Showcase of Medical Discoveries

A Focus on Natural Products

Wednesday, June 1, 2016
4:30—6:00 p.m.

A Wine & Cheese Reception Featuring UAMS Investigators Discussing their Research and Discoveries.

Winthrop P. Rockefeller Cancer Institute—10th Floor Rotunda

UAMS
COLLEGE OF MEDICINE
UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES

UAMS Office of Research

Poster #1

A Useful Rice By-product for Radiation Protection

Kimberly J. Krager, Mary Kordsmeier, Luke Howard, Philip J. Breen, Cesar M. Compadre, Martin Hauer-Jensen, Nukhet Aykin-Burns

Radiation therapy is frequently used to treat malignant conditions either alone or concomitant with other modalities. Skin tissue is a major recipient of significant collateral damage during radiotherapy, 95% of patients who have had radiotherapy suffer from both acute and chronic side effects. Thus, despite discoveries in radiation biology and improvements in radiation technology, there is still a significant need for a safe and effective radioprotector/radiomitigator compound to alleviate the side effects of radiotherapy on skin tissue.

One of the few, and most promising, alternatives is the vitamin E analogs δ-tocotrienol (DT3) and g-tocotrienol (GT3). These compounds have shown significant radioprotectant and radiomitigator activities with minimal side effects. Unfortunately, both compounds are in short supply and very expensive to purify. Rice bran oil deodorizer distillate (RBODD), a byproduct in the refinement of rice oil, contains large amounts of tocotrienols. In this study, we hypothesized that RBODD can protect mitochondrial function and oxidative metabolism and thus safeguard skin cells from radiation injury.

We have compared the effects of DT3 and RBODD against radiation induced mitochondrial dysfunction and oxidative stress in human skin cells. Our data demonstrated that IR exposed cells treated with 5 uM DT3 or RBODD preserved the clonogenic cell survival and mitochondrial function. Furthermore, treatment of skin keratinocytes with 5 µM DT3 or RBODD protected the cell morphology and improved migration rates observed in scratch assays, even though the cells were treated with tocotrienols 4hr. following IR exposure. These preliminary results indicate that RBODD is a potentially viable source for tocotrienols that can be used as radioprotector.

UAMS

UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES

Poster #2

Goldenseal (Hydrastis canadensis L.) Extract Dose-Dependent Growth Inhibition of H. pylori Bacteria and Effects of H. pylori on Gastric Cell Free Amino Acid Concentrations

Nimasha Fernando, Aime T. Franco, Howard P. Hendrickson

Gastric cancer is the third deadliest form of malignancy, responsible for 723,000 deaths worldwide in 2012. The greatest identifiable risk factor for gastric cancer development is infection with *H. pylori* which increases the risk of disease. Due to H. pylori's estimated presence in two-thirds of the human population, prevalence in underdeveloped regions, and elevated risk of associated gastrointestinal complications, natural supplements are being investigated for their antibacterial efficacy. Goldenseal extract has demonstrated potent *H. pylori* growth inhibition effects; however, standardization of plant-based supplements is challenging. We plan to determine Goldenseal's effects on *H. pylori* growth and gastric cancer cells' free amino acid metabolism as a means of standardization and to better understand Goldenseal's mechanism of action. First, 7.13WT *H. pylori* was cultured with varying Goldenseal extract concentrations to monitor dose-dependent growth inhibition effects. We hypothesize that decreased concentrations of non-essential amino acids utilized by *H. pylori* for survival, will be observed in the presence of Goldenseal. These changes in free amino acid concentrations may also alter cellular downstream pathways and provide further insight regarding H. pylori's mechanism of pathogenicity and carcinogenic potential. It also hoped that Goldenseal can provide a viable treatment option for this prevalent disease.

Poster #3

Transarterial Chemoembolization with Parthenolide in a Rat Liver Tumor Model induces Tumor Regression without any detectable Liver or Systemic Toxicity

Zheng Chen, Peter A. Crooks and Michael J. Borrelli

PURPOSE: *In vitro* studies with hepatocellular carcinoma (HCC) cells and normal hepatocytes demonstrated that the natural drug parthenolide (PTL) inhibits the growth of liver tumor cells without causing normal cell toxicity. Consequently, a 24 rat study was initiated to determine if transarterial chemoembolization (TACE) with PTL would inhibit the growth of Walker 256 rat liver tumors.

