242nd ACS National Meeting, Denver, CO

Program Chair, Shana Sturla

Sunday, August 28, 2011

General Papers (08:00 AM - 12:00 PM) Room 207, Colorado Convention Center

08:00 AM

Carcinogenic nitrosamines in U.S. cigarettes: Three decades of remarkable neglect by the tobacco industry

Irina Stepanov PhD, Aleksandar Knezevich, Liqin Zhang PhD, Clifford H. Watson PhD, Dorothy K. Hatsukami PhD, Stephen S. Hecht PhD. Masonic Cancer Center and Tobacco Use Research Programs, University of Minnesota, Minneapolis, MN, United States; Centers for Disease Control and Prevention, Atlanta, GA, United States

More than thirty years ago it was already known that modification of tobacco curing methods and other changes in cigarette manufacturing techniques could substantially reduce the levels of tobacco-specific nitrosamines (TSNA), a group of potent carcinogens, in cigarette smoke. We examined the levels of these carcinogens in tobacco filler and smoke of 17 various brands of cigarettes purchased in April of 2010 from retail stores in Minnesota. In all brands, the sum of two potent carcinogenic TSNA - 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and *N'*-nitrosonornicotine - averaged 2.54 (±1.05) μg/g in the filler and 215.1 (±84.3) ng/cigarette in the smoke. Transfer rate of TSNA from cigarette filler to smoke, the apparent lack of effort to control the levels of these carcinogens in cigarette tobacco and smoke by the cigarette manufacturers, as well as the most recent studies supporting the role of carcinogenic TSNA in human cancers will be discussed. In light of the recently granted regulatory authority to the FDA over tobacco products, regulation of TSNA levels in cigarette tobacco should be strongly considered to reduce the levels of these potent carcinogens in cigarette smoke.

08:25 AM

Progress towards personalized chemotherapy: Correlation of Pt-DNA adduct levels and clinical response in bladder and lung cancer patients undergoing platinum-based chemotherapy

Paul Henderson PhD. Department of Internal Medicine, Division of Hematology and Oncology, University of California Davis, Scaramento, CA, United States

Drug-DNA damage formation is a critical step in cytotoxicity of platinum (Pt) chemotherapy. We hypothesize that Pt-DNA damage induced by nontoxic "microdoses" (1/100th of therapeutic dose) is predictive of chemoresistance. Accelerator mass spectrometry (AMS) is an ultrasensitive method for measuring radiocarbon. We initiated a microdosing clinical trial using [^{14}C]carboplatin in order to determine if chemoresistance can be identified with AMS before patients receive toxic Pt-based chemotherapy. The eligibility criteria include patients with clinical diagnosis of lung or bladder cancer who are scheduled to have biopsy/resection and to receive Pt-based chemotherapy. Up to 80 patients will be recruited. Drug-DNA damage and gene expression data and correlations to several endpoints of response will be presented for the first ten patients. The data will be discussed in the context of demonstrating the feasibility of a larger clinical trial.

08:45 AM

Quantitation of DNA adducts using high resolution mass spectrometry

Peter W Villalta PhD, Silvia Balbo PhD, Stephen S Hecht PhD. Cancer Center, University of Minnesota, Minneapolis, Minnesota, United States

Liquid chromatography tandem mass spectrometric methods can provide sensitive, specific and accurate quantitation of DNA adducts. However the sensitivity/specificity of these techniques is compromised when significant chemical noise and/or coeluting substances are present which often occurs when quantifying smaller analytes (<300 amu) in complex matrices. High resolution mass spectrometry can reduce

chemical noise and coeluting signal allowing for dramatic improvements over the traditional “low resolution” approach. We have developed methodology using nanoflow HPLC and tandem mass spectrometry operating at a resolution of >30,000 and mass accuracy of ≤2 ppm. The methodology optimization was done with 7-ethyl-guanine (m/z 180) detection in DNA from animals treated with ethylating agents and resulted in limits of detection and quantitation of 10 and 50 attomole on-column, respectively. Detection of 7-ethyl-guanine from human leukocyte DNA was demonstrated at levels below the limit of detection of our triple quadrupole based method.

09:05 AM

Protein targets of alkynyl analogs of 4-hydroxy-2-nonenal and 4-oxo-2-nonenal in a human monocyte cell line

Simona G Codreanu PhD, Daniel C Liebler PhD. Department of Biochemistry, Vanderbilt University, Nashville, TN, United States

Endogenously formed lipid electrophiles initiate toxic responses by modifying proteins and triggering specific adaptive and stress responses. We have recently demonstrated the utility of alkynyl-tagged probes with post-labeling biotinylation using a photocleavable azido-biotin-linker via Click chemistry. Human THP-1 monocytic cells were treated with either 0, 5, 10, or 20 mM alkynyl-4-oxononenal (alkynyl-ONE) or alkynyl-4-hydroxynonenal (alkynyl-HNE) and adducted proteins selectively enriched by streptavidin capture and photocleavage. Liquid chromatography-tandem mass spectrometry analysis identified 1980 protein groups as alkynyl-HNE targets at an FDR of 5% and of 1880 as alkynyl-ONE targets. Identification of protein targets required a significant concentration-dependent relationship between electrophile concentration and protein spectral counts. Comparison at a linear FDR of 0.05 revealed 288 common protein targets, as well as a similar number of targets unique to each electrophile. Application of this approach dramatically expands the inventory of protein targets at biologically relevant electrophile concentrations. (Supported by NIH grant ES013125.)

09:25 AM

Hole injection and migration in nucleosomal DNA is different than in free DNA

Dr Yang Liu, Prof Nicholas E Geacintov, Prof Vladimir Shafirovich. Department of Chemistry, New York University, New York, New York, United States

A method for the site-specific, oxidative injection of holes and the propagation of DNA damage in nucleosomes has been developed. The nucleosomes were reconstituted from recombinant histones and the 147 base pair-long 601 DNA sequence (first described by Lowary and Widom) containing the DNA damage reporter sequence 5'-d(T[2AP]TGTGGGTTGGGTTTGGGT). The site-specific injection of holes into nucleosomal DNA was initiated by the selective two-photon ionization of 2-aminopurine (2AP), a nucleic base analog paired with T, with intense nanosecond 308 nm XeCl excimer laser pulses. The 2AP radical (“hole”) rapidly and selectively oxidizes guanines positioned on its 3'-side. The holes (guanine radicals) were trapped by reactions with superoxide radicals that were derived from the trapping of the hydrated electrons by molecular oxygen. The distributions of the guanine lesions were monitored as alkali labile strand breaks by gel electrophoresis. The wrapping of the DNA duplex around the histone core has a significant impact on the distance of hole migration that is significantly lower in nucleosomal than in free DNA in the same solution. Supported by NIEHS Grant 2R01 ES011589-09

09:45 AM

Identification of damage lesions derived from the 3'-deoxy-C3'-thymidinyl radical

Amanda C Bryant-Friedrich PhD, Buthina Abdallah, Suaad Abdallah, Cheryl Ann Love, Kevin Trabbic. Department of Medicinal and Biological Chemistry, University of Toledo, Toledo, OH, United States; Department of Chemistry, Oakland University, Rochester, MI, United States

Single and double strand breaks result from the interaction of DNA with low-energy (~10 eV) electrons (LEE). A primary event believed to initiate strand scission underthese conditions is the homolytic cleavage of the sugar-phosphodiester C-O bondresulting in the formation of a C3'/C5'-deoxyribose radical. Sugar radicals are known to undergo degradation in the presence or absence of oxygen delivering damage lesions of varying types at the site of stand cleavage. It is the goal of this work to determine the structure and reactivity of DNA damage fragments resulting from 3'-bond scission, through

the site-specific generation of a 3'-Deoxy-C3'-thymidinyl radical. Several precursors have been successfully synthesized which, upon photochemical activation, produce the desired C3'-radical intermediate.DNA oligomers containing precursors of this reactive intermediate havealso been obtained and utilized in the elucidation of the structure of damageproducts derived from this sugar radical under both aerobic and anaerobicconditions.

10:05 AM
Title: Intermission

10:20 AM
LC-MS/MS for assessing the formation, repair, and transcription mutagenesis of purine cyclonucleosides

Jin Wang, Changjun You, Jianshuang Wang, Candace Guerrero, Yinsheng Wang. Chemistry, University of California Riverside, Riverside, CA, United States

Reactive oxygen species (ROS) can be induced by both endogenous and exogenous sources and maintaining cellular ROS homeostasis is essential for normal cellular function; excess generation of ROS can cause damage to lipids, proteins and DNA. Despite significant amount of work has been carried out for the replication and repair of single-nucleobase lesions induced by ROS, not much is known about bulky DNA lesions are repaired in human cells. In this presentation, I will describe the use of an LC-MS/MS method for assessing the formation and repair of two pairs of ROS-induced bulky DNA lesions, i.e., 8,5'-cyclo-2'-deoxyadenosine (cyclo-dA) and 8,5'-cyclo-2'-deoxyguanosine (cyclo-dG) in mammalian cells. We also found that the deficiency in a nucleotide excision repair protein (XPA) or base excision repair enzyme (NEIL1) both gave rise to elevated formation of endogenous cyclo-dA and cyclo-dG in mammalian cells. H_2O_2 treatment stimulated the formation of these DNA lesions in mammalian cells. More importantly, deficiency in XPA or NEIL1 led to marked decrease in the rate for the repair of these DNA lesions from H_2O_2-treated human cells. Furthermore, we found that both cyclo-dA and cyclo-dG blocked substantially the transcription in mammalian cells, and transcription bypass efficiency is much lower in XPA-deficient cells than repair-proficient cells, suggesting again the compromised repair of these lesions in XPA-deficient cells.

10:40 AM
DNA alkylation with quinolinium quinone methide: Mutagenic or suppressive?

Prof Qibing Zhou. College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, Hunang, China

Quinone methides (QMs) are involved in the metabolism of many carcinogens as reactive intermediates to forms nucleobase adducts that associate with mutagenicity. On the other hand, QM is utilized in the DNA alkylating agent to modify nucleobases as a potential antitumor agent. The dilemma of QMs as either the carcinogenic or antitumor agent is yet to be clarified. In this report, *N*-methylquinolinium QM was investigated to reveal the biological consequences of the formation of QM nucleobase adducts in a DNA target. Alkylation with *N*-methylquinolinium QM on a DNA target produced mostly a stable N^2-dG adduct, which was found to cause extensive stops in the primer extension with high fidelity DNA polymerase T7 and even low fidelity error prone Dpo4. Further biological impact of the DNA alkylation with QMs was assessed by comparing the expression level of a green fluorescence protein plasmid in cells under treatment of QMs.

11:40 AM
Conformational mapping of arylamine-DNA adducts: Structure-function-relationships

Bongsup Cho, Satyakam Patnaik, Vipin Jain, Vaidyanathan Ganesan. Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI, United States

Arylamine mutagens are implicated in the etiology of human cancers. The prototype arylamines 2-aminofluorene and 4-aminobiphenyl produce C8-substituted DNA adducts *in vivo*: AAF, AF, and ABP. AAF differs from AF in that the nitrogen at the linkage is acetylated, thus limiting the glycosidyl flexibility in DNA. ABP is structurally similar to AF, but lacks one methylene bridge and thus non-coplanar. We have shown that these adducts exist in an equilibrium of major groove (B), stacked (S), and wedge (W) conformers. Here, we report a conformational mapping of the three bulky lesions on a complete series of fully complementary NG*N/NCN duplexes (G*=AAF, AF, ABP). The results show that the S/B/W-

conformational heterogeneity is markedly dependent on both adduct structures and flanking sequences. The strong tendency for AAF to adopt a syn-glycosidyl S/W-conformation, while AF and ABP can adopt B/S and B, respectively, may explain why AAF blocks polymerases much more efficiently and provides a greater propensity to mutation and repair.

Founders Award (01:30 PM - 05:00 PM), Room 207 Colorado Convention Center

01:30 PM
Introductory Remarks. D. Liebler.

01:35 PM
Title: Award Presentation. F. Beland

01:40 PM
Control of cytochrome P450 catalysis by the proximal iron ligand

Santhosh Sivaramakrishnan PhD, Hugues Ouellet PhD, Paul R. Ortiz de Montellano PhD. Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA, United States

Cytochrome P450 catalysis is mediated by a ferryl [Fe(IV)=O] coupled to a ligand radical. The cysteine iron ligand is a critical determinant of the formation and reactivity of this intermediate and thus of cytochrome P450 catalysis. Replacement of the cysteine by weaker electron donating ligands yields inactive proteins, but its replacement in CYP119 and CYP125 by selenocysteine, a stronger electron donating ligand, produces catalytically active proteins. Studies with *meta*-chloroperbenzoic acid as a surrogate oxygen donor show that the substitution destabilizes the ferryl intermediate. However, a stable, catalytically inactive intermediate is formed from which the active protein can be regenerated by mild reduction. CYP125 oxidizes the cholesterol side-chain terminus to the alcohol, aldehyde, acid and small amounts of other products. Changes in product distribution with seleno CYP125 indicate that enhanced electron donation favors ferryl reactions over those mediated by its ferric hydroperoxy ([Fe(III)-OO-] precursor. Supported by NIH grants GM25515 and AI074824.

02:15 PM
Mechanism and protection against acetaminophen-induced hepatotoxicity

Andrew D Patterson, Yatrik M Shah, Frank J Gonzalez. Laboratory of Metabolism, National Cancer Institute, Bethesda, MD, United States

Acetaminophen (APAP) has been widely studied and is a paradigm for chemically-induced liver toxicity. APAP hepatotoxicity was probed using metabolomics leading to determination of the complete metabolic map of APAP including the discovery of new metabolites of the drug. Toxicity related endogenous metabolites were also uncovered revealing a role for mitochondrial damage in APAP-induced hepatotoxicity. For example, wild-type mice treated with APAP have high serum acylcarnitine levels that are dependent on metabolism of APAP and production of NAPQ1; serum acylcarnitine is not elevated in the nuclear receptor peroxisome proliferator-activated receptor α (PPARα)-null mice. These data indicate that APAP causes hepatic mitochondrial dysfunction and suggest a role for PPARα in the mechanism. Another connection between PPARα and APAP toxicity was established when it was discovered that pretreatment with clofibrate, a PPARα activator, protected mice against APAP-induced hepatotoxicity and that this protection was PPARα-dependent. The mechanism of this protective effect will be discussed.

