nude mice. These xenografts grew multifocally in both lungs and all lobes of the lung and were resistant to intravenous Cisplatin treatment, closely mimicking the NSCLC patients who have unresectable and chemo-resistant tumors. Our therapeutic approach included an efficient nonviral gene delivery system (ENGD) that is composed of multiple cationic polymers at an optimal combination ratio, and a therapeutic gene (*badp*) that is a modified proapoptotic gene, *bad*, that carries mutatant Ser/Thr phosphorylation sites.
Results: *In vitro*, the ENGD-carried mutant *bad* (ENGD-*badp*) significantly induced apoptosis in human NSCLC cell lines H322, H358, H460, and A549. The apoptotic index of cells treated with *badp* was 2- to 6-fold higher than that of the cells treated with wild-type *bad* under the same experimental conditions. *In vivo*, intratracheal injections of ENGD-*badp* effectively inhibited the growth of H358 (%TGI = 61%) and A549 (%TGI = 78%) xenografts in nude mice. In contrast, iv Cisplatin at the maximum tolerated dose was not effective. Moreover, the combination of the ENGD-badp and Cisplatin further increased the average lifespan of the tumor-bearing mice by 60% to 210% compared with the single-agent therapeutics alone (68% vs 7% and 219% vs 7%).
Conclusions: Our studies support the hypothesis that locoregional administration of a proapoptotic gene could effectively inhibit the local chemo-resistant NSCLC tumors and sensitize them for further chemotherapy.

611 POSTER

Introduction of specificity into cytotoxic drugs and improvement of therapeutic index by kinase-mediated trapping

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Non-specific cytotoxic agents continue to play a major role in cancer therapy. In addition to their traditional role, they are essential partners for optimal activity of signal transduction inhibitors in most solid tumor settings and may also be useful in metronomic anti-angiogenic regimens. Despite the continued and potentially expanded use of these agents, their activity is constrained by dose-limiting side effects. Some cytotoxic drugs have been improved via the use of extracellular targeting and pro-drug approaches. However, improvements have been highly drug and disease specific, and suffer from drawbacks with respect to the efficiency of cellular uptake and drug release. We have developed broadly applicable methods for engineering selectivity into non-specific cytotoxic drugs. Our approach takes advantage of well-validated drug discovery targets, i.e. kinases that are aberrantly activated or overexpressed in tumor cells and tumor associated endothelium. Instead of making inhibitors of these cancer-causing enzymes, we have developed methods to covalently conjugate protein and small molecule kinase substrates to cytotoxic drugs. The resulting peptide and small molecule conjugates retain both drug and kinase substrate activities, are stable in serum, and are able to diffuse across cell membranes. We have proposed that selective phosphorylation of the conjugate by an elevated or aberrantly activated kinase can trap the conjugate in the disease or disease-associated cell, preventing exit by passive diffusion and increasing therapeutic index. We have produced bifunctionally active conjugates of paclitaxel and vinblastine with peptide substrates of Src tyrosine kinase and Akt serine/threonine kinase. We have also produced paclitaxel-thymidine conjugates. The conjugates retain 50 to >100% of the parent drug activity and 35 to >100% of the substrate phosphorylation potential. Furthermore, peptide and small molecule conjugates were produced that are stable in serum, exhibit cytotoxic EC50s within 5 to 10-fold of the values obtained for the parent drugs, and in the case of paclitaxel-peptide conjugates, are water soluble. The therapeutic index of each conjugate was determined by comparing cytotoxic EC50s against normal fibroblasts to those obtained with breast, lung and colon carcinoma cells, as well as normal endothelial cells. Conjugates from 4 different drug-substrate classes were obtained that exhibit a 4 to 31-fold increase in therapeutic index, relative to parent drug. Our results demonstrate that it is possible to significantly increase the cell-based therapeutic index of a non-specific cytotoxic agent by linking it to the substrate of a disease-causing kinase. This approach appears to be broadly applicable to non-specific drugs used for the treatment of cancer and many other diseases caused by chronic or undesirable activation of kinases.

