the resistance to blood flow inherent to the dynamics of tumor growth. Such vascular remodeling can offer selective targets to pharmacologically modulate tumor perfusion and thereby improve the efficacy of conventional anti-cancer treatments. Radiotherapy and chemotherapy can, indeed, take advantage of a better tumor oxygenation and drug delivery, respectively, both partly dependent on the tumor blood supply.

Here, we showed that isolated tumor arterioles mounted in a pressure myograph have the ability, contrary to size-matched healthy arterioles, to contract in response to a transluminal pressure increase. This myogenic tone was exquisitely dependent on the endothelin-1 pathway since it was completely abolished by the selective ET_A antagonist BQ123. This selectivity was further supported by the large increase in endothelin-1 abundance in tumors (5 to 15-fold according to tumor models) and the higher density of the ET_A receptors in tumor vessels. We also documented by using laser doppler microprobes and imaging that administration of the ET_A antagonist led to a significant increase in tumor blood flow whereas the perfusion in control healthy tissue was not altered. Finally, we provided evidence that acute administration of BQ123 could significantly increase cyclophosphamide delivery to the tumor. The tumor response to low-dose, clinically-relevant fractionated radiotherapy was also significantly improved by the concomitant administration of the ET_A antagonist as anticipated by the observed increase in tumor oxygenation, as determined by EPR oximetry. The dose-dependency of the ET_A antagonist effects and the consistency of these findings in various mouse tumor models further emphasized the relevance of our data.

Thus, blocking the tumor-selective increase in the vascular endothelin-1/ET_A pathway unravels an important reserve of vasorelaxation which can be exploited to selectively increase tumor response to chemotherapy and radiotherapy.

601 POSTER

Increased transport of nanoparticle albumin-bound paclitaxel (ABI-007) by endothelial gp60-mediated caveolar transcytosis: a pathway inhibited by Taxol

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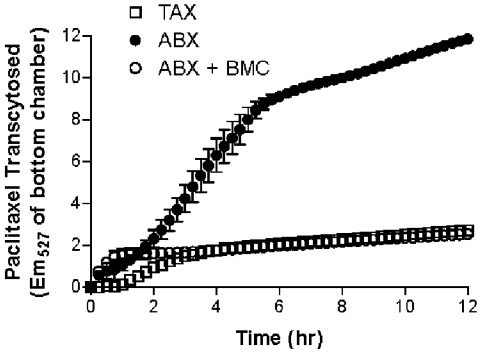
Background: Paclitaxel (P) albumin nanoparticles (Abraxane, ABX or ABI-007) demonstrated improved response rate over Taxol (TAX) in a phase 3 metastatic breast cancer trial (33% vs 19%, p<0.0001) (SABCS, O'Shaughnessy, 2003). Cremophor in TAX entraps P in micelles in plasma, reducing the P available for cellular partitioning (Sparreboom, Cancer Res 1999;59:1454). Studies in athymic mice have shown 30–40% higher intratumor P concentrations with ABX compared to equal doses of TAX (SABCS, Desai, 2003). Albumin is transported across endothelial cells (EC) by specific receptor (gp60)-mediated caveolar transport (John, Am J Physiol 2001;284:L187). Albumin-bound P in ABX may be transported across tumor microvessel EC by gp60, and this mechanism may be particularly active for ABX as compared to TAX. A series of studies were performed to evaluate binding and transport of P by human umbilical vein endothelial cells (HUVEC) and human lung microvessel endothelial cells (HLMVEC) for ABX and TAX.

Methods: ABX and TAX were formulated with fluorescent (FL) P. EC monolayers were grown to confluence on 96-well plates or transwell chambers. Binding was measured after incubation (1 hr @ 37ºC) with FL ABX or TAX in 96 well plates. For transport, FL ABX or TAX was added to the top chamber. P crossing the EC to the lower chamber was monitored for 12 hrs using a fluorometer in the presence or absence of selective inhibitors of transport.



Transcytosis of paclitaxel across EC monolayers.