02:50 PM
Drug metabolism: A critical element of contemporary drug safety assessment

Dr. Thomas A Baillie PhD, DSc. School of Pharmacy, University of Washington, Seattle, WA, United States

Over the past 20 years, drug metabolism has become an indispensible component of both research and development activities in the pharmaceutical industry, where it has played an increasingly important role in support of the safety assessment of new chemical entities (NCEs). In response to recent regulatory guidance on the safety evaluation of metabolites of candidate drugs in man, novel approaches have been adopted to define the identities and levels of circulating metabolites in the plasma of both human subjects and the animal species employed in safety assessment programs. In considering the factors that need to

be taken into account in assessing the safety of metabolites of NCEs, it is appropriate to classify biotransformation products into two categories based on their chemical reactivity, *viz*. 'stable' and 'chemically reactive'. The different experimental approaches employed to study these two groups are outlined, and the implications of the findings for lead optimization efforts in drug discovery, and for later phase clinical development programs, are discussed in the context of establishing the human safety of the NCE.

03:25 PM
Title: Intermission

03:40 PM
Insights into efficiency and specificity of bypass synthesis by Y-class DNA polymerases from X-ray crystallography

Professor Martin Egli PhD. Department of Biochemistry, Vanderbilt University, Nashville, Tennessee, United States

Among the 19 known human DNA polymerases (Pols), four belong to the trans-lesion or Y-class family, Pol eta, Pol iota, Pol kappa and REV1. These Pols exhibit different activities and abilities to replicate past a flurry of lesions. A unique feature of Y-class Pols, compared with the common right-handed arrangement of palm, thumb and finger subdomains of all Pols (i.e. A-class), is a 'little finger' subdomain that plays a crucial role in lesion bypass. To better understand the abilities of Y-class Pols to synthesize past damaged DNA, we are studying crystal structures of binary and ternary complexes between adducted DNAs and the human Pol eta, Pol iota and Pol kappa enzymes as well as the Dpo4 DNA Pol from Sulfolobus solfataricus (a Pol kappa homolog). The talk will summarize insights from recently determined structures as well as experiments probing the ability of Pols to discriminate between different nucleotide sugar conformations.

04:15 PM
Interactions of damaged DNA with DNA polymerases

Professor F. Peter Guengerich PhD. Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee, United States

Work on cytochrome P450 enzymes in this laboratoryled to studies on their roles in the activation of carcinogens, which then led to the study of DNA adducts. Further investigations have been focused on structural and functional aspects of how these lesions cause miscoding, via individual DNA polymerases. Recent investigations have involved N^2,3-ethenoguanine, utilizing an approach with a stabilized isostere. Another line of investigation involves the mechanism of base pair mutation by bis-electrophile crosslinked peptides (e.g. glutathione) and proteins to DNA. Work with *Sulfolobus solfataricus* DNA polymerase Dpo4 and adducts has been expanded to the four DNA polymerases of the organism, as a model for DNA fork traffic at adduct-stalled replication forks. (Supported by USPHS R01 ES010375, R01 ES010546)

Monday, August 29, 2011

Young Investigator Symposium (08:00 AM - 11:40 AM), Room: 207

08:00 AM
Title: Introductory Remarks. S. Balbo

08:05 AM
Use of an aldehyde reactive probe to capture protein carbonyls

Charles G Knutson PhD, Ujjal Sarkar PhD, John S Wishnok PhD, Joshua R Korzenik MD, Steven R Tannenbaum PhD. Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States; Crohn's and Colitis Center, Massachusetts General Hospital, Boston, Massachusetts, United States

Cellular exposure to lipid peroxidation products yields protein modifications from reactive carbonyls. However, the abundance of protein carbonyl adducts in biological samples is generally low relative to background. To more efficiently enrich the recovery of modified proteins from complex biological matrixes,

an aldehyde reactive probe was synthesized containing an oxo-amine “warhead”, a PEGylated linker region, and an azide tag. The azide tag can then be captured on alkyne-derivatized sepharose resin by copper-catalyzed 1,3-cycloaddition chemistry (Click chemistry). The crosslinking of protein carbonyls to the sepharose resin allows for stringent washing (1% SDS) and near-complete removal non-specifically bound protein. Tryptic digest of captured proteins from the resin produces a peptide fraction with good signal to noise properties.

08:20 AM

Base structure and sequence factors influence DNA duplex stability of oligonucleotides containing O^6-alkylguanosine-pairing synthetic nucleoside probes

Mr. Rahul R Lad, Prof. Shana J Sturla. Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN, United States; Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland

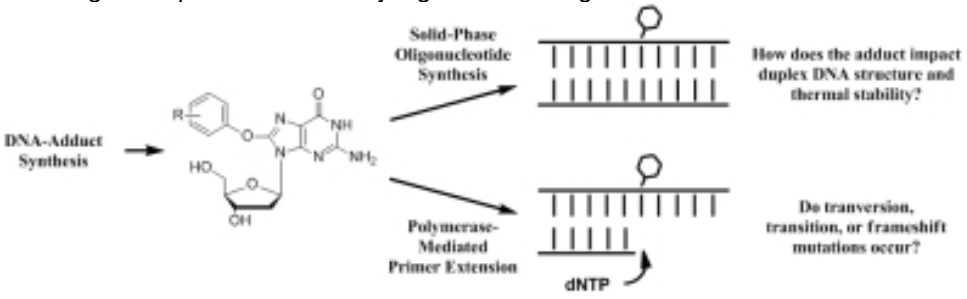
Despite their low abundance and potentialelimination by enzymatic repair, O^6-alkylguanosineDNA adducts can cause mutation and cancer. Synthetically derived hybridizationprobes for this class of adducts may provide a chemical basis for testing theformation and consequences of O^6-alkylguanosineadducts in cancer-relevant genes. In the present study, influences of nucleosidechemical structure on DNA duplex stability, as reflected in duplex meltingtemperatures (T_m), was evaluated by testing combinations ofsynthetic nucleoside probes, which differ in steric properties and hydrogenbond forming capacities, paired with DNA adducts or canonical bases. Sequencecontext, position of modification and neighboring base identity was found toimpact duplex stability when synthetic nucleosides were paired with adducts in systematicallydesigned sequences. Finally, detection limits for modified duplex interactionswere tested by titration with adducted oligonucleotides in the presence ofunmodified controls. Data suggest that modulation of structure and sequencecontext is critical in developing hybridization probes.

08:35 AM

Oxygen-linked 8-phenoxyl-deoxyguanosine nucleoside analogs

Heidi A. Dahlmann, Prof. Shana J. Sturla PhD. ETH Zurich, Switzerland

Nucleobase adducts, which form in vivo by the nucleophilic attack of nucleobases on exogenous electrophilic species, can impact the conformation and biochemical processing of the adducted nucleoside. Many nucleoside adducts have been shown to lead to mutations which may play a role in the development of cancer. Our aim is to evaluate the chemical and biological relevance of *O*-linked 8-phenoxy-purine adducts. An explicit limitation for studying these adducts is that synthetic methods for preparing them are considerably less developed than for their well-studied *N*-linked 8-arylamine-purine counterparts. Thus, we have developed a synthetic methodology to prepare a series of *O*-linked 8-phenoxyl-dG adducts with a variety of electron-donating, electron-withdrawing, and sterically demanding phenols; such adducts are expected to be useful as probes for further understanding the structural and toxicological impacts of DNA major groove-binding events.



08:50 AM

Unusual furanose “West” puckering of (5'*S*)-8,5'-cyclo-2'-deoxyguanosine in DNA

Dr. Hai Huang, Rajat S. Das, Prof. Ashis Basu, Prof. Michael P. Stone. Department of Chemistry, Vanderbilt University, Nashville, TN, United States; Center in Molecular Toxicology, Vanderbilt University, Nashville, TN, United States; Center for Structural Biology, Vanderbilt University, Nashville, TN, United States; Department of Chemistry, University of Connecticut, Storrs, CT, United States

8,5'-Cyclopurines represent an important class of tandem DNA damage induced by ionizing radiation, which might play a role in the etiology of neurodegeneration in xeroderma pigmentosum. These lesions block transcription and inhibit gene expression, and are subject to repair by NER. Structures of (5'*S*)-8,5'-cyclo-2'-deoxyguanosine (*S*-cdG) containing DNA duplexes were investigated by NMR spectroscopy. The duplexes were destabilized when *S*-cdG was placed opposite dA, dC or dT. In all circumstances, the *S*-cdG deoxyribose adopted the unusual O4'-exo (West) puckering and caused helical distortions at the lesion sites. The helical twist and base pair shifts at the lesion sites and the 5'-neighbor dC·dG base pairs were perturbed. Remarkably, the incorporation of *S*-cdG only destabilized the 3'-neighbor dT·dA base pair. These perturbations may be responsible for the genotoxicity of *S*-cdG and its ability to be recognized by NER repair. Supported by NIH grants CA-55678 (M.P.S.) and ES-013324 (A.K.B.).

09:05 AM

Structural investigation of exocyclic deoxyadenosine adducts induced by 1,2,3,4-diepoxybutane

Ewa A Kowal, Uthpala Seneviratne, Susith Wickramaratne, Natalia Tretyakova, Michael P Stone. Department of Chemistry, Center in Molecular Toxicology, Vanderbilt University, Nashville, TN, United States; Department of Medicinal Chemistry and Masonic Cancer Center and Department of Chemistry, University of Minnesota, Minneapolis, Minnesota, United States

Two exocyclic deoxyadenosine adducts of diepoxybutane (DEB), a carcinogenic metabolite of 1,3-butadiene were identified: N^6,N^6-(2,3-dihydroxybutan-1,4-diyl)-2'-deoxyadenosine (**1**) (*R,R*-N^6,N^6-DHB-dA), (**2**) (*S,S*-N^6,N^6-DHB-dA). These adducts prevent Watson-Crick base pairs, and they are anticipated to be pro-mutagenic lesions. NMR spectroscopy was utilized to determine how these adducts alter the structure and dynamics of DNA. Thermal melting studies indicated reduced stabilities for duplexes containing either adducts **1** or **2**. The NOESY spectra of duplexes containing adducts **1** or **2** were similar as compared to the unmodified duplex, suggesting localized structural changes at the lesion sites. The structure of the duplex containing adduct **1** or **2** shows that the T^6 base, which is complementary to the adduct, flips out from the duplex. These results confirm that **1** and **2** adducts destabilize duplex by disrupting Watson-Crick hydrogen bonding and displacing the opposite base into major groove which might be related to their mutagenecity.

09:20 AM Intermission

09:30 AM

Translesion synthesis across N^2-3-ethenoguanine by microbial and human DNA polymerases

Linlin Zhao PhD, Plamen P Christov PhD, Ivan D Kozekov PhD, Albena Kozekova MS, Carmelo J Rizzo PhD, F. Peter Guengerich PhD. Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN, United States; Department of Chemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN, United States

N^2-3-Etheno(ε)guanine(G) is an important DNA lesion resulting from exposure to xenobiotics (e.g., vinyl chloride) or from endogenous sources, e.g. lipid peroxidation. It has a reported mutagenic potential of G to A transitions; however previous approaches are rather indirect due to the labile glycosidic bond. Two 23-mer oligonucleotides containing the N^2-3-εG were successfully synthesized utilizing a 2'-fluoroarabinose strategy to retard glycosidic cleavage; 2'-fluoroarabino-G did not perturb DNA polymerase recognition. We systematically explored the miscoding properties of N^2-3-εG using several microbial and human DNA polymerases. In all polymerases examined, T incorporation was found to be the only miscoding event, with varying misincorporation frequencies. Klenow fragment, Dpo4, and human pol κ showed T miscoding frequencies of ≤30%; human pol ι preferred T to C incorporation by 3-fold. Mass spectrometric analysis of the extended products from the Dpo4 reactions identified major products from both C and T incorporation. Thus, the DNA polymerases studied here showed different miscoding properties across N^2-3-εG and varying miscoding frequencies for different template sequences, indicating different chemistry in the binding pockets. (Supported by USPHS R01 ES010375, R01 ES010546, PO1 ES05355, and P30 ES00267)

09:45 AM

Human DNA polymerase κ utilizes a WMSA mechanism for the nucleotidyl transfer reaction

Lee Lior, Lihua Wang PhD, Shenglong Wang PhD, Suse Broyde PhD, Yingkai Zhang PhD. Department of Chemistry, New York University, New York, NY, United States; Department of Biology, New York University, New York, NY, United States

The molecular mechanism of the DNA polymerase catalyzed nucleotidyl transfer reaction has elicited significant interest in recent years but a universal mechanism has been elusive. We have previously employed state-of-the-art simulations to determine a water-mediated and substrate assisted (WMSA) mechanism in which the hydrogen on the 3'-OH primer terminus is transferred through crystal and solvent waters to the gamma phosphate of the dNTP in a rate limiting step, followed by the nucleotidyl transfer step. The mechanism is energetically preferred for the low fidelity Y family polymerase Dpo4 and the high fidelity replicative polymerase from phage T7. Here we have utilized recently developed ab initio QM/MM-MD simulations with umbrella sampling to thoroughly explore the nucleotidyl transfer reaction for the human Y family DNA polymerase κ. Our results reveal that the WMSA mechanism is energetically favored for this case as well, suggesting the plausibility of its broad importance.Supported by NIH Grants R01-CA75449 and R01-CA-28038 to S.B., NIH R01-GM079223 and NSF CHE-CAREER-0448156 to Y.Z., and computations are supported in part by the NSF through TeraGrid resources under grant number TG-MCB060028N.

10:00 AM

Replication studies of carboxymethylated DNA lesions induced by *N*-nitroso compounds

Ashley L Swanson, Changjun You PhD, Jianshuang Wang PhD, Yinsheng Wang PhD. Environmental Toxicology Graduate Program, University of California, Riverside, Riverside, CA, United States; Department of Chemistry, University of California, Riverside, Riverside, CA, United States

N-nitroso compounds (NOCs) are known to cause DNA damage and are both mutagenic and carcinogenic. Humans are exposed to NOCs from environmental and endogenous sources, and the latter accounts for 45-75% of the total NOC exposure. NOCs have been shown to produce carboxymethylation of nucleobases through a diazoacetate intermediate. This research seeks to examine how these carboxymethylated DNA lesions compromise DNA replication by hindering TLS polymerases, including yeast pol h, and inducing mutations. This is accomplished using steady-state kinetic assays to assess the efficiency and fidelity of DNA polymerases to incorporate nucleotides opposite these lesions. The steady-state kinetic results revealed that yeast pol h can be error-prone or error-free. We also began to assess how these DNA lesions perturb the efficiency and accuracy of DNA replication in mammalian cells. The above results suggest that these lesions may bear important implications in the etiology of NOC-induced tumor development.

