



**Trip Report:
American Chemical Society Meeting
Washington, D.C.
August 28 – September 1, 2005**

Vadim Mozhaev, Ph.D.

Bioscience / Fermentation Department
Albany Molecular Research, Inc.
21 Corporate Circle
Albany, NY 12212

***Abstract:** Semi-annual American Chemical Society Meeting was held in Washington, DC, August 28 – September 1, 2005. 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.*

At the Meeting, I attended poster sessions in Biology, Medicinal Chemistry, and Organic Chemistry and the following oral sessions: “The Role of Drug Metabolism in Drug Discovery” and

“Chemical Toxicity and Drug Safety Prediction” at Division of Chemical Toxicology, “Adventures in Protein Chemistry Lilly Award Symposium” at Division of Biology, “Biologically Enabled and Bio-Inspired Polymers” at Division of Polymeric Materials: Science and Engineering, “General Oral Session” at Division of Medicinal Chemistry, “Tetrahedron Prize for Creativity in Organic Chemistry” at Division of Organic Chemistry. Especially interesting were the papers presented in Division of Chemical Toxicology.

Papers Presented at Division of Chemical Toxicology

In the opening address, Dr. J. Scott Daniels (*Millenium*) pointed out that there is less than 10% of probability now for a preclinical candidate to make it to the market. Primary causes of failure are: low drug efficacy (28%), safety issues related to different kinds of toxicity (44%), business-related problems (18%), and ADMET issues (9%). Due to significant progress in developing of different ADMET assays, the last number has reduced from 30% in recent years and, according to existing predictions, this number will still be going down to less than 5% in several next years.

According to the comments made by the other Organizer of the session, Dr. Kaushik Mitra (*Merck*), the most significant ways to increase efficacy of drug candidates are anticipate and resolve chemically significant drug-drug interactions and assess the role of metabolites in drug toxicity.

In the introductory part of the paper “Role of Contemporary Drug Metabolism in Drug Discovery”, Dr. Thomas A. Baillie (*Merck*) presented a list of molecular characteristics which a chemical compound should possess in order to become a drug. Among the most important properties are: good aqueous solubility, acceptable pharmacokinetics, minimal dependence on polymorphism of oxidative enzymes, “balanced” clearance from the body, i.e. metabolism to a limited number of products, low inhibition, low propensity to induce drug metabolism enzymes. Some, but not all reactive metabolites are toxic to the cell. ADMET scientists can deduce probable structure of electrophilic metabolites, but can not predict which ones are toxic. Human liver contains 5-10 mM L-glutathione (tripeptide L- γ -Glu-L-Cys-Gly), and molecular conjugates with L-glutathione are excreted in bile, whereas the degradation products, which present conjugates with L-cysteine, are excreted in urine.

Case study #1 (published in *Chem. Res. Toxicol.* 18, 934-945, 2005): 2,3-diaminopyridine-containing bradykinin B₁ receptor antagonists. Three adducts of the compounds with L-glutathione were detected and characterized by MS/MS. One adduct with $\Delta m/z = -2$ was identified as a product of substitution of F atom by OH in a reaction catalyzed by CYP.

Case study #2 (published in *Chem. Res. Toxicol.* 18, 675-685, 2005): metabolic activation of dihydrobenzoxanthin selective estragon receptor modulators (SERM). Work on significant minimization of metabolic modifications in two Merck compounds was successfully fulfilled as a result of identification of modifications sites in different part of the drug candidate molecules. Generation of quinone forms of the molecules studied is very often responsible for the formation of reactive metabolites.

In Introduction to the paper “Assessing Bioactivation in Lead Optimization Stages of Drug Discovery”, Dr. Cyrus Khojasteh (*Genetech*) mentioned that the pharmaceutical industry reinvests more money in R&D (18.5%) than any other industry.

