: S.c. tumors derived from ACHN cells were inhibited by GEM+/-IFN-α in a more sufficient way than by VBL+IFN-α. Xenografts of SN12C cells at s.c. sites did not respond to any treatment, while GEM+IFN-α was effective to decrease pulmonary metastasis. Moreover all animals that underwent a remission recieved GEM+/-IFN-α exclusively. In conclusion GEM has been shown to be more effective to treat xenografts from human renal cell carcinoma than VBL+IFN-α. In combination with IFN-α, GEM recommeds itself as an most interesting chemotherapeutic drug for patients with advanced renal cell carcinoma, thus an already pre-existing clinical trial (phase II) should be extended.