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Trip Report: American Chemical Society Meeting San Francisco, California September 9 -14, 2006

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***Abstract**: The semi-annual American Chemical Society Meeting was held in San Francisco, CA, September 9 – September 14, 2006. This symposium covered a wide range of topics in different areas of chemistry. This report highlights select material from the lectures and poster presentations most closely related to scientific topics covered by Metabolism and Biotransformations and R&D Groups at AMRI.*

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Poster Presentation: "Synthesis of Trace Drug Metabolites on Multi-Milligram Scale. Production of 32-Hydroxy-Rifalazil as an Example of Metabolite Synthesis of a Poorly Water-Soluble Compound."

We presented a poster at the Divisions of Biological Chemistry poster session (subdivision "Chemistry and Metabolism") on September 12, 2006. The poster describes development of a novel reaction system for the production of metabolites of compounds poorly soluble in water using mammalian liver microsomes as reaction catalyst. The system is based on the use of hydrophobic polymeric resin serving as a reservoir for the hydrophobic parent compound and its metabolites. The utility of the extractive biotransformation approach was demonstrated for the production of a low yielding hydroxylated metabolite of antibiotic rifalazil, 32-hydroxy-rifalazil, using mouse liver microsomes. In order to address the problem of the very low solubility of rifalazil in the predominantly aqueous microsomal catalytic system, a variety of strategies for the enhanced delivery of hydrophobic substrates were tested, including the addition of mild detergents, polyvinylpyrrolidone, glycerol, bovine serum albumin, and hydrophobic polymeric resins. The latter strategy (extractive biotransformation) was identified as the most suitable for the production of 32-hydroxy-rifalazil, resulting in up to 13-fold enhancement of the volumetric productivity compared to the standard aqueous system operating at the solubility limit of rifalazil. The production process was optimized for a wide range of reaction parameters; the most important for the volumetric productivity included the type and amount of the polymeric resin, type of the cofactor recycling system, concentration of the catalyst and rifalazil, reaction temperature, and agitation rate. The optimized reaction system was used for the synthesis of 32-hydroxy-rifalazil on a multimilligram scale. The structural identity of the synthesized product was confirmed by LC/MS characterization and comparing the HPLC behavior with authentic reaction products produced by human and dog liver microsomes. The research was sponsored by ActivBiotics, Inc.

Metabolite production for organic and natural compounds, candidates to drugs, is one of the hot topics for medicinal chemistry and ADMET research in particular. Our poster, which described a new approach to increasing productivity for the synthesis of metabolites for poorly water-soluble compounds, attracted significant interest of scientists from industry and academia and instigated interesting scientific discussion. Our colleagues admitted that the novel methods used in this study may appear very useful for improvement of synthetic yields in related biocatalytic systems. During the discussions, we also managed to establish good scientific and personal relations with a number of scientists who are working in the same scientific area. Some of them may potentially become our customers.

Papers Presented at Division of Analytical Chemistry

"**Screening for reactive drug metabolites during early pharmaceutical drug discovery,"**
John R. Soglia (Pfizer Global Research and Development).

Dr. Soglia gave a brief overview on reactive drug metabolite research conducted in the past decade focusing on high throughput screening for reactive metabolites during early drug discovery. Reactive drug metabolites could play a role in in-vivo toxicity events such as tissue necrosis,

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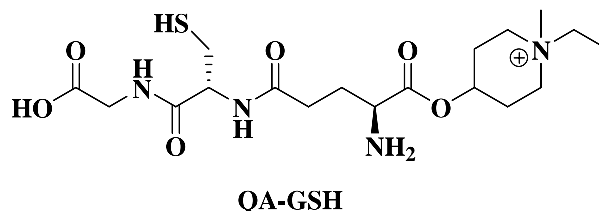
mutagenicity and teratogenicity. Although a direct link between reactive drug metabolite formation and the onset of certain toxicities has yet to be identified, a large number of cases are reported where the toxicity finding is accompanied by reactive drug metabolite formation by drug compounds. Dr. Soglia presented a novel in vitro semiquantitative reactive metabolite detection assay developed and implemented at Pfizer. The assay incorporates NADPH-supplemented human liver microsomes, a novel quaternary ammonium glutathione analogue conjugating agent (QA-GSH) containing a fixed positive charge, and LC-MS/MS for detection.



