National Animal Ethics Advisory Committee

Occasional Paper No 13

Animal models and drug discovery: How can we improve the outcome?

ISSN 1173-6763 (print)
ISSN 1173-6828 (online)
ISBN 978-1-77665-524-3 (print)
ISBN 978-1-77665-523-6 (online)

February 2017

Foreword

Many of the drug discovery achievements in modern medicine are the result of using animals during the research phase[1]. While technology increasingly offers alternatives, the study of the complex biological interactions involved in treating disease still frequently relies on the use of animals as the only way for the research to progress.

Laboratory animals, with an existing, inbred or induced disease or injury that is analogous to a human condition, are sometimes used by researchers as 'models' for that condition. By using animal models, researchers can test potential drugs, and treatments and avoid the ethical dilemma of administering untested drugs to humans. The short generation interval of laboratory animals also increases the rate of progress in the development phase, especially in cases where a disease has a relatively low incidence in the extant human population.

While acceptance is not universal, in general terms, society believes that where the use of animals for research offers considerable benefits, it should be permitted. That tacit approval however, carries with it the qualification that meticulous review must confirm it is both necessary and there are no alternatives. Rigorous scientific review of experimental protocols will ensure that the minimum number of animals required to produce a meaningful result are used[2]. A similar level of rigour can then be applied by the animal ethics committee to protect the welfare of those animals.

To achieve the best outcome in terms of animal use and welfare, researchers must be sure of the validity of the model(s) that they are proposing to use. In some cases, traditional models may need to be replaced by a model/models with more appropriate pathophysiology. Adoption of a collaborative approach to development and/or further characterisation of lesser used animal models, and the sharing of those models, would underpin the best interests of medical science, the patients and the animals.

Grant Shackell
Chair, NAEAC

[1] https://royalsociety.org/topics-policy/publications/2015/animals-in-research/

[2] http://www.understandinganimalresearch.org.uk/about-us/uar-position-on-the-use-of-animals-in-research/

NAEAC OCCASIONAL PAPER SERIES

1 *Underreporting of the Three Rs deployment that occurs during the planning of protocols that precedes their submission to animal ethics committees,* D J Mellor, J C Schofield and V M Williams, September 2008

2 *Regulation of animal use in research, testing and teaching in New Zealand – the black, the white and the grey,* L A Carsons, April 2009

3 *Regulation of animal use in research, testing and teaching: Comparison of New Zealand and European legislation,* N Cross, L A Carsons and A C D Bayvel, October 2009

4 *Compliance monitoring: The University of Auckland approach,* J Stewart, October 2009

5 Monitoring methods for animal ethics committees, D Morgan, October 2010

6 *Planning for refinement and reduction*, D Fry, R G Das, R Preziosi and M Hudson, January 2011

7 *Avoiding duplication of research involving animals*, D Morgan, March 2011

8 *Research on Vertebrate Pesticides and Traps: Do Wild Animals Benefit?* B Warburton and C O'Connor, August 2012

9 *Ensuring regulatory compliance in the use of animals in science in New Zealand – the review process*, August 2012

10 *How to improve housing conditions of laboratory animals: The possibilities of environmental refinement*, V Baumans and P Van Loo, February 2014

11 *Use of animals in the registration of veterinary medicine products in New Zealand,* K Booth, September 2015

12 *The blind leading the blind: animal facility staff and researchers working together to reduce bias in animal research*, Dr Jack Rivers-Auty, February 2017

Animal Models and Drug Discovery: How Can We Improve the Outcome?

Associate Professor Bronwen Connor, Department of Pharmacology and Clinical Pharmacology, Centre for Brain Research, School of Medical Science, Faculty of Medical and Health Sciences, The University of Auckland.

Introduction

The discovery of new therapeutics is predicated on the use of animal models both to identify new drug targets and to perform preclinical trials of drugs before using them in patients. However, even after animal studies suggest that a treatment will be safe and effective, only 10–15 percent of drugs that enter Phase 1 clinical trial result in FDA approval[1]. One of the challenges researchers face to correct this issue is to develop and/or utilise models that better recapitulate the disorder under investigation. Specifically, to ensure that a preclinical study is being correctly designed researchers must undertake rigorous assessment of the physical and biochemical traits of an animal model in terms of human disease, characterise when disease symptoms and death occur, and create a mathematical model to aid experimental design, including how many animals must be included in a study[2]. However, the choice of the most appropriate animal model is not as simple or straightforward as it may seem. Choices often need to be made between more traditional models that reproduce the cardinal pathological features of the disorder by mechanisms that may not necessarily occur in human versus newer, less established models that are based on known pathophysiological mechanisms but may not reproduce all the features seen in patients. In addition, the recent advent of human cell reprogramming allowing for the generation of patient-derived *in vitro* disease models now allows for a human-specific alternative to the use of animal models for target identification, drug screening and toxicology. However, regardless of recommendations and the recent advances in both human-specific *in vitro* modelling and new pathophysiologically relevant animal models, we still see the more traditional models prevailing in experimental design. This resistance to change raises a significant issue in regards to animal ethics, in particular, regarding the 3Rs (Replacement, Reduction and Refinement). Using the neurological disorder Parkinson's disease as an example, the importance of consideration of the 3Rs in the choice of preclinical animal models used in experimental design will be discussed. Furthermore, the resistance of researchers to changing from conventional models to potentially more disease-relevant models will be considered. While this discussion focuses on animal models of Parkinson's disease, the overarching concepts regarding model suitability can be extended to the majority of disease models.

