European Journal of Internal Medicine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Original Article

Animal testing is still the best way to find new treatments for patients

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ARTICLE INFO

Article history:
Received 10 November 2016
Accepted 25 November 2016
Available online xxxx

Keywords:
Animal testing
Experimental research

ABSTRACT

Experimental research proceeds by hypotheses formulated on the basis of previous or new knowledge and then tested. If they are accepted, they serve as the basis for further hypotheses, and if they are rejected new hypotheses can be developed. In other words, when we are at the frontiers of knowledge the path is forged by "trial and error". When a trial shows a hypothesis is wrong, this is a step toward making fewer errors.
This process also applies to drug development. There is no magic formula at present to predict - at the pre-clinical level - the therapeutic value of a drug for people with a disease. However, pre-clinical studies are needed in order to formulate hypotheses that justify clinical trials. Without these preliminary studies *in vitro* and *in vivo* in selected animal species it would be unethical to test still unproven chemicals in humans.

1. Introduction

Experimental research proceeds by hypotheses formulated on the basis of previous or new knowledge and then tested. If they are accepted, they serve as the basis for further hypotheses, and if they are rejected new hypotheses can be developed. In other words, when we are at the frontiers of knowledge the path is forged by "trial and error". When a trial shows a hypothesis is wrong, this is a step toward making fewer errors.

This process also applies to drug development. There is no magic formula at present to predict - at the pre-clinical level - the therapeutic value of a drug for people with a disease. However, pre-clinical studies are needed in order to formulate hypotheses that justify clinical trials. Without these preliminary studies *in vitro* and *in vivo* in selected animal species it would be unethical to test still unproven chemicals in humans.

There has recently been a shift in drug development. Historically, drugs were discovered by identifying the active ingredient from traditional remedies or serendipitously. Later, series of chemicals were screened on intact cells, isolated organs and whole organisms (functional screening) to identify substances with a desirable therapeutic effect. The sequencing of the human genome has permitted rapid cloning and purification of large quantities of proteins, and it has become common to use high-throughput screening of large chemical libraries against isolated biological targets which hypothetically are disease-modifying, in a process known as reverse pharmacology (targeted screening). Hits from these screenings are then tested in cells and animals for efficacy. In recent years scientists have been able to see the three-dimensional structure of target molecules and use that knowledge to design drug candidates.

Independently from the procedure followed, the discovery of new drugs has always been based on a series of variable interactions among data collected in patients, tissues, organs or cell culture and different animal species. However, in a large majority of cases clinical studies have been preceded by studies in mice, rats and other animal species which have led to suggestions for drugs to be tested in patients. Not always have the animal results been translated into effective drugs but the failures themselves have helped to reformulate the model or the experimental conditions or the type of chemical. The problem is selecting, for a given human target or function, the animal species that most closely resembles man, which should in principle be possible, drawing on the diversity of the animal kingdom.

The use of animals has always aroused controversy on ethical or technical grounds. Since the ethical issue is not the subject of this article, we shall analyze how animal experiments could be improved in order to increase their probability of predicting useful clinical results.

Are *in vitro* experiments alternative or complementary to animal tests?

Modern technology enables us to cultivate *in vitro* almost every kind of cell from all animal species including man. These cells can provide very useful preliminary information or help us understand how chemicals interact on the cell metabolism or functions, such as secretion of proteins, motility or enzyme activity. A few comments are necessary about whether *in vitro* tests offer an alternative and can therefore replace *in vivo* experiments.

First, drugs are easily available to cells *in vitro* while *in vivo* this is not always the case. For example, isolated cancer cells are more sensitive to an anticancer agent than *in vivo* because the complexity of a solid tumor, with the presence of inflammatory cells, inadequate vascularization,

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http://dx.doi.org/10.1016/j.ejim.2016.11.013
0953-6205/

Please cite this article as: Garattini S, Grignaschi G, Animal testing is still the best way to find new treatments for patients, Eur J Intern Med (2016), http://dx.doi.org/10.1016/j.ejim.2016.11.013

fibrosis and other factors, limits the drug penetration into the cancer cells. Old studies already demonstrated that several anticancer agents are not distributed evenly in a growing tumor, reaching higher concentrations on the surface than in the center. More recent studies have shown that paclitaxel distributes unevenly in *ex vivo* slices of tumor, indicating that not all the targets may be available to the drug [1]; this situation is different from *in vitro* conditions where the drug is distributed more uniformly. The difference is extremely important when deciding about the probable efficacy of an anticancer agent and therefore its potential as a candidate for clinical trials.

Second, when a drug is given *in vivo* it encounters a number of barriers that are hard to reproduce *in vitro*. The blood-brain barrier is an example - it is supposed to protect the brain from exposure to exogenous chemicals. The barriers can sometimes be overcome because there are transport mechanisms. Another example is the intestinal barrier for drugs that are taken orally. In this case too it is difficult to mimic intestinal absorption *in vitro*. The drug may interact with the microbiome, affect intestinal motility, or be absorbed by fibers in food, metabolized by cytochrome P45 (Cyp) in the intestine, transported or rejected by the multidrug resistance (MDR) complex.

