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Biodistribution and antitumor activity of drugs encapsulated in thermosensitive liposomes in tumor bearing mice

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Purpose: Specific localization of antitumor drugs is a goal that, if achieved, may result in more effective cancer therapy. Liposomes, as drug carriers, can be designed to leak their contents due to an increase of permeability at the temperature of the gel-liquid crystal phase transition. By this method both a selective increase of drug in tumor areas, and the positive interaction between hyperthermia and certain drugs, can be used to increase the tumor cell killing.

Methods: Temperature sensitive liposomes were prepared using different proportion of the phospholipids DPPC, DSPC and DSPE, sphingomyelin (SM) and cholesterol (chol). Liposomes containing a fluorescent dye (calcein) or an antitumoral agent were injected i.p. in murine mammary carcinoma. To determine tissue calcein or drug concentrations the main organs were removed from bled mice and homogenized. Blood was collected in heparinized tubes. Calcein or drug extracted from plasma or tissues was quantitated fluorimetrically or fotometrically.

Results and Conclusions: We have studied and characterized the different thermal stability and the phase transition temperature of several lipid formulations in order to find out the formulation of thermosensitive liposomes exhibiting stability profile allowing a fast release of the encapsulated compound at hyperthermic temperature (43°C) as well as simultaneous high stability at 37°C in serum containing buffer. We obtained the best results with the lipid formulation DPPC:DSPC:chol = 5:4:2 (molar ratio) without or with the addition of SM (30% mol) or DSPE (10% mol) exhibiting a leakage suitable to reach the best hyperthermal release in "*in vivo*" experiments. At the present we are investigating the effects of liposome size and lipid composition on the stability, circulation time and accumulation in tumor using these thermosensitive liposomes in an animal tumor model. Furthermore we would like to test the antitumor activity of liposomes containing a drug, such as mitoxantrone or taxol.

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Drug-DNA interactions and extracellular metabolism of KW-2149: A novel mitomycin C analogue activated in serum

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Purpose: The aim of this study is to investigate the mechanisms of action of 7-N-{{2-{[2-(-glutamylamino)-ethyl}}mitomycin C (KW-2149), which is under investigation in clinical trials.

Methods: DNA sequence specificity of MMC, KW-2149 and its metabolites M-18 and M-16 was determined using the Taq polymerase stop assay. DNA interstrand crosslinking was measured using an agarose gel method and alkaline elution. To identify the fraction in serum which activates KW-2149 we used ion exchange chromatography, size fractionation and HPLC analysis.

Results: The cytotoxicity of KW-2149 *in vitro* was increased by a factor of approximately 200-fold by serum. Purification of serum has identified one fraction responsible for the metabolism of KW-2149 to M-18 and a different fraction responsible for the conversion of KW-2149 to a cytotoxic species. KW-2149, M-16 and M-18 show a similar DNA sequence specificity to MMC. KW-2149 and M-18 both crosslink DNA.

Conclusion: KW-2149 is metabolised in the presence of serum to a compound which enters cells more rapidly and crosslinks DNA to a greater extent than the parent compound. M-18 also requires activation by serum, and therefore is not the active metabolite. Further experiments are ongoing to determine the component(s) in serum which activate both KW-2149 and M-18.

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Correlation between BCL-2 expression and *ex vivo* chemosensitivity of advanced gynecologic cancers

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Purpose: High expression of bcl-2 is known to block apoptotic pathways and might thus contribute to chemoresistance. This study was performed to investigate the correlation between bcl-2 expression and *ex vivo* chemosensitivity of native tumor cells against paclitaxel (PTX), cisplatin (DDP), doxorubicin (DOX), PTX + DDP, and DOX + PTX.

Methods: A total of 22 samples derived from patients with various advanced gynecologic carcinomas (ovary: 18; breast: 2; endometrium: 2) were studied. Using three color flow cytometry, bcl-2 expression was measured on a cytokeratin defined tumor cell gate. Chemosensitivity was assessed by an ATP-based luminescence assay (ATP-TCA) using a sensitivity index (SI) and IC_{50} for a series of 6 drug concentrations.

Results: Specimens contained on average 43.3 ± 19.2% of tumor cells. The bcl-2 expression was 54.6 ± 29.2% with no difference between primary and recurrent tumors. Bcl-2 expression was not correlated with sensitivity against DDP, DOX, or DOX + PTX. However, weak but significant inverse correlations were found between bcl-2 expression and sensitivity against PTX ($r = -0.559$; $p = 0.007$) and PTX + DDP ($r = -0.455$; $p = 0.033$), respectively.

Conclusion: High bcl-2 expression which appears to adversely influence the activity of some PTX-based regimens is unlikely a predictor of an uniformly resistant phenotype expressed by advanced gynecologic malignancies.

