Results: The extent of the oxidative stress and lipid peroxidation is evidenced by significantly higher concentrations of MDA produced by membrane phospholipids in normal tissues adjacent to carcinoma than of normal tissues adjacent to fibroadenoma. Moreover, data obtained from TBA assay on free arachidonic acid (50 μM) after O.N incubation at 37ºC with H_2O_2 (1%) produced more high level of MDA than control. However analysis by ES-MS and MALDI-TOF spectrometry showed the presence of an intermediate radical product of arachidonic acid metabolism only in normal tissues adjacent to breast cancer.
Conclusions: Oxidations of arachidonic acid by reactive oxygen radicals generate a complex family of oxidized lipids known as isoeicosanoids. In our experiments demonstrated that in lipid chloroform extract by normal tissues adjacent to carcinoma presented a anomalous fragmentation pattern of arachidonic acid.

Marine compound

513 POSTER

Pharmacokinetic evaluation of a novel anti-tumor agent, PM01120

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Introduction: PM01120 is a new synthetic anti-tumor agent related to Variolins that were originally discovered in Antarctic sponge, *Kirkpatrickia variolosa*. PM01120 has demonstrated encouraging results against a panel of human leukemic, ovarian and colon carcinoma cell lines, and multi-drug resistant cell lines. Our recent efforts have focused on the pharmacokinetic evaluation of PM01120 in preclinical species.
Methods: The pharmacokinetic studies were conducted in CD-1 mice, SD rats, New Zealand white rabbits and beagle dogs following a single intravenous or oral dose of PM01120. Blood was collected and plasma sample was harvested for analysis. Brain tissues were also collected from mice PK study to explore the exposure of PM01120 in brain. The plasma samples were processed by liquid-liquid extraction (LLE); the brain tissues were homogenized in buffer and the homogenates were processed by LLE. A liquid chromatography/tandem mass spectrometry assay was used to determine PM01120 concentration in plasma samples or brain tissues. In vitro plasma protein binding of PM01120 was conducted using an ultracentrifugation method. In vitro metabolism study was performed in the liver microsomes from various species.
Results: In mice, PM01120 plasma concentration vs time profile indicated multi-compartmental kinetics after a 5-mg/kg i.v. administration. The mean C_{max} was 1.91 mg/mL and AUC was 0.57 mg hr/mL. The plasma clearance (CL_p) was 147.1 mL/min/kg, which is higher than mouse hepatic blood flow (~90 mL/min/kg). The volume of distribution at steady state (Vd_{ss}) was 2.3 L/kg, indicating moderate tissue distribution. The terminal half-life ($t_{1/2}$) was 6.27 hours. In mice, PM01120 rapidly transferred into brain and peak concentration in brain tissues was reached in less than 5 minutes after i.v. bolus dose. After peak time, brain PM01120 concentrations declined in the similar manner as in circulatory system. The values of C_{max} and AUC were ~1.5-fold higher in brain tissue, compared to PM01120 plasma level. PM01120 showed 39% absolute bioavailability in CD-1 mice after a 10-mg/kg oral administration. In rats, plasma PM01120 concentrations declined with $t_{1/2}$ of 4.32 hours after a 2.5-mg/kg i.v. dose. The mean CL_p was 16.62 mL/min/kg and Vd_{ss} was 0.61 L/kg. In rabbits, plasma PM01120 concentrations declined with $t_{1/2}$ of 13−15 hours after a 2.5-mg/kg i.v. dose. The mean CL_p was 22.58−30.04 mL/min/kg and Vd_{ss} was 1.41−1.42 L/kg. In dogs, plasma PM01120 concentrations declined with $t_{1/2}$ of 0.85−1.05 hours after a 0.5-mg/kg i.v. dose. The mean CL_p was 44.37−44.38 mL/min/kg and Vd_{ss} was 1.78 L/kg. The in vitro studies demonstrated that PM01120 was stable in plasma and the plasma protein binding of PM01120 was high in all species studied, ranging from 89 to 98%. In man, the %bound was about 98.91% and this binding was independent of the drug concentration (80 to 800 mM range). In vitro metabolic stability studies showed that PM01120 was metabolized in a moderate to high degree in the presence of NADPH-regenerating system; the in vitro half-lives were 17.93, 6.31, 33.83, 9.90, 9.28, 12.59, and 43.73 min in human, mouse, rat, guinea pig, rabbit, dog, and monkey liver microsomes. The availability of i.v. pharmacokinetic data from four species allowed interspecies scaling of PM01120 to be done, which produced the following relationship between plasma clearance and body weight: $CL_p = 30.506(\text{weight}^{1.0232})$. The extrapolated plasma clearance of 33.67 mL/min/kg was predicted for the average human (70 kg weight).
Conclusions: Pharmacokinetic properties were established for PM01120 in preclinical species. PM01120 showed good oral bioavailability and brain exposure in mice. Interspecies scaling projects a plasma clearance of 33.67 mL/min/kg in humans.

