Modelling human development and disease: The role of animals, stem cells, and future perspectives.

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Introduction

The 'scientific method' begins with a hypothesis, which is the critical keystone in forming a well-designed study. As important as it is to ask the correct questions to form the hypothesis, it is equally important to be aware of the available tools to derive the answers.

Experimental models provide a crucial platform on which to interrogate cells, tissues, and even whole animals. They broadly serve two important purposes: investigation of biological mechanisms to understand diseases and the opportunity to perform preclinical trials of new therapies.

Here, an overview of experimental models based on animals commonly used in research is provided. Limitations which may impact clinical translation of findings from animal experiments are discussed, along with strategies to overcome this. Additionally, stem cells present a novel human-derived model, with great potential from both scientific and clinical viewpoints. These perspectives should draw attention to the incredible value of model systems in biomedical research, and provide an exciting view of future directions.

Animal models – a palette of choices

Animal models provide a 'whole organism' context in studying biological mechanisms, and are crucial in testing and optimising delivery of new therapies before the commencement of human studies. They may be commonly referred to under the classification of invertebrates (flies, worms) and vertebrates (fish, rodents, swine, primates); or small animal (fish, rodents) and large animal (swine, primates, sheep).

Whilst organisms have their own niche area of research, the most frequently used is the humble mouse. Its prominence is attested by the fact that it was only the second mammalian species after humans to have its genome sequenced, demonstrating that both species share 99% of their genes. [1] Reasons for the popularity of mice as a choice include that mice share many anatomical and physiological similarities with humans. Other advantages include that they are small, hardy, cheap to maintain and easy to breed with a short lifespan (approximately three years), [2] allowing experiments to gather results more quickly. Common human diseases such as diabetes, heart disease, and cancer affect mice, [3] hence complex pathophysiological mechanisms such as angiogenesis and metastasis can be readily demonstrated. [2] Above all, the extraordinary ease with which mice are manipulated has resulted in the widespread availability of inbred, mutant, knockout, transgenic or chimeric mice for almost every purpose conceivable. [3] By blocking or stimulating the overexpression of specific genes, their role in developmental biology and disease can be identified and even demonstrated in specific organs. [4]

Humanised mice are another step closer in representation of what happens in the human body, thereby increasing the clinical value of knowledge gained from experiments. Humanised mice contain either human genes or tissue allowing the investigation of human mechanisms whilst maintaining an *in vivo* context within the animal. Such approaches are also available in other organisms such as rats, but are often adapted from initial advances in mice, and hardly mirror the ease and diversity with which humanised mice are produced.

Aside from the mouse, invertebrates such as the *Drosophila* vinegar fly [5] and *Caenorhabditis elegans* worm [6] are also widely used in

research of genetics or developmental biology studies. They are particularly easy to maintain and breed and therefore large stocks can be kept. Furthermore, there are fewer ethical dilemmas and invertebrates have a genome simple enough to be investigated in its entirety without being cost-prohibitive or requiring an exhaustive set of experiments. Their anatomies are also distinct and simple, allowing developmental changes to be readily visualised.

Another alternative is the Zebrafish, which shares many of the advantages offered by *Drosophila* and *C. elegans*. Additionally, it offers greater scope for investigating more complex diseases like spinal cord injury and cancer, and possesses advanced anatomical structures as a vertebrate. [7] Given the inherent capacity of the Zebrafish for cardiac regeneration, it is also of interest in regenerative medicine as we seek to harness this mechanism for human therapy. [8]

Large animals tend to be prohibitively expensive, time-consuming to manage and difficult to manipulate for use in basic science research. Instead, they have earned their place in preclinical trials. Their relatively large size and physiological similarity to humans provides the opportunity to perform surgical procedures and other interventions on a scale similar to that used clinically. Disease models created in sheep or swine are representative of the complex biological interactions that are present in highly evolved mammals; hence may be suitable for vaccine discovery. [9] Furthermore, transgenic manipulation is now possible in non-human primates, presenting an opportunity to develop humanised models. [10] Despite this, there are obvious limitations confining their use to specialised settings. Large animals need more space, are difficult to transport, require expert veterinary care, and their advanced psychosocial awareness raises ethical concerns. [9]

The clinical context of animal experimentation

A major issue directly relevant to clinicians is the predictive value of animal models. Put simply, how much of research using animals is actually clinically relevant? Although most medical therapies in use today were initially developed using animal models, it is also recognised that many animal experiments fail to reproduce their findings when translated into clinical trials. [11] The reasons for this are numerous, and require careful analysis.

The most obvious is that despite some similarities, animals are still animals and humans are humans. Genetic similarities between species as seemingly disparate as humans and mice may lead to assumptions of conserved function between humans and other animal species that

are not necessarily correct. Whilst comparing genomes can indicate similarities between two species such studies are unable to capture differences in expression or function of a gene across species that may occur at a molecular level. [12]

The effectiveness and clinical relevance of experimental animal trials is further complicated by epigenetics. Epigenetics is the modification of genetic expression due to environmental or other cues without actual change in DNA sequence. [13] These changes are now considered just as central to the pathogenesis of cancer and other conditions as genetic mutations.

It is also important to consider the multi-factorial nature of human diseases. Temporal patterns such as asymptomatic or latent phases of disease can further complicate matters. Patients have co-morbidities, risk factors, and family history, all of which contribute to disease in a way that we may still not completely understand. With such complexity, animal models do not encapsulate the overall pathophysiology of human disease. Animals may be too young, too healthy, or too streamlined in sex or genetics. [14] To obtain animals with specific traits, they are often inbred such that two animals in the same experiment will have identical genetic make-up - like twins, hardly representative of the diversity present in nature. Understandably, it can be an extraordinary challenge to incorporate all these dimensions into one study. This is especially so when the very principles of scientific method dictate that variables except for the one under experimentation should be minimised as much as possible.

