NC3Rs Crack-it Challenge: Rodent Big Brother

Challenger: AstraZeneca; September 2011 (transcript of DVD from Challenge Surgery)

The pharmaceutical industry has been struggling to maintain its output of novel medicines in recent years. Part of the reason for this is failures of candidate drugs at various stages of development, some due to lack of efficacy, others to inadequate safety. I work in preclinical safety assessment, so it is the safety failures that I'm trying to address.

There are at least three ways we can try to reduce safety-related attrition of candidate drugs. Each of these is intimately linked to a 3Rs benefit.

Firstly, we are trying to set up, validate and deploy early in vitro toxicity screens in a fully automated, high-throughput format. The aim is to de-risk compounds early, so that the compounds that go past this stage are less toxic. This embodies all three 3Rs benefits: replacement, and refinement, by which I mean the animals used in the regulatory toxicology studies are exposed to less toxic compounds, and if the compounds themselves are more likely to succeed, this may also lead to reduction in terms of the number of regulatory studies on compounds that are doomed to fail.

Secondly, we should try to increase the information content of the in vivo toxicology studies that we conduct routinely, as part of the regulatory submission. I'll come back to this as this is the theme of my CRACK-IT challenge.

Finally, we are trying to better understand the translation of preclinical in vivo signals to outcomes in clinical studies. In other words, how well do we predict the occurrence of adverse effects in human clinical trials. If we have a good grasp of this, we will know where we have to improve and where we might even want to drop some studies or measurements entirely. And of course it feeds into the confidence we have in our preclinical risk assessment. There are a number of inter-company initiatives looking at this.

OK, so returning to how we could improve the information content of existing studies. From a 3Rs perspective, what we're trying to do is capture more information – that is, relevant physiological and behavioural data – from existing studies, without using additional animals and without increasing the welfare burden on those animals.

In other words, can we do this without the animals actually being aware they are being monitored?

We are obliged by regulatory authorities to assess the adverse effects of drugs in a rodent and a non-rodent species, including by repeat-dosing, before proceeding into human clinical trials.

I'm going to focus on the rodent studies, which are predominantly in rats.

Most companies perform a 1-month repeat-dose toxicity study in rats, which is preceded by a dose-range finding study for 7 days or 14 days to check the dose levels are acceptable before going into the 1-month study.

Traditionally, this involves dosing the animals in the early morning, making some clinical observations, taking blood samples on Day 1 of dosing and towards the end of the study, food and water consumption, body weight, urine collection on one day, etc.

This leaves plenty of opportunity to make some additional physiological and behavioural measurements, but as the regulatory authorities don't require them, companies don't tend to do them.

As a pharmacologist, I consider this a missed opportunity.

So, my challenge is, how can we monitor activity and temperature in group-housed, freely moving rodents automatically, without any surgical implantation? Can we go beyond this as well, to measure individual food and water consumption, and even detect abnormal events such as convulsions?

The home cage in which we group-house rats is a Techniplast 2000P cage, L610 mm; W435 mm; H 215 mm, with a flat wire mesh lid. The rats are usually grouped 3 per cage. We might have 20 cages per rack.

Although this is the cage type we use, some toxicology labs and academic researchers use opaque cages. But let's start with this.

Rats are predominantly nocturnal, so they do move around a bit during daylight hours, but most activity, eating and drinking is at night.

We would want to collect data over the 24 hour period, possibly for up to a month. It doesn't have to be continuous – it could be sampled (say) every 5 minutes.

Activity is affected by the presence of observers in the room. So it would need to be hooked-up to a PIR sensor to detect the presence of humans, and also a light sensor, to accurately log when the lights go on and off at the start and end of the light cycle.

One possibility is to use RFID chips – radiofrequency identity microchips. These are the same as are used in veterinary practice to microchip your dog or cat or horse. However, they also do versions which measure temperature. These can be injected into the nape of the neck. We've found that the temperature of this area is close to core temperature, as it is the site of the interscapular brown adipose tissue, which is like a central heating boiler for rats (and mice).

However, currently this can only be measured with a hand-held reader. Could these measurements be collected automatically? Could the proximity of the rat to detectors at each pole of the cage, or under the cage floor, be monitored?

For detecting abnormal movements such as convulsions, could this be done by a subcutaneous accelerometer? There are papers on rats fitted with accelerometers externally, for example in a jacket. This wouldn't be suitable. The problem is, miniaturized accelerometers suitable for subcutaneous injection are not commercially available, at least to my knowledge, so would have to be developed.

An alternative would be videomonitoring. This would have to use miniature cameras that were perhaps aligned in their own rack behind the rack of cages, looking through each cage, with algorithms to assess behaviours. You would have to work out how to distinguish between individuals during the dark phase, using infrared lighting.

Whatever you do, here are some things to avoid.

1. It has to work with standard rodent housing cages.
2. We don't want a bespoke rack that needs to remain in a dedicated room.
3. We don't want spaghetti coming out of the cage rack.
4. The system needs to be just about plug-in-and-go – no complicated configuration files.
5. The software needs to be GLP-compliant.

So, finally, I'll go through the 3Rs benefits of this.

Currently, measurements of the effects of new drugs on activity require separate stand-alone studies, measuring the activity of rats placed singly in a novel cage, which they explore out of curiosity so to speak. So, Rodent Big Brother would reduce the number of rats used for this. We don't do it on every compound, but even so, there would be a reduction.

Similarly, we would tend to set up bespoke studies to investigate the effects of new compounds on body temperature. Again, this is only occasionally, where there is cause for concern, but even so, this does mount up.

Secondly, refinement. Measurement of temperature using a rectal thermocouple involves manual restraint and is invasive, so the use of microchips is a clear refinement.

There is also a refinement in terms of animal welfare. Occasionally rats in the high dose group of a repeat-dose toxicology study begin to deteriorate. Monitoring activity, temperature, and the occurrence of convulsions etc. could identify individuals at risk before the onset of clinical signs, and enable you to stop dosing them. Alternatively, monitoring of animals already identified at risk can inform you as to whether they have reached humane endpoints, or are on the road to recovery.

So, in summary, the ability to reach the first level – activity and temperature of group-housed rodents in their home cage – is within reach. The second level – identifying specific behaviours, especially convulsions, in group-housed rodents is more challenging.

Cracking even the first level would be an enormous leap forward; cracking the second level could transform the way we do behavioural monitoring, both in industry and academia.

Good luck!