EDITORIAL

Dissociation between Animal and Clinical Studies. Where Do We Go Wrong?

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Preclinical studies have always represented and will continue to represent one of the pillars of medical progress. From William Harvey's description of blood circulation, to elucidation of mechanisms underlying atherosclerosis, cardiac arrhythmias, or heart failure, and to development of heart transplantation, valve replacement, or coronary artery bypass grafting, all major medical breakthroughs relied on studies performed in laboratory animals. Whether we are talking about elucidation of physiological or pathophysiological mechanisms, identification of new therapeutic targets, evaluation of efficacy and safety of new therapeutic strategies, or simply about the organ and tissue resource that they represent, animals are indisputably an invaluable resource for human medicine progress.

Unfortunately, however, not all results obtained in animal studies end up being confirmed in humans. Studies have suggested that less than 40% of what we record in animals is fully confirmed, whereas almost 20% has no equivalent in humans.[1] Moreover, only about one-sixth of the therapies successfully tested in animals manage to pass the initial clinical trials and only about half of them survive through phase 3 clinical trials and get to be approved for clinical usage.[2] Many strategies that have been safe and effective in animal studies have indeed failed or even proved harmful in humans. After rather reassuring preclinical studies, in humans, rofecoxib use has resulted in more than 88.000 cases of myocardial infarction and more than 38.000 deaths.[3] Isoprenaline use has been linked with more than 3.500 deaths in asthma patients, and thalidomide with more than 20.000 cases of phocomelia before it was withdrawn from the market, although studies in multiple animal species, with doses higher than those used in clinical practice, supported the safety of all these compounds.[4,5]

The opposite can obviously also be true. Once a drug shows potential toxicity in animal studies, it virtually loses all hope of ever being used or even tested in humans. Fortunately, one could say. However, this also carries the risk for premature loss of potentially valuable molecules. We enjoy today an entire range of magnificent compounds only because they had the chance to escape the standards of modern pharmacology. Penicillin is lethal in guinea pigs, acetaminophen is toxic to dogs and cats, whereas aspirin causes embryonic toxicity in rats and rhesus monkeys.[6] If modern rigors were applied, none of these compounds would probably be on the market today, with invaluable costs for humanity.

These cases and many others have raised serious questions regarding the relevance of animal data for human medicine and brought to light numerous problems related to animal experimentation (Table 1).

Interspecies differences. Numerous animal models have been shown to reproduce extremely well a wide range of human diseases. However, there are also important interspecies differences that need to be taken into account, since even minor differences in genes or proteins expressions, or in the distribution and/or affinity of membrane receptors can lead to extremely

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Table 1. Main problems explaining the gap between clinical and preclinical research and their potential solutions.

Problems	Potential solutions
Interspecies differences (e.g., different genes and proteins expressions, distribution and/or affinity of transmembrane receptors, immunogenic responses, disease susceptibility, etc.)	- identification and usage of animal species that mimic the best the human conditions - validation of obtained results in several species and in multiple small and large animal models
Inadequate animal models (e.g., different phenotypic features, pathophysiological mechanisms, doses, routes, and timings of drug administration, lengths of follow-up, endpoints, etc.)	- utilization of more 'humanized' animal models - studies in animals of different species, ages, and genders, with different forms and phases of the disease, and clinically-relevant comorbidities and medications - administration of tested therapies using clinically-relevant doses, routes, and timings - evaluation of long-term, functional, clinically-relevant outcomes - close clinical-preclinical collaboration in animal model development - algorithms to help identify most clinically-relevant animal models
Methodological rigor (e.g., lack of randomization and blinding, inadequate sample sizes, lack of model standardization, inappropriate statistical analyses, publication bias, etc.)	- standardized experimental models - correct sample sizing - randomization and blinding - multicenter studies - adequate (including intention-to-treat) statistical analyses - correct and complete reporting of both positive and negative study results - pre-registration of animal studies (e.g., preclinicaltrials.eu) - performing multicenter, blind, randomized, controlled preclinical trials
'Sabotage' by clinical trials (e.g., different study designs, populations characteristics, doses, routes, and timings of drug administration, different endpoints, assessment of drugs with unconvincing preclinical results, etc.)	Clinical trials: - started only after completion of relevant preclinical studies - preceded by a correct and complete analysis of previous preclinical studies - testing only those strategies that provided convincing preclinical results

different results. The choice of the animal species on which preclinical assessment is made is therefore of critical importance. Mice, for example, are often used in oncology studies, although mice have been shown to develop most commonly sarcomas and leukemias, whereas humans most often develop carcinomas.[7] Mice are, on the other hand, excellent models of cerebral ischemia, replicating very well the changes that occur in the ischemic penumbra and the immunosuppression that usually accompanies stroke.[8] Mice, as well as most dog breeds, are extremely resistant to atherosclerosis, while primates are well-known for their resistance to myocardial infarction.[8] Pigs, on the other hand, have vascular structural and functional features very similar to those of humans and are therefore very suitable for mimicking human atherosclerosis.[9]

Inadequate animal models. An „ideal" animal model should reproduce as well as possible the human disease, etiologically, mechanistically, and as manifestations, should have similar diagnostic and predictive biomarkers, and similar response to therapy.[10] Perfect animal models can obviously not be achieved. However, the better a model accomplishes these criteria, the greater the chances for the results to be successfully translated to humans. Unfortunately, the reality is often farther away from the ideal than one would expect.