METHODS: Luciferase expressing, Walker 256- tumor cells were inoculated into the left lateral liver lobe of male Wistar rats. TACE was accomplished via catherization of the left hepatic artery. There were three treatment groups: saline, Lipiodol (embolizing agent) and PTL dissolved in Lipiodol (PTL-Lipiodol: 80 mM PTL). Magnetic resonance imaging (MRI) and bioluminescence imaging were used to monitor and measure tumor growth non-invasively. Body weight and liver blood biochemicals were measured to detect any normal liver toxicity.

RESULTS: PTL-Lipiodol, TACE-treated tumors exhibited a significant (10%) decrease in tumor volume during a 9-day post treatment period while those embolized with saline or Lipiodol increased 4.5-fold in volume. MRI images and blood enzyme/biochemical showed no evidence of normal liver toxicity associated with the PTL-Lipiodol TACE treatment. Histopathology examinations are ongoing. This study suggests that PTL-Lipiodol TACE can be a safe and effective treatment for liver tumors.

Poster #10

Comparative Pharmacokinetics of S-(-)-Nornicotine and S-(-)-Nicotine after Transdermal Application

Zaineb A. F. Albayati, Buchi Nalluri, Philip J Breen and Peter A. Crooks

S-(-)-Nornicotine (S-NN), a tobacco alkaloid and N-demethylated metabolite of nicotine, accumulates in rat brain in pharmacologically relevant concentrations following S-(-)-nicotine administration. Studies in rats have shown that S-NN decreases i.v. nicotine self-administration. The purpose of this investigation was to evaluate the *in vivo* absorption and pharmacokinetics (PK) of transdermally administered S-NN and to compare it to transdermally administered S-(-)-nicotine (nicotine patch) in Yucatan minipigs. The absorption and PK parameters for S-NN and S-(-)-nicotine were determined after topical application of transdermal patches in twenty male minipigs. S-NN at a dose of 0.6 mg/kg was delivered i.v. via a catheter inserted into the left ear vein with blood sampling from the right ear vein over a 24 h period. S-NN at a dose of 195 mg, or S-(-)-nicotine at a dose of 154 mg were each formulated as two separate 49 cm2 patches administered transdermally. Blood samples were collected over 80 hr and plasma samples were analyzed for S-NN and S-(-)-nicotine. The results indicated that significant amounts of S-NN and S-(-)-nicotine were delivered after transdermal application. The PK results indicated that S-NN had a relatively shorter t0.95, a significantly higher predicted Css value, a larger observed Cmax, and a larger AUC0-80 compared to S-(-)-nicotine following 24 hr of transdermal delivery under identical conditions of drug administration. The data suggest that S-NN may have a more favorable transdermal PK profile than nicotine, thus warranting further pre-clinical development of S-NN as a smoking cessation therapy.

Poster #9

The Vitamin E Analog Gamma Tocotrienol (GT3) Suppresses Ionizing Radiation- and/or Microgravity-Induced Cytogenetic Damage: Possible Role of RAD50

R. Pathak, A. Bachri, S. P. Ghosh, I. Koturbash, G. A. Nelson,
M. Boerma, and M. Hauer-Jensen

A major concern of long-term space missions is the combined exposure to radiation and microgravity, which may adversely affect cellular genomic integrity, resulting in the pathogenesis of several diseases, including cancer and cardiovascular diseases. The vitamin E analog gamma tocotrienol (GT3) is a strong dietary antioxidant and has been shown to play a critical role in radiation protection. We hypothesized that GT3 will suppress radiation- and/or microgravity-induced cytogenetic damage.

In vitro studies demonstrated that GT3 pre-treatment substantially suppressed gamma-ray-induced DNA double strand breaks, total structural chromosome aberrations and damages in human chromosome-1, -2 and -3. We also observed that simulated microgravity enhanced radiation-induced endothelial cell death. *In vivo* studies revealed that a single dose of GT3 administered 24 hours before irradiation significantly attenuated cytogenetic damage in mouse bone marrow cells. Moreover, we observed that GT3 reduced cytogenetic damage involving mouse chromosome-1, -2 and -3. Our Spectral Karyotyping (SKY) data revealed that GT3 also lessened aberration induction in irradiated mice. Finally, we found that GT3 inhibited radiation-induced RAD50 down-regulation in endothelial cells.