10:15 AM

Stereochemical aspects of the conjugation of butadiene diepoxide with glutathione by glutathione transferases

Sung-Hee Cho, F. Peter Guengerich. Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee, United States

Butadiene diepoxide is the most potent mutagenic metabolite of 1,3-butadiene, an important industrial chemical and environmental pollutant. Three butadiene diepoxide stereoisomers including *R,R*-, *S,S*-, and *meso*-butadiene diepoxide are generated by 1,3-butadiene metabolism. We previously reported that butadiene diepoxide forms a conjugate with glutathione (GSH), and the conjugate is considerably more mutagenic than several other butadiene-derived epoxides, including the diepoxide, in *Salmonella typhimurium* TA1535 (*Chem. Res. Toxicol*. *23*, 1544 (2010)). We examined steady-state kinetic parameters for the conjugation with the three butadiene diepoxide stereoisomers and GSH by six GSH transferases (GSTs): rat GST 5-5 and human GST T1-1, A1-1, A3-3, M1-1, and P1-1. The catalytic efficiency (k_{cat}/K_m) differed depending on the butadiene diepoxide stereoisomer and the GST, although all were active. These results are relevant to the mutagenicity of butadiene diepoxide in different organs. (Supported in part by USPHS grants R01 ES010546 and P30 ES000267)

10:30 AM

Toward the understanding of benzo[*a*]pyrene (B[*a*]P) metabolism in human bronchial epithelial cells (HBEC) by a stable isotope dilution tandem mass spectrometry method

Ding Lu, Ronald Harvey, Ian Blair, Anil Vachani, James Kreindler, Trevor Penning. Center for Excellence in Environmental Toxicology, University of Pennsylvania, Philadelphia, PA, United States; Center for Cancer Pharmacology, University of Pennsylvania, Philadelphia, PA, United States; Department of Pharmacology, University of Pennsylvania, Philadelphia, PA, United States; Division of Pulmonary, Allergy and Critical Care, Department of Medicine, University of Pennsylvania, Philadelphia, PA, United States; The Ben May Department of Cancer Research, University of Chicago, Chicago, IL, United States

Polycyclic aromatic hydrocarbons (PAHs) are major constituents of cigarette smoke and are carcinogenic in multiple organs and species. Benzo[*a*]pyrene (B[*a*]P), a representative PAH, has been designated as a Group 1 human carcinogen by the International Agency for Research on Cancer. B[*a*]P requires metabolic activation to exhibit its toxicity and carcinogenicity. The three major metabolic pathways involved include formation of radical cations (peroxidase mediated), diol-epoxides (P450 mediated) and o-quinones (aldo-keto reductase mediated). However, the engagement of different metabolic pathways in human lung and their contributions to lung cancer induction are unclear. We developed a sensitive stable isotope dilution atmospheric pressure chemical ionization tandem mass spectrometry method to quantitate B[*a*]P signature metabolites from each metabolic pathway. This method was applied to study the B[*a*]P metabolism in HBEC-KT (immortalized) cells and primary HBEC cells obtained by flexible bronchoscopy from patient donors[Supported by 1P30-ES013508 and 1R01-ES-15857 to TMP]

10:45 AM Intermission

10:55 AM

QCI/DFT studies of metabolic pathways of hypoxia: Selective heterocyclic di-*N*-oxide antitumor agent tirapazamine (TPZ) and analogs

Jian Yin, Professor Rainer Glaser PhD, Professor Kent S Gates PhD. Chemistry, University of Missouri, Columbia, MO, United States; Biochemistry, University of Missouri, Columbia, MO, United States

Tirapazamine (TPZ, **1**, 3-amino-1,2,4-benzotriazine 1,4-*N*-dioxide) can selectively damage DNA in the hypoxic cells of solid tumors. There is general agreement that the primary metabolite of TPZ, 1-hydroxyl-3-amino-1,2,4-triazine-1-oxide (**2**) is formed by enzymatic reduction and protonation. The N-OH homolysis has been considered to be the main reaction channel for **2** leading to **3** and hydroxyl radical, the reactive species responsible for the DNA strand breaks. Recently, it was suggested that **2** might undergo dehydration instead of •OH loss leading to benzotrizinyl radical **4** (BTZ) and/or aryl radicals **5**. To explore these alternatives, we applied high-level correlated methods (QCI/DFT) to study the thermodynamics and the kinetics of the pathways for TPZ decomposition. The results are discussed with reference to experimental results of TPZ and of TPZ analogs and with focus on the analysis of potential chemical options to control access to any one of these reaction channels by substrate design.

11:10 AM

Examining the diabetogenic effects of trivalent arsenicals in cultured C2C12 myotubes

Samantha Attard, Jenna Currier, Felecia Walton, Christelle Douillet PhD, Zuzana Drobna PhD, Miroslav Styblo PhD. Department of Nutrition, UNC Chapel Hill, Chapel Hill, NC, United States; Department of Toxicology, UNC Chapel Hill, Chapel Hill, NC, United States

Chronic exposures to inorganicarsenic (iAs) have been linked to the risk of diabetes mellitus. We examinedeffects of iAsIII and its mono- and dimethylated metabolites, MAsIII andDMAsIII, on basal and insulin-stimulated glucose uptake (BGU, ISGU) by cultureddifferentiated C2C12 myotubes. 72-

hourexposures to iAsIII and MAsIII inhibited BGU and ISGU; however, the inhibition wasclosely linked with inhibition of myogenic differentiation and loss of cellviability. In contrast, 1μM DMAsIIIinhibited both BGU and ISGU without affecting myotube viability (LC50 ~4μM) or differentiation.Speciation analysis of As in iAsIII- and MAsIII-treated cells revealed that myogenicdifferentiation increased the cell capacity to methylate these arsenicals,suggesting that myogenic differentiation stimulates the expression of arsenic(+3 oxidation state) methyltransferase and/or As transporters. In summary, ourdata suggest that DMAsIII can inhibit glucose uptake by skeletal muscle cells ina manner that is consistent with the diabetogenic effects of iAs exposure.

11:25 AM

Computational framework for characterizing biomarkers oforganophosphorus insecticide mixture exposure

Mr. Jaime H Ivy, Jesse M Wright, Justin Rogers, Arthur N Mayeno, Michael A Lyons, Brad Reisfeld. Chemical and Biological Engineering, Colorado State University, Fort Collins, CO, United States

The disposition and toxicological effects of xenobioticsfollowing exposure are governed by complex absorption, distribution,metabolism, and elimination; and pharmacodynamic (ADME+PD) processes.Characterizing the biomarkers of exposure and their relationship to ADME+PDwarrants the development of modeling tools capable of facilitating thesimulation and analysis of tissue dosimetry. We propose such a tool can both describedosimetry and reconstruct dose from biomarker data.Here we describe the development and usage of such amodeling tool and framework. This software package, DoseSim:OP,is centered around a sophisticated statistical modeling engine, allowing analysesthat includes Bayesian inference. Though we include an updated version ofTimchalk et al. (2008) organophosphate insecticide (OP) mixture PBPK/PD model,the modular architecture behind DoseSim:OP allows various simulation modelstructures to be imported into the modeling engine, allowing great flexibilityin the systems and toxins for ADME+PD analysis. This project is supported byEPA STAR Grant #RE83345101.

Human Exposure and Responses to Toxins from the Air and Water (01:30 PM - 03:45 PM), Room 207 Colorado Convention Center

01:30 PM Introductory Remarks

01:35 PM

Dose-response study of arsenic exposure, blood glutathione, and peripheral blood mononuclear cell DNA methylation in Bangladesh

Dr. Megan N Hall, Ms. Megan Niedzwiecki, Dr. Xinhua Liu, Ms. Julie Oka, Ms. Vesna Slavkovich, Ms. Vesna Ilievski, Ms. Diane Levy, Mr. A Siddiquie, Mr. Faruque Parvez, Dr. Joseph H Graziano, Dr. Mary V Gamble. Epidemiology, Columbia University, New York, NY, United States; Environmental Health Sciences, Columbia University, New York, NY, United States; Biostatistics, Columbia University, New York, NY, United States

The mechanisms through which chronic exposure to arsenic (As) causes disease in humans remain poorly understood. We conducted a cross-sectional study to evaluate the dose-response relationships between arsenic exposure and two intermediate health outcomes: 1) blood and plasma concentrations of glutathione, a critical component of the primary antioxidant defense system and the electron donor for As reduction, and 2) genomic methylation of peripheral blood mononuclear cell (PBMC) DNA. We recruited 379 Bangladeshi participants between the ages of 30 and 63 into one of 5 water arsenic categories: <10 (n=76), 10-100 (n=104), 101-200 (n=86), 201-300 (n=67), and >300 ug/L (n=45). Exposure was assessed using well water, blood, and urinary concentrations of As. Our findings show that As is associated with alterations in both blood glutathione concentrations and in genomic methylation of PBMC DNA, suggesting that both of these pathways may be relevant to As-associated disease development in humans.

02:15 PM

Biomarkers for the assessment of diabetes associated with chronic exposure to arsenic

Dana Loomis PhD, Luz Maria Del Razo PhD, Gonzalo García-Vargas, Zuzana Drobná PhD, Miroslav Stýblo PhD. Department of Epidemiology, University of Nebraska Medical Center, Omaha, NE, United States; Department of Toxicology, CINVESTAV-IPN, Mexico City, Mexico; Faculty of Medicine, Juarez University of Durango State, Gómez-Palacio, Mexico; Department of Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Our recent work in Zimapan and Lagunera regions (Mexico) has linked chronic exposure to inorganic arsenic (iAs) in drinking water to the risk of diabetes characterized by glucose intolerance and increased fasting blood glucose (FBG) levels. The risk of diabetes was positively associated with the urinary concentration of dimethylarsinite ($DMAs^{III}$), a toxic metabolite of iAs. Both $DMAs^{III}$ in urine and risk of diabetes were higher for carriers of the M287T variant of AS3MT, the methyltransferase that methylates iAs. Notably, fasting plasma insulin (FPI) and insulin resistance (HOMA-IR) were negatively associated with iAs exposure, suggesting that the mechanisms of iAs-induced diabetes differ from those underlying type-2 diabetes. Thus, the diabetic phenotype associated with iAs exposure is characterized by glucose intolerance and high FBG with seemingly normal HOMA-IR and FPI. The AS3MT/M287T carriers are more likely to develop this phenotype when exposed to iAs because they produce more $DMAs^{III}$ than wtAS3MT carriers.

02:55 PM Intermission
03:05 PM

Linking arsenic metabolism and toxic effects

David J. Thomas. National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, United States

Although arsenic has been long recognized as a toxicant and a carcinogen, the molecular basis for few of its adverse effects are well understood. Like other metalloids, arsenic undergoes extensive metabolism involving oxidation state changes and formation of methyl-arsenic bonds which affects its kinetic behavior and inherent toxicity. Hence, factors that modify capacity for arsenic methylation likely account for some of the variability among individuals and species in susceptibility to arsenic-induced diseases. Arsenicals are interconverted between oxy- and thio- forms affecting kinetic behavior and toxicity and complicating dosimetric and mode of action studies. Thus, the research challenge is to develop analytical approaches that quantify relevant metabolites so accurate cellular budgets for arsenicals can be developed. Application of these analytical tools will support definitive studies of modes of actions of arsenicals and better estimation of risk associated with exposure to arsenic. (This abstract does not reflect US EPA policy.)

Perspectives Lecture (04:00 PM - 05:45 PM) Room: 207 Colorado Convention Center

04:00 PM: Introductory Remarks
04:05 PM

ToxCast: Applicationof computational toxicology and high throughput screening to improve chemicalsafety assessment

Director, NCCT Robert Kavlock. Office of Research and Development, United States Environmental Protection Agency, United States

Evaluating the safetyof chemicals is challenged by the expense of the traditional approach ofanimal-based experimental models and resulting low throughput acquisition ofknowledge. As a result, we simply do not know the potential hazards ofmany chemicals in commerce. EPAsNational Center for Computational Toxicology was established in 2005 to helpaddress this issue. It has developed a comprehensive high throughputscreening program (ToxCast) that has profiled 1000 chemicals of environmentalinterest across more than 600 biological endpoints. The ToxCast approach primarilyemploys protein and cell based in vitro assays, and we have built a number ofdatabases and statistical approaches to understand the resulting screeninginformation in the context of biological pathways and disease outcomes. Allthe databases, screening results, and models are made available in atransparent manner so that others can analyze independently. The ToxCasteffort is helping to transform the conduct of toxicology.

05:00 PM Reception

3. Tuesday, August 30, 2011

Drug Safety (01:30 PM - 04:50 PM) Room**:** 207 Colorado Convention Center

01:30 PM
Introductory Remarks. F. Guengerich

01:35 PM
Idiosyncratic drug induced liver injury: From man to mouse to computer

Director Paul Brent Watkins MD. Hamner – University of North Carolina Institute for Drug Safety Sciences, Research Triangle Park, North Carolina, United States

Drug Induced Liver Injury (DILI) remains the major organ toxicity that leads to termination of clinical development programs and regulatory actions on approved drugs. The most problematic DILI is “idiosyncratic”, occurring in only a very small proportion of treated patients and often not detected until late in Phase 3 or post-marketing. Improvements in preclinical approaches to identify DILI liability will require better understanding of mechanisms. The Drug Induced Liver Injury Network supported by the National Institutes of Health has created a registry and tissue bank from patients who have experienced DILI. Genome wide association analyses and whole exome/ genome sequencing of DILI cases are providing clues to mechanisms. However, it has become clear that chemoinformatic and preclinical approaches are also needed to generate specific hypotheses that can be tested with the genetic data. DILIsim is an initiative to incorporate the new data into an in silico predictive model.

02:20 PM
Screening for drug induced liver injury potential

Dr J Gerry Kenna PhD. Safety Assessment UK, AstraZeneca, Alderley Park, Cheshire, United Kingdom

Drug induced liver injury is a leading cause of attrition during drug development and of withdrawal, cautionary labelling and restricted usage of licensed drugs. Although the underlying mechanisms are incompletely defined, an essential first step is chemical insult to cells within the liver. Important initiating events include reactive metabolite formation, mitochondrial toxicity, potent intrinsic cell toxicity and inhibition of biliary efflux transport proteins, which for at least some drugs may act in combination (Greer et al, Toxicology. 2009; 268(3):125-31). Within AstraZeneca, an *in vitro* Hepatic Liability Panel has been developed which provides quantitative data on these liabilities and can be applied during drug Discovery, when chemical choice is available. The goal is to identify and deselect compounds that have a high likelihood to cause DILI if progressed into preclinicial species or man, and thereby improve the quality of molecules that enter drug Development.