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Mitochondrial-mediated apoptosis is induced by cationic polymers used in gene transfer

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A wide range of synthetic polycations in linear, branched, or dendrimer form have been used to condense DNA into structures amenable to cellular internalization via endocytosis. Polycations can destabilize endosomal membranes or act as proton sponges; they buffer the low pH in the endosomes and potentially induce membrane rupture, resulting in the release of polycation/DNA complex into the cytoplasm. The polycationic nature of the gene-delivery vehicles can induce cytotoxicity, but the mechanisms are poorly understood. Therefore, cytotoxic gene-delivery systems may compromise transcription and translation processes and potentially limit protein expression. In order to understand the molecular basis of polycation induced cytotoxicity, we studied the effect of a number of commonly used polycations on mitochondrial functions in isolated mitochondria from rat liver as well as directly in Jurkat cells. Mitochondria are key integrators of a cell's life and death decisions since they play a major role in subcellular partitioning of death-regulating biochemical signals. For example, the Bcl-2-sensitive release of proteins such as cytochrome *c* from the mitochodrial intermembrane space into the cytoplasm is a critical early event in apoptosis. Upon permeabilization or rupture of the outer mitochondrial membrane, cytochrome *c* binds to Apaf-1, leading to allosteric activation of pro-caspase-9. This in turn proteolytically activates caspase-3, one of the principal proteases that participates in the execution of cell death. A decrease in mitochondrial membrane potential ($\Delta\varphi$) due to permeability transition is also an early event in several types of apoptosis. We have demonstrated that at very low concentrations, polycations can affect mitochondrial respiration and $\Delta\varphi$; these events were followed by cytochrome *c* release from mitochondrial intermembrane in mitochondrial suspensions and in Jurkat cells. Changes in mitochondrial $\Delta\varphi$ in Jurkat cells was confirmed by Mitosensor test. Detection of phosphatidylserine translocation to the cell surface using Annexin V, and activated caspase-3 further confirmed the initiation of a mitochondrion-mediated apoptotic programme in Jurkat cells. These observations provide a molecular explanation for the previously reported immediate or delayed cytotoxicity following gene transfer with polycations. The results from this study may help to design novel materials with high transfection efficiencies suitable for clinical gene therapy.

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Plasma and tissue distribution of selenium after 5-methylselenocysteine (MSC) or seleno-L-methionine (SLM) in mice bearing human tumor xenografts

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Background: We previously reported that MSC and SLM, organic selenium compounds, increase the cure rates of human squamous cell carcinoma of the head and neck xenografts (HNSCC), FaDu and A253, in mice when combined with irinotecan. FaDu xenografts were more responsive to MSC/irinotecan or SLM/irinotecan combination (100% cure rate) than A253 xenografts (60% cure rate). MSC and SLM also protect the animals from irinotecan induced toxicities and lethalities (Cao et al., Clin. Cancer Res., *10*:2561–2569, 2004). To help understand the selectivity of selenium action and its protective effects, we initiated this plasma and tissue distribution study for selenium after MSC and SLM.
Material and Methods: Nude mice bearing bilaterally established (200–250 mg) FaDu and A253 tumors were treated daily with oral MSC at different doses (0.005, 0.01, 0.05, 0.1, 0.2 mg/mouse/d $\times$ 7) or SLM at (0.01, 0.1, and 0.2mg/mouse/d $\times$ 7). Plasma, tumor tissue, and normal tissues (liver, kidney, small intestine, large intestine, and bone marrow) samples were collected at 2h post last dose. Samples were analyzed for selenium concentration using Atomic Absorption Spectrophotometry.
Results: The data show that the base level of total selenium in the plasma of untreated mice is 4.5$\pm$0.5 μM. This level increased to 14.2$\pm$5.1 μM, 23.21$\pm$7.0 μM, and 47.7$\pm$2.1 μM at 2h post SLM administration of 0.01 (the minimal dose for modulation effect), 0.1, and 0.2 mg/mouse/d $\times$ 7 respectively. 94–96% of selenium in plasma is protein bound. The concentration of total plasma selenium increased post administration of MSC (same doses above) to 5.1$\pm$0.56 μM, 9.9$\pm$0.7 μM, and 12.8$\pm$1.6 μM respectively, with 12–21% of total selenium in free form.

