786 POSTER

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.

787 POSTER

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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788 POSTER

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.

789 POSTER

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.

790 POSTER

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