A second area of concern is the sub-optimal rigour and research design of animal experiments. Scientists who conduct animal experiments and clinicians who conduct clinical trials often have different goals and perspectives. Due to ethical and cost concerns, the sample size of animal experiments is often kept to a minimum, and studies are prolonged no more than necessary, often with arbitrarily determined end-points. [14] Randomisation, concealed allocation, and blinded outcome of assessment are poorly enforced, leading to concerns of experimental bias. [11] Additionally, scientific experiments are rarely repeated due to an emphasis on originality, whereas clinical trials are often repeated (sometimes as multi-centre trials) in order to assess reproducibility of results. Furthermore, clinical trials are more likely to be published regardless of the nature of results; in contrast, scientific experiments with negative findings or low statistical significance often fail to be reported. These gaps highlight the fact that preclinical trials should be expected to adhere to the same standards and principles of clinical trials in order to improve the translatability of results between the two settings.

Although deficiencies in research conduct is a concern, the fundamental issue that remains is that even the best-designed preclinical study cannot overcome the inherent differences that exist between animal models and 'real' human patients. However, it is reassuring to know that we are becoming better at manipulating animal models and enhancing their compatibility with their human counter-parts. As such, this drive towards increasingly sophisticated animal models will provide more detailed and clinically relevant answers. Additionally, with the recognition that a single animal model is inadequate on its own, experiments may be repeated in multiple models. Each model will provide a different perspective and lead to the formation of a more comprehensive and balanced conclusion. A suggested structure is to start initial proof-of-principle experiments in small, relatively inexpensive and easily manipulated animals, and then scale up to larger animal models.

'Human' experimental models - the revolution of stem cells

Given the intrinsic differences between animals and humans, it is crucial to develop experimental systems that simulate human biology as much as possible. Stem cells are 'master cells' with the potential to differentiate into more mature cells, and are involved in the development and maintenance of organs through all stages of life from an embryo (embryonic stem cells) to adult (tissue-specific stem cells). [15] With the discovery of human embryonic stem cells [16] and other tissue-specific stem cells [17] it is now possible to appreciate the developmental biology of human tissues and organs in the laboratory. Stem cells may be studied under various controlled conditions in a culture dish, or even implanted into an animal to recapitulate *in vivo* conditions. Furthermore, stem cell transplantation has been used in animal models of disease to replace lost or damaged tissue. These methods are now commencing high-profile clinical trials with both embryonic stem cells [18] and tissue-specific stem cells. [19] Although stem cells hold great potential, translating this into the clinical environment has been hindered by several obstacles. Chiefly, tissue-specific stem cells are rare and difficult to isolate, while embryonic stem cells can only be created by destroying an embryo. In order to generate personalised embryonic stem cells for cell therapy or disease modelling, they need to be created via 'therapeutic cloning.' The considerable ethical quandary associated with this resulted in a field mired in controversy and political debate. This led to research coming almost to a standstill. Fortunately, stem cell research was rejuvenated in 2007 with the revolutionary discovery of induced pluripotent stem (iPS) cells - a discovery notable enough to be awarded the 2012 Nobel Prize in Physiology/Medicine.

Induced pluripotent stem (iPS) cells are created by reprograming mature cells (such as skin fibroblasts) back into a pluripotent 'stem cell' state, which can then re-differentiate into cells of any of the three germ layers irrespective of what its original lineage was. [20] Cells from patients with various diseases can be re-programmed into iPS cells, examined and compared to cells from healthy individuals to understand disease mechanisms and identify therapeutic opportunities. Rather than using models created in animals, this approach represents a 'complete' model where all genes contributing to a specific disease are present. Crucially, this enables the previously inconceivable notion of deriving patient-specific 'disease in a dish' models, which could be used to test therapeutic response. [21] It also provides unprecedented insight into conditions such as those affecting the heart [22] or brain, [23] which have been difficult to study due to limitations accessing tissue specimens and conducting experiments in live patients.

However, if a model system rests purely on stem cells alone this would relegate the approach to *in vitro* analysis without the whole organism outlook that animal experiments afford us. Accordingly, by combining this with rapidly evolving cell transplantation techniques it is possible to derive stem-cell based animal models. Although this field is flourishing at an exponential rate it is still in its infancy. It remains to be seen how the actual translation of iPS technology will fit into the pharmacological industry, and whether personalised drug screening assays will become adopted clinically.

Conclusion

Experimental models provide us with insight into human biology in ways that are more detailed and innovative than ever before, with a dazzling array of choices now available. Although the limitations of animal models can be sobering, they remain highly relevant in biomedical research. Their contribution to clinical knowledge can be strengthened by refining models to mimic human biology as closely as possible, and by modifying research methods to include protocols similar to that used in clinical trials. Additionally, the emergence of stem cells has shifted current paradigms by introducing patient-specific models of human development and disease. However, it should not be seen as rendering animal models obsolete, but rather a complementary methodology that should improve the predictive power of preclinical experiments as a whole.

Understanding and awareness of these advances is imperative in becoming an effective researcher. By applying these models and maximising their potential, medical students, clinicians and scientists alike will enter a new frontier of scientific discovery.

Conflict of interest

None declared.

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