The total intra-tumoral selenium concentration achieved at 2h post MSC (0.2 mg/mouse × 7) was higher in FaDu tumors than in A253. In normal tissues, total selenium increased post MSC (0.2 mg/mouse × 7) with the highest concentration in the liver then kidney, small intestine, large intestine, and bone marrow.
Conclusions: The data suggest that at least a 14.2 μM concentration of selenium is required after SLM and 5.08 μM concentration for MSC to achieve the optimal therapeutic modulation of anti-tumor activity and protection from irinotecan induced toxicity. The higher concentration of selenium in FaDu tumor (more responsive) than in A253 (less responsive) could be responsible in part for the observed selective increase in antitumor activity of irinotecan, when administered in combination with selenium containing compounds. The levels of selenium after MSC relative to their untreated control in bone marrow and small intestine may be related to its protective effect against irinotecan induced toxicities. The tissue distribution studies with SLM are ongoing.

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Increased tumor extravasation with an elastin-like polypeptide systemic thermal pump

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Achieving a therapeutic concentration of chemotherapeutics in solid tumors while minimizing systemic toxicity remains a critical problem in the treatment of cancer. Macromolecular drug carriers are an attractive method for delivery of cancer therapeutics because they passively target tumors through the enhanced permeability and retention effect and have longer plasma half-lives, which result in improved therapeutic efficacy. In our ongoing studies, we use a thermally responsive elastin-like polypeptide (ELP) as a macromolecular drug carrier. ELPs belong to a unique class of biopolymers that undergo an inverse temperature phase transition; they are soluble at temperatures below their transition temperature (T_t) but become insoluble and aggregate at temperatures above their T_t. In this abstract we investigate the feasibility of an ELP systemic thermal pump drug delivery strategy described as follows. First, a heat-sensitive ELP is designed with a T_t of about 40ºC and conjugated to an anticancer drug. Then the tumor is heated with externally focused hyperthermia (T_h = 42ºC) and the ELP is administered intravenously. Upon entering the tumor vasculature, the ELP will undergo its phase transition and form adherent aggregates within the tumor vasculature (T_t<T_h), therefore concentrating the ELP in the tumor vasculature although it is trapped in immobile aggregates. Next, the tumor temperature is reduced to normothermia (T_n=37ºC), which is accompanied by a transient increase in plasma concentration of the ELP as its aggregates dissolve (T_n<T_t). The selective increase in plasma concentration only in the tumor drives more ELP across the tumor blood vessel wall resulting in increased extravascular accumulation. By cycling the tumor temperature between hyperthermic and normothermic temperatures, we may be able to repetitively pump ELP drug carriers into the extravascular space of a tumor. It was our goal to directly assess the performance of the ELP systemic thermal pump in a dorsal fold window chamber model in combination with laser scanning confocal microscopy. In preliminary experiments, we have found that the ELP aggregates form rapidly in hyperthermic tumor vasculature and grow in size during the heat treatment. Upon return to normothermia (one thermal cycle), there is a significant increase in extravascular accumulation of a thermally responsive ELP compared with a thermally insensitive control ($P<0.05$ ANOVA). We observe an increase in plasma concentration of freely mobile ELP as the aggregates dissolve that is most likely the cause of increased in extravascular accumulation. In conclusion, the ELP systemic thermal pump is a viable drug delivery strategy in order to increase extravascular accumulation of macromolecular drug carriers.

