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.

791 POSTER

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.

792 POSTER

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.

793 POSTER

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.

794 POSTER

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.

795 POSTER

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.

Methods and Results: To study the uptake of rat serum albumin (RSA) by tumors we chose a conventional radioiodine label and in addition two residualizing radiolabels. It is known that residualizing ^{131}I-tyramine deoxisorbitol and ^{111}In-DTPA protein labels remain trapped at catabolic sites after lysosomal degradation of their carrier proteins. A Walker-256 carcinosarcoma with a tumor size of about 5% of the body weight accumulated more than 20% of the initially injected iv dose of ^{111}In-DTPA-RSA within 24 h. Tumor uptake rates for albumin exceeded those of the kidneys by about 5 times and those of the liver by about 3 times. It was estimated that one out of two albumin molecules trapped by an Ovarian-342 tumor must have been degraded during 72 h.

Conclusion: High uptake and degradation rates would make albumin an important nitrogen and energy source for these tumors. Albumin might also be an interesting carrier for delivering covalently attached chemotherapeutic agents into tumors by an alternative lysosomal route.

796 POSTER

Multidrug resistance modifiers: Molecular modelling using comparative molecular field analysis

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Purpose: The MDR modifiers differ in their chemical structure and main biological action, but they are supposed to share a common target site for reversal of MDR. In this study three-dimensional (3D) molecular models are derived integrating different classes of catamphiphilic drugs and yielding information about the regions around the molecules that are favorable or unfavorable for their anti-MDR activity.

Methods: 40 phenothiazines, thioxanthenes and structurally related drugs able to modulate MDR in doxorubicin (DOX) resistant human breast carcinoma cell line MCF7/DOX were used. The CoMFA method (Comparative Molecular Field Analysis) was applied to correlate the MDR overcoming activity with 3-D structural properties of the molecules.

Results: 3D-QSAR (Quantitative Structure-Activity Relationship) models were obtained for different classes of ligands using steric, electrostatic and lipophilic fields. All good CoMFA models include the lipophilicity potentials (mostly alone or in combination with the steric ones) and are able to predict 80–90% of the observed differences in anti-MDR activity of the modifiers.

Conclusion: The results obtained postulate the importance of the lipophilicity for anti-MDR activity of the drugs studied. They direct to the possibility for a more unspecific membrane-mediated binding mode of these MDR modulators.

797 POSTER

Characterization of multidrug resistant Ehrlich ascites tumor cells (EHR2) selected for resistance to mitoxantrone (MITOX)

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EHR2 was selected *in vivo* for resistance to MITOX. EHR2/MITOX was 6123, 33 and 30 fold resistant to MITOX, daunorubicin (DNR) and etoposide, but sensitive to vincristine. Compared with EHR2, Western blot analysis showed 5 fold increased expression of the multidrug resistance associated protein (MRP) in EHR2/MITOX, whereas P-glycoprotein (PGP) was decreased. Topoisomerase (TOPO) IIα was reduced to 1/3 in EHR2/MITOX relative to EHR2, whereas TOPO IIβ was present in EHR2 but absent in EHR2/MITOX. Net-accumulation (60 min) of DNR was reduced by 27% and the efflux was significantly increased in EHR2/MITOX. Flow cytometry showed that the nuclear/total cellular DNR fluorescence ratio was similar in EHR2 and EHR2/MITOX. EHR2/MITOX microsomes had a significant basal unstimulated ATPase activity and the apparent K_i value for inhibition by vanadate of the ATPase activity was not significantly different from the K_i value obtained for PGP-positive cells. However, verapamil (VER) inhibited the ATPase activity of EHR2/MITOX, whereas VER stimulated the ATPase activity of PGP-positive microsomes. In conclusion, the resistance in EHR2/MITOX appeared to be associated with 1) a quantitative reduction in TOPO II α and β, 2) increased expression of MRP, and 3) increased expression of a novel resistance protein with ATPase activity.

798 POSTER

Soft agar cloning evaluation of effects of amifostine on clonogenic growth of freshly explanted human tumors in short term exposure in vitro

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Purpose: The aims of our study were to examine the effects of amifostine (ami) in combination with the cytostatics cisplatin (cis), carboplatin (carbo) and doxorubicin (doxo) on growth of tumor cells from freshly explanted human tumors.

Methods: Single cell suspensions, prepared from freshly obtained solid human tumors or effusions as part of routine clinical measures, stored in liquid nitrogen or fresh, were exposed to different concentrations of ami for half an hour, and then for one hour in combination with cis, carbo and doxo as clinically used anti-tumor agents. Tumor cells were cultured in soft-agar in glass capillaries for 21–28 days and colony formation was evaluated using an inverted microscope.

Results: 51/56 tumors (91%) showed evaluable growth in controls.

Ami (1 h)	0.0	0 002	0 02	0.2	0.4 mmol/l
NaCl 0.9%	–	0%*	2%	12%	35%
Cis 0.2 μg/ml	63%	0%	4%	39%	75%
Carbo 0.3 μg/ml	73%	0%	2%	47%	86%
Doxo 0 04 μg/ml	84%	4%	12%	59%	84%

*% inhibited specimens (= colony growth < 0 5 × control)

Conclusion: The combination with ami partially reversed the inhibitoring effect of cytostatic agents at clinically relevant concentrations. This may be of potential importance for the use of ami in combination with chemotherapy.

799 POSTER

Role of chloroacetaldehyde for the cytotoxic efficacy of Ifosfamid: Examinations on human tumor and renal tubular cell lines

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Introduction: The Ifosfamide (IFO) metabolism consists of two different pathways, which lead to the alkylating metabolite 4-hydroxyifosfamide (4-OH-IFO) and to chloroacetaldehyde (CAA). CAA is supposed to cause neuro- and nephrotoxicity, but no direct antitumor effect was described so far.

Methods: Two human tumor cell lines (MX-1 and S-117) and a renal cell line (Landa Leiden LL) were exposed to 4-OH-IFO, CAA and their combination in concentrations akin to blood levels of patients treated with 5 g/m^2 Ifosfamide. Cell survival was measured using the MTT-Assay.

Results: Similar dose-response curves were found for both metabolites. IC_{50} values for S-117 cell survival reduction (4-OH-IFO:25.0 μmol/l, CAA:15.3 μmol/l) were nearly twice the concentration needed for the MX-1. Combination treatment resulted in an additive effect. Both metabolites exhibited similar toxic effects on the LL renal tubular cells.

Conclusion: Our results indicate that CAA has its own cytotoxic efficacy against tumor cell lines. Hence we conclude that the molecular mechanism of IFO cytotoxicity seems to be only in part an alkylating effect and that CAA may play a pivotal therapeutic role Preliminary results from experiments of xeno-transplanted MX-1 and S-117 tumors in the nude mice model, which were treated with CAA, seem to corroborate our in vitro findings.

800 POSTER

Antitumor activity of MTX-SA conjugates in rats

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Methotrexate-albumin conjugates differ favorably from native MTX in terms of plasma presence and of tumor uptake. The purpose of this study was to evaluate therapeutic efficacy of the novel conjugates in rodent tumor models.