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.

Methods and Results: The maximum tolerated dose (MTD) for MTX and MTX-albumin was determined (2 mg/kg based on MTX injected on day 1, 3, and 7). 100 SD-rats bearing Walker-256 tumors received injections of the MTD and of MTD/2 of MTX, MTX-albumin and of mixtures containing MTD/2 or MTD/4 of both MTX and MTX-albumin (MTX-MIX). In addition 30 Copenhagen rats bearing a MTX resistant slowly growing Dunning Hi prostate adenocarcinoma (Du HI) were treated with the MTD of MTX and of MTX-albumin. No side effects were observed. MTX-albumin conjugates were more effective than MTX alone in terms of growth retardation of the Du Hi tumor ($p < 0.001$). In the Walker-256 tumor bearing rats, cures and growth retardation were observed with the lowest rates for MTX alone, than for MTX-albumin, and with the best results for the combination of MTX and MTX-albumin. This was confirmed for the MTD and MTD/2 group. At 1 mg/kg MTX cured 2 out of 10 rats, MTX-albumin 3 of 10, whereas a mixture 0.5 mg/kg of MTX and of 0.5 mg/kg MTX-albumin cured 6 out of 10 rats and prolonged the surviving time from 4.7 days to 7.3 days compared to MTX.

Conclusion: MTX-albumin conjugates show therapeutic activity in vivo. In combination with MTX additive effects were observed. MTX-albumin conjugates performed significantly better than the parent compound in a slow growing rodent tumor.

801 POSTER

The antihypercalcemic action of gallium-nitrate is not due to inhibition of parathormone or parathormone-related protein secretion in rats

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Purpose: Gallium nitrate (GaN), the anhydrate salt of the naturally occurring heavy metal, is able to reduce hypercalcemia of malignancy in vivo. Furthermore, GaN is able to reduce parathormone (PTH) secretion in parathyroid cells in vitro. We tested whether GaN is able to reduce PTH or parathormone-related protein (PTHrP) secretion in vivo.

Methods: We used female Fisher rats, weighing 160–200 g. Humoral hypercalcemia of malignancy was induced by subcutaneous inoculation of 10^6 Walker carcinosarcoma (WCS) 256 cells. PTH secretion was examined in normal animals, after inducing hypocalcemia with 100 mg/kg EDTA intraperitoneally.

Results: 40 mg/kg GaN led to a significant reduction of WCS tumor growth (5.1 ± 1.8 vs. 7.4 ± 2.2 g) and hypercalcemia (3.6 ± 0.5 vs. 4.3 ± 0.6 mmol/l) at day 8.40 mg/kg GaN did not influence PTHrP serum levels in WCS bearing rats at day 8 (27.8 ± 11.5 vs. 25.9 ± 6.2 pmol/l), whereas osteoclast surface (OcS/BS) was significantly reduced (3.5 ± 1.3 vs. $6.2 \pm 2.3\%$). EDTA-stimulated induction of PTH secretion in normal rats was not significantly reduced by 40 mg/kg GaN (133.8 ± 45.1 vs. 136.6 ± 66.8 pg/ml).

Conclusion: The antihypercalcemic effect of GaN is due to osteoclast inhibition and is not due to inhibition of PTHrP or PTH secretion in vivo.

802 POSTER

Stealth liposome entrapped doxorubicin (SLED) and cisplatin (SLEC) versus head and neck xenograft tumours

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Purpose: To study the effect of SLED and SLEC, compared to unentrapped doxorubicin (UD) and cisplatin (UC), in head and neck cancer xenograft tumours (HNCXT).

Materials and Methods: Groups of 8–10 nude mice with HNCXT received single i.v injections of one of the following agents: SLED, SLEC, UD or UC. Control animals received no therapy. Tumour volume was assessed on the day of treatment (Vo) and then 2–3 times per week. Mice were killed when the tumour had tripled its original volume (3Vo). Time taken to reach 3Vo was used as a surrogate measure of survival.