Refinement

There is a wide array of rodent models available to study Parkinson's disease (PD) with the 6-hydroxydopamine (6-OHDA) rat and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse lesion models being the most prominent. Both of these traditional neurotoxin models generate an acute destruction of the dopaminergic nigrostriatal pathway in the brain and a loss of dopaminergic neurons in the substantia nigra pars compacta, resulting in impairment in locomotor and sensorimotor function. Indeed, the 6-OHDA rat model in particular has been the mainstay of PD preclinical research since its development in 1968[3] and is considered to be a good predictor of therapeutic efficacy in humans[4]. However, the mechanisms by which either 6-OHDA or MPTP induce dopamine cell death and produce PD-like symptoms is not relevant to the disease pathology of PD, therefore questioning the usefulness of these models in predicting the effectiveness of new drugs.

Currently, the major challenge in trying to advance the treatment of PD lies in dealing with the progression of the disease process and in preventing or reducing dopaminergic neuronal cell loss. The use of the popular 6-OHDA model to test the efficacy of neuroprotective strategies appears to be inappropriate as therapies that have been shown to reduce nigral dopaminergic cell loss in the 6-OHDA rat have not been successfully translated to the clinic[5-7]. For example, adeno-associated virus (AAV)-mediated intra-striatal delivery of the neurotrophic factor neurturin restored nigral cell numbers in MPTP-treated primates[6] and protected against 6-OHDA lesions in the rat[8]. Unfortunately, this preclinical success was not recapitulated in clinical trials where intracerebral delivery of AAV-mediated neurturin failed to show efficacy[7]. Indeed, many potentially neuroprotective compounds have been identified in rodent models and it is worrying that so far none has proved effective in man. This clearly indicates that if the aim is to study the pathways involved in neuronal cell death and to develop neuroprotective strategies, the mechanism of action of the pathogenic event is important[4,9]. Killing dopaminergic neurons through a neurotoxic insult may provide suitable animal models for testing the effects of symptomatic treatments but does not reflect the pathogenic events occurring in man. As such, the use of the 6-OHDA or MPTP rodent models of PD for the identification and testing of neuroprotective agents is not necessarily scientifically or, in theory, ethically appropriate. Ideally, the model used for preclinical testing of novel neuroprotective therapies should reproduce pathological mechanisms that are targeted by a drug. However, a model need not reproduce behavioural features identical to those seen in people to provide valid information. Behavioural phenotypes in animals will be, by nature, different from clinical manifestations in humans.

Along these lines a number of genetic models of PD have been developed that model the molecular mechanisms of the disease using information obtained from research into familial PD and the identification of susceptibility genes[10]. The objective of these models is to generate a phenotype that is progressive, that reflects the molecular processes of the disease more closely and the widespread pathology associated with the disease in man, including the early stages of the disease. While most of these models have strong construct validity, they are limited as they do not reproduce the broad pathology seen in PD and predominantly show pathology only in the nigrostriatal pathway. Furthermore, many of these models have yet to be widely utilized for examining neuroprotective or restorative strategies due to the continued peer-support or "pressure" to use the 6-OHDA or MPTP rodent models. Indeed, it appears that if a model is considered to be predictive, such as the 6-OHDA or MPTP lesion models, those interested in producing therapeutic agents will use it, even if it has limited or no similarity to the known pathophysiology of the disease. That is, predictive validity appears to take precedence over pathophysiological validity.

So how does this sit in regards to the consideration of refinement within the 3Rs? As previously stated, researchers are under ethical obligation to consider the pathophysiological or construct validity of their choice of animal model rather than just the predictive validity. They need to refine and justify the choice of their disease model based on the therapeutic target and/or desired outcome. This may require researchers to use multiple disease models to confirm the therapeutic efficiency of their new agent. In some situations it may even need to be strongly considered whether animal models are actually appropriate to simulate human diseases[11].

Replace and Reduce

Alternative strategies to model human disease do of course exist with recent focus falling on the advent of human cell reprogramming technologies. Reprogramming allows mature cells, such as skin or blood, to be taken from patients and genetically transformed back to the state

of an embryonic stem cell (induced pluripotent stem cell; iPSCs). Just like embryonic stem cells, human iPSCs can generate a full lineage of the tissue/organ types found in the human body. This *in vitro* technology therefore provides an important tool for the study of a wide range of disorders and diseases using live human systems, allowing detailed investigation of molecular and cellular disease pathways specific to subtypes of cells/tissues, and potentially the identification of new drug targets[12]. In addition, as reprogramming technology enables the study of human tissues/organs during development, disease-specific pathways can be investigated prior to and during disease onset. Detecting disease-specific molecular signatures in live human cells, as opposed to late-stage post-mortem human tissues, introduces possibilities for the development of early intervention therapies and new diagnostic tools. Finally, through the use of reprogramming technology it is also now feasible to obtain tissues/organs that capture the genetic material from the patient, including not only the mutated gene(s), but also the genetic modifiers that play an important yet largely unknown role in the pathology of human diseases. Further advances in this field include the generation of 3 dimensional tissue organoids from somatic or pluripotent stem cells[13]. Organoid cultures derived from diseased tissues provide additional systems for better characterization of the disease phenotypes, basic research into disease mechanisms, personalization of treatment and primary compound screens aiming at developing new therapeutic interventions. Cultures obtained from somatic cells can be used as a reference for functional, genetic and toxicity studies.

