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

nM affinity. Pharmacokinetic studies and antitumor evaluation indicate that efficacy is dependent on sustained plasma concentrations rather than high C_{max} levels followed by rapid elimination.
Methods: A study was conducted to assess the potential toxicity and toxicokinetic profile of ECO-4601 when administered to cynomolgus monkeys for 14 consecutive days by continuous intravenous infusion (CIV). This route and schedule of administration was chosen to achieve sustained plasma concentrations and is similar to that planned for the clinical Phase I trials. ECO-4601 was administered to cynomolgus monkeys by CIV at doses of 5, 15 and 30 mg/kg/day, during a 14-day period, followed by a 14-day recovery period.
Results: The highest dose level (30 mg/kg/day) was very well tolerated. This dose resulted in sustained drug plasma concentrations of 10−20 μM, which is well above expected therapeutic and target drug concentrations to be achieved in human (2–5 μM). Furthermore, when treatment was stopped, drug plasma concentrations declined quickly and there was no persistence in tissues. There were no effects on body weight, blood pressure and electrocardiographic activity, and no treatment-related ocular or neurologic abnormalities. Treatment-related changes observed were limited to: (1) occasional inappetance; (2) a modest degree of regenerative (reversible) anemia with no other hematologic abnormalities noted; (3) elevations in serum cholesterol and triglycerides, and a decrease in serum albumin (all reversible); and (4) diffuse vacuolization of hepatocytes and accumulation of foamy histiocytes in the spleen, which appeared to reflect clearance of the vehicle.
Conclusions: An adequate margin of safety for ECO-4601 was established under clinically relevant dosing conditions in monkeys, which supports advancement into clinical trials.

520 POSTER

Marked inhibition of tumor growth, MMP secretion and invasion by a nutrient mixture on head and neck squamous carcinoma cell line FaDu: in vitro and in vivo studies

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Background: Head and neck squamous cell carcinomas (HNSCC), the sixth most common malignancy in the United State, are known for their aggressive growth and propensity to invade and metastasize. We investigated the effect of a novel nutrient mixture (NM) containing ascorbic acid, lysine, proline, and green tea extract on human HNSCC cell line FaDu *in vitro*, evaluating viability, MMP secretion, invasion and morphology. *In vivo* studies were carried out in athymic nude mice bearing HNSCC FaDu xenografts.
Methods: After one week of isolation, 5–6 weeks old athymic male nude mice were inoculated with 3×10^6 FADU cells subcutaneously and randomly divided into two groups; group A was fed a regular diet and group B a regular diet supplemented with 0.5% NM. Four weeks later, the mice were sacrificed and their tumors were excised, weighed, and processed for histology. We also tested the effect of NM *in vitro* on FaDu cells, measuring cell proliferation by MTT assay, invasion through Matrigel, morphology by H&E staining, and secretion of MMPs by gelatinase zymography. Cells were also treated with PMA for MMP-9 induction.
Results: NM strongly inhibited the growth of tumors by 50%. *In vitro*, NM exhibited dose response toxicity with maximum toxicity of 50% over the control at 100 μg/ml. Zymography showed only a faint band representing MMP-2 and PMA-induced MMP-9. NM inhibited secretion of both MMP-2 and MMP-9 in a dose dependent fashion, with virtual total inhibition at 1000 μg/ml. Invasion through Matrigel was inhibited at 50, 100 and 500 μg/ml by 75%, 85% and 100% respectively. H&E staining did not indicate changes even at the highest concentration.
Conclusions: In conclusion, NM has a great potential for therapeutic use in the treatment of HNSCC by suppressing tumor growth and significantly inhibiting MMP secretion and invasion of HNSCC cells *in vitro*.