QA-GSH

MS/MS was selective and sensitive for the QA-GSH conjugating agent and successful quantitation of QA-GSH-reactive metabolic conjugates was performed using QA-GSH standards added to samples prior to analysis. The assay was tested with a number of model drugs including acetaminophen, clozapine, and flutamide, which were bioactivated to afford reactive metabolites. The results show that the QA-GSH assay is a viable method for quantitatively assessing the bioactivation potential in vitro and is well-suited for use in early drug discovery high throughput screening.

"The analysis of basic drugs by LC/MS/MS with high pH mobile phases,"
Tivadar Farkas (Phenomenex, Inc).

The selection of mobile phase is an important factor in achieving maximum sensitivity in LC/MS when electrospray ionization (ESI) is applied. The current practice is to use low pH buffers containing volatile weak acids or salts to enhance the ionization of basic compounds in positive ion mode. Various publications have discussed the benefits of performing chromatographic separations of basic compounds in mobile phases at high pH. Under such conditions basic molecules are uncharged, their retention increases significantly in reversed-phase, and they elute with symmetrical peaks. One possible drawback could be a decrease in sensitivity with electrospray MS detection under conditions that suppress analyte ionization in solution. The researchers at Phenomenex have evaluated the responses of a large number of basic drugs covering a wide range of polarity (log P 0.4-7.6) and pKa values (6.5-10) in ESI+ LC/MS/MS, in low and high pH mobile phases. Contrary to common expectations, high pH mobile phases does not suppress basic compound ionization in ESI; positive ions were formed abundantly and analyte responses were comparable, or most often better in high pH compared to acidic mobile phases. Successful quantitation of twenty two drugs with good linearity, precision and accuracy was reported.

Papers Presented at Division of Biological Chemistry
Eli Lilly Award Symposium: Chemical Approaches to Neuroscience and Other Complex Systems

"A role for chemistry in stem cell biology,"
Peter G. Schultz (Department of Chemistry, The Scripps Research), La Jolla, CA.

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Chemical genomics and proteomics tools are being used to identify and characterize novel small molecules and genes that affect embryonic and adult stem cell self renewal and differentiation. Major advantage coming from the technology developed in the author's laboratory is very efficient phenotypic screening which allows for analysis of more than one million compounds a day. Specifically, tyrosine kinase screen provides for successful simultaneous screening for the enzymatic activity from seventy different cell lines in single experiment. The next important step is transition to direct unraveling molecular mechanisms of action of these hit molecules. A number of discussed examples included the differentiation of embryonic stem cells to neurons, cardiomyocytes and germ cells; the differentiation of various adult stem cells to osteocytes and neurons; the self renewal of murine embryonic stem cells; and the dedifferentiation of lineage committed cells to multipotent precursors.

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"The enzymatic regulation of endocannabinoid signaling,"
Benjamin F. Cravatt (Departments of Chemistry and Cell Biology, Skaggs Institute for Chemical Biology and Scripps Research Institute), La Jolla, CA.

Endogenous cannabinoids (endocannabinoids) constitute an emerging class of signaling lipids that act on both central and peripheral cannabinoid receptors, which also mediate the effects of Δ^9-tetrahydrocannabinol, the active component of marijuana. The magnitude and duration of endocannabinoid signaling are tightly controlled in vivo by the integral membrane enzyme, fatty acid amide hydrolase (FAAH), which is a member of the amidase signature family. FAAH in particular, is a potential drug target for the treatment of nervous system disorders as this enzyme regulates cannabioid activity and its inactivation produces major metabolic effects. The multidisciplinary research program aimed at understanding the molecular, cellular, and physiological functions of FAAH and its fatty acid amide substrates includes: 1) the recombinant expression, characterization, and structural determination of FAAH, 2) the generation and analysis of transgenic animal models with disrupted FAAH activity, and 3) the development and application of functional proteomic and metabolomic methods to globally interrogate the FAAH-fatty acid amide signaling system. The experimental approach is gene knock-out in mice and application of hot-plate test to the animals. Also using LC/MS, metabolites in different tissues (brain, liver, and kidney) in normal versus knocked-out mice are analyzed and compared in 3D plots: RT – mass – concentration. By using this approach, N-acetyl-taurins were found to differ in concentration significantly in normal versus knock-out cells. Mutant FAAH was found which shows 1000-fold difference in hydrolysis of N-acetyl-taurins. This enzyme will be introduced back into knock-out mice.

