Animal pharmacology

Automated in vivo sampling systems can provide more information, faster, from fewer lab animals.

BY PETER T. KISSINGER

The past few years have seen the rise of in vitro and even in silico technology that is purported to speed up the drug discovery process. Although much progress has been made, these approaches have a number of inadequacies, and it will take more time to understand how to use the data they generate and integrate it in a systemwide fashion. It clearly remains a fact that a mammal is not a linear assembly of its parts and that surprising things can happen when a drug candidate is first examined in vivo. Over the past several years, researchers have been exploring technology to enable them to quickly draw conclusions from initial screens in rodents. There are three essential components to this process: reducing the volume of blood inevitably required for pharmacokinetics (PK) and for biomarker determination, to facilitate automation once samples are in hand; improving data quality by automating the collection process, reducing the stress that can distort PK and pharmacodynamic (PD) data; and collecting PK, PD, and ADME data in parallel from individual animals on the same time axis.

Bioanalytical technology

Advances in LC-MS/MS coupled to 96-well sample preparation and autosampling are by now routine in most commercial laboratories. Immunoassays for various biomarkers are also evolving quickly, and high-field NMR spectroscopy on small-volume samples for metabonomics is making excellent progress. This means that it is no longer necessary to sacrifice a series of animals at given postdose time points to obtain biological fluid samples. When 1 mL or more of a biological fluid was required, there was certainly no alternative for a 30-g laboratory mouse, whose body contains only about 1.5 mL of circulating blood. Today, however, a great deal can be done with a 10-µL sample. Usually, 100 µL, a very generous donation from a 300-g rat, is a more-than-sufficient sample for a convenient data point on a PK curve. Since the mid-1980s, it has been possible to process in vivo microdialysis samples for markers such as neurotransmitters, amino acids, or glucose by using serial samples of 5 µL with no loss of body fluid from the animal at all. Thus, the need to sacrifice or even anesthetize animals is left to history.

Figure 1. Automatic blood sampler. (Image courtesy of Bioanalytical Systems, Inc.)

Because much in vitro bioanalytical work is now done in plates with the benefit of robotic technology, it is highly efficient to use the same or similar technology for in vivo work. For example, LC-MS/MS methods for monitoring the metabolites of new molecular entities for in vitro P450 studies can often be quickly adapted to the in vivo situation. There is much to be gained by getting to the in vivo case as quickly as possible and not waiting for thorough analysis of in vitro experiments. In fact, in vitro experiments can be better designed to explain in vivo observations rather than to avoid them. In any case, both will remain important.

Automating collection

When humans give blood in a clinical trial, they are not anesthetized, and they are never sacrificed. Nor do they interact with a technician who is 200 to 2000 times their weight, perhaps on the order of Godzilla. On the other hand, in traditional work with mice and rats, this is exactly what happens. Not surprisingly, it creates anxiety, raises heart rate and blood pressure, alters blood flow, and results in the dumping into circulation of hormones from the hypothalamic–pituitary–adrenal glands axis. When blood is collected by methods such as cardiac puncture, retro-orbital bleeds, or tail vein sampling with a syringe, it is hardly surprising that what we want to measure is affected by the way we measure it.

Even for animals with an indwelling catheter, where the pain of sampling is eliminated, substantial stress is associated with removing a subject from a home cage and constraining her in a length of plastic pipe. This sort of handling creates altered physiology and biochemistry that can put the meaning of bioanalytical data in doubt.

KEY TERMS: assays and screening, automation, drug delivery, informatics, money

A $500,000 mass spectrometer cannot fix this problem. A human walking into an animal study room in a vivarium will normally alert rodents to the possibility of upcoming trauma. This alters their physiology. The goal is to use allometric scaling of data from smaller animals to predict results in larger species. These facts suggest that good progress could be made if awake, freely moving animals with indwelling catheters could be sampled painlessly, over long periods of time, without human intervention.

