



Microdosing: safer clinical trials and fewer animal tests

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Human microdosing belongs to the group of new, advanced experimental approaches that meet 21st Century requirements. As suggested by the Centre for Medicines Research, without a new generation of product-development tools, it will be difficult to improve the 20-year low in the number of new medical therapies launched onto the market [1], despite more investment in biomedical research worldwide over the last two decades.

As mentioned in the ‘critical path’ report published by the US FDA [101], 92% of compounds that pass animal tests fail in Phase I clinical trials. A technique such as microdosing, which provides early data about the behavior of drugs in humans at very low doses, can only improve predictions of drug toxicity and efficacy, whilst also reducing the resources spent and the number of animal tests carried out.

In streamlining the drug-development process, human microdosing is also likely to reduce animal testing, a responsibility shared by companies, regulators and individual scientists worldwide. Legislation in Europe requires the Replacement, Reduction and Refinement (the ‘Three Rs’) of animal experiments wherever possible [2]. Similar policies are implemented in many countries, including the USA.

In the EU, the Commission and the member states also have a legislative duty to encourage research into methods that could achieve equivalent objectives, but using fewer animals or none at all. The European Centre for the Validation of Alternative Methods (ECVAM) was established in 1992 for this reason and, since then, some 27 nonanimal models and assays have been scientifically validated as full or partial replacements for animal tests and 20 have gained regulatory approval [102].

More than 12.1 million animals were used in 2005 (latest available figures) in experiments in the EU, of which 528,189 were used for safety testing of pharmaceuticals [3]. As well as their use in studies of absorption, distribution, metabolism and excretion (ADME), animals are

used to assess longer-term toxicities of pharmaceuticals, such as subchronic and chronic toxicity (67,651 animals in the EU in 2005), developmental and reproductive toxicity (49,026 animals) and carcinogenicity (26,589). The wider use of human microdosing would minimize all these animal tests, which are conducted later in drug development, by providing human-specific ADME data and, thus, identifying early those compounds destined to fail later owing to suboptimal pharmacokinetics or metabolism [4]. A 10% improvement in identifying failing candidates before classic clinical trials could also save US\$100 million in development costs per drug [5].

The British government’s Animal Procedures Committee, which advises on animal experiments, recommended in its 2002 report on the use of primates that human microdosing should be further developed and resourced. The Committee recognised that microdosing has the potential to limit the use of primates in repeat-dose toxicity tests, pharmacokinetics and safety pharmacology studies [6]. In 2008, repeat-dose tests alone (subacute, subchronic and chronic) involved 2346 primates in Britain [7].

In contrast to the fairly extensive animal data required prior to a Phase I trial, single-dose rodent tests can be the primary support for microdose studies in humans and the European Medicines Agency recognizes that microdosing, among other kinds of exploratory clinical trials “can reduce overall animal use in drug development” [103].

The use of subpharmacological and subtherapeutic microdoses in early clinical studies also offers better protection for Phase I trial volunteers, without compromising the safety of microdose subjects. It has been suggested that a microdose study of TGN1412 might have prevented the tragedy that occurred in 2006, when Phase I trial subjects suffered life-threatening, unexpected side effects despite extensive pre-clinical tests on animals, including rhesus and cynomolgus monkeys.



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TGN1412 targets the CD28 receptor on T-cells, where there are differences between the rhesus monkey and human sequences. A microdose study with TGN1412, by systemic or dermal application, could have determined the amount of the antibody that bound to T cells in the whole human body without risk to the subjects, as well as providing pharmacokinetic and metabolic data relevant to the human species [8]. Safely obtained human microdose data may have been able to improve the design of the Phase I study.

In fact, the Duff report on the TGN1412 incident stated that “the view that higher-risk agents should be given at specified low dose levels, for example in the microgram or nanogram range (‘microdosing’ and ‘nanodosing’) may have value as a general guideline” [104].

The human relevance of microdosing applies to a wider field than drug toxicology, as illustrated by its proposed uses in oncology. For example, applying microdosing to measure interindividual variability in drug disposition caused by genetic and environmental factors is a promising application [9]. In addition, microdosing offers opportunities to conduct new study designs and investigate previously unanswerable questions in oncology [10].

The value of microdosing in other sectors has already been recognized. As a case in point, microdosing was considered by an ECVAM expert group as a potential replacement for most of the toxicokinetic and ADME data normally obtained from animal tests for chemicals used in cosmetics [11].

The role of nonanimal techniques

Many researchers, governments and regulators now agree that replacement techniques are often more relevant, reliable, sensitive, cost-effective and reproducible than experiments on animals [105]. The FDA’s report stated that a major problem is the failure to create and use novel tools to deliver “fundamentally better answers about how the safety and effectiveness of new products can be demonstrated, in faster time frames, with more certainty, and at lower costs” [101].

The FDA called for a greater emphasis on *in vitro*, clinical and computational tools. For example, using human cell lines to characterize drug metabolic pathways can predict human metabolism and help eliminate compounds with unfavorable metabolic profiles. This approach has meant that clinical failures due to drug-interaction problems are now far less likely.

Computational modeling is increasingly being applied to human disease simulation, to predict novel drug pharmacokinetics and to conduct ‘virtual’ clinical trials. In terms of ADME simulation, the mathematical relationship between drug doses, plasma concentrations, pharmacokinetics and pharmacodynamics can be characterized and patient covariates are included in the computational model. The systematic application of mathematical modeling could significantly improve drug development.

Analytical techniques have already replaced many animal tests and in so doing have improved sensitivity, reliability and precision [12]. For example, digitalis was once routinely tested for potency on guinea pigs and pigeons using a lethal method. In the late 1980s, this was replaced by a chemical colorimetric assay which directly measured the content of digitoxin. In 1980, insulin batch testing used 600 mice per test, but was later replaced by HPLC – a more rapid and precise method [13].

Where now?

The number of consortia and organizations working to replace animal tests in medicines development is growing fast. The European Partnership to Promote Alternative Approaches to Animal Testing was established in 2005 as a joint initiative between the European Commission, European trade associations from seven industry sectors (including pharmaceuticals) and individual companies. Its purpose is to promote the development and implementation of modern Three R approaches, including nonanimal methods, in the field of safety testing [106].

In 2009, an international memorandum of co-operation was signed by four agencies, in the USA, Japan, Canada and the EU, to co-ordinate the adoption of nonanimal testing methods. The agreement “will speed the adoption of new test methods based on advances in science and technology. Animal welfare will also be improved by the national and international acceptance of alternative test methods that reduce, refine and replace the use of animals”, according to William Stokes, an assistant surgeon general in the US Public Health Service and a leading scientist in the Three Rs field [107].

Although nonlinear absorption or disposition characteristics could affect the validity of microdose predictions of therapeutic-level pharmacokinetics, recent publications suggest that a concordance of approximately 80% can be anticipated [14]. Its direct and safe applications for humans, together with its economical and ethical benefits, bring microdosing to the



forefront of 21st Century technology. Combining human microdosing with other complementary nonanimal approaches clearly offers ethical, scientific and efficiency benefits for pharmaceutical development. Wider recognition of this should lead to faster progress towards that goal.

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