Automated blood sampling in drug discovery and development

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Introduction
In the past decade, a lot of progress has been made in predicting the basic properties of newly discovered compounds. New in silico methods, improved in vitro tests and high throughput screening in cell cultures or artificial membrane systems are available nowadays. Researchers in the pharmaceutical field have access to a whole series of different tests to predict pharmacokinetic (PK) properties such as absorption and metabolism of new chemical entities. Despite all these improvements, still 39% of the failures in drug development are due to poor PK.\(^1\)\(^2\) Therefore, in order to improve the characterization of new compounds, in vivo animal testing remains required. However, these experiments are costly (in terms of people, need of animal facilities, outsourcing), very time consuming, stressful for the animals (and sometimes for the researchers as well) and not very well accepted by the general public.

Reduction, Refinement and Replacement
It is the stated aim of all medical researchers to use as few animals as responsibly as possible. Indeed, in the last 30 years, the annual number of animals used has halved and the search for validated alternatives continues. Ultimately, it would be ideal if the use of animals could be totally replaced by non-clinical methods. Unfortunately, few of these currently exist and where they do, they are often not yet fully accepted by the world’s regulatory authorities. This means that the use of animals will continue for some time to come. However, the search for alternatives continues and is guided by the golden principle of the 3 R’s: Reduction, Refinement and Replacement.

Reduction means that the number of lab animals used in experiments should be reduced. Reduction can be achieved for example by cassette dosing, in which a series of compounds (instead of one single compound) is administered to one animal. With this technique, some practical problems may arise such as PK and/or analytical drug-drug interactions.

The principle of refinement requires a fine-tuning of the experiment: obtaining as much information as possible from one animal, for example by combining different techniques, but also reducing the stress and pain of the surgical procedure.

Replacement implies replacing animal experiments as much as possible by alternatives such as in silico and in vitro tests.

Influence of stress in animal testing
As already mentioned, stress is an important factor during animal experiments. In pharmacology studies, stress can influence several pharmacological parameters including glucose, insulin, prolactin, and dopamine plasma levels. As a result of stress, blood flow to critical organs (heart, lungs, brain, muscles) is increased while the peripheral blood flow (GI tract, liver) is decreased and the heart rate and blood pressure are changed. Stress can therefore result in a slower or less absorption of the oral dose and a longer metabolisation and excretion time and can influence some PK parameters. An additional effect on the function and expression of hormone-sensitive transporters may not be excluded.

PK experiments
In a classic preclinical in vivo PK experiment with a staggered design, 3 animals would be required to get one single full 24-hour PK profile (9 time points/drug, 3 blood samples/animal). Often, the last blood sample is taken through the abdominal aorta, implicating that animals are sacrificed at this time point, which might have an effect on plasma concentrations of the compound. Additionally, in some cases, anaesthesia is required to take the blood samples, which can also influence the outcome of the experiment. Typically, one or more lab technicians are present in the animal housing facility at all times. Their presence, together with being grabbed several times for blood sampling, is causing stress to the animals, possibly influencing the outcome of the experiment. All these factors can be eliminated by the use of automated blood sampling (ABS).
Automated blood sampling

Before applying ABS on an animal, surgery is required to introduce a catheter (into a vein or an artery). During two or more days of recovery in their cage, the catheters are kept patent either with a lock solution or by continuous infusion of a heparinised saline solution. After the recovery period, lab technicians insert the parameters for the PK experiment into the software protocol, connect the animal with the system and administer the compound under investigation (via the oral or intravenous route). The ABS-system will start collecting blood samples at the predefined time points during the entire experiment. The samples can be kept at a temperature of 4°C. Recently, ABS has been coupled with dried blood spot technique and mass spectrometry with very promising results.³

In a PK experiment with ABS, the animal is conscious at all times, and it can move freely in its cage. With this technique, the blood of only one animal is required to obtain a 24-hour PK profile thus minimizing the interanimal and physiological variations.⁴ This will result in a decrease of the number of animals used, as well as in the amount of compound needed for the experiment. The required blood volume can be taken from the animal very accurately and at the exact time points as required by the protocol. Another advantage is that animals that have been used for oral treatment can be used again (after a recovery and wash out period) for the intravenous treatment so that the oral bioavailability of the compound can be obtained in one and the same animal.⁵

Experiments in which stress was measured during ABS showed that a few days after the initial surgery, the corticosterone blood levels (as a stress-marker) decreased until they showed a diurnal variation with high levels during the evenings and low levels in the mornings. ABS itself did not result in a recordable increase in corticosterone plasma concentrations.⁶ However, Abelson and colleagues reported that the frequency of the blood sampling may also influence the corticosterone plasma levels.⁷ More research to clarify this issue is still required.

Available systems and applications

ABS systems are available in different formats and sizes. The main players on the market are Verutech (Accusampler), Basi (Culex) and Instech (ABS2). The first systems were focused on the use of rats in PK experiments. However, nowadays, ABS systems exist for mice (accurate blood samples of 15-25 µL are possible) and pigs. Some systems also exist in a dual blood sampling mode, allowing blood and bile collections and first pass effect studies. Additionally, several reports are available on the use of ABS systems in humans.⁸-¹⁰ Portable ABS systems are currently under development.¹¹

In combination with other techniques such as microdialysis¹²,¹³ and telemetry¹⁴,¹⁵ ABS offers a very powerful approach for pharmacological, PK/PD and toxicology studies.

Conclusion

ABS can be a solution to improve the quality of the data of animal experiments while applying the principle of the 3 R’s. Using this technique allows the scientist to refine the experiment and to reduce the number of animals without affecting the strength of the statistical evaluation of the data. Animal stress during the experiment can be reduced which is giving room for new applications.

References