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Orsolya E. Varga, Axel K Hansen, Peter Sandøe and I. Anna S. Olsson
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ABSTRACT

The use of animals to model humans in biomedical research relies on the notion that basic processes are sufficiently similar across species to allow extrapolation. Animal model validity is discussed in terms of the similarity between the model and human condition it is intended to model, but no formal validation of models is applied. There is a stark contrast here with non-animal alternatives in toxicology and safety studies, for which an extensive validation is required. In the present paper we discuss the potential and limitations of validating preclinical animal models for proof-of-concept studies using an approach similar to that applied to alternative non-animal methods in toxicology and safety testing. A major challenge in devising a validation system for animal models is the lack of a clear gold standard to compare results with. While a complete adoption of the validation approach for alternative methods is probably inappropriate for research animal models, key feature such as making data available for external validation and defining a strategy to run experiments in a way that permits meaningful retrospective analysis remain relevant.

Keywords: animal models, predictive validity, validation, ethics

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Comment: Validating animal models for preclinical research: a scientific and ethical discussion

Orsolya E. Varga[1,2], Axel K Hansen[3], Peter Sandøe[2] and I. Anna S. Olsson[1,2]

[1]Laboratory Animal Science, IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal
[2]Danish Centre for Bioethics and Risk Assessment, Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark
[3] Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark

Address for correspondence: Orsolya E. Varga, Laboratory Animal Science, Institute for Molecular and Cell Biology (IBMC), Universidade do Porto, Rua do Campo Alegre, 823, 4150-180 Porto – Portugal, Tel: +351 226 074 900, Fax: +351 226 099 137. E-mail: ovarga@ibmc.up.pt

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Introduction

The use of animals to model humans in biomedical research relies on the notion that basic processes are sufficiently similar across species to allow extrapolation. We discuss the potential and limitations of validating preclinical animal models for proof-of-concept studies using an approach similar to that applied to alternative non-animal methods in toxicology and safety testing.

While studies using animal models are an important part of biomedical research, the translation of results into treatments for human beings is far from straightforward (1). Both economic and ethical issues come into play when a potential therapy fails first-in-human or later trials (2). Better (use of) animal models is one way of reducing high attrition rate (3).

Animal model validity is discussed in terms of the similarity between the model and human condition it is intended to model, but no formal validation of models is applied. There is a stark contrast here with non-animal alternatives in toxicology and safety studies, for which an extensive validation is required.

Animal models and validity

Roughly speaking, the present approach to model development is based on similarities in the symptoms and/or aetiology of a disease in humans and animals. An animal model is described as valid if it "resembles the human condition in aetiology, pathophysiology, symptomatology and response to therapeutic interventions" (4). Usually, this general validity is broken down into three aspects: predictive validity (performance in the test predicts performance in the modelled condition), face validity (phenomenological analogy with the modelled condition) and construct validity (the model has a sound theoretical rationale) (5).

Over the last few years several initiatives have been launched to encourage the use of more accurate animal models in both industrial and academic research. European and US authorities have published guidelines which identify the key characteristics of an approved animal model and list criteria which, if met, demonstrate a model's suitability (cross-species comparison taking into account the target, its structural homology, distribution, signal transduction pathways and the nature of pharmacological effects); these are to be addressed by those seeking approval or a licence for drugs or biological products (6, 7). Several voluntary initiatives from researchers and industry point in the same direction, including the STRAIT initiative for more sophisticated, consensus-based validity criteria governing preclinical animal studies of stroke (8) and the ongoing MATRICS, TURNS and CNTRICS programmes to improve research into therapy for schizophrenia (9). Essentially, these initiatives promote a more sophisticated way of delivering construct and face validity. However, when the results of an animal study are intended to be translated into human treatments (preclinical research), the ultimate proof of a model's value is its predictive validity.

While face and construct validity are primarily theoretical considerations, predictive validity involves the calculation of a number of statistical parameters in a validation process. In a simple case predictive validity can be calculated in terms of reliability and

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relevance. Reliability is assessed by calculating intra- and inter-laboratory reproducibility and intra-laboratory repeatability. Relevance shows whether a model is meaningful and useful for a particular purpose, and the extent to which the model accurately measures or predicts the biological effect of interest (sensitivity and specificity) (10).

Process of validation – the alternative methods approach

The predictive validity of an animal model can be tested by systematic examination of the data from animal model studies, and by comparing these data with reference data obtained in humans. One way of doing this would be to follow the validation process for alternative methods. The process described here is used by the European Centre for the Validation of Alternative Methods (ECVAM) (11); a similar system has been adopted by OECD and North American organisations, which have harmonised their validation processes (12).

This process has five basic steps (10, 13). The first is test development. The fifth is formal regulatory acceptance. Actual validation, in the sense of generating, analyzing and assessing data, takes place in steps two, three and four:

2. Pre-validation: An inter-laboratory pre-validation study is conducted to optimize the protocol and assess its performance over three phases: phase I, where the protocol is refined in a single laboratory; phase II, assessing the transferability of the method to a second laboratory; and phase III, where the relevance and reliability of the test are assessed under blind conditions in two or more laboratories.