03:05 PM Intermission
03:20 PM
Kidney injury biomarkers in urine: From rats to regulatory approvals

David Gerhold, Frank Dieterle, Josef Ozer, Daniel Holder, Sean Troth, Warren Glaab, Wendy Bailey, Frank Sistare. NIH Chemical Genomics Center/National Center for Advancing Translational Sciences, present address: National Institutes of Health, Rockville, MD, United States; Safety Assessment/Molecular & Investigative Toxicology, Merck & Co. Inc., West Point, Pennsylvania, United States; Global Program Diagnostics, Novartis Pharma, Basel, Switzerland; Pharmacokinetics, Dynamics, and Metabolism, PGRD, present address: Pfizer, Andover, Massachusetts, United States; Department of Biometrics, Merck & Co. Inc., West Point, Pennsylvania, United States

Kidney toxicity is a frequent cause of failure in pharmaceutical drug development. Since traditional methods for diagnosis of kidney injury are notoriously insensitive, we set out to identify and evaluate emerging biomarkers of renal injury in urine and blood. Immunoassays were developed and validated for urinary biomarker proteins, including: KIM1, Clusterin, Trefoil Factor 3, Albumin, Total protein, b2-microglobulin, and Cystatin C. Biomarkers were evaluated in rat studies using >20 renal or non-renal toxicants; and found to respond to acute kidney injury affecting either proximal tubules or distal tubule

papillae.Through the Predictive Safety Testing Consortium, these seven biomarkers were approved as “valid” by the regulatory agencies: FDA, EMA, and PMDA. Additional translational efforts are ongoing to evaluate an expanded set of 20 biomarkers in human studies. Qualification of such kidney injury biomarkers is facilitating development of safe pharmaceuticals, and will ultimately improve clinical diagnosis of kidney injury.

04:05 PM

Role and influence of trans-membranetransporters in toxicity

William W Johnson. GlaxoSmothKline, United States

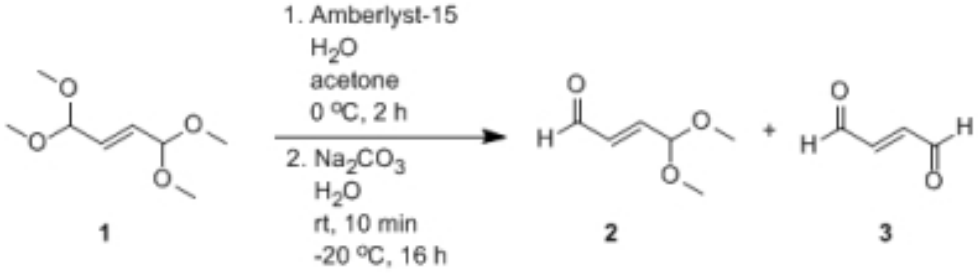
Hepatobiliary trans-membranetransport regulates intracellular exposure to xenobiotic and endogenouschemicals and metabolites. The mechanism for liver injury of several withdrawntherapeutics involves abrogation of the bile salt export pump (BSEP) functionand animal results do not correlate well with clinical hepatoxicity. BSEP impedance by drugs correlates with humanhepatoxicity; in some cases the function of P-glycoprotein and/or BCRP cancompensate for the abrogation of the BSEP transport of xenobiotics. Insufficientrouting of acyl-glucuronides due to ABC-C2 inhibition can result inhepatotoxicity. In addition to various hepatotoxicantsthat rely on transporters to influence the degree of severity there are manyneurotoxins that are precluded from the CNS/CNF by transporters at theblood-brain barrier. Uptake transporters such as OATPs and OCTs canenrich some xenobiotics to concentrations above the tolerability window. Certain drugs potently inhibit OATP1B1,causing clinically significant drug interactions.

General Posters (07:30 PM - 09:30 PM)
Hall D, Colorado Convention Center

Selective monohydrolysis of (*E*)-1,1,4,4-tetramethoxybut-2-ene

Yahua Liu, Lawrence M Sayre. Department of Chemistry, Case Western Reserve University, Cleveland, OH, United States

(*E*)-4,4-Dimethoxybut-2-enal (**2**) is a key intermediate for the synthesis of 4-hydroxynonenal (HNE) that has been extensively investigated for its role in Alzheimer's disease pathology. In the preparation of **2**, hydrolysis of (*E*)-1,1,4,4-tetramethoxybut-2-ene (**1**) resulted in both **2** and fumaraldehyde (**3**) in previous literature reports. We have developed a selective monohydrolysis method which gives **2** without forming **3**: A solution of **1** in mixed solvent of H_2O and acetone (1:3) with suspended Amberlyst-15 was stirred at 0 ºC for 2 h, filtered to remove Amberlyst-15, basified with Na_2CO_3, and stored at -20 ºC for 16 h.



Selective estrogen receptor modulators (SERMs) inhibit oxidative stress and malignant transformation in breast epithelial (MCF-10A) cells

L.P. Madhubhani P. Hemachandra, Judy L. Bolton, Gregory R. J. Thatcher. Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, College of Pharmacy, 833 S Wood Street, Chicago, Illinois, United States

The risk of developing hormone dependent cancers in women will increase with long term exposure to estrogens. Estrogen induced cell proliferation in estrogen receptor positive cells (hormonal pathway) and formation of reactive estrogen quinoids mediated by cytochrome P450s (chemical pathway) are believed to contribute to estrogen carcinogenesis. Estrogens are oxidized to the catechols, 2-hydroxyestradiol (2-OHE_2) and 4-hydroxyestradiol (4-OHE_2) by P450 1A1/1A2 and P450 1B1, respectively. Both catechols are further oxidized to form electrophilic *o*-quinones which can react with DNA and proteins. Selective

estrogen receptor modulators (SERMs) have been used for chemoprevention and in the treatment of post menopausal osteoporosis. The current study focuses on elucidating the influence of clinical and pre-clinical benzothiophene SERMs on oxidative estrogen metabolism in breast epithelial (MCF-10A) cells. MCF-10A cells are estrogen receptor negative non-tumorigenic human breast epithelial cells which can be transformed into a malignant phenotype with exposure to 17β-estradiol (E_2). After treatment of MCF-10A cells with E_2 and SERMs, estrogen metabolites were analyzed using LC-MS/MS. Among the SERMs tested, raloxifene and desmethylarzoxifen (DMA) showed a significant reduction in catechol estrogen formation whereas 4'F-DMA had little effect. E_2 induced oxidative stress and malignant transformation were also inhibited by raloxifene and DMA. These data suggest that raloxifene and DMA possess chemopreventive activity through inhibition of genotoxic estrogen quinone formation, in addition to their action via estrogen receptors.

Lesion topology and nucleotide excision repair: The mobile phenyl ring of C8-dG-PhIP allows NER susceptibility despite absence of a partner nucleotide in a deletion duplex

Hong Mu, Dara A. Reeves PhD, Konstantin Kropachev PhD, Yuqin Cai PhD, Shuang Ding PhD, Lihua Wang PhD, Alexander Kolbanovskiy, Marina Kolbanovkiy, Ying Chen, Assistant Professor Jacek Krzeminski PhD, Professor Shantu Amin PhD, Professor Suse Broyde PhD, Professor Nicholas E. Geacintov PhD. Department of Biology, New York University, New York, NY, United States; Department of Chemistry, New York University, New York, NY, United States; Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA, United States

The differences in the structural properties of DNA lesions that are either resistant to, or elicit efficient nucleotide excision repair (NER), are of considerable interest. The C8-dG-PhIP adduct, derived from a carcinogen in cooked foods, is very susceptible to human NER in a full duplex, while deletion of the nucleotide opposite the lesion reduces the efficiency of NER but does not eliminate it entirely. We performed molecular dynamics simulations for C8-dG-PhIP-modified full and deletion duplexes, and compared the results with the 10*R* (+)-*cis*-*anti*-B[*a*]P-N^2-dG adduct which is efficiently repaired in the full duplex, but is completely resistant to NER in the case of the deletion duplex containing the identical 10*R* (+)-*cis*-*anti*-B[*a*]P-N^2-dG lesion. In both cases the adducts adopt base-displaced intercalated conformations. Our simulations demonstrate the destabilizing features of both types of lesions in the full duplexes, explaining their high NER susceptibilities. The persistence of some repair susceptibility in the C8-dG-PhIP-modified deletion duplex, despite the absence of the partner nucleotide, stems from the mobility of its phenyl ring and the smaller number of aromatic rings as compared to the rigid, planar B[a]P-derived adduct, highlighting the conclusion that the absence of a partner nucleotide does not necessarily confer repair resistance to NER. This research is supported by NIH grants R01-CA-75449 and R01-CA-28038 to S.B. and R01-CA-099194 to N.E.G.

Insights into the susceptibility of the 10*R* (+)-*cis*-*anti*-B[*a*]P-N^2-dG adduct to human NER: Replacing the normal partner base C by an A mismatch dramatically decreases the efficiency of repair

Hong Mu, Hong Zhang, Shuang Ding PhD, Yuqin Cai PhD, Lihua Wang PhD, Dara A. Reeves PhD, Konstantin Kropachev PhD, Alexander Kolbanovskiy, Marina Kolbanovkiy, Ying Chen PhD, Professor Shantu Amin PhD, Professor Suse Broyde PhD, Professor Nicholas E. Geacintov PhD. Department of Biology, New York University, New York, NY, United States; Department of Chemistry, New York University, New York, NY, United States; Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA, United States

A DNA duplex containing 10*R* (+)-*cis*-*anti*-B[*a*]P-N^2-dG (*cis*-B[*a*]P) and a normal partner dC is base-displaced intercalated and is highly susceptible to repair by the human NER apparatus. However, when this *cis*-B[*a*]P adduct is mismatched with dA in the partner strand, human NER is nearly abolished, although spectroscopic evidence indicates that the polycyclic *cis*-B[*a*]P residue remains intercalated. We used molecular dynamics simulations to elucidate the reasons underlying the remarkable repair resistance to NER of the duplex with an A, instead of the normal C, positioned opposite the lesion in the complementary strand. Our results show that stacking of the B[a]P ring system in the intercalation pocket is better with partner A than partner C, and hydrogen bonding of adjacent base pairs is also less perturbed. The enhanced stacking and better hydrogen bonding with partner A can account for the diminished intrusion of the XPC recognition factor's β-hairpin and, as a consequence, the low

susceptibility to NER with the mismatched duplex. This research is supported by NIH grants R01-CA-28038 to S.B. and R01-CA-099194 to N.E.G.

Generation of guanine-thymidine cross-links by peroxynitrite, a chemical mediator of inflammation

Dr Byeong Hwa Yun, Prof Nicholas E Geacintov, Prof Vladimir Shafirovich. Division of Environmental Health Sciences, Wadsworth Center, NYS Department of Health, Albany, New York, United States; Deapartment of Chemistry, New York University, New York, New York, United States

Nitrosoperoxycarbonate derived from the combination of carbon dioxide and peroxynitrite, is an important chemical mediator of inflammation. In aqueous solutions, it rapidly decomposes to form carbonate and nitrogen dioxide radicals. Exposure of oligonucleotides and native DNA generates cross-linked guanine-thymine product G*-T* with a covalent bond between the C8 (G*) and thymine N3 (T*) atoms together with known nitration/oxidation products of guanine such as 8-nitroguanine, 5-guanidino-4-nitroimidazole, 8-oxoguanidine and spiroiminodihydantoin. The yields of these products, after enzymatic digestion with P1 nuclease and alkaline phosphatase to the nucleotide level, and reversed phase HPLC separation, were compared with those obtained with the isotope labeled standards of these lesions. The difference in the distributions of the end products in oligonucleotides and native DNA is discussed in terms of the competition between nucleophilic addition reactions to guanine radicals (followed by the formation of the G*-T* cross-links), and radical-radical combination reactions (followed by the formation of nitroproducts). Supported by NIEHS Grant 2R01 ES011589-09

Inflammation-mediated nitrosative deamination of RNA: Resistance of adenosine and partition of guanosine to xanthosine and oxanine

Vasileios Dendroulakis, William M. Deen, Peter C. Dedon. Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States; Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States

The gaseous free radical nitric oxide (NO) plays an important role in innate immunity, with activated macrophages generating high levels of NO at sites of inflammation. Among reactive nitrogen species derived from NO, nitrous anhydride (N_2O_3) is believed to be the major nitrosating species in cells and tissues, yielding xanthine (X) and oxanine (O) from guanine, hypoxanthine (I) from adenine and uracil (U) from cytosine in DNA and RNA. Previous studies revealed that, while NO caused similar high rates of deaminaton of G, A and C in DNA in vitro, the cellular environment protected DNA from significant deamination. We have now undertaken studies of NO-induced deamination of RNA in an effort to assess RNA as an index of nitrosative deamination in cells, estimate the steady-state levels of N_2O_3 in cells, and define the utility of RNA deamination as a biomarker of inflammation. We exposed purified RNA from human lymphoblastoid TK6 cells to NO and O_2 at constant levels of 1.7 µM and 210 µM, respectively. Kinetic studies revealed that adenosine was resistant to deamination compared to guanosine, which is significantly different from the equal reactivity of A and G observed in DNA. Further, NO caused significant formation of oxanine in addition to xanthine. The deamination rate constants for inosine, oxanine and xanthosine formation were found to be 3×10^4, 8×10^5 and 9×10^5 $M^{-1}s^{-1}$, respectively. We then exposed cultures of TK6 cells to 4-5-fold higher NO doses, using a novel delivery system. While there was little detectable deamination of RNA in exposed cells, reduction of glutathione by incubation of cells with buthionine sulfoximine prior to NO exposure revealed increased accumulation of xanthosine and oxanosine, but not inosine, in RNA. This highlights the role of endogenous reductants in affecting nitrosative damage in the cellular milieu.

Quantitation of 7-ethylguanine in human leukocyte DNA by liquidchromatography-nanospray ionization-high resolution mass spectrometry

Silvia Balbo, Peter W Villalta, Stephen S Hecht. Masonic Cancer Center, University of Minnesota, Minneapolis, MN, United States

There is convincing evidence for an uncharacterized ethylatingagent in cigarette smoke. To further investigate the role of ethylatingagents in smoking-induced cancer we developed a LC-nanospray ionization-highresolution--mass spectrometry method for the analysis of 7-ethyl-Gua in humanleukocyte DNA. [$^{15}N_5$]7-Ethyl-Gua was used as internal standard. After hydrolysis and SPEpurification, the DNA was analyzed using an Eksigent-NanoLC-Ultra-HPLC system interfacedwith a ThermoScientific LTQ-Orbitrap-

Velos mass spectrometer. We monitored theaccurate mass of the fragment in the transition *m/z* 180→ *m/z* 152.05669 for 7-ethyl-Gua and *m/z* 185→ *m/z* 157.04187 for the internalstandard. Recovery, accuracy and precision were tested. The LOD was 10 amol andthe LOQ was 50 amol. DNA from 30 smokers and 30 nonsmokers was analyzed. Nodifference was observed between the two groups (p = 0.346). However, theseresults clearly demonstrate that 7-ethyl-Gua is detectable and quantifiablein leukocyte DNA. Further investigationis required to clarify its relationship, if any, to cigarette smoking.