514 POSTER

Antitumor activity of aplidin® in human neuroblastoma tumors

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Background: Aplidin® (APL), a natural compound originally derived from the Mediterranean tunicate *Aplidium albicans*, is an anti-cancer agent currently in Phase II clinical trials for multiple indications in Europe and the USA. In these trials a suggestion of activity was observed in pediatric neuroblastoma. This observation prompted us to evaluate the cytotoxicity of Aplidin against a broad spectrum of pediatric neuroblastoma human tumor cell lines.
Materials and Methods: All cell lines were seeded at two densities in 96 well microtiter plates at 10,000 and 15,000 cells per well. Plates were incubated for 24 hours at 37 degree C prior to treatment for 72 hours with Aplidin® at a concentration range from 10 uM to 26 pM. The degree of cytotoxicity was determined by MTS assay (Tetrazolium), which is based on metabolic reduction of MTS to formazan product that is soluble in the tissue culture medium. The quantity of formazan is measured by the amount of 490 nm absorbance and it is proportional to the number of living cells. The IC_{50}, which is an approximate equivalent of IG_{50} (50% growth inhibition) was calculated and converted that to molar concentration by dividing by the molecular weight of Aplidin®.
Results: Aplidin® demonstrated significant nanomolar or lower potency in all neuroblastoma cell lines tested (table).

Cell line	IC_{50} M/10,000 cells	IC_{50} M/15,000 cells
SK-N-AS	2.6 to 1.3 nM	4.4 to 1.1 nM
SK-N-DZ	2.4 nM	4.6 to 1.1 nM
SK-N-MC	13 pM	2.9 to 4.8 nM
SK-N-SH	1.9 to 0.87 nM	2.2 to 1.1 nM

This level of *in vitro* activity was followed up in xenograft studies. Several human neuroblastoma cell lines were implanted subcutaneously into athymic nude mice. Tumors were allowed to grow *in situ* until they reach a size of approximately 100 mm^3 at which time animals were randomized into either treatment or control groups.
A detailed analysis of preclinical data with APL will be presented at this venue.

515 POSTER

Transcriptional profiling of palmerolide A, a putative inhibitor of V-ATPase, indicates perturbation of cholesterol biosynthesis

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Palmerolide A (NSC 730282), a 20-membered macrocyclic polyketide bearing carbamate and vinyl amide functionality, was isolated from the tunicate *Synoicum adareanum* collected from the vicinity of Palmer Station on the Antarctic Peninsula. Palmerolide A was tested in the NCI 60 cell line screen and displayed differential growth inhibition (GI_{50} ranging from 10 nM to 30 μM) and potent toxicity towards several of the melanoma cell lines. The NCI COMPARE algorithm correlated the toxicity profile of palmerolide to several vacuolar ATPase inhibitors, and ongoing studies have demonstrated that palmerolide A inhibits V-ATPase with an IC50 of 2 nM. Two melanoma cell lines (UACC62 and LOX) were treated with 100 nM and 10 nM palmerolide for 6 and 24h, then transcriptional profiles were measured on 35K spot, whole genome, oligonucleotide microarrays (NCI, ATC, Gaithersburg MD), and the data analysed through the mAdb bioinformatics website of the NCI's Center for Cancer Research. Overall, 10 nM (24h) and 100 nM (6h) palmerolide treatment had a limited response on the transcriptome. Gene expression profiles of UACC62 and LOX cell lines after duplicate palmerolide treatments (100 nM) for 24h were well reproduced. A drug-induced change of >1.8-fold in gene expression selected a group of 991 genes which correlated with a Pearson correlation coefficient (PCC) = 0.941 between replicate samples in the UACC62 cell line and PCC = 0.628 in the LOX cell line. The LOX cell line was less sensitive to the cytotoxicity of palmerolide and this was borne out by the array analysis where the magnitude and the number of genes dysregulated by palmerolide in LOX was less than in UACC62. Analysis of these 24h treatment data indicated a subset of 169 genes altered >3 fold by 100 nM palmerolide. Functional analysis

