apparatus and a pressure gradient is applied across the membrane by raising the fluid level in the tube above that of the bottom chamber. Convective fluid flow is determined by weighing the medium in the bottom chamber as a function of time. Doxorubicin was added to the top chamber and its ability to cross the multicell layer was determined by HPLC in the presence and absence of a pressure gradient.
Results: Using a physiologically relevant pressure gradient of 28.8 mmHg, convective fluid flow progressively decreased as the thickness of the multicell layer increased. DLD-1 multicell layers with a thickness of 47.6±11.2 microns completely impeded convective fluid flow (<0.01 ml/min). Using a multicell layer of 12.9±3.0 microns and a pressure gradient of 28.8 mmHg, convective fluid flow was 0.192 ml/min. Under these conditions, the rate of penetration of doxorubicin across the multicell layer was 75 fold greater than when no pressure gradient exists.
Conclusions: This study demonstrates that the absence of convective fluid flow significantly reduces drug penetration through multicell layers. By increasing the thickness of the multicell layer and blocking convective fluid flow, this system provides an experimental model for evaluating strategies designed to re-establish convective fluid flow by targeting cell:cell or cell:matrix interactions.

428 POSTER

Targeting delivery systems mediated by a novel peptide for breast cancer therapy and imaging

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Anticancer drugs lack selective toxicity leads to their dose-limiting side effects which compromise clinical outcome. Targeting liposomes that bind to surface receptors of cancer cells is a recognized strategy for improving the therapeutic effectiveness of conventional chemotherapeutics. In this study, we isolated several ligands from a phage-displayed peptide library that bind to breast cancer cells. Targeting peptides were found to bind to breast cancer cells *in vitro* and breast cancer xenografts *in vivo*. The targeting peptide-linked liposomes were capable of translocating across the plasma membrane into endosomes through receptor-mediated endocytosis. Targeting peptides also recognized the tumor tissue in surgical specimens of breast cancer patients, with a positive rate of 90%. The tumor site fluorescent intensity in the mice treated with targeting peptide-linked quantum dots (QD) was around 28-fold of that in the mice treated with QD. When the targeting peptides were coupled to liposomes carrying doxorubicin, the therapeutic index against breast cancer xenografts was markedly enhanced. We conclude that the targeting peptides may be used to improve the systemic chemotherapy of breast cancer or to diagnose this malignancy.

429 POSTER

Design and development of nanocarrier for efficient drug delivery into the brain

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The success in the treatment of brain cancer or brain metastases by chemotherapy is very limited because of the efficient blood–brain barrier (BBB) which prevents most drugs from reaching tumour cells in the brain. An encapsulation of cytostatic drugs into liposomal nanocarrier may help to overcome the tight cell layer of the BBB and to enhance the therapeutic effect.
The aim of the study was to develop Trojan Horse Liposomes (THL) for an improved drug transport of anticancer drugs across the BBB to enhance the anti-tumour effect. In a first part we optimized the membrane properties of vesicles with respect to a better passive uptake by and transport through a tight cell barrier *in vitro*. In the second part, the most efficient liposomes were surface modified for an active targeting using a 19-mer peptide sequence (Angiopep) to increase specific uptake and transcellular transport. It was already shown that Angiopep passes the BBB by a physiological transcytosis process mediated by the low density lipoprotein receptor related protein (LRP) receptors expressed on the surface of the BBB and brain cancer cells.
In this study we could demonstrate *in vitro* that the liposomal nanocarrier with an optimised composition of the lipid membrane (L2) and the Angiopep ligand, bound to the liposomal surface, significantly improved cellular uptake by epithelial (MDCK II), endothelial (bEnd.3) and glioama (U373) cells. Transcytosis through a tight cell barrier using the MDCK II model demonstrated that the highest amount of calcein passed through the cell layer was induced by the THL-L2 formulation.
In vivo studies using a human xenograft brain metastasis model (MT-3 breast cancer) in nude mice showed already a significantly better anti-tumour effect of Mitoxantrone loaded L2-liposomes compared to the free drug. In addition, clearly fewer side effects like gastrointestinal complications, weight loss and dehydration were observed.
The therapeutic effect was further improved if THL-L2 liposomes were used, resulting in an additional tumour volume reduction as compared to L2.
Our results demonstrate that the obstacle in the chemotherapeutic treatment of brain tumours and metastases in the brain can be overcome by liposomal nanocarrier with carefully composed bilayer and surface modification with the Angiopep sequence to obtain an improved transport through the BBB.

