Testing for toxicity

Dr Colin Brown elucidates how the drawbacks of today’s approach to drug development have inspired him to create improved strategies for detecting toxicity in new chemical entities

Can you begin by introducing your research at the Institute for Cell and Molecular Biosciences at Newcastle University, UK?

The kidney – in particular, the first part of the nephron known as the proximal tubule – is really important in removing drug molecules from the body. The proximal tubule achieves this through a series of specialised proteins that transport drug molecules from the blood into the primary urine. My research at the Institute for Cell and Molecular Biosciences focuses on developing novel in vitro cell culture models of the kidney as tools to understand this process and identify why some drug molecules or combinations of these molecules result in kidney toxicity.

What led you to research in vitro screening of new chemical entities (NCEs)?

The drive behind our research was the realisation that the current testing regime and use of animal models in drug testing was poorly predictive of outcomes in humans. In fact, recent reports suggest that around 50 per cent of drugs that successfully pass animal safety tests fail in subsequent human trials. This is a huge financial burden to the pharmaceutical industry; is not sustainable in terms of the principles of replacement, reduction and refinement (the 3Rs) of animals used in research; and has an impact on human health by delaying the introduction of new drugs to the clinic.

How have you approached the development of new, highly predictive in vitro human and rat primary proximal tubule cell (PTC) models? To what extent are you utilising these to investigate species differences in drug handling?

We have developed highly differentiated cell culture models of the human and rat proximal tubule (aProximate™ cells), which closely recapitulate the physiology of the in vivo proximal tubule. Allied to a broad screen of measures of nephrotoxicity, these models will provide substantial new mechanistic and species-specific data about the nephrotoxic potential of new drug molecules. In essence, the strategy would replace ineffective preclinical screening of compounds in animals with effective screening in advanced cultured cell models. This would reduce the number of animals entering mandatory preclinical drug safety testing by identifying the compounds with the potential to generate human toxicity at the in vitro screening stage.

Progress in the pipeline

Exciting research from Newcastle University is bringing about a revolutionary new approach to drug development to reduce animal testing and ease the introduction of new treatments onto the market

TWENTY PER CENT of drug attrition during development is attributable to drug induced nephrotoxicity – the toxic effects of the new chemical entity (NCE) on the kidney. It is a major cause of acute kidney injury. Axing a potential toxic NCE before the preclinical phase of the development pipeline can offer several benefits, including easily saving a company over US $700 million in development costs.

Accurate prediction of human drug toxicity, however, is a major challenge for the pharmaceutical industry, as around half of the preclinical in vivo screenings of NCEs in rat models fail to predict toxicity in humans later down the line. Better models are needed to reduce the number of animals being wastefully used in inadequate screening processes and to ensure that more safe, effective and efficacious drugs are reaching the clinics.

NEW SCREENING STRATEGIES

Senior Lecturer at Newcastle University’s Institute for Cell and Molecular Biosciences, Dr Colin Brown, has spent the past five years developing the aProximate™ cell model, a highly predictive in vitro human and rat model of the primary proximal tubule cell (PTC), which is highly important for removing drug molecules.

It is currently a challenge to deduce the real impact of transporter proteins on the toxicity of NCEs because the current in vitro models used in drug development are mainly based on human or animal cells, transfected with a limited number of human transporters. With these models, scientists can compile a lot of information on an interaction between a drug and a transporter – or even interactions with two to three transporters in some cases – but it is not an accurate picture of the situation in vivo. “These approaches give you a ‘jigsaw piece’ but don’t tell you whether you have all the jigsaw pieces that make up the puzzle,” explains Brown. Harder still, using these models, there’s no way of knowing the importance each transporter has in relation to the entire process.

Brown’s PTC monolayers, on the other hand, allow for a holistic insight into drug interactions. Using transplant grade kidney tissue, Brown isolates PTCs using a collagenase...
Have you encountered any major roadblocks in generating predictive renal models that provide translatable results from animals to humans?

Initially, a major roadblock was obtaining a supply of human kidneys to develop the model. This was critical and it took substantial effort from Nathan Griffiths at Scievita Tissue Bank to achieve. Our second major hurdle was convincing the pharmaceutical industry that our in vitro models were robust and predictive. To do this, we spent a large amount of time building up a wealth of validation data to back up our claims to the advantages of using the model in their development and safety pipelines. We have been successful in doing this and have now worked with most of the major pharmaceutical companies in screening NCEs for renal liabilities.

Can you summarise the project’s most significant achievements to date?

For me, the key highlight was taking the project from the laboratory to the marketplace. I am proud that this approach has been successful and that many major pharmaceutical companies have tried it. Our approach has had real impact in providing for the first time an in vitro predictive model of the proximal tubule that has the potential to replace ineffective screening of NCEs with effective screening in an advanced in vitro cell culture model. To this point, I would like to thank the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) Crack It Solutions scheme, which helped us find commercial partners and promote the aProximate™ models to the pharmaceutical industry.

What is the predicted impact of your proposal on the guiding principles of the 3Rs of animal testing?

The 3Rs were immensely important to me and were a key driving force in developing our models. We are at the beginning of a period of real change. Over 350,000 rodents are used in drug safety screening every year. If our in vitro models identified even 10 per cent of NCEs that would fail at first in humans then that would represent a substantial benefit in terms of reduction in animal usage. And, if we realise predictive in vitro models of other key targets such as liver, cardiomyocytes and the blood brain barrier that are well advanced then I can easily see how the use of animals in drug testing will be more selective and ultimately more successful. We are fortunate to have the NC3Rs to drive forward this vision; it is funding a studentship in my laboratory.

In addition to the human model, Brown has developed what may be the first rat proximal tubule model to form monolayers and remain differentiated in culture for up to 14 days. In this predictive in vitro strategy, the compound of interest can be introduced to the human and rat PTC models in parallel to identify problem NCEs before in vivo animal screening takes place. Brown and his team have demonstrated that the in vitro models handle drug molecules in human and rat the same way as the in vivo models, and to date they have successfully identified drug-drug interactions for numerous molecules and deciphered the renal handling of around 50 NCEs.

Brown has teamed up with Solvo® Biotechnology, the leading provider of transporter-based assays to the pharmaceutical industry, to promote aProximate™ models within their renal transporter portfolio. With Solvo’s worldwide client base, the aProximate™ cell model could soon revolutionise the world of drug development, saving millions of dollars and putting an end to the unnecessary use of animals in preclinical testing.

**INTELLIGENCE**

**HUMAN PROXIMAL TUBULE CELL MONOLAYERS**

**OBJECTIVES**

- To develop novel predictive in vitro renal proximal tubule models as alternatives to animal testing currently used in risk assessment during the drug development process
- To replace ineffective preclinical screening of compounds in animals with effective screening in advanced cell culture models
- To reduce drug attrition earlier in the drug development pipeline

**KEY COLLABORATORS**

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**PARTNERS**

Solvö® Biotechnology
Scievita Tissue Bank

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**COLIN BROWN**

received a PhD in Physiology from St Andrews University, UK, in 1983. He then held a Royal Society European Fellowship at University of Zurich, Switzerland, and a Wellcome Trust Senior Research Fellowship at Manchester University, UK, before joining Newcastle University. His recent work has focused on developing in vitro kidney cell models as predictive platforms of renal drug handling and nephrotoxicity.