of these genes reveals a cohesive perturbation of the cholesterol and fatty acid biosynthesis pathway. Genes from this pathway (~21) were upregulated (>1.8-fold) and include 3-hydroxy-3-methylglutaryl-Coenzyme A (HMGCoA) synthase 1 and reductase, low density lipoprotein receptor (LDLR), and squalene epoxidase among others. As this was consistent with published gene expression changes for bafilomycin, we measured the transcriptional consequences of bafilomycin treatment on these cells, and found correlations between the ~900 genes dysregulated by the two drugs of PCC > 0.9 for UACC62 and PCC > 0.75 for LOX. Bafilomycin has been reported to inhibit cholesteryl ester synthesis through sequestration of free cholesterol in the endosomal/lysosomal compartment as a consequence of V-ATPase inhibition. The high degree of coherence between their gene profiles supports the premise that palmerolide is a V-ATPase inhibitor, and consequently has an effect on cholesterol sequestration.
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Natural products

516 POSTER

JNK-mediated p53 phosphorylation and stabilization contributes to the sensitization effect of luteolin on the anti-cancer effect of cisplatin

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Background: Luteolin is a flavonoid widely present in edible plants. Our previous studies have demonstrated the sensitization activity of luteolin on cancer cell apoptosis induced by TNFα or TRAIL. In this study we further investigate the synergistic effect of luteolin on cisplatin-induced apoptosis and the molecular mechanisms involved.
Material and Methods: Human cancer cells were pretreated with luteolin, followed by cisplatin. Apoptotic cell death, p53 protein level and JNK activation were determined using various methods.
Results: First, we provided evidence that apoptosis-induced by combined treatment of luteolin and cisplatin is p53-dependent: only p53 wild type cancer cells, such as HCT116 and HepG2, but not the p53 mutant cancer cells, such as HT29 and Hep3B, were sensitive to luteolin and cisplatin. Further, knock down of p53 protein level by siRNA made p53 wild type cancer cells resistant to luteolin and cisplatin, indicating a critical role of p53 in the sensitization process. Second, we observed significant increase of p53 protein level in luteolin-treated cancer cells, without increase of p53 mRNA level, indicating the possible effect of luteolin on p53 post-transcriptional regulation. Third, we found the critical role of c-Jun-N-terminal kinase (JNK) in luteolin-mediated p53 protein stablilization: luteolin activates JNK and JNK then stabilizes p53 via phosphorylation, leading to reduced ubiquitination and proteasomal degradation. Finally, by using an *in vivo* nude mice model xenografted with HCT116 cells, we confirmed that luteolin enhanced the cancer therapeutic activity of cisplatin via p53 stabilization and accumulation.
Conclusions: Data from this study demonstrate that luteolin enhances the anti-cancer activity of cisplatin via JNK-mediated p53 phosphorylation and stabilization. Our study thus supports the potential clinical application of luteolin as a chemosensitizer in cancer therapy.

517 POSTER

Preclinical development of novel betulinic acid derivatives as potent anticancer and antiangiogenic agents for systemic administration