Results: Median times to 3Vo were as follows: 7.3 days (control); 9.3, 5.4, and 9.7 days (UD 50, 100 and 200 μg); 16.1, 18.3 and 40.6 days (SLED 50, 100 and 200 μg); 6.9 and 15.3 days (UC 100 and 250 μg); 15.9, 21.5 and 34.0 days (SLEC 100, 250 and 500 μg). Durable complete response or stable disease (>60 days) was seen after 200 μg SLED in half the mice. 500 μg UC caused the death of all animals at 5 days. No toxicity was seen with single dose SLED or SLEC.

Conclusion: SLED and SLEC show significant activity in HNCXT. Both SLED and SLEC were more active than their unentrapped counterparts. Clinical trials of both agents in patients with head and neck cancers are planned.

803 POSTER

Effects of MTA (multi-targeted antifolate, LY231514) on intracellular folate and nucleoside triphosphate pools in CCRF-CEM cells

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Purpose: MTA (LY231514) is a novel pyrrolo[2,3-d]pyrimidine-based antifolate, presently in phase II trials for various solid tumours. It has been shown to inhibit thymidylate synthase (TS), dihydrofolate reductase and glycinamide ribonucleotide formyltransferase *in vitro*. Both thymidine and hypoxanthine are required to completely reverse the cytotoxicity of MTA (at $\geq$30 nM) in CEM cells. The present study examined the effects of MTA on intracellular folate, ribo- and deoxyribo-nucleoside triphosphate (rNTP and dNTP) pools.

Methods: Intracellular folates were pre-labelled by culturing CCRF-CEM cells in medium containing ^{3}H-leucovorin. After drug treatment, the folates were extracted, treated with conjugase and analyzed by HPLC. Total NTPs were extracted in 60% ethanol. rNTPs were analyzed directly on HPLC, and dNTPs likewise after per-iodate degradation of rNTPs.

Results: Treatment with MTA (300 nM) for 4 h resulted in no detectable accumulation of dihydrofolate. Over 24 h, MTA caused little change in levels of rNTP, but induced a rapid loss of TTP, dCTP and dGTP (to <15%), with a concomitant rise in dATP (~30%).

Conclusion: Our data qualitatively resemble those reported for TS inhibitors (*Biochem Pharmacol* 1995; *49:* 819), suggesting that inhibiting the thymidylate cycle is a key effect of MTA in CCRF-CEM cells. Studies on the anti-purine effect of MTA are in progress.

804 POSTER

Clofazimine and B4121 sensitize an intrinsically resistant human colon cancer cell line to paclitaxel and taxotere

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Purpose: To investigate the potential of clofazimine and a more active derivative B4121, to sensitize three intrinsically resistant human colon cancer cell lines ($CaCo_2$, ATCC HTB 37; COLO 320DM, ATCC CCL 220; HT-29, ATCC HTB 38) to vinblastine, doxorubicin, daunorubicin, paclitaxel, taxotere and cisplatin at a non toxic, therapeutically relevant concentration of 0.25 μg/ml. Cyclosporin A (CsA) multidrug resistant (MDR)-modifying agent at 5 μg/ml was included for comparison.

Methods: Cell proliferation and P-glycoprotein (P-gp) expression were measured by colorimetric and flow cytometric procedures.

Results: The cell line expressing high levels of P-gp, COLO 320 DM, was susceptible to chemosensitization by the experimental agents for the P-gp substrates (paclitaxel, taxotere, daunorubicin, vinblastine and doxorubicin) but not for cisplatin. Clofazimine, B4121 and CsA increased the sensitivity of COLO 320 DM cells for paclitaxel 7, 30 and 47 fold and taxotere 5, 10 and 1460 fold respectively. $CaCO_2$ cells expressed low levels of P-gp and were only marginally susceptible to sensitization by these drugs whereas the HT-29, a P-gp negative cell line, was unaffected.

Conclusion: The riminophenazines might prove useful for inclusion in taxotere or paclitaxel chemotherapy of P-gp expressing colon cancers.

805 POSTER

Mapping drug distribution patterns in solid tumors: Toward conformal chemotherapy for local tumor control

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Purpose: Chemotherapeutic efficacy depends on concentration and duration of drug exposure to tumor cells. Extending our ability to map and predict local drug exposure may lead to generation of treatment algorithms such that rational conformal chemotherapy of solid tumors similar to conformal