After preclinical testing which usually takes four years, pharma scientists do not have a luxury of making additional changes to the structure of their molecule, which goes to clinical studies, which

in turn last for more than eight years. A drug molecule can form stable metabolites, which are excreted and reactive metabolites, which react with proteins and other molecules that may result in toxicity. Covalent binding with reactive metabolites does not necessarily lead to toxicity. Molecule of *L-glutathione* presents major defense against soft electrophiles and *L-glutathione* is also used to assess formation of reactive electrophiles in vitro. The respective conjugate with *L-glutathione* is detected by MS in positive mode. A number of other thiol derivatives are also used to identify reactive electrophiles. These include: *glutathione ethyl ester* which sometimes gives higher response in MS, but is not as reactive as *L-glutathione*; *dansyl glutathione* which allows quantitation of conjugate formation by applying a sensitive method of fluorescence spectroscopy; a number of *sulfur non-glutathione compounds* which all satisfy a number of criteria such as high metabolic stability, chemical reactivity similar to *L-glutathione*, good solubility, and ability to generate strong MS signal. These substitutes for *L-glutathione* often give more than 20-fold increase in MS response.

Recently a plate method was developed in *Genentech* which uses *dansyl L-cysteine* as a reactive probe that allows formation of the conjugates which trap reactive metabolites. By adding specific resins, excess of unreacted *dansyl L-cysteine* is removed and fluorescence is read at 500 nm (excitation at 340 nm).

In the paper “Utility of Metabolite Identification Studies in Minimizing ADME Liabilities and Safety Concerns”, Dr. Amit S. Kalgutkar (*Pfizer*) touched the basis of reducing safety-related attrition of drug candidates. Adverse drug reactions occurring with very low frequency of 1:1,000 to 1:10,000 represent, however, one of the leading cause of attrition for drugs and the fourth to sixth leading cause of fatalities in the US. Often such reactions proceed according to molecular mechanisms of suicide or mechanism-based inactivation of CYPs. An approach to minimize these reactions is to reduce the amount of “structural alerts”, i.e. reactive functions susceptible to bio-activation. An example of a structural alert is aniline functionality. To reduce adversity, aniline can be substituted by other functionalities: an example is *ibufenac*, which was withdrawn from the market; it’s structural analog, *ibuprofen*, is a well-known generic drug. Chemistry behind the difference in toxicity is the formation of an aldehyde group in α -position to the carboxyl group of *ibufenac* molecule, which can further react with carbohydrates. However, existence of “structural alerts” in the molecules of potential drug candidates does not necessarily lead to adverse effects, as these effects can be significantly minimized by a number of approaches, the most efficient being dose reduction.

Reactive metabolites can be trapped for further characterization either by “soft” reagents (*L-glutathione*) or “hard” reagents (*suprofen* or *MPTP*). A case study reported in the paper presents development of a drug *meloxicam*, which only differs in additional CH_3 -group in comparison with the structure of *sudoxicam*, which has been previously withdrawn at Phase III due to its high toxicity.

The paper “Drug metabolism and drug toxicities” presented by Dr. Gerald Miwa (*Millenium*) emphasized possibility of reducing drug safety risks in humans by performing preclinical toxicology studies in animals. Frequently, species-specific toxicities are observed and their relevance to human subjects becomes a critical issue for the initiation of studies in the clinic. Two examples of the role of drug metabolism in drug toxicities were presented by the author, one being *ronidazole*, a genotoxic animal health product for swine dysentery and the other one *DuPont*’s anti-HIV drug *custira*.

Dr. F. Peter Guengerich (*Vanderbilt University*), organizer of the session “Chemical Toxicity and Drug Safety Prediction”, announced a workshop “Cellular and In Vitro Aspects of Toxicity” to be held on June 4-6, 2006. This scientific meeting is organized under the auspices of ACS Prospective Series of conferences and will accommodate 100-200 attendees.

In the paper “Toxicogenomics for drug discovery: Application to safety assessment”, Dr. William Foster (*Bristol Myers Squibb*) presented innovative research on genomic toxicity. Implementation of the program started at BMS several years ago (results of 15 studies have been reported) includes creation of standards, information and infrastructure, and development of databases, which allow classification of huge information clusters obtained from changes in more than 12,000 genes in different tissues in response to several doses of various toxic treatments.

Dr. John Leighton (a Supervisory Pharmacologist at *Food and Drug Administration*) discussed the concepts developed by FDA for submission of genomic data. Most of the recommendations could be found online at www.fda.gov/oc/initiatives/criticalpath in the document entitled Innovation.