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Introduction: Betulinic acid (BA) is a natural pentacyclic lupane-type triterpene shown previously to have potent anti-cancer activity in melanoma and neuroectodermal cancers. We have demonstrated the potential of novel C-2, C-3, C-20 and C-28 modified BA derivatives as broad-spectrum anti-cancer and anti-angiogenic agents.
Material and Methods: Cytotoxicity of novel derivatives was studied on a panel of human tumor cell lines using the tetrazolium-based MTT assay and *in vivo* efficacy was evaluated in melanoma and ovarian xenograft models. The *in vitro* anti-angiogenic effect was studied using human endothelial cells while anti-metastatic effect was tested in the mouse lung nodule assay. Predictive absorption, distribution, metabolism, elimination and toxicity (ADMET) studies were done using commercially available and validated software. Lead development studies like solubility, permeability, metabolic stability, cytochrome P450 inhibition and plasma protein binding were done using standard methods. Single dose pharmacokinetic study was carried out in rats and results analyzed using WinNonlin v5.0.1. Safety studies were done in rodents upon intravenous administration of potent derivatives.
Results: More than 1500 derivatives were screened and about 30 derivatives showed better broad-spectrum anti-cancer activity compared to BA in melanoma, glioblastoma, lung, and ovarian cancers with better selectivity for cancer cells and endothelial cells compared to normal cells. Modifications at C-3 position resulted in more potent derivatives. These derivatives, at non-cytotoxic concentration, significantly ($P < 0.05$) inhibited chemotaxis of endothelial cells towards angiogenic factors. Efficacy studies demonstrate that potent derivatives inhibit growth of human tumor xenografts and the formation of melanoma lung nodules in athymic mice. ADMET studies show that BA derivatives have poor solubility (<0.1 μg/ml), low to moderate permeability ($\log P_e < -5.0$) and high protein binding (>90%) suggestive of low/moderate bioavailability. A few derivatives had good *in vitro* metabolic stability (>90%). None of the derivatives inhibited key cytochrome P450 enzyme isoforms *in vitro* (IC_{50} > 10 μM) indicating less potential for drug interaction in combination therapy. The derivatives were safe in animals at the therapeutic dose and possess favorable properties of a systemically administered drug in the pharmacokinetic study.
Conclusion: Appropriate modifications in BA have resulted in more potent anti-cancer and anti-angiogenic compounds. ADMET studies indicate that BA derivatives have potential for development in a suitable anti-cancer formulation. Being natural-product derived compounds with good activity and low toxicity BA derivatives are potential anti-cancer agents.

518 POSTER

Curcumin inhibits tumor growth and angiogenesis in glioblastoma xenografts

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Background: Among the natural products that have shown chemopreventive and anticancer properties, curcumin is one of the most potent. In the current study, we investigated the effects of this natural product on growth of human gliomas U-87 cells xenograft in immunodeficient *nu/nu* mice.
Material and Methods: The anti-proliferative effect of curcumin on human glioma cell line U87 was studied in vitro by ^{3}H-thymidine incorporation methods. Tumor size and animal survival time were followed in curcumin treated mice with subcutaneous (s.c.) gliomas. Furthermore, *in vitro* proliferation, migration and tube formation were assayed on rat brain capillary endothelial cells to explore the effect of curcumin on angiogenesis.
Results: Curcumin was demonstrated to exert anti-proliferatif effects of human gliomas cells in a dose-dependent manner (IC50 = 12 μM). In addition, curcumin (50 mg/kg/day) exert significant antitumor effects on s.c. gliomas including slower tumor growth rate (up to 70%) and higher animal survival rate (up to 40%). Furthermore, treatment with curcumin inhibits angiogenesis, as indicated by the concentration of hemoglobin in the tumor. *In vivo* experiments revealed that curcumin decrease matrix metalloproteinase-2 (MMP-2) activation, whereas MMP-9 activation is unaffected by this natural product. Our study also shows that curcumin inhibited proliferation of endothelial cells *in vitro* (IC50 = 9 μM). In tube formation and cell migration assays using brain capillary endothelial cells, noncytotoxic doses of curcumin significantly inhibited formation of intact tube networks and reduced the number or migratory cells.
Conclusions: Our results indicate that, curcumin caused significant antitumor effects and inhibited angiogenesis in s.c. gliomas. Thus, curcumin might be helpful for the prevention and treatment of gliomas.

519 POSTER

Safety profile of ECO-4601, a novel PBR ligand anticancer agent, in primates

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Background: ECO-4601 is a structurally novel farnesylated dibenzodiazepinone (MW 462) discovered through Ecopia's Decipher® technology proprietary drug discovery platform. Initial *in vitro* assessment indicated cytotoxic activity against a wide panel of tumor cell lines, including several brain tumor cell lines. The mechanism of action of ECO-4601 is unknown at this time. However, the product binds selectively to the peripheral benzodiazepine receptor (PBR), preferentially expressed in tumors, with