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Lymphatic drug targeting with liposomal mitoxantrone for breast cancer – Results of a pilot study

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Purpose: Reaching a therapeutic sufficient dosage in lymphatic tissues by systemic drug application comprises a pharmacologically difficult problem in breast cancer. The maximum concentrations of mitoxantrone (MTX) in lymphatic tissue after systemic applications varies from 23 to 172 ng/g after cumulative dosage between 6 and 100 mg/m^2. We have evaluated the effect of liposomal drug targeting of MTX to the lymph nodes after intraparenchymatic application on the drug concentration in lymph nodes in-vivo.

Methods: 8 patients with N1 breast cancer received 48 h before operation 1 mg of MTX intraparenchymal at a defined location. 4 of 8 patients received the MTX encapsuled in liposomes. During operation samples of tumor site, breast tissue, axillary fat and lymph nodes were taken for determination of MTX concentration. Toxic reactions due to the drug application were not detected.

Results: The application of the free drug was accompanied by systemic concentrations between 0.36 and 1.56 ng/ml after 1 and 4 hours. After application of liposomal MTX only one patient showed a systemic concentration of 0.42 ng/ml. The concentration of mitoxantrone in lymph nodes showed values between 40 and 60 ng/g in lymph node tissue for the free drug; for the application of liposomal MTX the concentration in the lymph node varied between 90 and 6720 ng/g (mean value: 2730 ng/g).

Conclusion: Lymphatic drug targeting by liposomal MTX is able to improve drug concentrations in lymphatic tissue by locally administered medicaments.

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Augmentation of antitumor activity of 5'-deoxy-5-fluorouridine by IL-12 through the up-regulation of pyrimidine nucleoside phosphorylase in murine tumor models

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Purpose: 5'-deoxy-5-fluorouridine (5'-dFUrd, FURTULON®) and its derivative capecitabine are prodrugs of 5-FUra and activated by pyrimidine nucleoside phosphorylase (PyNPase) which is preferentially located in the tumor tissue. We have previously found that TNF-α, IL-1α, and IFN-γ up-regulate

PyNPase expression and increase the susceptibility of the tumor cells to 5'-dFUrd. In the present study, we examined the ability of IL-12 to augment the antitumor activity of 5'-dFUrd through the up-regulation of cytokines and PyNPase in murine tumor models

Results: 1) Administration of mIL-12 increased tumor levels of mIFN-γ, mIL-1α and PyNPase activity. The tumor level of mIFN-γ was higher than that of the serum level, indicating that mIFN-γ was produced in the tumor tissue. 2) Increases in tumor levels of mIFN-γ and PyNPase by mIL-12 were not observed in T-cell deficient mice, indicating that these processes were T-cell dependent. 3) Administration of mIL-12 and 5'-dFUrd in combination showed synergistic antitumor activity in the A755 mammary adenocarcinoma model. Furthermore, this combination induced remarkable prolongation of the survival and complete regression of the tumor.

Conclusion: IL-12, which up-regulate local cytokine production and PyNPase activity in the tumor tissues, would have additional therapeutic benefits in combination with 5'-dFUrd, as well as in combination with capecitabine.

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PGP and growth factor expression is cell cycle dependent; Expression and function is modulated by sequential TMX and IFN

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Purpose: Tamoxifen (TMX) has been shown to have a number of clinically relevant effects on hormone receptor and growth factor expression as well as on p-glycoprotein (PGP) expression and function. Interferons (IFN), at least in vitro, may potentiate these effects.

Methods: The effects of TMX and α-IFN on cell kinetics, growth factor expression and PGP expression and function in MCF-7 and MCF-7^{mdr} cells were examined. Cells were cultured in indicator free RPMI and stripped FCS in the presence of α-IFN $\pm$ TMX. Harvested cells were examined by immunocytochemistry (ICA) for ER, P24, PDGF, c-erbB-2 and PGP. Functional efflux and membrane vesicle studies were performed with ^{3}H-vinblastine (VB) utilising standard methodology.

Results: Expression of ER, P24 and PDGF was cell cycle related. TMX was growth inhibitory and modestly increased P24, PGP and c-erbB2 expression. Preincubation of cells with α-IFN prior to TMX exposure potentiated the effects of TMX on growth inhibition, P24, cerbB-2 and PGP expression, increased ER expression and led to decreased expression of PDGF. Short term exposure to TMX decreased VB efflux and was significantly increased by preincubation with α-IFN prior to the addition of TMX. The effects were ATP dependent, suggesting decreased efflux was due to modulation of PGP activity. TMX $\pm$ α-IFN increased PGP expression, but decreased function suggesting possible competitive inhibition.

Conclusions: Sequential α-IFN and TMX increases ER, P24 and c-erbB2 expression, decreases expression of PDGF and partially reverses the MDR-1 phenotype in vitro. Clinical studies examining the role of TMX and α-IFN in modulation of MDR-1 mediated drug resistance are indicated.