Third, when a drug is absorbed by the intestine it may bind to circulating proteins and distribute to various organs. The first pass is in the liver, where drugs can be profoundly metabolized to form several metabolites, depending on the Cyp system. These metabolites may have similar or different activities and in some cases they may be toxic or even counteract the action of the parent drug. Therefore *in vivo*, in contrast to the *in vitro* condition, the action of a drug may be related not just to a single chemical species but to a mixture of effects depending on other chemical species formed (the metabolites) and their interactions. To summarize, *in vitro* drugs are faced with a static system, but *in vivo* they are subject to a very dynamic condition where absorption, distribution, metabolism and excretion change with time.

Fourth, cells or tissue cultures cannot mimic the complexity of a living organism where cells are assembled in organs, under the influence of nerve, hormonal, immunological and circulatory systems. In particular, interactions between drugs and functional activities such as blood pressure, sleep or cognitive activities cannot at present be studied *in vitro*.

Fifth, the animal species employed for preclinical tests have many features similar to man. They have similar organs: brain, lung, heart, liver, *etc*., similar functions such as circulation, hormonal set-up, peripheral nervous system, immunological functions. The genomic organization too is common, although with different degrees of complexity; animal proteins are in most cases homologous to man; metabolic processes are similar.

All the reasons that distinguish the complexity of living animals from *in vitro* conditions also apply to the various animal species and strains, which can differ in their absorption, transport, distribution, metabolism and excretion as well as in the way in which they respond to the same drug. All these considerations imply that while cells or tissue culture are useful for studying drug activity they are complementary, not alternative, to *in vivo* studies. At present, animals are still the best model - however imperfect - to predict activity in man.

Why does translation from animals to man sometimes fail?

Hackman and Redelmeier [2] analyzed this question in a quantitative manner. Out of 76 animal studies retrieved from top journals, 37% were replicated in clinical randomized trials, 18% were contradicted and 45% remained untested. It is logical to assume that for therapies against bacterial, fungal or viral infections the translation from animals to man is more likely to be effective. Vaccines against poliomyelitis, meningitis and rotaviruses are outstanding examples, as are a number of antibiotics and the recent agents against HIV and hepatitis C viruses. They illustrate the striking concordance between animal results and human benefits. Translation has also been fairly good with agonists or antagonists of chemical mediators. Beta-adrenergic blockers for the treatment of tachycardia, alpha-adrenergic blockers for hypertension, and beta-adrenergic agonists for asthma are also good examples of concordant results between animals and man. Similarly, serotonin antagonists have antiemetic activity and serotonin-uptake inhibitors act on some symptoms of human depression. Antihistamines are useful for the treatment of allergic reactions. Drugs acting on metabolism, such as insulin, oral anti-diabetic agents and cholesterol-lowering drugs have been successfully translated from animals to man. The history of today's therapeutic armamentarium has always involved animal testing.

At the other extreme, however, we cannot overlook the poor correlations between results in animals and man in several diseases such as stroke, amyotrophic lateral sclerosis (ALS) and Alzheimer disease. A recent study found that the success rate of new drugs entering phase 1 clinical trials for diseases of the central nervous system is only 8% [2,3].

Several analyses have set out to understand why in many cases the results in animals and man differ. One obvious reason is the difference not so much in organ composition and functions but the greater complexity of man compared to all the animal species that could be used. For logistic and economic reasons mice and rats are the most widely used laboratory animals partly because their genomic, proteomic and metabolomic profiles as well as their organic functions and behavior are better known than for other species. The significant number of rodent strains extends the range of experimental testing. In addition, mice can be genetically mutated so we can investigate the functional role of single and combined genes. Mutated human genes responsible for or involved in human diseases can be transferred into mice, and recently “humanized” mice have been developed, which are useful models to reproduce some aspects of neonatal sepsis [4,5]. All these and future improvements may enable researchers to reproduce at least some features of most human diseases awaiting better treatments.

It must be admitted that an important area of discrepancy is the poor quality of some animal investigations [6]. For instance, amlodipine was tested in 22 trials of cerebral hemorrhage in man, with negative results - in apparent contrast with animal findings. However, when the animal results were systematically reviewed, it became clear that there was no benefit, indicating that animal findings did in fact overlap those in man. Similarly, out of nine drugs found effective in an animal model of ALS only one, riluzole, actually appeared to prolong patients' survival – to a limited extent. But then, when these nine drugs were tested in mice by the ALS Therapy Development Institute, all of them, with the partial exception of riluzole, were inactive. Therefore, the discrepancy was due to the fact that the borderline results had been interpreted optimistically. In general, there is a regrettable tendency to over-rate the value of products that in fact show only marginal efficacy for a pathology that has no treatment.

It must be stressed that the progress made in controlling bias in clinical trials has not been translated to animal trials. Although animal publications far exceed the number of clinical trials, systematic reviews and meta-analyses are ten times less frequent for animals than for clinical research. Bias related to randomization, double blinding, surrogate end-points, calculation of sample size, statistical analysis, and non-publication of negative results still greatly limits the extrapolation of animal findings to man. The over-enthusiastic attitude of scientists, together with economic interests, have in several cases led to premature clinical tests.

In some cases the treatment schedule is inadequate, or blood and tissue concentrations are too low to affect a target or too high to be tolerated in man. In addition, the target in man may not follow the same pathway of events, with a different functional effect from that seen in pre-clinical studies. Frequently, for instance in experimental cancer research, treatments are preventive or are tried at too early a stage of tumor development - differently from the clinical condition. Therefore the inadequacy of the model or the time and doses of the treatment – as well as a critical evaluation of results – may explain the discrepancy rather than the translation itself.

How could we improve *in vivo* studies? There may be at least four essential approaches.

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