Alfred Bader Award Symposium: Protein Folding, Unfolding, and Misfolding

"Sequence dependence of amyloid formation and toxicity,"
Carlos Dotti, Alexandra Esteras, Nico Kümmerer, Manuela López de la Paz, M. Teresa Pastor, Vanessa Schubert, ***Luis Serrano*** *(European Molecular Biology Laboratory), Heidelberg, Germany.*

Understanding the molecular events underlying the conversion of normally soluble proteins into amyloid fibrils is fundamental for prevention or cure of amyloid-related disorders. Conversion of a soluble non-amyloidogenic protein into an amyloidogenic prone molecule can be triggered by the presence of a short amyloidogenic region in a protein. Saturation mutagenesis of a de novo designed hexapeptide allows the identification of an amyloidogenic pattern. Using the pattern, short amyloidogenic stretches were identified in six relevant amyloid proteins that form amyloid fibrils *in vitro*. Prefibrillar aggregates (spherical aggregates + short protofibrils) formed by these 6-residue peptides and by longer polypeptides share a common morphology and are responsible for cell death and impairment in PC12 cells and primary hippocampal neurons. Initially, prefibrillar aggregates bind to cell membranes and localize in neuron synapses re-organizing actin cytoskeleton. In both cell systems, prefibrillar aggregates are further internalized which leads to cell death through an apoptotic mechanism, that does not involve large pore formation. Prefibrillar aggregates are proposed to exert their cytotoxic effect through a common cell death mechanism related to their structure, which is independent of polypeptide sequence, length and peptide chirality, but dependent on membrane microdomains and composition.

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"Protein folding, misfolding and cancer: The tumor suppressor p53,"
Alan R. Fersht *(Cambridge University and MRC Centre for Protein Engineering), Cambridge, UK.*

Systematic mutagenesis is one of the most powerful tools for elucidating the principles of protein folding and stability. Mutation is also the principle cause of cancer, and the technology for studying simple folding can be transferred to the study of proteins involved in cancer. The homo-tetrameric tumor suppressor p53 consists of 1588 residues, some of which are in well-structured domains but others are natively unfolded. Some 50% of human cancers have mutations that inactivate the core domain of p53, frequently just by lowering its melting temperature. The structure of the core domain was solved in solution by state-of-the-art NMR methods and structural reasons for its instability were found. A more stable variant, which is biologically active was engineered and the crystal structures of oncogenic mutants in this framework was solved.

Papers Presented at Division of Biochemical Technology
Emerging Technologies: Nanobiotechnology

"Protein folding and stability at nanoparticle surfaces: What are the important factors?,"
Jonathan S. Dordick, Joseph H. Nuffer, ***Wen Shang****, and Richard W. Siegel (Rensselaer Nanotechnology Center and Department of Materials Science and Engineering, Rensselaer Polytechnic Institute), Troy, NY.*

A fundamental understanding of the property changes of biomolecules upon their attachment to nanoscale surfaces is a prerequisite for the fabrication of functional bioconjugated nanosystems. The adsorption of proteins onto solid surfaces induce changes of their conformation. Recent studies have indicated that nanoscale surface curvature plays a key role in influencing the structure and properties of adsorbed proteins. Ribonuclease A (RNase A) was chosen as a model system for analysis of protein folding/unfolding behaviors on silica nanoparticle surfaces and urea denaturation of RNase A adsorbed onto silica nanoparticles was studied by CD. While no significant structural changes in the folded states at pH 7.4 could be detected for the adsorbed RNase A on silica nanoparticles with different sizes, the stability of RNase A varies with the nanoparticle sizes. There was increased destabilization of RNase A attached to nanoparticles with increasing sizes.