Results: Binding of P to HUVEC was 10X higher for ABX than TAX. The transport of P from ABX across EC monolayers was enhanced 2–3 fold and 2–4 fold for HUVEC and HMVEC, respectively, as compared to TAX. Transport was dependent on albumin. Transport of P from ABX was inhibited by anti-SPARC antibody, known to bind gp60, the receptor required for caveolar albumin transcytosis. Known inhibitors of calveolar transcytosis, NEM and β-methyl cyclodextrin (BMC), also inhibited the transport of P from ABX across the endothelial monolayers (Figure). Inhibition of caveolar transport decreased ABX transport to the level of TAX.

Conclusion: P from ABX was actively transported across EC by gp60-mediated caveolar transcytosis, a process that was inhibited by TAX. P from ABX was transported at a 2–4 fold higher rate than TAX, which relied on a non-caveolar mechanism, presumed to be paracellular. Utilization of this pathway by albumin-bound paclitaxel may be responsible in part for increased intratumoral concentrations of P seen for ABX relative to TAX.

602 POSTER

SMA-pirarubicin micelles: highly efficient tumor targeting and therapeutic effects without apparent toxicity

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Material and Methods: Present SMA-micelles were prepared through multiple steps: (a) hydrolysis of styrene-co-maleic anhydride copolymer to generate poly (bicarboxylic acid), SMA, (b) mixing of SMA and pirarubicin in water, and shifting pH to low (~5), and then high (~10), and neutralization, (c) followed by purification through ultrafiltration using an exclusion size of 10kDa. Analysis was carried out by UV and fluorescence spectroscopy, TLC, molecular sieve chromatography (Sephadex G-50 and G-150) and elemental analyses. In vitro biological activity was tested with use of human breast cancer MCF-7, human colon adenocarcinoma SW480, HeLa and human epidermal carcinoma KB cells. For in vivo activity and safety studies, ddY and C57BL/6 mice bearing sarcoma S-180, adenocarcinoma Colon 38 tumor models were used respectively.

Results: Present micelle was found to bind to plasma albumin and showed an apparent MW of 94 kDa. In vitro cytotoxic activity of SMA-pirarubicin micelle measured by MTT assay showed 85–100% activity of equivalent molar concentrations of pirarubicin. The micelle at a total dose of 20-mg/kg pirarubicin equivalent, given over four aliquots (5mgx 4/kg) i.v., showed 100% tumor regression of S-180 tumor in all of tested animals, and 80% regression of colon 38 tumor bearing mice. The drug was safe up to 100mg/kg (given 25 mgx4/kg) body weight of pirarubicin equivalent in ddY mice and rats (15 fold more than LD_{50} of pirarubicin). Treated animal showed extended survival for more than 2 years of follow-up without tumor recurrence.

Conclusion: SMA–pirarubicin micelle appears potentially very interesting polymeric drug, exhibiting remarkably excellent antitumor effect and very high safety margin that warrants clinical evaluation.

603 POSTER

Effect of membrane transport proteins on the disposition of vincristine in brain-tumor bearing mice

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Vincristine [VCR] is a natural product microtubule inhibitor that is used to treat a variety of CNS tumors. Recently, we reported that P-glycoprotein [Pgp] influenced the penetration of paclitaxel into brain tumors [*Cancer Res*63:5114, 2003]. However, the impact of Pgp on brain tumor penetration of other agents, and the influence of other pumps on this process, is unknown. In the present study we use a mouse genetic approach to investigate how brain tumor concentrations of VCR, a substrate of both Pgp and multidrug resistance protein-1 [MRP1], is affected by these two pumps which are known to be expressed at the blood-brain barrier (Pgp) or the choroid plexus (Pgp and MRP1). Pharmacokinetic investigations were conducted in wild-type [wt], Pgp knockout [KO, mdr1a$^{-/-}$/1b$^{-/-}$], and MRP1 KO [mrp1$^{-/-}$] mice bearing intracerebral tumors derived from B16 melanoma cells. Mice had implanted jugular vein cannulas for drug administrations and carotid artery cannulas for serial collection of blood samples. First, pharmacokinetic [PK] parameters (total drug clearance [CL], volume of distribution at steady-state [Vss] and elimination half-life [t1/2]) were determined for each strain (4 mg/kg intravenous bolus). Based on the CL and Vss, a second series of studies were performed under steady-state VCR plasma concentrations to assess drug distribution, enumerated as tissue/plasma concentration ratios. VCR was measured in plasma, normal brain, brain tumor and bone marrow [BM] by a LC/MS technique. The Table below summarizes the PK data for VCR. These preliminary data show: i) Pgp KO mice exhibited a significant reduction in total clearance, consistent with the known contribution of Pgp to hepatobiliary elimination; ii) there is a large and unexpected increase in Vss in MRP1 KO mice, which may be