3. Validation: The formal validation study can be thought of as an extended version of the phase III stage of pre-validation in which an inter-laboratory blind trial (involving at least three laboratories) is conducted to assess whether tests can be shown to be relevant and reliable for one or more specific purposes. This inter-laboratory trial is followed by data analysis and an evaluation of the outcome of the study in comparison with predefined performance criteria.

4. Independent assessment: Validation study results are published in peer-reviewed journals and considered by independent assessment panels working under the auspices of appropriate national or international organisations. The panel review of the data and peer review recommendations are published.

The validation process, from test development to regulatory acceptance, need not be unidirectional; retrospective data analysis is also common. This helps to reduce both economic and ethical costs: the repetition of animal research or human clinical trials is obviously wasteful when the necessary data is already available. On the other hand, retrospective data is often less reliable, and its interpretation can be challenging (11, 14), and therefore the prospective approach is usually preferred.

Could the alternative methods approach be used to validate animal models?

Validation has two principal aspects: how well a test method compares with itself when repeated under identical as well as different conditions (e.g. with

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different test substances and in different laboratories); and how well a test method compares with a reference method. These two aspects present somewhat different challenges in terms of data required, but there is no theoretical obstacle to their application to animal models in biomedical research.

How well an animal model compares with itself under different conditions can be evaluated using animal data alone. The evaluation requires data to be available using the same model, ideally both in several replications with identical conditions (to estimate repeatability(15)) and under controlled conditions, where one factor is varied while others are kept constant (to estimate reproducibility(15)).

Evaluating how well an animal model compares with reference data is more challenging. This is a practical challenge because it requires data from humans and is thus only possible when a substance, or other type of therapy, has advanced through preclinical stages to human trials. An even more fundamental challenge is presented by the difference between the repetitive nature of testing and the innovative nature of research. When non-animal alternatives in safety testing are validated there is a clear gold standard in the form of the animal test to be replaced (although it should be remembered that this gold standard is only a proxy measure of the real parameter of interest – the human reaction to a substance – against which it has in fact never been validated). In proof-of-concept studies in research, there is no gold standard. Depending on the intended target of drug action, different types of research approach require different models, and a model with proven predictive validity for a particular compound may not in fact be sensitive to the effects of a different type of compound that acts on different targets (9). Efforts to validate against a standard in the form of a proven successful treatment may give rise to a system that will only detect “me-too” treatments, that is those based on the same principle of action (16), and hence unduly restrict necessary innovation. This does not mean that the analysis of the correspondence of results of animal and human experiments is impossible or of no value. Indeed it is precisely this type of retrospective analysis which, in recent studies, has helped to identify inconsistencies in animal and human studies (e.g. in dosage, administration method, parameters, and method of assessing effect) that are likely, at least in part, to underlie poor translation of results.

The validation of animal models potentially carries monetary as well as ethical costs. Validation is time consuming (2-6 years for the alternative methods), costly, and financial returns may be more difficult to secure, since intellectual property rights over animal models are more restricted than they are for alternative methods. Ethical concerns may also arise over the use of animals for the sole purpose of validation. However, validation that is based on the re-analysis of existing data may partly overcome these concerns, and if validation results in more effective research, both animal numbers and costs may be offset by savings in later research. Thus, we argue, there is reason to consider partial adoption of the validation procedure.

Conclusions and suggestions

Over the last few years, a number of recommendations and guidelines have been published to encourage more accurate use of animal

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models (6, 7). Against that background, what benefits would accompany the application of the alternative methods approach to the validation of animal models? We identify two key gains: retrospection and publication.

Guidelines for better animal experiments take a primarily prospective view, but if lessons are to be learned from previous mistakes retrospective analysis and the re-assessment of data are vital. A recurring obstacle here is the difficulty of accessing an unbiased and complete dataset. Data from many experiments simply do not enter the public domain, either because the results are negative and therefore difficult to publish (publication bias) or because they are compiled in pharmaceutical companies and only, if at all, presented to authorities for drug approval.

The type of prospective validation favoured for non-animal alternatives is ethically problematic when living subjects – animals or humans – are involved. The challenge therefore is to produce a system in which data are made available for external validation, and to define strategies for running experiments which will allow more meaningful retrospective analysis. Within the validation system for alternatives there is unique experience in dealing with this in a systematic way. Making these analyses available in peer-reviewed journal – the fourth step in the alternative methods validation procedure – is also crucial if knowledge is to disseminate to the wider scientific community.

Successfully learning from experience also means being able to accept new data that challenge old paradigms. Old models and methods must be abandoned, or suitably revised, if systematic analysis of replicability, repeatability and correspondence with reference data indicate that their performance is not up to standard.

The validation of animal models and tests is a shared responsibility in which academic research, the pharmaceutical industry, regulatory authorities and ethics committees/IACUCs all play a part. That this issue is taken seriously and validation integrated into the research process is both a scientific and ethical imperative. As scientists, we need to reassure those who have concerns about animal use in research that we are using animals in the best possible way to make progress on the treatment of human diseases. Validation can underwrite that reassurance. Specifically, where an animal model results in a drug moving from preclinical to first-in-human trials when better preclinical trials would have prevented that, animals are used needlessly, economic resources are wasted, and human volunteers are exposed to risks to no avail. Conversely, the abandonment of a drug development programme where the drug would have proven effective in clinical trials is not only a waste of resources, but also a loss for patients.

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