Investigation of cytotoxic and mutagenic effects of the minor groove adduct O^2-methylthymine

Nisana Andersen, Jianshuang Wang, Yinsheng Wang. Department of Chemistry, University of California Riverside, Riverside, CA, United States

Human genomic DNA is susceptible to modifications induced by alkylating agents, which have the ability to transfer an alkyl group to all oxygen atoms in nucleobases in DNA. Little work has been done to assess the cytotoxic and mutagenic properties of the O^2-alkyl derivatives of thymine, where the modification occurs in the minor groove of DNA. Previous studies revealed that DinB DNA polymerases, i.e. pol IV and human pol k, are capable of accurately and efficiently bypassing some minor-groove N^2-modified dG derivatives. Whether these DinB DNA polymerases are capable of accurately bypassing O^2-alkylthymidines has yet to be explored. Initial experiments show that human pol k-mediated nucleotide incorporation opposite O^2-methylthymidine is neither accurate nor efficient. The emphasis of this work is to investigate how O^2-methylthymine can perturb the efficiency and fidelity of DNA replication in cells and which translesion synthesis DNA polymerase is involved in bypassing this lesion in cells.

Structural and conformational insights into nucleotide excision repair of 2-acetylaminofluorene-dG adducts by UvrABC

Vipin Jain, Benjamin Hilton, Yue Zou, Paul Chiarelli, Bongsup Cho. Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI, United States; Department of Biochemistry and Molecular Biology, East Tennessee State University, United States; Department of Chemistry, Loyola University, United States

2-Acetylaminofluorene (AAF) is a well-known model arylamine carcinogen and an excellent substrate for nucleotide excision repair (NER). The *E. coli* UvrABC system removes the dG-C8-AAF adduct present at different guanines in the *Nar*I sequence (5'-CTCTCG$_1$G$_2$CG$_3$CCATCAC-3') with different efficiencies (G$_3$≥G$_1$>G$_2$). To obtain the structural/conformational insight into this sequence-dependent NER, we conducted ^{19}F NMR/ICD/DSC studies on three different 16-mer *Nar*I duplexes, in which each guanine was modified with the fluorinated probe FAAF. The results show that the lesion exists in equilibrium between three distinct conformations: major groove (B), stacked (S), and wedge (W), but that the ratios differ depending on the orientation of adjacent G:C base pairs. The FAAF at G$_3$ and G$_1$ produced relatively larger populations of S- and W-conformers, resulting in greater DNA distortion and thermodynamic instabilities. Taken all together, our data supports a correlation between conformational/thermodynamic instability and repair efficiencies in the *E. coli* UvrABC system.

Conformational mapping of DNA duplexes containing dG-acetylaminofluorene adduct

Satyakam Patnaik, Paul Chiarelli, Bongsup Cho. Department of Biomedical and Pharmaceutical Sciences, Uinversity of Rhode Island, Kingston, RI, United States; Department of Chemistry, Loyola University, United States

The environmental pollutant 2-nitrofluorene can produce two major C8-substituted dG adducts, AAF and AF. AAF differs from AF in that the nitrogen at the linkage is acetylated. Despite the wealth of data accumulated for AAF thus far, the relationship between conformational factors and their mutational and repair consequences remains elusive. Here, we report a systematic conformational mapping of AAF on a complete NG*N/NCN duplex series. ^{19}F NMR/ICD results show that the bulky AAF lesion exists in equilibrium between major groove (B)/stacked (S), and wedge (W) conformers, but the population ratios differ depending on the nature of flanking sequences. The polarity changes on the 3'-base sequence from A:T to T:A and from G:C to C:G greatly increase S-conformation in the *N*G*A and *N*G*G series, respectively. A similar trend was observed for the 5'-A to T change in the AG**N* series. Comparative mapping analyses suggest a strong preference for AAF to adopt the S/W-conformers, which may explain why AAF blocks polymerases efficiently and exerts a greater propensity to mutation and repair.

Kinetics of O^6-pyridyloxobutyl-2'-deoxyguanosine adduct repair by O^6-alkylguanine DNA alkyltransferase using capillary HPLC-ESI MS/MS methodology

Delshanee Kotandeniya, Prof. Anthony E Pegg, Prof. Sreenivas Kanugula, Prof. Natalia Y Tretyakova. Department of Medicinal Chemistry and the Cancer Center, University of Minnesota, Minneapolis, Minnesota, United States; Department of Cellular and Molecular Physiology, Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, Pennsylvania, United States

O^6-alkylguanine-DNA alkyltransferase (AGT) is an important DNA repair protein that protects cells against alkylation damage, such as O^6-[4-oxo-4-(3-pyridyl)butyl]guanine (O^6-POB-G) and O^6-methylguanine (O^6-Me-G) lesions induced by tobacco specific nitrosamine 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK). We have developed an HPLC-ESI$^+$-MS/MS based methodology to investigate the second-order kinetics of O^6-POB-G repair by AGT. O^6-POB-G adducts were placed within synthetic DNA duplexes derived from frequently mutated regions of the human *p53* tumor suppressor gene and rat *H-ras* protooncogene. Following incubation with recombinant AGT protein for defined periods of time, the unrepaired O^6-POB-G adducts were quantified by isotope dilution HPLC-ESI$^+$-MS/MS, while POB group transfer to the protein active site was monitored by HPLC-ESI$^+$-MS of tryptic digests. We found that AGT-mediated repair of O^6-Me-G lesions is affected by the local sequence context, endogenous cytosine methylation, and the identity of the base in the opposite strand. O^6-POB-G repair in human bronchial epithelial cells treated with NNK diazohydroxide was also investigated.

Solution structure studies of aminofluorene-DNA adduct complexed with Klenow fragment and DNA polymerase ß

Vaidyanathan Ganesan, Fengting Liang, David Shock, William Beard, Samuel Wilson, Bongsup Cho. Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI, United States; Laboratory of Structural Biology, NIEHS, National Institutes of Health, United States

As part of our program on arylamine mutagenesis, we have been focused on the structure-function relationships of dG-C8-AF, a major DNA adduct derived from the prototype carcinogen 2-aminofluorene. We have extensively reported on the sequence-dependent AF-induced conformational equilibrium of the B-type (B), stacked (S), and wedge (W) conformers. Though insightful, the results from these studies were obtained in DNA polymerase-free solutions. When polymerases interact with damaged/modified nucleotides, they are likely to impose structural constraints that will influence the conformational equilibrium of the adduct, and thereby the fidelity of translesion DNA synthesis. We now conduct ^{19}F NMR, SPR, and EMSA studies on an AF-template/primer bound to a Klenow fragment (exonuclease deficient) and DNA polymerase ß in both the absence and presence of non-hydrolyzable α,β-methylene-dNTPs. These polymerases serve as model enzymes from alternate DNA families. The idea was to analyze AF-induced S/B/W-conformational heterogeneity in both the binary-DNA and ternary-DNA-dNTP complexes. In addition, we performed steady state kinetics and fluorescence assays. The results provide valuable structural/conformational insights into the delicate balance in binding characteristics of AF and dNTP in the active site of the enzymes.

Synthesis and biological evaluation of site-specific DNA lesions of 1,3-butadiene

Susith Wickramaratne, Srikanth Kotapati, Prof. Natalia Tretyakova PhD. The Masonic Cancer Center and the Departments of Medicinal Chemistry and Chemistry, University of Minnesota, Minneapolis, MN, United States

Known human carcinogen 1,3-butadiene (BD) is metabolized to epoxide intermediates that react with DNA to form promutagenic nucleobase lesions and toxic DNA-protein cross-links (DPCs). Mutagenesis studies suggest that BD adducts formed at adenine bases may be critically important, as it induces large numbers of A to T transversions. In the present study, a series of synthetic oligodeoxynucleotides containing regio- and stereo-specific BD-adenine adducts, N^6-(2-hydroxy-3-buten-1-yl)-dA, 1,N^6-(2-hydroxy-3-hydroxymethylpropan-1,3-diyl)-dA and N^6,N^6-(2,3-dihydroxybutan-1,4-diyl)-dA, were synthesized using a post-oligomerization approach. Structurally stable synthetic DPC lesions were constructed using click chemistry and reductive amination strategies. NMR, CD and UV melting studies of structurally modified duplexes revealed that BD lesions have varying effects on DNA structure. The ability

of the AlkB protein and its human homologues hABH2 and hABH3 to recognize and directly repair BD-DNA adducts was investigated. Polymerase bypass and site-specific mutagenesis studies have shown that BD-DNA adducts can block replication and affect DNA polymerase fidelity.

Conformational analysis of the effect of a single C8-arylguanine mutation on the B/Z-DNA equilibrium: An implication of arylhydrazine carcinogenesis

Brian C. Train, Vorasit Vongsutilers, Daniel J. Phillips, Peter M. Gannett. Department of Pharmaceutical and Pharmacological Sciences, West Virginia University, Morgantown, West Virginia, United States; Department of Chemistry, Bethany College, Bethany, West Virginia, United States

Arylhydrazines are carcinogens that form C8-purine DNA adducts, however it is unclear how this leads to their carcinogenicity. We have previously demonstrated that doubly modified oligonucleotides, that contain an arylhydrazine DNA adduct on each strand of the duplex, drive Z-DNA formation. Here, a hairpin-turn oligonucleotide has been designed to contain only one modification to compare with previous studies. The conformational effects were determined and quantitated by NMR and circular dichroism, respectively. The B/Z-DNA equilibrium in oligonucleotide sequences with one or two modified bases are nearly identical indicating that the two modified purines exert their conformational effects independently. This work suggests that one arylhydrazine DNA adduct can drive Z-DNA formation in oligonucleotide sequences near physiological conditions, which may provide a novel carcinogenic mechanism. Future work will examine the interactions between Z prone modified oligonucleotides and Z-DNA binding proteins (Supported by a fellowship to BCT HEPC.dsr.09013 and NSF EPS-1003907).

Effects of co-administration of γ-glutamylcysteine (GGC) and conjugated linoleic acid (CLA) on oxidative stress in human endothelial cells

Postdoctoral Fellow Yukiko K Nakamura Ph.D., Research Scientist Michael A Dubick Ph.D., Professor Stanley T Omaye Ph.D.. Department of Nutrition, University of Nevada, Reno, Reno, Nevada, United States; U.S. Army Institute of Surgical Research, San Antonio, Texas, United States

In our recent study, γ-Glutamylcysteine (GGC), a dipeptide and precursor of glutathione (GSH), appears to protect against oxidative stress independently by modulating expression of glutathione synthetase (GSS). CLA reduces oxidative stress by inducing GSH synthesis. We investigated effects of GGC (100 μmol/L) alone or co-administrated with CLA (0, 10, 50, 100 μmol/L; 24 hrs) on oxidative stress in human umbilical vein endothelial cells (HUVEC). Increases in 8-epi-$PGF_{2\alpha}$, GSH, and GSS protein levels occurred at 10 μmol/L of CLA, compared to the control with no GGC and CLA, suggesting prooxidant effects of CLA. At 50 μmol/L of CLA, we observed protective effects from oxidative stress with decreases in thiobarbituric acid reactive substance (TBARS), NF-κB DNA binding, and GSH levels, similar to GGC treatment alone. Cytotoxicity, approximately 40% cell death, was observed at 100 μmol/L of CLA. In conclusion, co-administration of GGC and CLA was as protective against oxidative stress as GGC alone under specific conditions, but concerns over CLA-induced cytotoxicity should limit doses used.

Species differences for the stereoselective carbonyl reduction of triadimefon to triadimenol and resulting stereoselective inhibition of cytochrome P450 enzymes

John F Kenneke PhD, Christopher S Mazur, Megan R Holton, Thomas J Sack. National Exposure Research Laboratory, U.S. Environmental Protection Agency, Athens, GA, United States; Region 4, U.S. Environmental Protection Agency, United States; Senior Service America Incorporated, United States

1,2,4-triazoles are used extensively in agriculture and medicine to control fungal infections. Recently, exposure to 1,2,4-triazoles has been shown to adversely impact mammalian steroid biosynthesis, and in some cases (e.g., triadimefon) has been linked to tumor formation in rodents. While many 1,2,4-triazole fungicides are metabolized via an oxidative, cytochrome P450 (P450)-mediated pathway, we have shown that triadimefon is not. Based on results from studies conducted with microsomes from 15 vertebrate species, including human, triadimefon is metabolized via the stereoselective reduction of its prochiral carbonyl to yield four stereoisomers (diastereomer A and B) of triadimenol: (A: 1R,2S; 1S,2R; B: 1R,2R; 1S,2S). Significant differences in stereoisomer formation were observed across species, but not genders. Human microsomes produced significantly more of the toxic triadimenol A diastereomer than either rat or mouse. The stereoisomers were also found to differentially inhibit ten P450s. These results suggest that stereochemistry needs to be considered in risk assessment.

O^6-alkyl-guanine hybridization probes: Mono- and bi-cyclic3-deazacytosine analogs

Dr. Todor Angelov, Prof. Shana Sturla. Institute of Food, Nutrition and Health, ETH Zürich, Zürich, Switzerland

O^6-Alkyl-guanine adducts arehighly mutagenic and persistent DNA lesions, formed in vivo in reactions betweenvarious endogenous alkylating species, food derived substances andenvironmental pollutants, and cellular DNA. A panel of 2'-deoxyribonucleosidesof mono- and bi-cyclic 3-deazacytosine analogs was synthesized with the aim ofdeveloping hybridization probes for sequence-specific detection andquantification of O^6-alkyl-guanine in DNA.The hybridization properties of a corresponding panel of 3-deaza-2'-deoxycytidineanalog containing oligonucleotides, obtained through solidphase synthesis using phosphoramidite chemistry, were characterized. The influence of 3-deaza-2'-deoxycytidine analog presence in different sequence contexts on thethermodynamic stability of O^6-alkyl-guaninecontaining and adduct free double-stranded DNA was investigated. The results ofthis study provided important insights regarding the relative importance ofsteric bulk, shape complementarity, hydrogen bonding and pi-stacking indesigning of high-affinity and high-specificity O^6-alkyl-guaninehybridization partners.

Ethnic differences in metabolism and DNA adduct formation by 1,3-butadiene

Mr. Srikanth Kotapati, Mr. Dewakar Sangaraju, Dr. Loic Le Marchand MD, PhD, Dr. Natalia Y Tretyakova PhD. Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, United States; Cancer Centre, University of Hawai'i, Honolulu, Hawai'i, United States

1,3-butadiene (BD) is a known human carcinogen that is abundant in cigarette smoke. Upon inhalation, BD is metabolically activated to 3,4-epoxy-1-butene (EB), hydroxymethylvinylketone (HMVK), and 3,4-epoxy-1,2-butanediol (EBD), which can alkylate DNA base to form promutagenic DNA adducts. Alternatively, the electrophilic metabolites of BD can be detoxified *via* glutathione conjugation and excreted in urine as 1-hydroxy 2-(N-acetylcysteinyl)-3-butene (MHBMA), 1,2-dihydroxy-4-(N-acetylcysteinyl)-butane (DHBMA), 1,2,3-trihydroxy-4-(N-acetylcysteinyl)-butane (THBMA), respectively. We have developed sensitive and specific isotope dilution HPLC-ESI-MS/MS methods to quantify N7-trihydroxybutylguanine adducts induced by EBD in human leukocytes; and BD-mercapturic acids MHBMA, DHBMA and THBMA in human urine. These methods were applied to analyze samples obtained from smokers and non-smokers belonging to European American and African American ethnic groups in an effort to identify any differences in the metabolic activation and detoxification of BD that may contribute to the observed ethnic differences in lung cancer incidence.