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Biophysical and Biomolecular Symposium: Protein Engineering

"Protein engineering of 4-methyl-5-nitrocatechol monooxygenase from *Burkholderia* sp. strain DNT for enhanced degradation of nitroaromatics,"

Glenn Johnson, Thammajun Leungsakul, ***Thomas K. Wood*** *(Department of Chemical Engineering, Texas A&M University), College Station, TX.*

The flavoprotein 4-methyl-5-nitrocatechol (4M5NC) monooxygenase (DntB) catalyzes the second step of 2,4-dinitrotoluene degradation by converting 4M5NC to 2-hydroxy-5-methylquinone by removing the nitro group. DntB has a very narrow substrate range. Error prone PCR was used to create variant DntB M22L/L380I that accepts two new substrates: 4-nitrophenol (4NP) and 3-methyl-4-nitrophenol (3M4NP). For 4NP, the initial rate of the purified variant expressing M22L/L380I enzyme was 10-fold higher than that of the wild-type enzyme. In addition, the variant M22L/L380I enzyme has 4-fold higher activity toward 3M4NP. Saturation mutagenesis at the individual positions M22L and L380I did not show appreciable enhancement in 4NP activity which suggested that these two mutations should be evolved together; simultaneous saturation mutagenesis led to the identification of the variant M22S/L380V with 20% enhanced degradation for 4NP compared to the variant M22L/L380I. This is the first report of protein engineering for nitrite removal by a flavoprotein.

"Designed divergent evolution of enzyme function,"

Jay D. Keasling, ***Yasuo Yoshikuni*** *(University of California), Berkeley, CA.*

It is generally believed that proteins with promiscuous functions divergently evolved to acquire higher specificity and activity, and this process was highly dependent on the ability of proteins to alter their functions with a small number of amino acid substitutions (plasticity). The application of this theory of divergent molecular evolution to promiscuous enzymes may allow design of enzymes with more specificity and higher activity. Many structural and biochemical analyses have identified the active or binding site residues important for functional plasticity (plasticity residues). To understand how these residues contribute to molecular evolution and thereby formulate a design methodology, plasticity residues were probed in the active site of the promiscuous sesquiterpene synthase, γ-humulene synthase, as a model. Identified plasticity residues were then systematically recombined based on a mathematical model in order to construct seven novel terpene synthases, each catalyzing the synthesis of one or a few very different sesquiterpenes.

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"Development of a novel phosphite dehydrogenase based NAD(P)H regeneration system for industrial biocatalysis,"

Tyler Johannes, Michael McLachlan, Ryan Woodyer, ***Huimin Zhao*** *(Department of Chemical and Biomolecular Engineering, University of Illinois), Urbana, IL.*

Enzyme-catalyzed reactions that require NADH and NADPH as cofactors, require in situ cofactor regeneration for preparative applications. A novel enzymatic system was developed based on phosphite dehydrogenase (PTDH) to regenerate NADH and NADPH that is more efficient than the currently used formate/FDH system. Both rational design and directed evolution were used to engineer PTDH variants that exhibit increased stability, activity, and cofactor specificity. A PTDH mutant with relaxed cofactor specificity showed a 3.6-fold higher catalytic efficiency for NAD^+ and a 1000-fold higher efficiency for $NADP^+$. Directed evolution was used to enhance the solubility, expression, and activity of PTDH and a mutant with 2-fold higher turnover rate toward NAD^+ and 3-fold better expression than the wild type enzyme was obtained. Thermostability of PTDH was also improved and a mutant was obtained whose half-life of thermal inactivation at 45°C is >22,000-fold greater than that of the parent PTDH. The final mutant PTDH was compared to FDH for cofactor regeneration in the industrially relevant production of L-tert-leucine and xylitol in a membrane bioreactor. This novel enzymatic NAD(P)H regeneration system has recently been licensed to BASF(Germany) and Biocatalytics (Pasadena, CA).