Natural products, new cytotoxics, clinical trials

430 POSTER

Dose finding of inecalcitol, a new VDR agonist, in combination with docetaxel–prednisone regimen for castrate-resistant prostate cancer (HRPC) patients (pts)

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Introduction: Inecalcitol is a novel Vitamin D Receptor (VDR) agonist which shows high antiproliferative effects in human cancer cell lines and a 100-fold lower hypercalcemic activity than calcitriol the natural ligand of VDR.
Methods: In this study, escalating dosages of inecalcitol were combined to chemotherapy in chemonaive CRPC patients. Safety and efficacy were evaluated in groups of 3−6 patients receiving inecalcitol every other day (qod), once a day (qd) or twice a day (bid) on a 21-day cycle in combination with docetaxel (75 mg/m^2 q3w) and oral prednisone (5 mg bid). Biphosphonates were prohibited during the first cycle. Patients received up to six cycles unless unacceptable toxicity or disease progression. Primary endpoint was Dose Limiting Toxicity (DLT) defined as grade 3 hypercalcemia within the first cycle. Calcemia, creatininemia and CBC were assessed weekly; biochemistry, ECG and PSA every 3 weeks. Efficacy endpoint was PSA response defined as ⩾30% decline within 3 months.
Results: Eight dose levels from 40 to 8000 μg have been evaluated in 54 pts; 83 % had bone metastases, 12% had visceral disease only. Median age was 71 years [range, 49−87], median Gleason score (Gs) 7 [42% Gs 10−8, 58% Gs 7−6] and median PSA 31.7 ng/mL [range, 0.8–962.4]. 5 patients had PSA level <2ng/mL.
Up to the daily dose of 4000 μg no significant changes in calcemia have been observed. Only hypercalcemia grade1 of short duration occurred in 24% of patients.
DLT (hypercalcemia G3) occurred in 2 out of 4 patients receiving 8000 μg/day (4000 μg bid). DLT was observed after 1 week and 2 weeks of treatment respectively. In both cases, calcemia normalized in few days after interruption of treatment. The 2 other patients treated at this dose level experienced hypercalcemia G2 and were switched to 4000 μg qd.
The Maximum Tolerated Dose (MTD) is defined at 4000 μg qd since none of the patients treated at this dose level experienced hypercalcemia > G1 even after more than 3 cycles of treatment. Blood levels of inecalcitol reached antiproliferative concentrations without inducing hypercalcemia.
Most of the adverse events (AES) reported were grade 2 except hematological toxicities which were not increased or decreased with the addition of inecalcitol. Asthenia (48%), constipation (33%), diarrhea (31%), nausea (17%), headache (12%), vomiting (7%), mucitis/mucositis (7%), anorexia (7%), fever (7%) were the most frequent AEs. None was considered related to inecalcitol. Frequency of AEs related to docetaxel did not seem to be modified.
Efficacy analyses have been performed on 47 pts treated up to the dose of 2000 μg. PSA responses with this combination are encouraging. 83% of the treated patients had ⩾30% PSA decline within 3 months of treatment since in historical data around 65% are responder with docetaxel as a single agent. Moreover, 50% PSA decline was observed in 67% of pts and time to biochemical relapse was 169 days.
Conclusion: High antiproliferative daily dose of inecalcitol, a new VDR, agonist has been safely used in combination with docetaxel in HRPC patients. This combination treatment shows encouraging PSA response with 83% of responder. A multicenter randomized double blind Phase 3 study is forecasted to confirm these results.