

Systematic reviews improve clinical research – can they help improve animal experiments?

Animal rights campaigners have long argued that animal research is a flawed science. Few in the scientific community would agree with such a sweeping generalisation. But, of course, we have to interpret the results of all research with caution. And for ethical reasons, we must look critically at the use of animals – not just accept it.

We have had three major independent inquiries in the UK into animal research in the past four years (carried out by a House of Lords Select Committee, the Animal Procedures Committee and the Nuffield Council on Bioethics). The reports considered the scientific arguments in depth. They all concluded that animal research provides information which can be of relevance to humans and does lead to medical benefits.

Nonetheless, the report of the NCOB pointed out that at present, there are a relatively limited number of useful systematic reviews and meta-reviews that address the question of the scientific validity of animal experiments and tests. They went on to say that it would be desirable to undertake further such studies to evaluate more fully the predictability and transferability of animal studies. The NCOB recommended the major players in the scientific community should consider ways of funding and carrying out these reviews.

RDS has been in touch with one group who are working in this field. We have invited them to contribute their ideas to *RDS News*.

Physicians have long used the observation of animals to make inferences about human physiology in health and disease. There are now many specific animal models for a wide variety of human diseases, which permit exploration of disease pathophysiology and testing of potential treatments. In many countries, the ethics of experiments on animal are being actively debated. For the most part, public opinion accepts the need for animal experimentation, provided it informs our understanding or treatment of human disease and is conducted within a well-regulated framework.

Those opposed to animal experimentation cite two chief areas of concern. Firstly, some believe that it can never be ethical to harm animals in the

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pursuit of improved human health, no matter how small the harm or how large the health improvement. Secondly there are those who believe that animal experiments have not and will not lead to improvements in human health.

While the first represents a moral position, the second is a more practical judgement of the extent to which knowledge gained from animal experiments might translate to treatments that improve human (or indeed to animal) health. The process of taking the many treatments that appear effective in pre-clinical studies including animals and identifying the few that are safe and effective in man is long, complex and inefficient. We believe that applying the technique of

systematic review might improve the efficiency of the process and hence increase the number of effective treatments available for use in man.

Clinical studies as part of the research cycle

By the late 1980s there had been over 20 randomised trials of clot busting treatment for heart attacks. Because individual trials had been small their results were contradictory. Systematic review – assembling all the relevant evidence – avoided the tendency of previous narrative reviews, to cite only those trials which supported the author's own prejudice. Meta-analysis provided a more precise estimate of the treatment effect because it made use of all the available data. This suggested that clot-busters reduced death by about one fifth.

This result led to two very large studies that were designed reliably to confirm or refute the estimate from the meta-analysis; these trials did indeed confirm the efficacy of clot-busters, which are now in widespread use. Thus, a systematic review provided a less biased and more precise assessment of existing research evidence, justified the need for further studies, and in particular, identified that study sample sizes needed to be much greater than had previously been thought necessary.

Identifying key features of clinical research design

Systematic reviews of research methodology showed that several aspects of research design had a significant impact on estimates of treatment effect: poor allocation concealment (when the clinician could predict whether the next subject would be allocated experimental or control treatment)¹; whether or not funding came from the pharmaceutical industry²; and the use of blinding (preventing the person measuring outcome know-

ing whether the patient had received experimental treatment or control)³. These findings have been incorporated into the CONSORT statement⁴, which sets standards for the reporting of clinical trials and has been adopted by many major medical journals.

Systematic reviews of animal data in stroke

Stroke places a huge burden on patients, families and health care systems all over the world. The neuroscience community and the pharmaceutical industry have invested huge efforts and billions of pounds of money in the search for an effective treatment. Over 1,000 drugs have been evaluated as treatments for stroke, of which 883 have been tested in animal models (of focal cerebral ischaemia). And, of those 883, 97 have been tested in clinical trial in humans.

But of the many 'neuroprotective' (protecting neurons from the effects of ischaemia, rather than thrombolytic – reversing the ischaemia itself) treatments, only one drug remains in clinical development⁵. So here is the challenge – how can it be that so many drugs appear protective in animal studies, but so few make the transition from bench to bedside and none have proven to be effective and safe in man? One possibility is that the biology of laboratory animals is so far removed from human biology that animal models are irrelevant to human disease. On the other hand it is plausible either that the clinical trials may have been falsely negative, or that animal studies were falsely positive.

Tirilazad is a 21-amino steroid which can protect against neurotoxic reactive oxygen species (ie it is 'a free radical scavenger'). In 1990 it was shown to have neuroprotective effects in a model of focal cerebral ischaemia in the rat, and on the basis of this and

Research design for experimental work?

Useful links

National Council on Bioethics report *The Ethics of Research Involving Animals*
<http://www.nuffieldbioethics.org/go/ourwork/animalresearch/introduction>

Systematic review of animal data in stroke
http://www.camarades.info/index_files/Page332.htm

subsequent animal work, a number of large scale clinical trials – involving in total over 1,700 patients - were conducted in the late 1980s and early 1990s. Synthesis of data from those clinical studies demonstrated beyond very much doubt that tirilazad was not effective in human stroke.

A synthesis of the animal data confirmed that while tirilazad does indeed have substantial efficacy in experimental stroke, improving outcome by almost 40% (see http://www.camarades.info/index_files/Page332.htm), the review identified a crucial difference between the human and animal data. The mean interval between stroke onset and treatment in clinical trials was 5 hours (with a range of up to 24 hours), but the mean interval between stroke onset and treatment in the animal studies was less than one hour. The clinical trials may therefore have been negative not because the drug doesn't work, but because it was administered to stroke patients far too late to have any chance of being effective.