Upstream Processing: Biocatalysis

"Lipases for biocatalysis: Discovery, engineering and production,"

Kim Borch, Barbara Cherry, Haiyan Ge, Janine Lin, Suzie Otani, Sham Paktar, ***Debbie S. Yaver*** *(Novozymes, Inc.), Davis, CA.*

One of the challenges faced in developing a biocatalytic method is the limited number of commercially available enzymes that are useful in biocatalysis and that can be provided in bulk quantities. Most industrial enzymes are normally developed and formulated to work in a given application such as in laundry, baking, or brewing. However, there are examples of enzymes that have been used for biocatalysis (Novozyme 435, a lipase from Candida antartica, and the Thermomyces lanuginosa lipase). Many methods have been used to discover new lipases with novel properties. A new approach is the identification of putative lipases using bioinformatics followed by cloning, expression and characterization of the enzymes. Several fungal lipases homologous to T. lanuginosa lipase and a lipase from Geotrichum candidum were cloned, expressed and characterized. Another approach for developing new enzymes is protein engineering and a few examples of engineering of lipases were presented.

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"Thermophilic chaperones for maintaining enzyme structure and function in organic co-solvents,"

Lisa M Bergeron, *Douglas S Clark, Cecilia Lee (Department of Chemical Engineering, University of California at Berkeley), Berkeley, CA.*

The use of enzymes in solutions containing high concentrations of water-miscible organic co-solvents is often limited by enzyme stability. Some molecular chaperones can function to prevent irreversible unfolding in organic co-solvents as well as maintain enzyme activity under solvent-denaturing conditions. A single subunit isolated from the thermosome of the hyperthermophile *Methanocaldococcus jannaschii* functions in the presence of several water-miscible organic co-solvents. By comparison, its mesophilic homologue, GroEL, exhibits loss of secondary structure and therefore loss of activity in identical conditions.

"Activation of enzymes in nonaqueous solvents by incorporating them in amphiphilic polymer conetworks,"

Nico Bruns, *Stephan Dech, Reinhild S. Ladisch, Joerg C. Tiller, Olena Yurchenko, Frank Zander (Freiburg Materials Research Center, University of Freiburg), Freiburg, Germany.*

A novel technology based on enzymes entrapped in amphiphilic polymer conetworks was developed to enhance the activities of enzymes such as lipases, peroxidases and α-chymotrypsin by several orders of magnitude in heptane and in supercritical CO_2. This high activation is due to the conetworks phase separation with domain sizes between 2 and 25 nm and their peculiar swelling properties. Enzymes diffuse into the water-swellable hydrophilic phase and substrates into the organo-swellable hydrophobic phase. In organic solvents the enzymes can be accessed by their substrates via the extremely large interface of the network.

Papers Presented at Division of Chemical Toxicology

"Cytochrome P450 and chemical toxicology,"

F. Peter Guengerich *(Dept. of Biochemistry & Center in Molecular Toxicology, Vanderbilt University), Nashville, TN.*

The most general process catalyzed *in vivo* by cytochromes P450 is that of detoxication and about 75% of all metabolic reactions are provided by CYPs, while other catalysts present UGT, esterases FMO, NAT, and MAO. About 25% of P450s are devoted to steroids, although for a number of CYPs the substrate is unknown. Although activation of chemicals to reactive electrophilic species is less common, P450s are also the major catalysts, and many examples of toxicity of drugs and other chemicals are understood in this context. Now the major issue for termination of drug candidates is animal and human toxicities, which are responsible for 1/3 of all cases. Roles of P450s in oxygen radical damage have also been proposed but are less clear. Much is now known about catalytic specificity of animal and human P450s, although a number of questions about the molecular basis of substrate specificity and catalysis are still unanswered and a priori predictions are still difficult. The most abundant CYP 3A4 does not present a big substrate hole accommodating any substrate and it has significant stereo- and regio-selectivity (6β-hydroxylation of testosterone). Five P450s, including 1A2, 2C9, 2D6, 2E1, and 3A4) are major contributors to activation of carcinogens. Now understanding of some aspects of toxicity is really good; however, our prediction ability for human toxicities remains very poor. Major resasons for lung cancer

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toxicity: biotransformation-related (37%), target-related (28%), ion chanel inhibition (18%), immune-mediated (7%). Understanding the chemistry of oxidations by P450s has been important in rationalizing biotransformation of chemicals to reactive and other products.