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DNA alkylation and interstrand crosslinking by treosulfan

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Purpose: The antitumour drug treosulfan (L-threitol 1,4-bismethane-sulfonate, Ovastat) is used clinically primarily in the treatment of advanced ovarian cancer and the lack of significant non-haematological toxicity suggests treosulfan as a candidate for high dose chemotherapy regimens with autologous stem cell reinfusion. The present study investigates the molecular mechanism of action of treosulfan.

Methods: Cytotoxicity was assessed in human tumour cells using the MTT assay. DNA interstrand crosslinking was measured in plasmid DNA using an agarose gel based method and in cells using alkaline elution. DNA sequence specificity was measured using a Taq polymerase stop assay.

Results: The pH-dependent, non-enzymatic conversion of treosulfan to epoxide species is required for cytotoxicity *in vitro*. Alkylation and interstrand crosslinking of plasmid DNA is observed following treosulfan treatment, again produced via the active epoxide species. Alkylation is sequence specific occurring at guanine bases with a preference for runs of contiguous guanines, as observed previously with alkylating agents such as nitrogen mustards. In treosulfan-treated human leukaemic K562 cells DNA crosslinks form slowly, reaching a peak at approximately 24 hours. Incubation of cells with the pre-formed epoxides shows faster and more efficient crosslinking. The sensitivity of cells to treosulfan was not determined by levels of either guanine-O6-alkyltransferase or glutathione.

Conclusion: The prodrug treosulfan acts as a DNA crosslinking agent following conversion to epoxide species.

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In vivo evaluation of the irinotecan-oxaliplatin combination

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Purpose: Irinotecan (Campto®, CPT-11) and oxaliplatin (Eloxatine®) are two new agents approved for the treatment of colon cancer. The goal of this study was to evaluate them in combination in tumor bearing mice.

Methods: Dose-response studies were performed following the intermittent i.v. administration of irinotecan, oxaliplatin, and their simultaneous combination, to B6D2F$_1$ mice bearing subcutaneous Glasgow osteogenic sarcoma (GOS). This model was chosen as it was found the only model with similar sensitivity to both agents. Efficacy was determined at the highest non toxic dose in each arm of the trial. The end point used was the log cell kill (tumor growth delay in days/3.32 $\times$ tumor doubling time in days).

Results: The single agents were found active at their respective highest non toxic dose, irinotecan: 349.8 mg/kg with a 2.1 log cell kill, and oxaliplatin: 10.2 mg/kg with a 2.3 log cell kill. Host recovery occurred within 10 and 6 days for Irinotecan and oxaliplatin, respectively. The optimal combination (irinotecan: 226.8 mg/kg and oxaliplatin: 10.8 mg/kg) was also very active with a 2.3 log cell kill. Full host recovery was obtained 10 days post therapy.

Conclusion: At equitoxic dosages, the simultaneous administration of i.v. irinotecan and oxaliplatin to GOS bearing mice produce a similar activity to that produced by each of the single agents.

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Kinetics of MTX-albumin conjugates in rats

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Pharmacokinetics, organ distribution and tumor uptake of methotrexate-albumin conjugates, derivatized at a molar ratio of 1:1, were compared with the properties of the native carrier protein and with native MTX.

Methods and Results: Rats bearing W-256 tumors received iv injections of residualizingly radiolabeled MTX-albumin or of residualizingly radiolabeled albumin or tritiated MTX. Pharmakokinetics of all compounds were determined by radioactivity, MTX-albumin and MTX were also measured by an immunologic assay (EMIT MTX) in plasma. After tumor and organ removal uptake rates were recorded. The distribution pattern of MTX-albumin was identical with that of native albumin. Area under curve calculations for plasma concentrations of MTX-albumin exceeded those of MTX by 120 fold. After 1 h about 4.2% of the injected dose of MTX-albumin had accumulated in the tumor compared to 0.11% of MTX. After 24 h tumor uptake rate of MTX-albumin increased to about 14%, whereas MTX declined to 0.04%. The liver uptake rate was 7.6% for the conjugate and 1.8% for MTX after 24 h.

Conclusion: Conjugation of MTX to albumin will dramatically alter MTX pharmacokinetics. Advantages of MTX-albumin conjugates are a very long plasma presence comparable to native albumin and high tumor accumulation rates.

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Albumin catabolism by tumors

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Albumin dominates the nitrogen and energy resources in blood. However, only limited data is available on its accumulation and catabolism by tumors. This was caused by the lack of suitable radiolabels for long-term follow-up of protein catabolism in-vivo. Conventional radiolabels like radioiodine are metabolically unstable. Tumors with high metabolic activity evade detection.