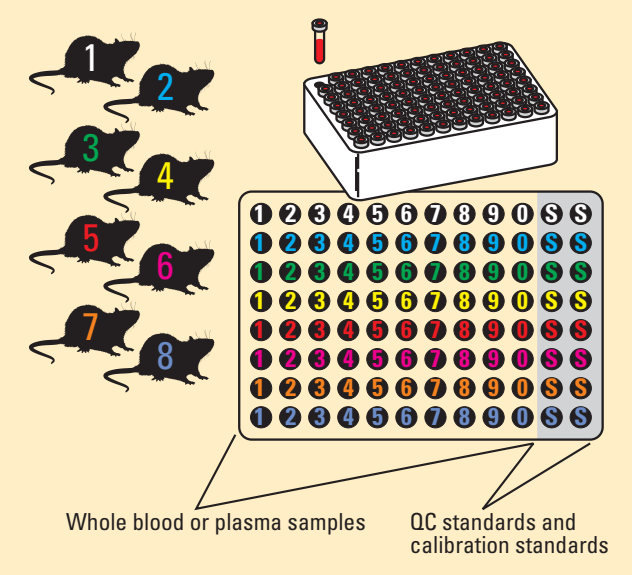

Figure 2. High-throughput sample collection. All of the samples for PK screens using 8 animals can be placed in 300-µL sealed vials on one 96-well plate. (Image courtesy of Bioanalytical Systems, Inc.)

The enabling technology for mice, rats, and guinea pigs is an interactive caging system called the Raturn (*www.culex.net*), which was originally developed by researchers at Bioanalytical Systems, Inc. (*www.bioanalytical.com*) to eliminate liquid swivels from in vivo microdialysis sampling. The purpose of this device is to enable study subjects to be tethered and connected to the outside world with more than one fluid or electrical line. Infusions, blood collections, and microdialysis can be carried out simultaneously with electrical measurements without the problem of lines becoming twisted as a result of animal motion. In fact, animal motion (counter- and clockwise turning and rearing) can be used to advantage as behavioral data, accumulated digitally throughout experiments. Analysis of this data can provide early indications of neurotoxicology or neuropharmacology. The animal containment system likewise allows for monitoring food and drink and collecting urine and feces, if desired.

Figure 1 illustrates just one of many configurations researchers have used to screen drug leads and to conduct detailed pharmacology studies later in development. This particular arrangement includes two refrigerated microfraction collectors, one for blood and the other for brain microdialysis. This instrument provides for unattended sample collection and PD monitoring over long periods (Figure 2). The blood sample you wanted to collect at 3 a.m. is now waiting for your arrival the next morning.

Data acquisition

It is not uncommon in pharmaceutical companies to find silos of expertise across a range of disciplines. Behavioral information is obtained by behaviorists, neurotransmitters are monitored by neurochemists, PK work is done by pharmacokineticists, ADME information is collected by drug metabolism people, and so forth. Electrocardiograms (ECGs), electroencephalograms, and measurements of glucose, blood pressure, and other parameters fit the same pattern.

In the standard business model, each of these activities is pursued by a different set of people with a different set of animals, often over many months, because of difficulties in scheduling the work. What we normally look for in pharmacology is how these data correlate on the same time course. How does the change in a neurotransmitter in the brain vary with the time course of the pharmacokinetics in systemic circulation? We cannot know precisely if the data are obtained from different subjects at different times. We try to make up for this deficiency by using larger numbers of animals to be more confident in the statistics. But this is costly and labor-intensive, and the informatics becomes complex to organize.

What if we could pick from a menu of measurements appropriate to the therapeutic area of interest? I personally have been interested in neuroscience and therefore want to see behavioral and neurotransmitter data tracked on the same time axis as drug concentration in blood. Others might select blood pressure, temperature, or ECGs as the key PD parameter. Those developing drugs for diabetes might add insulin and glucose to their menu. Drug metabolism and PK people might well focus on blood, urine, feces, and bile collections. They likewise might wonder about bioavailability and want to compare oral and intravenous dosing or try something like transdermal or intramuscular dosing, or even dose directly into the duodenum via a catheter. Clearly, not everything can be done at once, but many measurements can be fully automated, and they are all feasible with the same basic system, which will undoubtedly evolve further.

By applying the same automation principles used for in vitro experimentation to in vivo analysis, researchers have the opportunity to ensure the accuracy and believability of their results. In the process, they will reduce not only the number of experiments but also the number of test subjects.

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Further reading

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