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Preliminary results of a phase I/II study of inhaled doxorubicin combined with docetaxel and cisplatin for advanced non-small cell lung cancer

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The addition of molecular-targeted agents to the initial treatment of NSCLC has failed to improve results seen with chemotherapy alone. Targeted delivery of agents to the lung through inhalation has been used in selected situations. Preclinical studies support the use of inhaled therapeutic agents for the treatment of cancer. We have reported the results of a phase I study of inhaled doxorubicin/ Resmycin™ (DOX) in patients with advanced cancers affecting the lungs. 7.5 mg/m^2 was the recommended phase II dose in this study, with minimal systemic toxicity. Dose limiting toxicity was found in the lungs at higher doses. Using the Oncomyst® CDD-2a delivery device we designed a combination trial of DOX, docetaxel (D) and cisplatin (P) administered every three weeks. Patients were required to have acceptable organ function, including PFT entry criteria of ⩾50% predicted FVC, $FEV_{1.0}$ and DLCO, and resting and exercise oxygen saturation of ⩾90% and 85%, respectively. D/P were administered at 75 mg/m^2 with routine premedications and hydration. Since DOX had not been combined with other agents, we performed a 2-level phase I portion starting at 6.0 mg/m^2 of DOX, given prior to D/P, with escalation to 7.5 mg/m^2 if this was safe. To date, we have treated 9 patients (28 cycles at level 1, 9 at level 2). 1 patient experienced febrile neutropenia during cycle 1, and had their D reduced by 25% for subsequent cycles. 1 patient had grade 3 nausea and vomiting after cycle 2 and had 25% reduction of P. One patient (at 6.0 mg/m^2) had possible interstitial infiltrates (on lung CT scan) prior to cycle 2 (unchanged PFTs), but also had worsening of tracheal narrowing that may have led to hypoventilation. This patient was taken off study. An additional 3 patients were treated at this dose level with no further pulmonary problems. To date three patients have been treated at the 7.5 mg/m^2 dose level without pulmonary toxicity during cycle one. Overall, 2 patients had progressive disease and required radiation therapy without undue complications. 2 patients had partial responses, and 4 patients had stable disease for up to 6 cycles of treatment. 1 patient is unevaluable for tumor response. Several patients have had asymptomatic 20% decreases in DLCO 6–8 weeks following their last cycle of treatment with further PFT monitoring. We conclude that it is safe to administer DOX with IV D and P in patients with advanced NSCLC.

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Elimination of liposomes by apheresis-techniques

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Background: Using highly toxic drugs like chemotherapeutic agents, therapeutic success is often limited by severe side effects. Most often these side effects occur in other than the target tissue and are caused by a part of the administered dose, that does not reach the target tissue at all. This part of the total dose has no therapeutic value, but must be detoxified and excreted by the patient as well. To lower the side effects of chemotherapy, liposomes can be used as drug-delivery system. Thereby the toxic profile of the encapsulated chemotherapeutic agent is shifted, but detoxification of drugs not reaching the tumor still remains an obstacle. In some respects, liposomes are very similar to low-density-lipoproteins (LDL). LDL can be efficiently eliminated by LDL-apheresis-systems, which are used in therapy for years. If the excess of administered liposomes circulating in the blood could be eliminated by apheresis, side effects of chemotherapy may be lowered or minimised. As a first step towards this goal, the elimination of liposomes by different apheresis-techniques was investigated in vitro.
Methods: The different seperation principles used in LDL-Apheresis were examined to eliminate appropriate liposomes out of a liposomal suspension. The separation principles used were double membrane filtration, precipitation with heparin, adsorption chromatography by the use of either heparin coupled sepharose or polyacrylic acid coupled to polyacrylamide-beads.
Results: Liposomes can be effectively eliminated out of a liposomal suspension by the use of differential filtration, precipitation and adsorption by heparin coupled sepharose. Polyacrylic acid coupled polyacrylamide-beads are not able to adsorb liposomes effectively.
Conclusion: In general, liposomes can be effectively eliminated out of liposomal suspensions by the separation principles used in LDL-apheresis. Among the apheresis-systems used in clinical practice, MDF (membrane differential filtration) and H.E.L.P. (heparin induced precipitation) may be particularly useful, while DALI (adsorption by polyacrylic acid) has to be modified at least.