In humans, cardiovascular diseases most often occur in elderly patients, of both genders, with profoundly inhomogeneous genetic background, with multiple comorbidities and medications and questionable compliance to treatment. Meanwhile, laboratory animals are usually young and healthy, most often of a single gender, have homogeneous genetic background, are free of comorbidities and associated medications, and have impeccable treatment compliance. Thus, it is not surprising that the mechanisms by which the disease develops in these animals are often fundamentally different from those encountered in humans. If the atrial fibrillation patient is most often elderly and has intensely remodeled heart, experimental atrial fibrillation is most often induced by rapid electrical stimulation of a young, structurally normal heart. Myocardial infarction, most often associated in humans with inflammation and intense thrombogenic status, is usually induced in animals by external occlusion of a structurally and functionally normal coronary artery, whereas diabetes mellitus, usually associated in humans with multiple risk factors and peripheral insulin resistance, is most often induced in the laboratory by selective chemical destruction of pancreatic beta-cells in young, otherwi-

se healthy animals, with no comorbidities and no insulin resistance.[11] Many animal models therefore ignore even the most critical pathophysiological aspects encountered in humans.

The way in which the tested therapies are administered to those animals is also often rather irrelevant from a clinical perspective. Administered doses are often much higher than those that can be used in clinical practice, therapies are often administered to anesthetized animals, although they are intended for use in unanesthetized, conscious patients, or they are administered much too early, at the beginning or even prior to an event that is, by definition, acute.

The outcomes evaluated in animal studies are also often of questionable clinical relevance. Whereas most clinical trials focus on medium- or even long-term outcomes, most commonly with major clinical impact, in animals the follow-up is usually short (i.e., days or weeks) and often focused on surrogate markers. In atrial fibrillation, the antiarrhythmic potential of new therapies is most commonly assessed not by evaluating the occurrence of spontaneous arrhythmic events, but the inducibility of the arrhythmia by burst pacing, or even based on atrial electrophysiological or histological parameters.[11] Bleeding risk associated with novel antithrombotic drugs usage is most often evaluated based on bleeding time and almost never on bleeding events per se. Many of the most widely used animal models therefore continue to have limited ability to mimic the human conditions.

Insufficient methodological rigor. In the case of clinical trials, methodological rigor has increased considerably over the years. Although progress has been made, animal studies continue to present major methodological issues. Preclinical studies are rarely blind and randomized, sample size is often insufficient and rarely calculated a priori, experimental models are often little or not at all standardized, and the quality of the statistical analyses is often questionable. Randomization and blinding seem to be particularly important, since unblinded and non-randomized preclinical studies appear to be up to 10 times more likely to obtain positive results than those with more rigorous design.[12] In addition, there is also the problem, possibly even more than in the case of clinical trials, of an obvious and important publication bias. As many of the negative studies often remain unpublished,[13] the balance is obviously artificially inclined towards those experimental studies that provide favorable results.

„Sabotage" by clinical trials. One would expect clinical trials to aim to validate in humans the positive results obtained in previous preclinical studies. In reality, the design of clinical trials is often extremely different, however, than that of their precursor preclinical studies:[14] they often include patients with much less severe disease, use different doses, routes, and timings of administration, and assess different outcomes, although it is widely accepted that even minor between-model differences can lead to completely different results. In addition, clinical trials often test strategies that were not at all convincing in the previous preclinical studies,[14] or even ignore completely the relevant preclinical studies, often running in parallel with their corresponding preclinical studies, without waiting for their completion.[13]

Time for a paradigm shift in animal experimentation. An entire list of issues (Table 1) can therefore explain why a gap continues to exist between clinical and experimental studies, and why animal data do not always correspond with those obtained in humans. Efforts should therefore be made to correct these issues in order to increase the translational value of animal models (Table 1).

Interspecies differences can obviously not be corrected. However, efforts can be made to identify those animal species that best mimic the human condition. Moreover, the results can be validated in several species, using both small and large animals. Although this approach does not guarantee clinical relevance, obtaining similar results in several animal models and in several animal species considerably increases the chances for successful translation of animal data to humans. This was the case with PCSK9 inhibitors for instance, which showed positive results in a number of animal species before proving their effectiveness in humans.[15]

It is also the time for a paradigm shift regarding animal models. To achieve truly clinically-relevant results, basic scientists will have to start using more „humanized" experimental models and to investigate animals of different species, ages, and genders, with different forms and phases of the disease, that associate clinically-relevant comorbidities and medications. Tested therapies will have to be administered using clinically-relevant doses, routes, and timings, and studies will have to focus on assessing long-term, functional, truly clinically-relevant outcomes. As the physician-basic scientist, who has solid training in basic science re-

search, as well as solid clinical knowledge, is unfortunately an „endangered species", and the involvement of clinicians in animal experimentation is also on the drop, development of clinically-relevant animal models is becoming increasingly difficult. Algorithms that help basic scientists select those animal models that best incorporate the most relevant features of human diseases have been developed.[16] However, it is unlikely that such algorithms will become relevant competitors to clinical-preclinical collaboration, which remains a critical point in medical research and innovation.

Time has come to bring the rigor from clinical to preclinical studies as well. More efforts should be made to size samples correctly, randomize the animals and evaluate all parameters blindly, make multicenter studies and perform adequate statistical analyses, and report, correctly and completely, both positive and negative study results. Using the model already used for clinical trials, pre-registration of animal studies should also be encouraged. Briefly, the clinical trials model could be fully „plagiarized" and multicenter, blind, randomized, controlled preclinical trials could become the new norm.

Finally, if they want to achieve positive results, clinical trials will have to make some efforts too. They will have to wait for the completion of relevant preclinical studies, start with a correct and complete analysis of previous preclinical studies, and test only those strategies that have provided truly convincing preclinical results.

If we will manage to solve at least part of these problems, we will certainly succeed together, basic scientists and clinicians, to achieve our common goal: bring into clinical practice new therapies, with much better efficacy and safety profiles.

Compliance with ethics requirements:

The authors declare no conflict of interest regarding this article. The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

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