"Tobacco carcinogen biomarkers: Development and application,"
*Steven G. Carmella, **Stephen S. Hecht**, Yanbin Lao, Irina Stepanov, Pramod Upadhyaya, Peter W. Villalta, Mingyao Wang (University of Minnesota Cancer Center), Minneapolis, MN.*

Smoking causes all possible cancers and 30% of all cancer deaths in developed countries. Tobacco carcinogen biomarkers include DNA adducts, hemoglobin adducts, and urinary metabolites. These quantitative measurements can be extremely informative with respect to mechanisms and prevention of tobacco induced cancer. The focus for research is on three classes of carcinogens in tobacco products: tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons (PAH), and aldehydes. Urinary metabolites of tobacco-specific nitrosamines such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides have provided important information about the uptake of the tobacco-specific lung carcinogen compounds in humans. This biomarker has been widely applied in studies of carcinogen uptake in smokers, smokeless tobacco users, and non-smokers exposed to environmental tobacco smoke. Methods for the analysis of other tobacco-specific nitrosamine metabolites in human urine and for their DNA adducts have been developed. The uptake and metabolic activation of PAH differs widely among individuals, and the authors have developed phenanthrene metabolite ratios to probe these differences. The relationship between phenanthrene metabolite urinary phenotype and variants in PAH metabolizing genes has been investigated in smokers. Acetaldehyde and formaldehyde occur widely in the human environment including cigarette smoke.

"Quinoids formed from estrogens and antiestrogens: Role in carcinogenesis?,"
***Judy L. Bolton** (Dept. of Medicinal Chemistry & Pharmacognosy), University of Illinois at Chicago, Chicago, IL.*

Estrogens and antiestrogens have been implicated in hormone dependent cancers (breast cancer) and osteoporosis; however, carcinogenic mechanisms remain both controversial and elusive. Most common mechanism involves metabolism of these compounds to reactive intermediates such as quinones, quinone methides, and di-quinone methides which leads to oxidation and/or alkylation of DNA and proteins. Four SERMs including tamoxifen, raloxifene, acolbifene, arzoxifene all are metabolized by P450 to quinone methides and some cases o-quinones. In order to determine if these quinoids could cause protein modification a novel raloxifene covert oxidatively activated tag (COATag) was synthesized in which the SERM was linked to biotin. This COATag facilitated the isolation and identification of covalently modified proteins following metabolic activation of the labeled raloxifene by rat liver microsomes. These data show that oxidative metabolism of raloxifene produces reactive intermediates of sufficient lifetime to covalently modify proteins in microsomes and that this would be an expected general feature of SERMs currently in use.

"Arsenic: The king of poisons, the poisons of kings, and the bane of investigators,"
HV. Aposhian, Mihaela D. Avram, Uttam K. Chowdhury, George Tsaprailis (Dept. of Molecular and Cellular Biology, The University of Arizona), Tucson, AZ.

Arsenic-contaminated drinking water is a great problem for some regions on Earth (Bangladesh and West Bengal are the worst) and is the cause of the greatest public health calamity of the last 25

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years. Yet, there is still a mystery as to the molecular mechanisms of inorganic arsenic toxicity. The human metabolizes inorganic arsenic via a number of methylation steps. Recently, the established pathways have been questioned and new ones have been proposed by Hayakawa et al. (2005). Schematically, arsenic metabolism can be presented as:

As(V) → As(III) → methyl-As(V) → reduction to give As(III) → dimethyl-As(V) → reduction to give dimethyl-As(III)

Newer proteomics techniques have allowed the investigation of proteins whose syntheses are inhibited by arsenic species as well as proteins whose syntheses appear to increase and may be non-toxic reservoirs for arsenic. The relationships of many of these proteins with arsenic metabolism and toxicity are being identified for the first time by using proteomics techniques combined with LC-MS/MS analysis.