attributable to the absence of MRP1-mediated efflux from many tissues of the body; and iii) VCR uptake into brain tumors was not only elevated in Pgp KO mice, but also in MRP1 KO mice. We speculate that the surprising and prominent increase in brain tumor/plasma ratios in MRP1 KO mice may be related to the ability of MRP1 to attenuate the CSF drug concentrations. Finally, MRP1, and not Pgp, had a pronounced effect on BM distribution of VCR. The substantial effects of Pgp and MRP1 on VCR tissue distribution warrant further investigations, and may provide insights into how transport modulators may be utilized with therapeutic intent.

Mouse strain	Systemic PK parameters[a]		Steady-state tissue/plasma ratios	
	CL [L/min/kg]	Vss [L/kg]	Tumor/plasma	BM/plasma
Wt	0.091 (0.011)	19.2 (4.0)	2.28 (1.21)	4.48 (1.06)
P-gp KO	0.069 (0.024)	19.2 (6.1)	3.91 (2.25)	4.75 (0.52)
MRP1 KO	0.097 (0.025)	37.4 (14.3)	8.10 (0.79)	8.0 (2.48)

[a]Mean (SD), n = 4 -10/group.

604 POSTER

Secretory phospholipase A2 as tumour specific trigger for targeted delivery of a novel class of liposomal prodrug anticancer etherlipids

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Cancer treatment using traditional chemotherapeutics is often problematical due to severe side effects. These side effects could be diminished using specific tumour targeting of the drugs, thereby increasing the drug concentration in the tumour area and lowering the systemic exposure. Liposome based drug delivery has been thought to alleviate these problems, but so far no tumour specific release mechanism has been demonstrated.

We have designed a new generation of liposomes that are specifically degraded by secretory phospholipase A2 type IIA (sPLA2), which is secreted into the tumour microenvironment in a broad range of human tumours. Accumulation of the prodrug liposomes in the tumour is facilitated by the leaky tumour vasculature, known as the enhanced permeability and retention effect.

As a result of the high levels of sPLA2 in the tumour, sPLA2-mediated degradation of the prodrug liposomes results in the production of a free fatty acid and an anticancer etherlipid (AEL). AELs belong to a group of anticancer drugs possessing an anticancer effect both *in vitro*and *in vivo* (e.g. Edelfosine and Miltefosine) without causing mutagenic effects. Thus, the liposomes are built of proAEL lipids, which are converted to active AEL drugs by sPLA2 leading to a site-specific anticancer drug release in the tumour area.

Novel AELs were synthesized and tested for *in vitro* activity. Promising candidates were synthesized as proAELs and liposomal preparations of the proAELs were tested *in vitro* for sPLA2 mediated degradation and activation. Human tumour cell lines secreting sPLA2 were growth inhibited in a dose dependent nature (IC50 20–50 μM) whereas non sPLA2 secreting cells were unaffected up to 200 μM. The dependency of these effects upon sPLA2 was further reinforced by the lack of proAEL cytotoxicity when a specific sPLA2 inhibitor was present.

These data demonstrate that our novel prodrug liposome based drug delivery concept triggered by sPLA2 in human cancer is a promising approach for targeted delivery of anticancer ether lipids as well as conventional chemotherapeutics encapsulated in the liposomes. We are currently evaluating suitable mouse and rat models for in vivo proof of principle.