521 POSTER

Influence of new analogs and complexes of genistein on the expression of αvβ3 integrins on the A498 cell line

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Integrins comprise a large family of heterodimeric cell-surface receptors that present in many species. They are expressed on the wide variety of cells and mediate cell–cell and cell–extracellular matrix interaction. Dysregulation of the β3 integrins is involved in the pathogenesis of many diseases including cancer and transplant rejection.
The aim of our study was to investigate the influence of new analogs of genistein IFG-027 (7-O-alkenyl) and IFG-043 (7-O-arylmethyl) and its two polysaccharide complexes: schisophillan–genistein (SCH) and xyloglucan–genistein (XYL) on the expression of αvβ3 integrins.
Human kidney carcinoma A498 cell line was used (ATTC). Cells were incubated for 72 hours with tested compounds in concentration of 10 μg/ml. The cells were then labeled by αvβ3-specific antibodies conjugated with FITC and expression of integrins was analyzed by flow cytometry (Becton Dickinson, San Jose, CA, USA).
We have found that genistein, its new analogs and complexes have antiproliferative effect against many human cancer cell lines. We have also showed in our studies that these compounds had also influenced the expression of αvβ3 integrins. Genistein and XYL complex decreased the expression of integrins by 20%, whereas IFG-027 analog and SCH complex decreased it by 38%. IFG-043 analog revealed only low influence on the expression of the integrins (decrease by 10%).
We suggest that potential antitumor (antimetastatic) properties of genistein and its derivatives IFG-027, SCH, and XYL are worth of further research.
This work was supported by the Foundation for Development of Pharmaceutical Sciences (grant 8/FB/2004, Poland).

522 POSTER

Selective action of reveromycin A, a novel anti-resorptive agent, on osteoclasts

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Background: Bone destruction by osteoclasts plays an important role in the establishment and progression of osteolytic bone metastasis, which causes pain, pathologic fractures, and hypercalcemia. Therefore, osteoclasts are the ideal therapeutic target of osteolytic bone metastasis. Recently, we found that reveromycin A (RM-A), a polyketide-type natural product with three carboxylic groups in its structure, inhibited bone resorption through inducing apoptosis specifically in osteoclasts in vitro and in vivo. Moreover, we showed that RM-A inhibited the formation of bone metastasis in an experimental multi-organ metastasis mouse model of human lung cancer cells. Here, we investigated the mechanism of selective action of RM-A on osteoclasts using tritium-labeled RM-A ([^{3}H]RM-A).
Methods: [^{3}H]RM-A was prepared from RM-A by oxidation of C-5 OH followed by 1,2-reduction of the resultant enone using NaB[^{3}H]$_4$ and $CeCl_3$.
Results: RM-A inhibited the survival of osteoclasts with an IC50 value of 0.2 μM, and the ED50 of RM-A on bone marrow cells, osteoblasts, and a number of other cell lines, was at least 100-fold higher than that for osteoclasts. [^{3}H]RM-A was selectively incorporated into osteoclasts among various cells, and the uptake of [^{3}H]RM-A was prevented by disruption of the acidic microenvironment, a prominent characteristic of osteoclasts. [^{3}H]RM-A was dramatically incorporated into murine monocytic cell line RAW264 in acidic culture medium (pH 5.5), but not in neutral culture medium (pH 7.5). In addition, the apoptotic effect of RM-A was also increased under acidic conditions in RAW264 cells. RM-A inhibited protein synthesis in osteoclasts by selectively blocking enzymatic activity of isoleucyl-tRNA synthetase.
Conclusions: These results suggest that the specific sensitivity of osteoclasts to RM-A is due to their acidic microenvironment, which increases cell permeability of RM-A by suppressing the dissociation of protons from the carboxylic acid moieties, and the inhibitory effect of RM-A on bone resorption is caused by apoptosis through the inhibition of isoleucyl-tRNA synthetase in osteoclasts. This unique mechanism suggests that RM-A may represent a new type of therapeutic agent against osteolytic bone metastasis.

523 POSTER

The anti-angiogenic properties of Mistletoe extracts is associated with endothelial cytotoxicity

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Background: Viscum album (VA) preparations are used as adjuvant therapy in cancer patients. Angiogenesis plays an important role in the growth and sustenance of the tumors and their metastasis. Inhibition of angiogenesis is explored as a new therapy for cancer. We hypothesize that the anti-angionenic properties of VA extracts are due to their cytotoxic properties.
Materials and Methods: *In vitro angiogenesis assay*: Unpolymerised matrigel (10 mg/ml) was placed in the wells (400 μl/well) of a 24-well plate and allowed to polymerize for 1 h at 37ºC. EA-hy926 cells (Endothelial cell line) were then seeded (50×10^3 Cells/well). After the incubation with VA