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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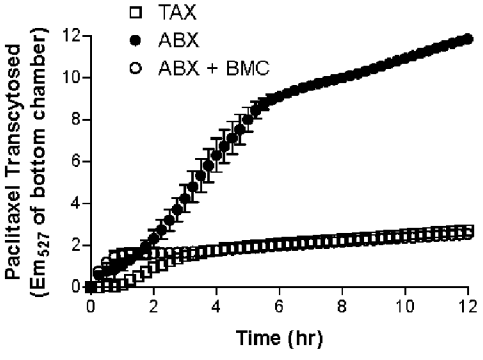
Background: Paclitaxel (P) albumin nanoparticles (Abraxane, ABX or ABI-007) demonstrated improved response rate over Taxol (TAX) in a phase 3 metastatic breast cancer trial (33% vs 19%, p<0.0001) (SABCS, O'Shaughnessy, 2003). Cremophor in TAX entraps P in micelles in plasma, reducing the P available 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