特別講演 2

DISCOVERIES IN BASIC ENZYMOLOGY AND APPLICATIONS TO PROBLEMS IN DRUG METABOLISM AND TOXICOLOGY

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Drug metabolism research has changed, not only in type of studies that are done but also in terms of its role in the overall process of drug discovery and development. Historically drug metabolism studies were done at a relatively late stage in the course of pharmaceutical development. Most studies were heavily focused on pharmacokinetics in experimental animals, and relatively little effort was made to link results with clinical phenomena. Many drugs with good animal efficacy failed in humans. Further, failures often followed protracted and expensive animal testing.

During the past 15 years there has been a continuous but dramatic shift in emphasis in drug metabolism in the pharmaceutical industry. A key development was understanding details of the human enzymes involved in drug metabolism. This involved three major research efforts: purification, cloning, and heterologous expression. Much of the focus has been on the cytochrome P450 (P450) enzymes because of their dominant role, but much has been done with some of the other so-called Phase I and Phase II enzymes now, too. It should also be emphasized that the increased availability of human tissue samples, particularly liver, has been important in the development of *in vitro* systems. Our own laboratory has been fortunate to be able to participate in the development of this area (*e.g.*, *Cytochrome P450*, 2nd ed., P. R. Ortiz de Montellano, Ed., pp. 473-535, 1995).

Today it is usually relatively straightforward to determine: (i) which P450s are most dominant in the metabolism of a new drug, (ii) which drugs will inhibit metabolism of the new drug, and (iii) whether or not the drug induces any of the major P450s. *In vitro* methods that can be used to address the first question include correlations, chemical and antibody inhibition, and heterologous expression. Some of these approaches (*e.g.* correlation and chemical inhibition) can be extended to *in vivo* work. Several non-invasive markers have been developed, and assaying for induction is

probably more direct *in vivo* than *in vitro*. *In vitro* information with a new drug can be used to predict: (i) possible interactions with other drugs, (ii) bioavailability, and (iii) toxicity (at least genotoxicity). The information regarding possible drug interactions can be used to decrease the number of clinical interaction studies that are required. As a result of all of this, drug metabolism groups are now often fully integrated in the discovery process and have major ties to medicinal chemistry and basic pharmacology, as well as safety assessment. Indeed, with the development of more rapid synthesis and pharmacological screening, drug metabolism and safety assessment can now be limiting factors in getting new drugs into clinical trials.

In a certain sense, the new approaches have made the areas of drug metabolism and toxicology more fully appreciated. However, major new challenges now confront the field: (i) How can we screen bioavailability more rapidly? (ii) Many *in vitro* expression systems are now available and which should a drug metabolism department invest in? (iii) The recent development of combinatorial methods for generating new chemical entities has dramatically increased the demand for drug metabolism groups to handle chemicals even faster.

Some future needs for development are outlined below:

1. Improved and more inexpensive expression systems. These are needed not only for P450s but for many of the enzymes as well. Most of the systems currently available make certain large scale screening approaches impractical or prohibitive and require the use of highly sensitive, slow throughput assays.
2. Use of recombinant systems for production of new entities. One need is for rapid and practical large scale production of the products (metabolites) of new drug candidates. These need to be produced quickly to facilitate characterization, and they should also be available for conducting new pharmacological screens. Another concept is to develop modified P450s (*e.g.*, by random mutagenesis) that can produce a great variety of new products from a drug, can be screened for activities.
3. Novel uses of high-throughput screens. Today much of the focus on expediting screening is on using automation and robotics to do existing technology, rather than developing totally new systems. More assays could be developed to facilitate use of microtiter plates and other large-scale screening methods.

What would be extremely useful would be an assay that could couple the oxidation by a P450 to an alternative assay to product formation. Of course, the "obvious" choices NADPH and O₂ are usually not very well-coupled.

4. Improved *in vitro/in vivo* correlations. Some efforts are underway to make better quantitative comparisons. Another continuing problem is how to measure enzymes such as P450 3A4 in different tissues and make intelligent predictions with new drugs.
5. Basic aspects of enzyme mechanisms. We cannot expect to be able to make completely intelligent assessments of enzymes *in vivo* if we do not understand them *in vitro*. With some enzymes (*e.g.*, glutathione (GSH) transferases), X-ray structures are known. With mammalian P450s this is not the case and the details of substrate interaction are only postulated. Ultimately crystal structures are needed. Other aspects of catalysis and kinetics remain to be further explored. As an example, we have studied the seemingly unusual situation of a change in the K_m of substrates for P450 2E1 imposed by deuterium substitution. This has been explained by the demonstration that product release is the rate-limiting step (*Biochimie* **77**, 573-580, 1995). The issue is raised as to what a K_m generally is, other than an experimental parameter. Another outstanding issue is the homotropic and heterotropic cooperativity of P450 3A4, the major human P450 (*Biochemistry* **36**, 370-381, 1997). The basis of this may be allosteric. The *in vivo* relevance has some basis but it has not yet been proven. Moreover, the generality is unknown. Finally, one can ask the general question of what steps in the catalytic cycle govern rates of oxidation of particular P450 substrates. How much generality is there in this regard?

Most of the discussion here has been on P450s. In many respects the GSH transferases have been easier to study and may point the way to more uses of P450s in bacteria. Our own work with GSH transferase expression in *Salmonella typhimurium* indicated that such systems could be very useful in genotoxicity experiments (*Proc. Natl. Acad. Sci. USA* **90**, 8576-8580, 1993). The expression was relatively straightforward and simple vectors could be used to express human P450 1A2 (*Cancer Res.* **55**, 799-802, 1995). Further work with GSH transferase expression has been done using linkage with an SOS/*umu* system (*Carcinogenesis* **17**, 297-302, 1996). In principle, such systems can be modified to introduce P450s. Recently we have developed a

bicistronic vector system for co-expression of both human P450s and NADPH-P450 reductase together, catalytically active, in *Escherichia coli*. Such systems may be useful in genotoxicity test systems. All of these (and other) developments imply that further success is possible in the development of high-throughput bacterial systems. (Supported in part by United States Public Health Service grants R35 CA44353 and P30 ES00267.)