N^6-Formylation of lysine: A pathological secondary modification of histone proteins

Bahar Edrissi, Koli Taghizadeh, Peter C. Dedon. Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States; Center for Environmental Health Sciences, Massachusetts Institute of Technology, Cambridge, MA, United States

We recently discovered N^6-formylation of lysine as an abundant endogenous secondary modification of histone proteins. Its resemblance to lysine N^6-acetylation of histones raises the possibility that lysine formylation will have effects on regulation of gene expression. To further define the formation and biological effects of N^6-formyl lysine, we developed an ultrasensitive and specific LC-MS/MS based method for quantifying this adduct in proteins. One series of studies with stable isotope-labeled formaldehyde revealed that formaldehyde represents a major source of N^6-formyl lysine, in addition to transfer of the formyl moiety from the 3'-formylphosphate residue of 5'-oxidation of 2-deoxyribose in DNA. In vitro studies revealed that histone deacetylases that reverse lysine acetylation do not remove the formyl group from N^6-formyl lysine, while a pulse chase study in TK6 cells, using isotopically labeled lysine, indicated that the formyl lysine adduct has a half-life equivalent to the cell doubling rate. Both results suggest that the abundant formyl lysine adducts (~0.01% of total lysines) are not repaired in cells and that lysine formylation could interfere with the function of conserved acetylation and methylation sites in histones. Thus, N^6-formylation of lysines in histones may represent an epigenetic mechanism of disruption of cell function leading to cancer and other diseases.

Redox reactions between cysteines of tubulin and glyceraldehyde-3-phosphate dehydrogenase: Implications for oxidative stress and neurodegeneration

Lisa M Landino PhD, Tara D Hagedorn. Department of Chemistry, The College of William and Mary, Williamsburg, Virginia, United States

Recent reports implicate the glycolytic enzyme, glyceraldehyde-3-phosophate dehydrogenase (GAPDH) in neuronal cell death. In the case of GAPDH, the mechanism of cell death involves oxidative stress and disulfide-bonded GAPDH aggregates. We observed greatly enhanced cysteine oxidation of both mammalian brain tubulin and rabbit muscle GAPDH by H_2O_2 in vitro when both proteins were present relative to when each individual protein was oxidized. We will provide evidence to support our hypothesis that the cysteines of one protein may be oxidized by H_2O_2 and then undergo thiol/disulfide exchange with a cysteine on the other resulting in enhanced oxidation of each and transient disulfides between them. The addition of high salt decreases the extent of oxidation of each protein by H_2O_2 suggesting an ionic interaction between tubulin and GAPDH facilitates oxidation. The effects of each protein, in both its oxidized and reduced forms, on the other's activity will also be presented.

Detoxification of Benzo[a]pyrene-7,8-dione by human recombinant SULTs via sulfation of B[a]P-7,8-catechol

Dr. Li Zhang, Prof. Trevor M Penning, Prof. Ian A Blair. Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania, Philadelphia, PA, United States

Polycyclic aromatic hydrocarbons (PAH) are ubiquitousenvironmental pollutants occurring in tobacco smoke and residues of fossil fuelcombustion. Metabolic activation of intermediate PAH trans-dihydrodiols byaldo-keto reductases (AKRs) leads to o-quinones that are redox-active and maycontribute to human lung carcinogenesis. We investigated whether sulfation ofPAH catechol by human sulfotransferases (SULTs) is feasible for thedetoxification of benzo[a]pyrene-7,8-dione, a representative PAH o-quinone. RT-PCRshowed that human lung cells (A549, HBEC-KT, H358, BS2B) expressed SULT1A1, 1A3 and 1E1. B[a]P-7,8-dione was thenreduced to B[a]P-7,8-catechol by dithiothreitol under anaerobic conditions andthen further sulfated by the corresponding human recombinant SULTs in thepresence of 3'-phosphoadenosine 5'-phosphosulfate as a sulfate group donor. Theformation of the B[a]P-7,8-catechol monosulfate was detected by HPLC-RAM-UV andLC-MS-MS. It is concluded that human SULTs may play a critical role in thedetoxification of PAH o-quinones [Supported by 1R01-CA-39504,and P30-ES13508awarded to TMP].

Oxidation of furan to a reactive metabolite by human cytochrome P450 enzymes

Leah A Brus, Ding Lu PhD, Lisa A Peterson PhD. Division of Environmental Health Sciences, School of Public Health, & Masonic Cancer Center, University of Minnesota, Minneapolis, MN, United States

Furan is a liver toxicant and carcinogen in rodents. It is classified as a possible human carcinogen, but the human health effects of furan exposure remain unknown. The oxidation of furan by cytochrome P450 (CYP) is necessary for furan toxicity. The product of this reaction is the reactive α,β-unsaturated dialdehyde, *cis*-2-butene-1,4-dial (BDA). To determine if humans can metabolize furan to its toxic metabolite, we screened recombinant human liver CYP enzymes and human liver microsomes for their ability to catalyze furan oxidation to BDA, which was trapped with N-acetyl-lysine and N-acetyl-cysteine. This NAL-BDA-NAC conjugate was then quantified by LC-MS/MS. Our results indicate that CYP2E1 is the most active human liver furan oxidase, followed by CYP2D6 and CYP3A4. Human liver microsomes also converted furan to BDA. These results suggest that humans are capable of oxidizing furan to its toxic metabolite, BDA, and may be susceptible to its harmful effects [Funded by ES-10577].

Naturalantioxidants act synergistically to inhibit lipid peroxidation

Martha A. Hass PhD, Alaa M. Hammad. Arts & Sciences, Albany College of Pharmacy and Health Sciences, Albany, NY, United States

The individual and synergistic antioxidant activity of α-tocopherol (α-TOC), ascorbic acid (ASC), lipoic acid (LA) and LA derivatives (lipol (LOH) and dihydrolipoic acid (DHLA)) were evaluated by measuring their respective inhibitory effects on the formation of thiobarbituric reactive substances (TBARS) from ferrous ion stimulated lipid peroxidation of rat liver microsomes. The IC_{50} measured for α-TOC(0.08mM), ASC (4.32mM) and DHLA (14.6mM) were significantly lower than for LA (0.065mM) and LOH (0.08mM).

The antioxidant activity of α-TOC in combination with ASC, LA and LA derivatives (LOH andDHLA) was then investigated to determine if these combinations exhibit synergistic activity. The combined antioxidant activity of α-TOC with LA, LOH, ASC and DHLA significantly exceeded the expected additive effect of the two antioxidants. These data support our hypothesis that α-TOC in combination with other natural antioxidants act synergistically to limit lipid peroxidation.

Pyridyloxobutyl DNA adducts and their relationship to tumor formation in the A/J mouse lung model

Anna M Urban MPH, Pramod Upadhyaya PhD, Lisa A Peterson PhD. Divison of Environmental Health Sciences, University of Minnesota, Minneapolis, MN, United States; School of Public Health, University of Minnesota, Minneapolis, MN, United States; Masonic Cancer Center, University of Minnesota, Minneapolis, MN, United States

The nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) generates pyridyloxobutyl DNA adducts. The pyridyloxobutyl adducts O^6-[4-3-(pyridyl)-4-oxobut-1-yl]deoxyguanosine (O^6-pobdG) and O^2-[4-3-(pyridyl)-4-oxobuty 1-yl]deoxythymidine (O^2pobdT) are thought to contribute to the mutagenic and tumorigenic properties of the pyridyloxobutylation pathway. O^6-Alkylguanine-DNAalkyltransferase (AGT) is known to repair O^6-pobdG, while nucleotide excision repair may be important in the repair of O^2pobdT. To determine the role of pyridyloxobutyl DNA adduct formation and repair in the tumorigenic properties of NNK, A/J mice weretreated with the model pyridyloxobutylating agent 4-(acetoxymethyl-nitrosamino)-1-(3-pyridyl)-1-butanone. DNA adduct levels and tumor yield weremeasured in the lung. Our findings indicate that O^2pobdT accumulates in lung DNA. AGT depletion does not significantly affect O^6-pobdG repair or tumor formation. These data suggest that O^6-pobdG is repaired by pathways other than AGT, and that formation and persistence of O^2pobdT may be important for tumorigenesis. Studies are underway to investigate other possible repair pathways of O^6-pobdG [Supported by CA-115309 and CA-138338].

Protein targets of lipid electrophiles from alkynyl linoleic acid

Hye-Young H. Kim PhD, Keri A. Tallman PhD, Ned A. Porter, Daniel C. Liebler PhD. Chemistry, Vanderbilt University, Nashville, TN, United States; Biochemistry, Vanderbilt University, Nashville, TN, United States

Studies with alkynyl analogs of specific lipid electrophiles enable identification of their targets through biotinylation via Click chemistry and liquid chromatography-tandem mass spectrometry (LC-MS/MS) of captured proteins. To better understand the roles of endogenous lipid electrophiles, we synthesized omega-alkynyl-linoleic acid (*a*LA), which was incorporated into human monocyte THP-1 cells with 0, 50, 100, and 250 μM for 20 h. Incubation of *a*LA-treated cells with 50 ng/mL for 6 h of bacterial lipopolysaccharide induced oxidation of aLA to electrophiles, which subsequently adducted cell proteins, as detected by streptavidin immunoblotting. Adducted proteins were captured on streptavidin following Click derivatization of alkynyl adducts with a photocleavable azido-biotin tag. After washing and photorelease, adducted proteins were digested with trypsin and analyzed by LC-MS/MS. These analyses identified approximately 300 target proteins as putative targets of *a*LA-derived electrophiles. Targeted analysis of adducted peptides is directed at identifying *a*LA-derived electrophiles that form adducts. (Supported by NIH grant ES013125)

Sequence-dependent structural perturbations induced by the β-anomer of the aflatoxin B_1 formamidopyrimidine (FAPY) adduct in DNA

Grad Student Liang Li, Dr. Kyle L Brown, Michelle Y Wang, Amanda Meyers, Professor Michael P Stone. Department of Chemistry and Center in Molecular Toxicology, Vanderbilt University, Nashivlle, TN, United States; Department of Chemistry, Augustana College, Rock Island, IL, United States

Aflatoxin B_1 (AFB_1) is the predominant mutagenic fungal metabolite produced by *Aspergillus flavus*. The genotoxic metabolic product, AFB_1 epoxide, alkylates DNA regioselectively at N7-dG. The initially formed N7-dG adduct may subsequently rearrange to a N7-dG formamidopyrimidine (FAPY) derivative. In DNA, the AFB_1-FAPY derivative equilibrates between α and β deoxyribose anomers, and also undergoes conformational interconversions involving both the C5-N^5 bond and the formyl group. Using NMR, we examine the hypothesis that the conformation of the AFB_1-FAPY modified dG is dependent on the 3′-neighbor nucleotide. We compared the 5′-XC-3′ and 5′-XA-3′ sequences (X= AFB_1-FAPY lesion). The 5′-

XA-3′ sequence predominately exists one conformational species as the b anomer and the Z rotamer about the formyl bond, whereas the 5′-XC-3′ sequence shows multiple subspectra. This might be attributed to the co-existence of *E* and *Z* conformations of the formamide moiety in the latter sequence. Supported by NIH grant R01 CA-55678 (M.P.S.).

Inhibition and mechanism based inactivation of human cytochrome P450 2A6 and 2A13

Valerie M Kramlinger, Linda B von Weymarn PhD, Sharon E Murphy PhD. Biochemistry, Moliecular Biology and Biophysics, University of Minnesota, Twin cities, Minnesota, United States; Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, United States

Nicotine, the primary addictive compound in cigarettes, is metabolized in humans by cytochrome P450 2A enzymes. The hepatic enzyme responsible for the metabolism of nicotine in smokers is P450 2A6. P450 2A13, which shares 94% primary sequence homology with P450 2A6, also catalyzes the metabolism of nicotine and is present in the lung. Loss of P450 2A activity is correlated with modified smoking behavior and addiction. Inhibition of these enzymes decreases nicotine metabolism and may be of benefit in smoking cessation.We investigated the relative potency of (-)-Menthol (menthol), (R)-(+)-Menthofuran (menthofuran), and β-nicotyrine as inhibitors and mechanism-based inactivators of both P450s 2A6 and 2A13. All three compounds inhibit P450 2A6 and 2A13 activity. In addition, menthofuran and β-nicotyrine mediate mechanism-based inactivation ofP450 2A6 but not 2A13. Adducts to the apo-protein were characterized using LC/MS/MS and we are in the process of identifying the structure and site of the modification.

Nevirapine metabolism to 4-formyl-nevirapine: Possible role in nevirapine toxicity

Alexandra M.M. Antunes PhD, Ana L.A. Godinho MSc, Sofia A. Pereira PhD, Gordon Surratt, Gon • alo Gamboa da Costa PhD, Frederick A. Beland PhD, M. Matilde Marques PhD. Centro de Química Estrutural, Instituto Superior Técnico; Universidade Técnica de Lisboa, Lisboa, Portugal; Departamento de Farmacologia, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal; Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR, United States

Nevirapine (NVP), a non-nucleoside reverse transcriptase inhibitor used against HIV-1, is associated with liver and skin toxicities. NVP metabolism to 4-hydroxymethyl-NVP (12-OH-NVP) is thought to play a role in these processes but other pathways could be involved. 12-OH-NVP undergoes further metabolism to 4-carboxy-NVP *via* the aldehyde, 4-formyl-NVP. This species may bind to biomacromolecules but has so far eluded detection. To investigate this pathway, we incubated NVP and 12-OH-NVP with rat and human liver microsomes and rat liver cytosol. In all instances, LC-ESI-MS confirmed the formation of 4-formyl-NVP, trapped as a hydrazone derivative. Incubation of 4-formyl-NVP with Human serum albumin, followed by reduction and enzymatic hydrolysis, yielded a lysine adduct, identified by LC-ESI-MS through comparison with a synthetic standard. Likewise, incubation of 4-formyl-NVP with Human hemoglobin yielded an adduct with the N-terminal valine, characterized upon reduction and N-alkyl-Edman degradation. These results suggest that 4-formyl-NVP could play a role in NVP-induced toxicity.