While cell reprogramming technology and the development of cell-based human disease models is still in its infancy, the use of *in vitro* disease models that resemble conditions in human patients increases the efficiency and accuracy of drug screening and toxicology tremendously, as the targets can then in principle be scaled up for high-content production. Ideally, the use of disease-specific human tissues in pre-clinical testing will significantly enhance the successful translation of new therapeutics to the clinic. It is unlikely that human cell reprogramming will remove the use of animal models of disease, but the use of disease-relevant live human tissue systems should greatly improve the specificity and efficacy of new compounds prior to transfer into appropriate animal models. This should therefore lead to a reduction in the number of animals required due to a replacement of drug screening and toxicology studies in reprogrammed human tissues rather than in animal models.

Conclusion

In order for new and improved therapeutic agents to be developed, it is imperative that researchers strongly consider the validity of the animal model they are proposing to use based on the scientific or clinical question being asked. This may require researchers to move away from the traditionally used models and instead use models with more appropriate pathophysiological validity. This may also require laboratories to develop or further characterise lesser used animal models. Although the costs to achieve this are meagre compared with those of clinical trials, the investment required in time and funds is far beyond what any one laboratory should be expected to do. This burden and the resulting animal models should be shared. At the very least, researchers should place new animal models in a public repository so that other teams can repeat the characterisation, and share the costs of doing it well. Furthermore, public and private agencies should fund characterisation studies as a specific project. Competitive bidding and milestone-driven payments could persuade qualified groups to perform the necessary experiments and to make results publicly available. This is unglamorous work that will never directly lead to a breakthrough or therapy, and is hard to mesh with the aims of a typical grant proposal. However, without these investments, more patients and funds will be squandered on clinical trials that are uninformative and disappointing. Hopefully, if researchers make a

concerted effort to use animal models that have pathophysiological validity over predictive validity, combined with the use of human disease-based *in vitro* models, we will see an increased proportion of new agents being successfully transferred to the clinic.

References

1. Hay, M., Thomas, D. W., Craighead, J. L., Economides, C. & Rosenthal, J. Clinical development success rates for investigational drugs. *Nat Biotech* 32, 40-51, (2014).
2. Perrin, S. Preclinical research: making mouse studies work. *Nature* 507, 423-425, (2014).
3. Understadt, U. 6-hydroxydopamine induced degeneration of central monoamine neurons. *European Journal of Pharmacology* 5, 107-110 (1968).
4. Duty, S. & Jenner, P. Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. *British Journal of Pharmacology* 164, 1357-1391, (2011).
5. Mittermeyer, G. *et al*. Long-term evaluation of a phase 1 study of AADC gene therapy for Parkinson's disease. *Human Gene Therapy* 23, 377-381, (2012).
6. Kordower, J. H. *et al.* Delivery of neurturin by AAV2 (CERE-120)-mediated gene transfer provides structural and functional neuroprotection and neurorestoration in MPTP-treated monkeys. *Ann Neurol* 60, 706-715, (2006).
7. Warren Olanow, C. *et al.* Gene delivery of neurturin to putamen and substantia nigra in Parkinson disease: A double-blind, randomized, controlled trial. *Ann Neurol* 78, 248-257 (2015).
8. Gasmi, M. *et al.* AAV2-mediated delivery of human neurturin to the rat nigrostriatal system: long-term efficacy and tolerability of CERE-120 for Parkinson's disease. *Neurobiol Dis* 27, 67-76 (2007).
9. Bové, J. & Perier, C. Neurotoxin-based models of Parkinson's disease. *Neuroscience* 211, 51-76 (2012).
10. Fleming, S. M., Fernagut, P.-O. & Chesselet, M.-F. Genetic mouse models of parkinsonism: Strengths and limitations. *NeuroRX* 2, 495-503, (2005).
11. Perel, P. *et al*. Comparison of treatment effects between animal experiments and clinical trials: systematic review. *British Medical Journal* 334, 197-197, (2007).
12. Ebert, A. D., Liang, P. & Wu, J. C. Induced Pluripotent Stem Cells as a Disease Modeling and Drug Screening Platform. *Journal of Cardiovascular Pharmacology* 60, 408-416, (2012).
13. Schweiger, P. J. & Jensen, K. B. Modeling human disease using organotypic cultures. *Current Opinion in Cell Biology* 43, 22-29, (2016).

National Animal Ethics
Advisory Committee

New Zealand Government