"Oxidative stress induced macromolecule modification and cell signaling,"
Koji Uchida *(Laboratory of Food and Biodynamics, Nagoya University Graduate School of Bioagricultural Science), Nagoya, JP.*

Primary targets in proteins are SH-groups, which are oxidized to SOH-groups and S-S bonds. Lipid peroxidation generates active aldehyde species, such as short-chain, unesterified aldehydes. It is now recognized that acrolein is an endogenous electrophile that could be formed in cells via lipid peroxidation, amino acid oxidation, and polyamine metabolism. Its high reactivity makes this aldehyde a dangerous substance for the living cell. Specifically, acrolein shows the damaging effects on the tracheal ciliatory movement and the pulmonary wall and also initiates urinary bladder carcinogenesis in rats. In the reaction of BSA with this carcinogenic aldehyde, a novel acrolein-lysine adduct, *N*ε -(3-formyl-3,4-dehydropiperidino)lysine (FDP-lysine), was identified as the major product. Also FDP adduct is a thiol-reactive electrophile, which reacts with sulfhydryl groups to form thioether adducts. Furthermore, glutathione-dependent detoxification of FDP adducts in the oxidized LDL is possible. Specific antibody kit on acrolein is developed, which allows detection of acrolein conjugates with proteins, for example in liver.

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"Role of reactive metabolites and other risk factors in determining susceptibility to drug-induced liver disease,"
Lance R. Pohl *(Molecular and Cellular Toxicology Section, NIH National Heart Lung and Blood Institute), Bethesda, MD.*

Liver failure is the major reason for withdrawing drugs from the market. Drug-induced liver disease (DILD) is thought to be caused by both allergic and non-allergic mechanisms of pathology that are mediated by reactive metabolites of drugs and endogenous biochemicals. Most cases are idiosyncratic (incidence of 10^{-4} to 10^{-5}) and almost impossible to predict due in large part to insufficient knowledge of predisposing risk factors. Examples for idiosyncratic toxicity are presented by such anesthetics as acetaminophen, chloroform, and halothane, which are dangerous as they may cause toxicity. Recent murine model studies, however, have led to the discovery of several potential susceptibility factors that may predispose individuals to DILD. For example, mice deficient in interleukin (IL)-4, IL-6, IL-10, IL-13, or cyclooxygenase-2 are more susceptible to DILD than wild type mice, while deficiencies in other factors including macrophage migration inhibitory factor and osteopontin make mice less susceptible to DILD. Other potential risk factors for DILD have been discovered by proteomic and genomic analyses when protein and mRNA levels in livers of susceptible and resistant strains of mice were compared before and after drug treatment. These findings suggest that polymorphisms and/or environmental factors that affect the activities of both hepatoprotective and hepatotoxicant factors may contribute to the incidence of DILD.

"Strategies to reduce drug candidate failure due to off-target activities,"
Bruce D. Car *(Discovery Toxicology Department, Bristol-Myers Squibb Co), Princeton, NJ.*

Drug candidate failure comes from on-target toxicity (a mechanism-related phenomenon caused by binding to the same receptor in a wrong tissue - example of statins) or off-target toxicity (example of terfenadin). To optimize safety profiling, a 14-year retrospective analysis of drug attrition due to toxicity (85 cases) was undertaken. Over 80% of liabilities were detected in toxicology studies of 2-week's duration. Such studies complemented by screens for target selectivity and counter screens for known liabilities would identify nearly all toxicity. Attrition due to toxicity was detected as target-based (23%), biotransformation-related (22%), and immune-related (11%). Leading off-target causes of attrition included cardiovascular (34%), hepatic (13%), C/PNS (10%) and hematologic (10%). Most cardiovascular liabilities resulted from drug and metabolite interactions with single or multiple cardiac ion-channels or GPCRs, including hERG, L/T-Ca^{2+}, Na^{+}, 5HT2A, and others. Different variants if hERG assays are used such as in silico analysis, binding displacement, electrophysiology with standard transfectant, analysis of transfectant mutants, FLIRP, and QT interval. Obviously IC50 value of 1 µM in hERG assay for the compound with the target efficacy of 1-5 nm does not represent a significant risk to a patient. Multiple ion channel effects (K^{+}, Na^{+}, Ca^{2+}, Pacemaker currents, and others) are also common. Sometimes Na^{+} channel is a more risk to a patient than hERG channel. Particular attention is given to avoiding the inappropriate termination of molecules or chemotypes based on isolated in vitro results, with considerations of in vivo corroborative findings (sometimes in vivo and in vitro results disconnect!), seriousness or reversibility of liabilities and the risk-benefit of the drug indication all weighing into the consideration of a molecule's advancement.

