tumor model. Inhibition of both aromatase and sulfatase activities should offer a new therapeutic approach to the treatment of hormone-dependent breast cancer.

509 POSTER

Evaluation of in vitro toxicity and efficacy of ferutinin, a natural promising chemopreventive compound

C. Severini[1], M.G. Mascolo[2], E. Morandi[1], P. Silingardi[1], W. Horn[1], S. Perdichizzi[1], M. Vaccari[1], A. Colacci[1]. *[1]Environmental Protection and Health Prevention Agency-Emilia-Romagna Region, Excellence Environmental Carcinogenesis, Bologna, Italy; [2]University of Bologna, Department of Experimental Pathology-Cancer Research Section, Bologna, Italy*

The biological activity of the natural phytoestrogen ferutinin have not been extensively examined as yet, in spite of the interest about plant-derived products as possible chemopreventives. In this study, the efficacy of ferutinin on several in vitro experimental endpoints correlated with tumour onset and progression has been compared to that of the well characterized soy isoflavone genistein. Effects of ferutinin and genistein have been examined on cell proliferation and growth inhibition, anchorage-independent growth and Matrigel invasion, cell growth in estrogen depleted media, programmed cell death. Like genistein, ferutinin acts as an estrogen agonist in the E-screen assay and exerts a biphasic effect on cell growth and proliferation in ER-positive MCF-7 cells, with an induction of proliferation at lower concentration (1 μM) and an antiproliferative dose-response effect at higher concentrations (10–100 μM). In MDA-MB-231 ER-negative cells, the dose-related inhibition of cell growth induced by ferutinin or genistein was evident, even if no biphasic effect was shown. In the agar clonogenic assay, ferutinin did not induce any significant increase in colony growth of MCF-7 cells at the assayed doses, while it showed a strong dose-related antiproliferative activity at high concentrations (10–100 μM). The biphasic effect of genistein on anchorage-independent growth was evident. The effect of the phytoestrogens on the malignant phenotype was evaluated in the in vitro Matrigel invasion assay. Ferutinin (1−100 μM) induced a dose-dependent inhibition of the invasive ability of MDA-MB-231 cells. The effect of ferutinin on cell death was also evaluated. The morphology of dying cells suggested the induction of a different mechanism of cell death induced by ferutinin, possibly alternative to apoptosis. The monodansylcadaverine (MDC) assay for autophagic cell death revealed some MDC-positive structures, which could be classified as autophagic vacuoles, in cells treated with high ferutinin concentrations (80 and 40 μM). The results show that ferutinin is more effective than genistein in the assayed in vitro endpoints in human breast cancer cells, suggesting a possible use of this natural compound as a chemopreventive or chemoterapeutic drug, even if the mechanisms of action and the benefit/risk ratio need to be further evaluated.

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510 POSTER

Estrogenic effect of ellagic acid in the estrogen sensitive breast cancer cells

R. Lee, H. Kim. *College of Medicine, Ewha Womans University, Seoul, Surgery, Seoul, Korea*

Background: Ellagic acid is a dietary polyphenol present in abundance in strawberries and pomegranate. The antiproliferative activity of ellagic acid is documented and has been extensively studied in colorectal cancer, prostate cancer, and endometrial cancer cells. Ellagic acid exerts its effects via activation of various signaling pathways. Some study showed that the antiproliferative effect of ellagic acid is through mitochondrial pathway in colorectal cancer cell line. But another study reported that ellagic acid has antiproliferative effect by estrogen receptor α. We hypothesized that ellagic acid could be used as a new anticancer drug or new selective estrogen receptor modulator to manage the breast cancer. In the present in vitro study, we have compared the effect of ellagic acid on the proliferation of estrogen receptor negative or positive human breast cancer cells. In addition, the effect of ellagic acid on estradiol-induced stimulation of receptor-positive breast cancer cells was studied. Next, we evaluate the expression of pS2 and c-fos which is the down stream gene of ER.
Material and Methods: The receptor-positive breast cancer cell line MCF-7 and the receptor-negative cell line MDA-MB231 were used. The ellagic acid were tested in the concentration range of 10 um to 100 um. In MCF-7, 17α-estradiol and the mixture of ellagic acid and 17α-estradiol were also evaluated. Cell proliferation was measured after 0 hours, 24 hours, 4 8hours and 72 hours using the MTT assay. Apoptosis was confirmed by flowcytometry. The western blotting for pS2 and c-fos was done.
Results: Ellagic acid was able to significantly inhibit the cell proliferation of MDA-MB-231. But it caused cell proliferation in MCF-7. Flowcytometry showed that ellagic acid caused apoptosis in MDA-MB-231. It provokes cell-cycle arrest in S phase in MDA-MB-231. The western blotting for pS2 and c-fos was done to evaluate the estrogenic effect of ellagic acid on estrogen-receptor positive cell line. The expressions of pS2 and c-fos were higher in MCF-7 which was treated by ellagic acid.
Conclusions: The present data indicate that ellagic acid can inhibit the proliferation of receptor-negative human breast cancer cells. But it also stimulates the proliferation of receptor-positive breast cancer cells. Ellagic acid has different pathway in ER positive and ER negative cell lines. It might be used as anticancer drug in ER negative breast cancer, but it would be forbidden in ER positive breast cancer.