Together, our data clearly indicate that GT3 plays an important role in suppressing radiation- and/or microgravity-induced cytogenetic damage, possibly by modulating RAD50 expression.

Poster #4

Natural compounds in the prevention and treatment of radiation-induced heart disease

Marjan Boerma, John Seawright, Preeti Singh, Maohua Cao,
and Viji Mohan Seenivasan

We used a pre-clinical animal model to test whether natural compounds can reduce radiation injury in the heart. Rats receive local image-guided irradiation of the heart and were followed for 6 months to develop late manifestations of radiation-induced heart disease. We tested whether Tocomin SupraBio®, an extract from palm oil that is enriched in tocotrienols, can prevent late manifestations of radiation-induced heart disease when administered orally 24 hours before local heart irradiation. We also tested POLY-MVA®, a mixture of lipoic acid mineral complexes and vitamins, when given orally once a day from week 16 until 22 after irradiation. We found that both products reduced the effects of radiation on mitochondria, but did not modify the effects of radiation on cardiac function or collagen deposition. Therefore, while both products seem to have beneficial effects in the irradiated heart, they may have to be given in combination with other compounds to provide full prevention or mitigation of radiation-induced heart disease.

Poster #5

Development and Commercialization of Tocol-containing Pharmaceuticals Derived from, or Inspired by, Natural Products

Philip J. Breen, Nukhet Aykin-Burns, Shraddha Thakkar,
and Cesar M. Compadre

Delta and gamma tocotrienol, vitamers of Vitamin E, are potent radioprotectants, but have short elimination half-lives and are expensive to synthesize. Tocol Pharmaceuticals, LLC, has developed plant-based tocols with equal efficacy to these pure compounds. These results include complete protection of mice exposed to a lethal dose of radiation, as well as restoration of normal mitochondrial bioenergetics in irradiated cells or cells exposed to high concentrations of peroxide. Moreover, we have designed and synthesized an NCE, tocoflexol, which has improved binding to a liver protein ATTP, which will result in an increased residence time in the body. Applications of these products include enhancement of safety against radiation exposure, whether occupational, accidental or due to terrorism, as well as, potentially, the protection of normal, but not cancer, cells during radiotherapy.

Poster #8

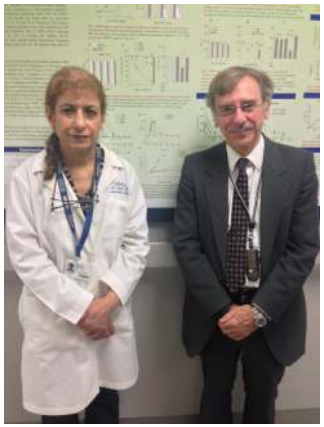
Metformin and Soy Bioactives Limit the Frequency of the $CD133^+CD44^+$ Epithelial Sub-population in Human Colon Cancer Cells

Maria Theresa E. Montales, Adam R. Brown, Rosalia C.M. Simmen,
and Frank A. Simmen, Ph.D.

Colon cancer is the third leading cause of cancer-related deaths worldwide. Obesity and diabetes, due in part to high-caloric diet and sedentary lifestyles, underlie increased colon cancer risk. Epidemiological studies suggest that the biguanide Metformin (Met), commonly used as a first-line treatment for Type 2 diabetes, may exhibit anti-cancer activity by reducing blood glucose and hence insulin levels that fuel growth of cancer cells and by activating the AMPK pathway that regulates their metabolic state. While Met has been evaluated for its ability to inhibit the malignant potential of colon cancer cells, studies addressing the effects of dietary factors with health benefits and their combined effects with Met have not been reported. Herein we performed *in vitro* studies to evaluate whether Met and the soy bioactive components genistein (GEN) and Lunasin (LUN) alone and in combination inhibit the tumor potential of the metastatic human colon cancer line HCT116. The abundance of the $CD133^+CD44^+$ epithelial sub-population of HCT116 cells, that form non-adherent colonies (termed colonospheres) under anchorage-independent conditions, is an *in vitro* measure of tumor formation. Met (5 µg/ml) decreased cell viability, induced cell apoptosis, decreased the frequency of sphere formation, promoted tumor suppressor PTEN expression and inhibited pro-tumorigenic FASN transcript levels in HCT116 cells. Similar to Met, GEN (2µM) and the soy peptide LUN (2 µM) reduced cell viability (GEN>LUN>Met), inhibited colonosphere formation, and enhanced PTEN mRNA expression. The effects of Met on colonosphere formation were enhanced by GEN but not by LUN; co-addition of all three reduced colonosphere numbers. To evaluate if Met limits colonosphere formation partly through KLF9, Met-treated cells were evaluated for KLF9 mRNA expression, and siKLF9-targeted HCT116 cells were assessed for proliferation. Met reduced KLF9 and FASN gene expression by 2h and 6h, respectively, suggesting a temporal relationship, and siKLF9 targeting decreased proliferation. Results suggest that Met, when used in conjunction with dietary bioactives, may limit the expansion of colon tumor-initiating cells. The intriguing possibility of mechanistic linkages among Met, KLF9 and FASN in colon tumorigenesis warrants further study.