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“Structural basis of drug binding to hERG K^+ channels,”
Michael C Sanguinetti *(Department of Physiology and Nora Eccles Harrison Cardiovascular Research & Training Institute), University of Utah, Salt Lake City, UT.*

Block of hERG K^+ channels by a surprisingly large number of drugs prolongs the QT interval of the body surface electrocardiogram and increases the risk of cardiac arrhythmia. These drugs (CNS drugs, antiarrhythmics, antimicrobials/antimalarials etc.) block open pores of hERG channel (not with bound K^+). This side effect is a major hurdle to the development of new drugs. The molecular determinants of hERG channel block have been identified using a site-directed mutagenesis approach. hERG channels are formed by coassembly of 4 subunits, each containing 6 α-helical transmembrane domains. Two aromatic residues, Tyr652 and Phe656 located in the S6 domain of hERG, and 2 polar residues, Thr623 and Ser624, are critical for interaction with structurally diverse drugs. By contrast, hERG channel agonists bind to specific residues located between the S5 and S6 domains. Blocker potency is well correlated with hydrophobicity of Phe656 and an aromatic side group at position 652, suggesting the importance of a cation-pi interaction between Tyr652 and a basic N of the drug.

“Properties of the hERG potassium channel that promote promiscuous binding of small organic molecules,”
Ramy Farid, Richard Friesner, ***Robert Pearlstein*** *(Richard Novartis Institutes for BioMedical Research), Cambridge, MA.*

The hERG potassium channel is a key cardiac ion channel that terminates the plateau phase of ventricular repolarization. The inactivated form of hERG promiscuously binds small organic compounds in the ion conduction pathway, leading to adverse effects. In the absence of co-crystal structures, overall binding mode, bound conformation, and interactions have yet to be determined, and the basis for promiscuity explained. A model of the homo-tetrameric pore domain of hERG was created based on its homology with known K^+ channels, and a set of known blockers docked. It is suggested that the pore domain has symmetry, and multiplicity of key side chains identified from mutagenesis, are responsible for the propensity of the cavity to bind compounds containing aromatic and basic groups. In response to signals (particularly voltage) the channel cycles between open, close, and inactivated states and conducts unidirectional transport of K^+. Part of the channel function is to reject Na^+. Selectivity filter mediates K^+ dehydration. Unlike other K^+ channels, the binding site is not very selective to molecule structure, and many blockers are trapped upon channel deactivation. In the absence of the channel, K^+ transport costs +60 kcal/mol, whereas with the channel, this is -8.5 kcal/mol. This tremendous gain in energy is due to specific electrostatics in the channel cavity: pore helix dipoles stabilize K^+ and water. Drug-like compounds containing branched chains and aromatic rings are proposed to bind according to “ring stacking” mechanism. Compounds containing both embedded hydrophobic cation and aromatic rings bind according to “dual binding” mechanism.

Papers Presented at Division of Medicinal Chemistry

Dr. **Junying Yuan**, Professor of Cell Biology, Harvard Medical School, presented the studies on alternative forms of regulated cell death. The researchers found a connection between apoptosis (programmed cell death) and necrosis (unavoidable lethal breakdown) and demonstrated that a general process exists to allow cells to die by necrosis when apoptosis fails. The team called the new cell death pathway “necroptosis”. Apoptosis needs energy and stops when there is not enough energy available. That’s the case in strokes, which are caused by a lack of blood and oxygen in the

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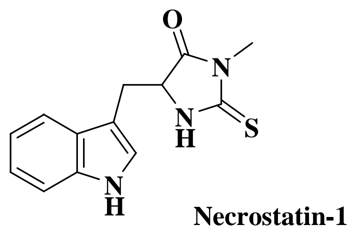
brain. In such cases necrotic mechanism might take over apoptosis. Dr Yuan's group found a compound that can block necrosis without interfering with apoptosis. The scientists have screened about 15,000 compounds before scored a hit named necrostatin-1.



Necrostatin-1

Necrostatin-1 limits brain damage in mice undergoing strokes by inhibiting necroptotic death. It was also found that Necrostatin-1 operates in many cell types involved with different deseases. Currently the group is conducting studies to determine how necrosis–blocking might inhibit conditions such as brain injuries caused by trauma and the nerve cell degeneration typical of Alzheimer's and Parkinson's diseases.

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