Selective enrichment and profiling of low-abundance serum serine hydrolases using click chemistry

Ujjal Sarkar PhD, postdoc associate Charles G. Knutson PhD, John S. Wishnok Ph.D, Professor Steven R. Tannenbaum PhD. Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, United States; Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States

Inflammation is associated with arthritis, diabetes, heart, bowel, and neurodegenerative disease. A variety of serin hydrolases are involved in chronic inflammation. Thus, comprehensive profiling of serine hydrolases and other serum proteins is needed for the discovery of potential biomarkers of inflammation. Initially we took an approach to isolate hydrolases from human serum by using HiTrap Benzamidine FF column. This approach was not sufficiently sensitive and selective, whichled us towards developing a method based on covalent modification and clickchemistry. We utilized trypsin and a recently synthesized polyethyleneglycol (PEG)-azide containing fluorophosphonate probe to initiate the activity-based proteinprofiling study in serum. By utilizing Cu(I) mediated click chemistry, we wereable to selectively

enrich adducted trypsin spiked in serum samples. Results will be presented from examination of serum samples from both patients and mouse models with inflammatory bowl disease.

Identification of myeloperoxidase-catalyzedoxidation of tyrosine residues in human serum proteins for a potential inflammatorydisease biomarker

Pilsoo Kang, Yu Zeng, John S Wishnok, Steven R Tannenbaum. Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States; Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States

Targeted quantification of specific oxidativepost-transitional modifications of serum proteins arising fromoxidative-stress-related reactive chlorine can be exploited to discover novelbiomarkers in inflammatory disease progression.During inflammation, reactive nitrogen and oxygen species can oxidativelydamage proteins, resulting in a variety of adducts. Hypochlorous acid and other reactive chlorinespecies are also generated by neutrophils via myeloperoxidase (MPO)-catalyzedreaction of hydrogen peroxide and chloride, leading in turn to chlorinatedamino acids, e.g, 3-chlorotyrosine. Chlorinatedtyrosine is a unique and stable marker for MPO-catalyzed oxidation althoughchlorination of tyrosine may not be a major product of oxidative stress. Protein adducts in biological fluids are goodtargets for an assay of disease biomarkers.MPO-catalyzed chlorination on specific tyrosine sites of serum proteins havebeen identified through proteolytic digestion and peptide sequencing by liquidchromatography-tandem mass spectrometry.Quantitative proteomic analysis for MPO-catalyzed chlorination of atarget protein site is being carried out by multiplex selected ion monitoringand isotope dilution mass spectrometry. This method may prove suitable forfuture inflammatory disease biomarker investigations.

Synthesis of modified uridine as a radical precursor for the study of oxidative damage to RNA

Raziya Shaik, Amanda Bryant Friedrich PhD. Department of Chemistry, University of Toledo, Toledo, Ohio, United States

The majority of studiesinvolving radical induced nucleic acid damage have been limited to DNA.Investigations focusing on RNA damage have increased after studies implicatedthis phenomenon as an underlying cause in the early development ofneurodegenerative diseases. Like DNA, RNA is susceptible to oxidative damage byexogenous or endogenous sources. In order to understand the relationship betweendisease development and radical formation, it is essential to understandradical initiated damage to nucleic acids. The goal of this project is to study radicalinduced RNA damage. 5'-Pivaloyluridine was successfully synthesized and utilized for thegeneration of the ribosyl 5'-radical through Norrish type I photocleavage.Photolysis under anaerobic conditions using glutathione or tri-n-butyltinhydride as H-atom donors delivered several products that were analyzed by HPLC.Formation of the expected reduction product uridine as well as the baseelimination product uracil was identified. Further investigation of 5'-radicalderived products will be discussed.

Styrene and its metabolites exhibit potential for multiple-site interaction with CYP2E1

Jessica H Hartman, Gunnar Boysen PhD, Grover P Miller PhD. Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR, United States; Department of Chemistry, University of Arkansas at Little Rock, Little Rock, AR, United States

CYP2E1 detoxifies and activates many small potentialcarcinogens. Thus, it is critical to understand and predict its role inmetabolism. We hypothesize that CYP2E1 binds monocyclic compounds through catalyticand effector sites that influence metabolism. To test this hypothesis, weinvestigated the inhibitory mechanisms for styrene (ST) and its metabolites, styreneoxide (SO), 4-vinylphenol (4VP) and styrene glycol (SG), toward CYP2E1 oxidationof 4-nitrophenol. Initially, we screened their inhibitory potency through IC_{50}studies. ST and SO were the most potent (IC_{50} 34 and 55 μM,respectively), while 4VP and SG were relatively weak (IC_{50} 290 and6000 μM, respectively). Subsequent efforts demonstrated these compounds mayinteract through two sites; however, the affinity for the effector site wasoften too weak assess accurately. These findings contrast with the positivecooperativity mechanism reported for xylene by CYP2E1 and suggest selectivityof the effector site may impact its catalytic importance.

Non-competitive inhibition of UDP-glucose dehydrogenase by 6-thiopurine and 6-thiouric acid and quantification of the enzymatic formation of UDP-glucuronic acid by UDP-glucose dehydrogenase

Richard M Hyslop PhD, Ryan J. Rafferty. Chemistry and Biochemistry, University of Northern Colorado, Greeley, CO, United States

UDP-Glucose Dehydrogenase (UDPGDH) is an enzymeresponsible for the conversion of UDP-glucuose to UDP-glucoronic acid (UDPGA)which is required for bilirubin excretion. 6-Thiopurine, a common drugfor the treatment of cute lymphocytic leukemia, and is primarily metabolite 6-thiouricacid have been thought to be inhibitors of UDPGDH. Thus, inhibition of this enzyme has a direct effect uponbilirubin excretion resulting in jaundice, a condition most patients developwhen treated with 6-thiopurine.Kinetics data supporting the theory of UDPGDH inhibition by 6-thiopurineand its primarily metabolite will be present as well as a method for thequantification of UDPGA that can be used to *invitro* to access UDPGDH activity.

Thiol-mediated reactivation of oxidatively-inactivated protein tyrosine phosphatase 1B: Kinetics studies

Zachary D. Parsons, Kent S. Gates. Department of Chemistry, University of Missouri, Columbia, Missouri, United States; Department of Biochemistry, University of Missouri, Columbia, Missouri, United States

Protein tyrosine phosphatases represent a family ofenzymes which regulate a diverse array of cellular processes. Protein tyrosinephosphatase 1B (PTP1B) is the prototypical member of this family, and serves asa negative regulator of the insulin and leptin signaling pathways. For itscrucial role in modulating metabolic activity, it has garnered much attentionas a potential drug target for the treatment of type II diabetes mellitus.PTP1B has been shown to be redox-regulated *in vitro* and *in vivo*by chemical modification of the catalytic cysteine residue. Though the kineticsassociated with oxidative inactivation of PTP1B by endogenous and exogenousoxidants have been well-studied, the kinetics of the reverse process (reductivereactivation) have not yet been detailed. Herein, we report on the kineticsassociated with thiol-mediated reactivation of PTP1B by biological andexogenous thiols, and “trapping” of the oxidized form of the enzyme bynucleophilic reagents.

Optical properties of amylose-encapsulated chromophores

Adjunct professor Sung-Jae Chung Ph.D, student Suk-Won Chung, student Kevin D Ko, student Kim Hyunsoo. School of Arts & Sciences, Marymount University, Arlington, VA, United States; Centreville High School, Centreville High School, Clifton, VA, United States; science and Technology, Thomas Jefferson High School, Alexandria, VA, United States; Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, United States

We have investigated photophysical properties of the chromophores in various ratio solutions of H_2O and DMSO with amylose encapsulation and free of encapsulation for reference. Amylose-encapsulated chromophores showed over 100 times the fluorescence intensity of a non-encapsulated chromophores. At less than 40% DMSO, the chromophore had a stable encapsulation with amylose and showed very high fluorescence intensity.

Inflammation induced changes to the serum metabolome

Erin G. Prestwich, I. Ramesh Babu, Koli Taghizadeh, Peter C. Dedon. Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States; Center for Environmental Health Sciences, Massachusetts Institute of Technology, Cambridge, MA, United States

The chronic inflammatory release of cytokines and reactive oxygen and nitrogen species by immune cells is linked to human disease. To examine inflammation-promoted changes to the serum metabolome, we utilized the SJL mouse model of nitric oxide over-production that mimics human inflammatory processes. Deproteinized serum from control and inflamed animals was analyzed via LC-MS and LC-MS/MS methods and the resulting spectra were compared on the basis of m/z values and retention time. Statistical analyses revealed significant differences between the control and inflamed animals with changes in several lipids and amino acids, including lysophosphotidylcholines and members of the tryptophan, proline, and arginine metabolic pathways. Additionally, a subset of animals fed the nitric oxide synthase inhibitor N-methyl-arginine exhibited altered metabolite levels. The prospective application of these and other metabolites as biomarkers of inflammation will be discussed.

Determination of *p53*mutation pattern and spectrum due to B[*a*]P radical cations in a yeast basedreporter system

Sushmita Sen PhD, Zahidur Abedin PhD, Jeffrey Field PhD. Department of Pharmacology, University of Pennsylvania, Philadelphia, PA, United States

p.MsoNormal, li.MsoNormal, div.MsoNormal { margin: 0in 0in 0.0001pt; font-size: 12pt; font-family: "Times New Roman"; }div.Section1 { page: Section1; }Benzo[*a*]pyreneis an ubiquitous air pollutant released as a result of fossil fuel combustionand cigarette smoke. Mutagenic effect of B[*a*]Pradical cations formed by action of CYP peroxidases has not been studied in *p53* oncogene which is commonly mutatedin lung cancer. We have used a yeast based reporter system to study *p53* mutagenesis due to B[*a*]P radical cations. Since B[*a*]P radical cations are short-livedspecies, they were generated *in situ*enzymatically using HRP, CuOOH as HRP substrate and 50uM B[*a*]P as the electron donor. Mutagenic effects of *in situ* generated radical cation werecompared with B[*a*]P 7, 8 dione(250nM). B[*a*]P radical cations werenot as mutagenic compared to (+)-*anti*-BPDEor ROS generated due to B[*a*]P 7,8-dione in the yeast assay. The frequencyof mutation increased when the *Apn1*gene was knocked out suggesting that the mutations maybe caused due toformation of AP sites.

Occurrence of algal toxin *Microcystin-LR* in source waters and its removal by *Moringa oleifera* seed extract

Pinar Omur-Ozbek PhD, Victor Sam. Department of Civil and Environmental Engineering, Colorado State University, Fort Collins, co, United States

Cyanobacteria blooms are becoming more frequent around the world due to changes in climate, decreased ice cover in surface waters and increased eutrophication. During blooms several types of algal metabolites are released. Algal toxins, such as *Microcystin-LR*, are very toxic to humans and a guideline of 1 µg/L for drinking water is set by the World Health Organization to prevent adverse health effects such as cancerous tumors or liver and kidney failure leading to death. Treatment of toxins require costly advanced methods, however, sustainable and cost effective solutions should also be investigated for smaller utilities and the developing world. Effectiveness of *Moringa oleifera* tree seed extract is being investigated byjar tests for algal toxin removal from drinking water. It has been shown thatthe extract has good coagulation properties and is effective in removingorganic pollutants, turbidity and hardness from water.

Rapid estimation of activation enthalpies for cytochrome-P450-mediated hydroxylations

Dr. Arthur N. Mayeno PhD, Jonathan L. Robinson, Dr. Brad Reisfeld PhD. Department of Chemical & Biological Engineering, Colorado State University, Fort Collins, Colorado, United States; Department of Chemical & Biological Engineering, Princeton University, Princeton, New Jersey, United States

Cytochrome P450 (CYP) enzymes play a critical role in detoxication and bioactivation of xenobiotics; thus, the prediction of biotransformation rates and regioselectivity of CYP enzymes toward substrates is an important goal in toxicology and pharmacology. We have applied the semi-empirical quantum chemistry method SAM1 to rapidly estimate relative activation enthalpies ($\Delta H^{\ddagger}$) for the hydroxylation of aliphatic carbon centers of various substrates. The $\Delta H^{\ddagger}$ were determined via a reaction path calculation, in the reverse direction (RRP), using the iron-hydroxo-porphine intermediate and the substrate radical. The SAM1 $\Delta H^{\ddagger}$, calculated via unrestricted Hartree-Fock (UHF) and configuration interaction (CI) formalisms, were compared with density functional theory (DFT) B3LYP activation energies and showed R^2 ranging from 0.69 to 0.89. SAM1 RRP calculation times were, on average, over 200 times faster than those for the corresponding forward reaction path DFT calculations, suggesting that this approach can rapidly estimate DFT activation energy and substrate hydroxylation rates.

Lack of estrogenic potential of monomers utilized in Eastman Tritan™ Copolyester

James Deyo DVM, PhD, Emmett O'Brien PhD, Steve Green PhD. Product Safety and Health, Eastman Chemical Company, Kingsport, TN, United States; Specialty Plastics Technology, Eastman Chemical Company, Kingsport, TN, United States

Eastman Tritan™ Copolyester, a novel plastic from Eastman is manufactured utilizing three monomers, di-methylterephthalate (DMT), 1,4-cyclohexanediemthanol (CHDM), and 2,2,4,4-tetramethyl-1,3-

cyclobutanediol (TMCD) in various ratios. As with most any polymer, the monomers along with the high molecular weight oligomers, whose toxicity is most commonly represented by the monomers, make up the predominate amount of free chemicals available for leaching into the environment and/or foods. In light of the high level of public concern about the presence of estrogenic activity ascribed to certain plastics and chemicals in the environment, Tritan's™ monomers were evaluated using a battery of *in vitro* and *in vivo* techniques to understand whether they might also pose such a concern. When these data are coupled with other *in vivo* data developed to assess systemic toxicity, and developmental and reproductive toxicity the weight of evidence clearly indicates that these monomers do not pose an estrogenic risk to humans.

Estimation of dermal permeability in untested vehicles based on measured permeability in a reference vehicle

Heather J. Avens, Ken M. Unice, Jennifer Sahmel. ChemRisk, Boulder, CO, United States; ChemRisk, Pittsburgh, PA, United States

Dermal permeability is commonly reported for neat or aqueous solutions; however, most dermal exposure scenarios involve chemical mixtures. Since it is infeasible to test all possible vehicles, we sought to predict a chemical's permeability in one vehicle based on knowledge of its permeability in another previously tested vehicle. The vehicle can alter permeability by changing the chemical's thermodynamic activity or by affecting the diffusive properties of the membrane. For vehicles that do not alter the skin's diffusivity, comparison of the activity coefficients of the chemical in an untested vehicle and a reference vehicle provides an efficient approach to estimate dermal permeability in the untested vehicle. Although some preliminary studies have been published which investigated the correlation between activity coefficients and permeability, only a few vehicles have been investigated. We present a meta-analysis of published permeability data to identify vehicles for which thermodynamic activity may be used to estimate permeability.