Result-specific publication bias

A systematic review of a set of animal studies of a drug might overestimate the effects of a drug for several reasons. Publication bias arises because positive studies are more likely to be published (and hence more likely to be included in research syntheses). Each experiment provides only a single estimate of the 'true' efficacy of the drug in question. Where there are multiple studies these estimates should be normally distributed. If a systematic review suggests a non-normal distribution (with fewer studies suggesting benefit than expected), this suggests publication bias. A review of the animal studies of the neuroprotectant FK506 suggested that the distribution of the estimates of effect was consistent with a significant proportion of the 'negative' studies being

missing, and hence that result-specific publication bias was indeed present⁶.

Identifying key features of animal experimental design

Animal evidence might also falsely overestimate efficacy if the design or conduct of individual studies introduced bias. We used methods adapted from research synthesis of clinical trials to explore the impact of potential sources of bias in animal stroke models. Ketamine anaesthesia and a failure to control body temperature, both already known to influence outcome, were identified as powerful sources of bias^{7,8}. Failure to use blinded assessment of outcome and the failure to use animals with relevant co-morbidities (stroke patients are often elderly and suffer from other conditions such as hypertension or diabetes) also led to overestimation of benefit.

We applied a 10-point scale to assess the quality of research design and found study quality was inversely associated with estimate of benefit (the higher the quality of the research design, the smaller the estimate of benefit). The magnitude of these influences can be estimated using regression modelling; the bias introduced by the combined effects of using ketamine anaesthesia, animals without co-morbidity and not using a blinded assessment of outcome increased the estimate of benefit by 40%.

Applicability to different forms of experimental work

Systematic reviews are most easily applied where multiple studies test the same hypothesis (eg the efficacy of drug X in a model of condition Y). However, where the research involves the testing of a sequence of related but developing hypotheses, systematic review may be less easily applicable (though, even here, it is now recognised that systematic reviews of clinical

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cal studies of the impact of genotype on phenotype have demonstrated that publication bias and random error mean that small scale studies can substantially overestimate the impact of genotype⁹.

It may be much easier to apply these techniques to pre-clinical drug development studies with 'hard' numerical outcomes than it is say to fundamental research or results from a western blot or a DNA sequence. Nonetheless, a systematic (rather than non-systematic) approach is more likely to limit bias. It also seems difficult to avoid the fact that several aspects of study design, including truly random allocation to experimental or control condition, allocation concealment, and blinded assessment of results probably have a significant impact on estimates of effect derived from studies in animals, and should become accepted standard in experimental research design.

The associations described above are with study quality as reported in published work. This may be substantially lower than actual study quality. There is continuing pressure from the journals to 'optimise' space, often by shortening 'Methods' sections, but our work argues strongly against this move.

Locating unpublished data

Finally, systematic reviews have been applied to information in the public domain rather than to unpublished

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Can systematic reviews help animal experimental work?

Useful links

Cochrane Collaboration
www.cochrane.org
CAMARADES collaboration
www.camarades.info

data held by investigators (or the pharmaceutical industry). To date the industry has been somewhat reluctant to allow systematic reviewers access to their data. It may be that they have procedures in place which render them immune to such sources of bias. If so, it would be very helpful for these to be identified (and what better way than by systematic review and meta-analysis?), to inform the practice of those of us who work in other environments.

CAMARADES collaboration

The Cochrane Collaboration is an international not-for-profit organisation for the preparation and dissemination of systematic reviews of the effects of health care interventions (published on-line in the Cochrane Library www.cochrane.org). We are proposing that a similar international collaborative approach might be constructive in animal research.

To improve the design, conduct and reporting of animal experiments requires the support of those who fund, regulate, design, carry out, report, publish, read and use data from animal experiments. The Collaborative Approach to the Meta-Analysis and Review of Animal Data in Experimental Stroke (The CAMARADES collaboration; see www.camarades.info) has been established to facilitate this process for experimental stroke, and much of what is true for stroke modelling will be relevant to other disease models.

The CAMARADES Manifesto

- All those involved in animal experiments – funders, regulators, scientists, peer reviewers, publishers – should engage in a concerted attempt to eliminate known sources of bias.
- Journals might agree a common set of required standards similar to the CONSORT statement for clinical trials.

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- Further analyses of existing published data should seek to identify others sources of bias.
- Journals need to be convinced of the scientific and ethical importance of publishing negative studies.
- Funding agencies, regulatory agencies and publishers might explore a requirement for the pre-registration of experiments in order that systematic reviewers can identify all potential sources of evidence.
- Systematic review requires unbiased identification of all relevant data – the techniques for achieving this have been borrowed from the clinical trial literature, and need to be refined and revalidated in the animal literature.
- Meta-analytical techniques have been adapted from the clinical sphere, and the best techniques, and the sensitivity to using different techniques, needs to be established.
- Funding agencies, drug companies, clinical research ethics committees and clinical trialists should require a systematic review and meta-analysis of animal data before embarking on a clinical trial.

We are embarking on a very long journey, but with apologies to Mao we are beginning to take single steps in the right direction. While change is not always comfortable, we have the chance to bring about substantial improvements in the quality and utility of animal experiments. No one group can hope to bring about this change, but by working together we have the opportunity to deliver real improvements in both animal and human health.

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