511 POSTER

Tamoxifen induces degradation of the o6-methylguanine DNA methyltransferase protein via the ubiquitin-dependent proteosome pathway in human cancer cells

C.-C. Kuo, J.-F. Liu, J.-Y. Chang. *National Health Research Institutes, Institute of Cancer Research, Taipei, Taiwan*

Background: Tamoxifen is a synthetic nonsteroidal anti-estrogen triphenylethylene compound. It is part of a class of anti-cancer drugs known as selective estrogen receptor modulators (SERMs), and could block tumor growth by mimicking estrogen and filling up estrogen receptors which prevents the cancerous growth. However, the current view is that the action of tamoxifen is not only mediated by its anti-estrogenic properties. Previous study have demonstrated that a combination chemo/hormonal therapy regimen for the treatment of patient with neoplastic diseases, for example, the combination of the tamoxifen with the CNU-type alkylating agents, leads to synergistic cytotoxic effects. However, the mechanism of action of combined effect had not been elucidated.
Material and Methods: MGMT activity assay, Western blot analysis, Northern blot analysis, and Immunoprecipitation were used in this study.
Results: Here, we demonstrated that treatment of human colorectal HT-29 carcinoma cells with tamoxifen decreased the expression level of MGMT protein in a dose- and time-dependent manner. This inhibition, independent with estrogen receptor status, was also shown in other common cancer types tested. No difference between MGMT mRNA levels before or after tamoxifen treatment was found. However, MGMT protein half-life was markedly decreased in the presence of tamoxifen compared with that of control. Moreover, the MGMT protein was found to increase its ubiquitinated species after tamoxifen treatment. This tamoxifen-induced MGMT degradation could be reversed by proteosome inhibitors lactacystin and MG-132.
Conclusion: We conclude that tamoxifen induced reduction of MGMT protein levels by accelerating protein degradation via the ubiquitin-dependent proteosome pathway. This result provides the evidence for the combination benefit in chemo/hormonal therapy.

512 POSTER

Alteration of arachidonic acid metabolism in human breast cancer

P. Stiuso[1], A. Dicitore[1], D. Cassese[1], P. Ferranti[2,3], G. Picariello[3], S. Canonico[4], A. Santoriello[4]. *[1]Second University of Naples, Biochemistry and Biophysic "F. Cedrangolo", Naples, Italy; [2]University of Naples 'Federico II', Science of Foods, Naples, Italy; [3]CNR, 3 Institute of Science of Foods, Avellino, Italy; [4]Second University of Naples, Gerontology, geriatric and metabolic disease, Naples, Italy*

Background: The plasma membranes of most cells contain both polyunsaturated and monounsaturated lipids, which are susceptible to oxidative damage by free-radical processes or electrophilic addition reactions, e.g. reaction of hydroxide ion (OH^-) with a double bond. Oxidative stress elevate levels of free radicals that can directly target arachidonic acid, an important mediator of inflammation, bound to phospholipids. This generates a complex mixture of oxidized products, known as isoeicosanoids, that can be cleaved off. In mammary gland tissues lipid peroxidation promotes the production of linoleic acid-derived arachidonic acid, a fatty acid that is most susceptible to lipid oxidation and formation of malondialdehyde (MDA). In this research we have studied alteration of arachidonic acid metabolism, both in model systems and in normal tissue adjacent to breast cancer and not in order to understand features of phospholipids fragmentation pattern in breast cancer.
Materials and Methods: Membrane phospholipids fractions from normal human tissue adjacent to breast cancer and fibroadenoma were extracted with chloroform/methanol (2:1), then sonicated on ice. The lipids chloroform extract was used for thiobarbituric acid assay (TBA) to induce a fragmentation pattern of lipids and evidenced the formation of aldehydic products as malondialdehyde (MDA). Moreover the lipids chloroform extract obtained after TBA assay was analyzed by ES-MS and MALDI-TOF spectroscopic techniques.

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

J. Yin[1], P. Aviles[2], M.J. Guillen[2], C. Ly[1], W. Lee[1], A. Soto[2], S. Munt[2], C. Cuevas[2], G. Faircloth[1]. *[1]PharmaMar USA, Inc., Cambridge, MA, USA; [2]PharmaMar S.A., Colmenar, Madrid, Spain*

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

H. Sasak[1], D. LePage[1], W. Grant[1], M. Elices[1], B. Rinehart[1], T. Caylor[1], L. Cheney[1], K. Halley[1], P. Aviles[2], G. Faircloth[2]. *[1]PharmaMar USA, Pharmacology, Cambridge, MA, USA; [2]PharmaMar SA, R&D, Madrid, Spain*

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

A. Monks[1], N. Reifsnider[1], R. Shoemaker[1], J. Cardellina[1], B. Baker[2]. *[1]NCI-Frederick, Screening Technologies Branch, Frederick, USA; [2]University of South Florida, Department of Chemistry, Tampa, USA*

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