Poster #7

Rapid Pharmacokinetic Screening of Lobelane and Lead Lobelane Analogs in Rats via Cassette Dosing



ZAF Albayati, M. Sunkara, A.J. Morris, L. Dwoskin, and P. A. Crooks

Methamphetamine is an addictive psychostimulant drug and chronic abuse may cause neural damage in humans with deleterious effects on cognitive processes such as memory and attention. Research on lobelane, a minor alkaloid isolated from *Lobelia inflate*, and systematic structural modification of the lobelane molecule has led to the discovery of molecules that may provide more potent and selective treatments for methamphetamine addiction. This work was aimed at developing a rapid pharmacokinetic (PK) screen of lobelane and 4 lead lobelane analogs emerging from *in vitro* assays to quickly eliminate compounds with poor drug-likeness properties by utilizing fast throughput cassette dosing, i.e. the "N-in -one technique". Lobelane and analogs GZ-745A, GZ-282A, GZ-757A, and GZ-793A, and propranolol (of known PK profile, used as a positive control drug) were mixed together in a dosing "cocktail", and then co-administered to a single catheterized male rat at an oral dose of 10 mg/kg for each compound. The data obtained were compared to an i.v. injection of a cocktail containing 1 mg/kg of each of the 6 compounds utilized. Plasma samples were collected at specified time points and analyzed using a convenient LC/MS/MS method. Following oral administration, bioavailabilities (*F*) were as follows: lobelane: 4.6%, GZ-757A < 0.1%, GZ-745A: 1.50%; GZ-282A: 4.2%, and GZ-793A: 10.2% propranolol had an oral bioavailability of 35.5% (Lit. 30%-40%, Lee & Ku, 1999). This method enabled the identification of the potential clinical candidate GZ-793A with the best PK properties in this *in vivo* single animal screen.

Poster #6

Dehydroleucodine a Sesquiterpene Lactone from Gynoxys verrucosa Demonstrates Cytotoxic Activity Against Human Leukemia Cells

Paola E. Ordóñez, Krishan K. Sharma, Laura M. Bystrom, Maria A. Alas, William F. Reynolds, Raul G. Enriquez, Darcy C. Burns, Omar Malagón, Darin E. Jones, Monica L. Guzman, and Cesar M. Compadre

The Sesquiterpene lactones dehydroleucodine (1) and leucodine (2) were isolated from *Gynoxys verrucosa* Wedd., a species used in traditional medicine in southern Ecuador. The activity of these compounds was determined against eight acute myeloid leukemia (AML) cell lines and compared with their cytotoxicity against normal peripheral blood mononuclear cells (PBMCs). Compound 1 showed potent cytotoxic activity against the tested cell lines, with LD50 values between 5.0 and 18.9 µM. Compound 2 was inactive against all of the tested cell lines, demonstrating that the exocyclic methylene in the lactone ring is required for cytotoxic activity. Importantly, compound 1 induced less toxicity to normal blood cells than to AML cell lines and was active against human AML cell samples from five patients, with an average LD50 of 9.4 µM. To develop analogs of compound 1 with increased water solubility, three amino adducts (4-6) were synthesized. Compounds 4-6 showed very little antileukemic activity. The structures of all of the compounds were unambiguously established by spectroscopic methods and single-crystal X-ray diffraction. Mechanistic assays suggest that compound 1 has a similar mechanism of action to parthenolide (3). Although these compounds have significant structural differences, their lipophilic surface signatures show striking similarities.