605 POSTER

Encapsulation of vinorelbine in sphingomyelin/cholesterol liposomes enhances the tumor exposure and antitumor activity of vinorelbine in human mammary and non-small cell lung cancer solid tumor models

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Vinorelbine (VRL) is a cell cycle specific, anti-mitotic drug that is clinically approved for the treatment of advanced non-small cell lung cancer (NSCLC) and metastatic breast cancer. *In vitro* studies have demonstrated that VRL cytotoxicity can be improved through increased duration of exposure. To evaluate whether increased tumor exposure would translate into an *in vivo* benefit, we have developed a sphingomyelin/cholesterol (SM/Chol) liposome formulation of vinorelbine (INX-0125) that has optimized drug release properties *in vivo*. The pharmacokinetics, tumor accumulation and anti-tumor activity of INX-0125 and VRL were evaluated in nude mice with subcutaneous human MX-1 mammary carcinoma tumors. Liposome encapsulation of VRL resulted in an approximately two order of magnitude increase in plasma AUC, as well as increased drug levels in MX-1 tumors compared with VRL, consistent with the increased circulation time of INX-0125. The tumor AUC of INX-0125 injected intravenously at 20 mg/kg was approximately 10-fold higher than the same dose of VRL and resulted in significant improvements in anti-tumor activity. MX-1 bearing mice treated with INX-0125 at 20 mg/kg showed complete tumor regression, with all animals being tumor free at the end of the study (day 91). In addition, 20 and 10 mg/kg doses of INX-0125 resulted in tumor growth delays (T-C) of >60 and 32.4 days respectively, whereas the equivalent doses of VRL exhibited a T-C of 28.5 and 7.3 days, respectively. Empty SM/Chol liposomes were inactive in this model. Significant improvements in anti-tumor activity were also observed for INX-0125 relative to VRL in several other mammary and NSCLC solid tumor models. The combination of significantly improved pharmacokinetic properties, increased drug delivery to solid tumors and the improved anti-tumor activity of INX-0125 relative to VRL in human mammary and NSCLC xenograft models justifies further development of this promising liposomal formulation of vinorelbine.

606 POSTER

Phase I and pharmacological study of AP5346, an HPMA copolymer-linked DACH platinum therapeutic, in patients with solid progressive tumors

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AP5346 consists of a cytotoxic 1,2-diamino-cyclohexane platinum complex (DACH-Pt) linked to a water-soluble, biocompatible copolymer, N-(2-hydroxypropyl)methacrylamide (HPMA), which, in animal models, has been shown to significantly increase platinum (Pt) accumulation in tumors, presumably via the enhanced permeability and retention (EPR) effect. The main objectives of this Phase I study were to determine the maximum tolerated dose (MTD), dose limiting toxicities (DLTs), the recommended dose (RD) for phase II and pharmacokinetic (PK)/pharmacodynamic (PD) parameters. Dose escalation was based on the accelerated titration method followed by modified Fibonacci method. AP5346 was administered as a 1-h IV infusion days 1, 8 and 15 of each 28-day cycle. The starting dose was 40 mg Pt/m^2. Pt analysis was performed in plasma and plasma ultrafiltrates by AAS and DNA adducts by a ^{32}P-postlabeling assay. To date, 15 patients have been enrolled (8 female, 7 male), median WHO PS 1 (range 0–2), median age 51 (range 32–74). AP5346 was administered at the following dose levels: 40, 80, 160, 320, 640 and 1280 mg Pt/m^2. Probable drug related toxicities seen to date at all dose levels included CTC grade 1 or 2 nausea and vomiting requiring anti-emetic prophylaxis at high doses, anorexia, asthenia and diarrhea. Two patients experienced a dose-limiting Grade 4 neutropenia during the first cycle at 1280 mg Pt/m^2. One patient was withdrawn from the study after 2 cycles at 640 mg Pt/m2, and another patient was delayed for a 3rd cycle, both due to renal toxicity. A pre-hydration and urine alkalinization protocol has been implemented at the 1280 mg Pt/m^2 dose level. In addition, two patients at the lower dose levels of 160 and 320 mg Pt/m^2 experienced hypersensitivity reactions to the drug. PK analysis revealed a linear relationship between dose and AUC of total Pt. Estimated terminal half-life of the total Pt in plasma was 78±17 h. One patient with melanoma of the auricular area and metastasis to the lung experienced a partial response by RECIST criteria after two cycles of treatment (1st cycle at 1280 mg Pt/m^2, reduced to 640 mg Pt/m^2 for 2nd cycle) and is undergoing a 4th cycle. In conclusion, the MTD of one cycle of AP5346 has been reached and enrollment in the study is continuing at a lower dose level. Updated results will be presented at the meeting.