"Just in Time" derivatization workflow for GC-MS metabolomics studies

Colleen McNaney, Dr. Serhiy Hnatyshyn PhD, Dr. Michael Reily PhD, Dr. Dieter Drexler PhD. Department of Applied and Investigative Metabolomics and Department of Discovery Analytical Science, Bristol-Myers Squibb, Wallingford, CT, United States; Department of Applied Investigative Metabolomics and Department of Discovery Analytical Science, Bristol-Myers Sqibb, Princeton, NJ, United States

Metabolomics is an emerging technology for biomarker discovery that has the potential to facilitate the elucidation of drug toxicity mechanisms and disease processes. Metabolomics studies employ various analytical techniques to measure the large chemical diversity of endogenous metabolites. One of the major analytical technologies for studying the composition of biological sample is Gas Chromatography-Mass Spectrometry (GC-MS). It enables qualitative and quantitative measurement of a variety of classes of endogenous molecules. Prior to analysis, compounds are derivatized to improve sensitivity and volatility. Derivatization is a highly manual multi-step process which is time consuming and adds potential entry points for error. Typically, larger sample sets are processed in batches which can cause a significant time interval between the analysis of the first and last sample thus raising the question of sample stability and data reliability.Enhancements to the autosampler hardware and software allow the automated addition of reagents at pre-programmed time intervals for “just in time” derivatization of the sample prior to analysis. Such technical improvements have enabled us to streamline GC-MS analysis workflow with the benefit of increased sample throughput capacity and enhanced data reproducibility.

Tailoring microsensor-array composition and operation for biomarkers in simulated exhaled breath

Dr Phillip H Rogers PhD, Dr. Kurt D Benkstein PhD, Dr. Steve Semancik PhD. Materials Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, Maryland, United States

Monitoring breath composition can be an approach for early diagnoses of diseases and other conditions resulting in homeostatic imbalance. Here we demonstrate the use of microsensor-based devices for detecting select biomarkers in simulated exhaled-breath as a step toward enabling fast and inexpensive breath-screening technology. Microhotplate elements functionalized with various chemiresistive metal oxide films were used to acquire data in simulated breath containing single targets [(5 to 20) µmol/mol ammonia, methanol and acetone], as well as complex mixtures of those species. Devices were operated

with a fast temperature program consisting of dynamic temperature segments (ramps) interlaced with isothermal segments where film conductances were measured. All sensing films were operated with identical ramps, but isothermal values varied for each oxide material to take advantage of temperature-dependent target selectivity and oxide conductance. Methods used in determining sensing material selectivity and contribution to overall target discrimination and mixture quantification will be discussed in detail.

Wednesday, August 31, 2011

Human Exposure and Responses to Toxins from the Air and Water (08:30 AM - 11:50 AM), Room: 207 Colorado Convention Center

08:30 AM
State-of-the-Art in Research on Endocrine-Active Chemicals and Perspectives. K. Thayer

08:35 AM
Introduction of Speakers. D. Doerge

08:40 AM
Reproductive and chronic toxicity testing of endocrine-active compounds in rodents

PHARMACOLOGIST Barry K. Delclos Ph.D.. Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR, United States

Since EPA was charged with assessing the endocrine-related toxicity of environmental chemicals in the mid-1990's, there has been considerable research and debate as to the most comprehensive yet efficient means to evaluate potential toxicity mediated through endocrine mechanisms. By far the major thrust of the research to this point has focused on toxicities mediated through estrogen and androgen receptor signaling. While *in vitro* assays to detect receptor binding and transcriptional activation have played significant roles in research and testing, animal models, and particularly rodent models, remain for the foreseeable future the main experimental and regulatory tools for the evaluation of these compounds. This reliance on animal models is due in large part to the critical nature of metabolism and to organ system interactions in endocrine-related toxicity. The timing of exposure, route of exposure, and timing, nature, and specificity of endpoint assessment are important factors in the design of studies to evaluate the toxicity of endocrine-active compounds. Factors such as species and strain sensitivities, dietary modulation of response, and the need to assess a broader dose range than has traditionally been used in toxicity studies have been much debated and studied. The most definitive test for the evaluation of reproductive toxicants has long been the multigenerational study, and enhancements of the basic study design have been added in some cases to better assess treatment-related effects on reproductive development and function and long term toxicity. Alternatives to these lengthy and costly assays have also been considered. These issues will be addressed and discussed using examples from the recent literature.

09:15 AM
Perinatal PCB exposure and deficits in cognitive function: Parallels between animals and humans

Professor Susan L. Schantz Ph.D.. Department of Comparative Biosciences and Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, IL, United States

Appropriately designed studies in animal models provide important data that can increase our confidence in the findings from human epidemiological research. Our laboratory studies assessing the impact of early developmental exposure to polychlorinated biphenyls (PCBs) on later cognitive function in the Long Evans rat provide a useful illustration of this concept. This talk will highlight parallels between the cognitive deficits associated with prenatal PCB exposure in humans and cognitive deficits we have observed in perinatally PCB-exposed rats. For the sake of brevity, results from tests of two aspects of cognitive function—cognitive flexibility and response inhibition—will be presented as examples. Recently we reported that perinatal exposure of rats to an environmental PCB mixture resulted in impaired performance on an operant schedule that assesses inhibitory control and timing ability. Other researchers have reported a remarkably similar pattern of responding in children exposed to PCBs during early

development. This pattern of responding is indicative of impaired response inhibition. PCB-exposed rats and human children also show similar patterns of perseverative responding on tests that measure cognitive flexibility. These examples illustrate that findings from studies in animal models can add to the weight of evidence for associations between chemical exposures and specific cognitive outcomes in epidemiological studies. The more directly laboratory animal studies model actual human exposure scenarios, the more useful they will be in this regard. Similarly, the more closely the cognitive tests used in animal studies parallel those used in humans in terms of the functional domains assessed, the more useful these studies will be.

09:50 AM

Human biomonitoring of environmental chemicals

Dr. Antonia M. Calafat Ph.D.. Division of Laboratory Sciences, Centers for Disease Control and Prevention, Atlanta, GA, United States

In modern societies, humans may be exposed to a wide spectrum of environmental chemicals. Data are limited on the potential human health effects of many of these chemicals, but several of them have demonstrated toxicity in experimental animals. We have developed biomonitoring programs to assess human exposure to select environmental chemicals. One such program, the National Health and Nutrition Examination Survey (NHANES), conducted by the U.S. Centers for Disease Control and Prevention, is designed to collect data on the health and nutritional status of the general U.S. population. NHANES data can be used to establish reference ranges for select chemicals, provide exposure information for risk assessment (e.g., set intervention and research priorities, evaluate effectiveness of public health measures), and monitor exposure trends. We have used state of the art analytical methods to analyze many thousands of urine and serum samples collected from NHANES participants. NHANES data have shown that in the general population, exposure to certain environmental chemicals is prevalent. We have also observed differences in concentrations of some of the chemicals by sex, age, and race/ethnicity, all of which probably reflect lifestyle differences. But one NHANES limitation is its exclusion of persons under 1 year of age. Further, NHANES by design does not include population groups that might be highly exposed or the collection of urine from persons younger than 6 years. Therefore, biomonitoring efforts other than NHANES are needed. Such efforts should focus on a) identifying the analytes best suited for use as biomarkers; b) improving understanding of these biomarkers toxicokinetics in different populations; and c) studying targeted populations with known exposure source(s) to better relate internal exposure to potential health effects.

10:25 AM Intermission

10:40 AM

PBPK modeling of endocrine active chemicals: Linking animal toxicity studies with human exposures

TOXICOLOGIST Jeffrey W. Fisher Ph.D.. Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR, United States

Physiologically Based Pharmacokinetic (PBPK) models or Biologically Based Dose Response (BBDR) models can play an important role in translating exposure or administered dose of endocrine active chemicals to internal dose that can be associated with endocrine disruption and target organ toxicity or conversely, no endocrine disruption and toxicity. The ultimate goal is to extrapolate model predictions of dosimetry, endocrine disruption and toxicity in rodents to humans. This talk discusses the current status on the development and use of two animal and human models, one for perchlorate and the other, bisphenol A. Both bisphenol A and perchlorate are widely distributed in the environment, thus public exposure to these chemicals is common. The sensitive populations for both chemicals are the fetus, infant, and child. Bisphenol A is a weak estrogen active chemical that is rapidly detoxified by metabolism, while perchlorate is a potent thyroid active chemical that is not metabolized and is excreted in urine. Many toxicological effects of bisphenol A are reported in the literature, while few are reported for perchlorate. Model structure and predictions are presented with a discussion of inherent challenges in extrapolation of dosimetry and endocrine disruption from animals to human for perchlorate and bisphenol A.

11:15 AM

U.S. safety assessment of food contact substances

TOXICOLOGIST Michelle L. Twaroski Ph.D.. Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD, United States

The U.S. Food and Drug Administration has regulatory authority over the premarket approval of food additives, direct and indirect (currently referred to as food contact substances) as delegated in the Federal Food, Drug and Cosmetic Act. Food contact substances (FCS) are defined as any substance intended for use as a component of materials used in manufacturing, packing, packaging, transporting, or holding food if such use is not intended to have any technical effect in such food. Moreover, these substances are used in the manufacturing of food (assembly line components), assist in the preservation of food (food cans, multi-laminate pouches) or allow for consumer conveniences (plastic bottles, paper food boxes, etc.). As a result of the chemistries involved, these industrial compounds do migrate to food at usually low levels under their conditions of use; therefore, resulting in consumer exposure. FDA evaluates these compounds for safe use based on an exposure based tiered toxicology data recommendation paradigm using the reasonable certainty of no harm definition. In the past decade, a few of the compounds regulated for uses as FCSs have been reported to have endocrine disrupting activity, most notably phthalates and bisphenol A. This presentation will provide an overview of the FDA's regulatory authority, how FCSs are evaluated for safety, the use of hazard identification tools, as well as current challenges as a result of recent review activities/concerns for endocrine active compounds and/or infant products.

Bioanalytical Platforms in Biomarker Discovery and Development (01:00 PM - 05:30 PM) Room: 207 Colorado Convention Center

01:00 PM: Introductory Remarks

01:05 PM

Metabolomics techniques for biomarker discovery

Professor Chris Beecher PhD. Pathology, University of Michigan Medical School, Ann Arbor, MI, United States

Metabolomics examines the biochemical components of a biological sample in order to understand its physiological status. Diseased tissues generally demonstrate significant deviations in their metabolism from non-diseased. Thus, Metabolomics is often a very good technique to use to find biomarkers for disease or other abnormal response states. We will present a number of biomarker discoveries that the techniques are quite sensitive and relatively simple to apply. Since the biochemicals that are being measured are extremely well understood it is often possible to interpret the mechanism by which a biomarker acts.

01:55 PM

Emerging field of lipidomics

Oswald Quehenberger PhD. Departments of Medicine and Pharmacology, University of California, San Diego, La Jolla, CA, United States

By far, the largest number of distinct molecular species in cellular metabolism lies in the lipids, where tens of thousands of distinct molecular species exist in cells and tissues. As part of the LIPID MAPS Consortium [LM/Nature Lipidomics Gateway www.lipidmaps.org], our laboratory has developed a comprehensive approach to the lipidomic analysis of fatty acids, fatty amides and inflammatory eicosanoids. We will discuss the application of lipidomic analysis to characterize cellular lipid signaling in endotoxin stimulated macrophages as models of inflammation. We will also illustrate the application of lipidomics to the analysis of lipid signaling in an animal model of infection, which demonstrates a unique lipidomics profile of both pro- and anti-inflammatory lipid molecules. Finally, we have now profiled the human plasma lipidome and anticipate that the future practice of clinical medicine will be significantly impacted by the identification of lipid biomarkers in plasma and other tissues in specific disease states.

02:45 PM

Biomarkers of toxicity: Discovery through NMR-based biochemical profiling

Nelly Aranibar. Bioanalytical and Discovery Analytical Sciences, Bristol-Myers Squibb, Princeton, NJ, United States

NMR-based biochemical profiling or metabolomics, can be a powerful tool in the discovery and identification of small molecule metabolic biomarkers of toxicity. Some examples will be illustrated in which metabolomics, the simultaneous quantification of many metabolites in biofluids, translated into biochemical and physiological knowledge of mechanisms of toxicity and resulted in the identification of small molecule biomarkers.In the instance of a drug-related, preclinical myotoxicity event, organic acid urinary excretion in a pattern similar to a known congenital disorder, led to hypothesis generation and confirmation for the molecular mechanism of the toxic insult, as well as the discovery of a biomarker translatable into the clinic. In a second example, urinary biomarkers were identified which led to the monitoring of P450 enzyme induction. A third case will show the application of NMR to the identification of excreted methyl-histidines' derivatives as response to myofribillar damage related to statin treatment.NMR-based biochemical profiling of biofluids can be applied in an investigative manner to generate mechanistic hypotheses, by relating the changes in the composition of biofluids to metabolic pathways and, in parallel, to identify small molecule biomarkers.

03:50 PM

Steroid analysis by LC-MS to monitor toxicity and support toxicological model development

Kara Pearson, Thomas Griffiths II, Yi Yang, Louise Saldutti, Neetesh Bhandari, Alema Galijatovic-Idrizbegovic, William Schaefer. Safety Assessment and Laboratory Animal Research, Merck & Co. Inc., West Point, PA, United States

Clinical labs have begun to implement LC-MS assays for steroid analysis to overcome some of the issues that affect the variability and non-specificity of immunoassays, especially at low concentrations. We have implemented three LC-MS assays for analysis of steroids and their metabolites for use in Safety Assessment. First, the plasma testosterone assay has been qualified for its sensitivity through analysis of samples from in vivo studies with model compounds (androgens and testicular toxicants.) Second, the assay for hydroxylated metabolites of testosterone has been employed to assess CYP3A4 activity in development of metabolically competent in vitro and human-relevant in vivo models. Finally, the assay to monitor glucocorticoid metabolites in urine provides a means to monitor changes in rat corticosterone metabolism without the complications of diurnal variation and sample collection stress for plasma or serum analysis.

04:40 PM

Lipid tracing and metabolic flux for translatable biomarker development in drug discovery

Director Thomas P Roddy PhD. Discovery and Preclinical Sciences, Merck & Co., Rahyway, NJ, United States

From first in man to registration, lack of efficacy is the primary reason for attrition of targets in drug discovery, and biomarkers help to address this issue. Lipid biomarkers are often translatable from preclinical species to humans because lipid analytical techniques are usually applicable to any species. With biomarkers like triglycerides, where the concentration is high and the turnover rate is relatively low, measuring steady state concentration changes after treatment is challenging. To address this, tracing with a stable-isotope labeled fatty acid quantifies newly made triglycerides, resulting in a robust target engagement assay. An alternative to fatty acid tracing is flux analysis in which a ubiquitously distributed tracer, like D_2O, is dosed, and deuterium is incorporated into newly synthesized lipids, proteins, and metabolites in the same experiment. In this presentation, examples of lipid tracing, lipid flux, and apolipoprotein flux will be discussed as dynamic biomarkers for lipid metabolism.