In the paper “Toxicogenomic Approaches to Biomarker Discovery and Predictive Toxicology” Dr. James L. Stevens (*Eli Lilly*) reviewed recent progress in evaluation of expression profiling in predictive toxicology. Between drug candidate selection and first approval, 83% of attrition occurs in Phase I toxicology trials. Few companies are engaged in widespread expression profiling of clinical candidates, but expression profiling can add mechanistic information early in the development pipeline. Expression profiling also provides value in areas such as biomarker discovery and mechanistic investigation. Recent work at *Eli Lilly* has identified novel biomarkers for gut toxicity associated with inhibition of γ -secretase, a key enzyme in processing of β -amyloid precursor proteins and a target for therapeutic intervention in Alzheimer’s disease. Recent work has also uncovered a novel biomarker candidate for bile duct hyperplasia and provides insights into mechanisms underlying the toxicity.

Dr. Chris Bradfield (*University of Wisconsin*) presented the paper “Use of Transcriptional Profiles to Classify Toxicants”, where he described the EDGE (Environment, Drugs & Gene Expression) database, which contains toxicogenomic data obtained mainly using mouse as a model on more than 18,000 DNAs, as of 2003. This database has an open access at www.edge.oncology.wisc.edu. New information to the database is added by using microarray technology developed in the author’s group to define transcriptional profiles that result from particular chemical exposures. The vast amount of data from these studies has led to the development of bioinformatic and statistical approaches that help to analyze and interpret this information.

Interesting Papers Presented at Other Divisions

In the paper “De novo Design of Metalloproteins,” presented by Dr. William DeGrado (*University of Pennsylvania*) at the Lilly Award Symposium “Adventures in Protein Chemistry,” an attractive approach of de novo design is described by example of metalloproteins. “If we truly understand proteins, we will be able to design them from the scratch”. A model diiron protein, Dueferri, a carboxylate-bridged binuclear protein with a cofactor similar to those observed in enzymes such as the R2 subunit of ribonucleotide reductase from *E. coli* and soluble methane monooxygenase, has

been designed. This protein provides an attractive model for various proteins that utilize oxygen for diverse processes including iron oxidation and storage, and hydrocarbon oxidation.

Dr. Richard A. Gross (*Polytechnic University at Brooklyn*) presented the paper “Lipase-catalyzed Polycondensation Reactions: Expanding the Box,” at the Division of Biopolymers. A number of polymerization reactions (for example, polymerization of macrolactones) and polycondensation reactions (for example, reactions between diacids and polyols or diacids and diamines) are catalyzed by hydrolytic enzymes, mainly lipases, and can produce polyesters or polyamides characterized by high crystallinity and narrow molecular weight distribution. A recently discovered reaction of oligopeptide synthesis, catalyzed by papain, is driven by low solubility of the product and results in formation of oligopeptide mixture with low dispersity. A number of radical polymerization reactions are catalyzed by oxido-reductases, for example horseradish peroxidase.

In the paper presented by Zhong-Liu Wu from the laboratory of Dr. F. Peter Guengerich (*Vanderbilt University*), an example of expanding the active site of cytochromes P450 in order to accommodate bulky substrates of hydroxylating enzymes is described. Error-prone PCR and site-directed mutagenesis led to the identification of the amino acid changes, N297Q and I300V, in CYP2A6 that achieve the goal of accommodating bulky-substituted indoles instead of the natural product, indole. For a number of derivatives of indole, monomeric oxidation products were isolated and characterized, three phenols and one quinone being the major ones.

Dr. Martin P. Mayhew (*BioCatalytics, Inc*) presented the poster “Human Cytochrome P450 Biocatalysts for the Synthesis of Metabolites” at Division of Biology. The approach for the synthesis of metabolites includes preparation of a biocatalyst by freeze-drying human cytochromes P450 and P450 reductase into a fully functional catalytic system, which contains NADPH cofactor, a cofactor recycling system, and stabilizers, that improve both the activity and stability of CYPs during synthetic reactions. Based on the presented data, the freeze-dried catalyst shows reaction turnovers comparable with pooled human liver microsomes and higher stabilities. Examples of generation of preparatively useful quantities of metabolites of several model compounds were discussed. However, from practical standpoint, application of BioCatalytics synthetic catalyst is more expensive than use of human liver microsomes.