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.