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Opportunities to minimise animal use in pharmaceutical regulatory general toxicology: A cross-company review

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ABSTRACT

Toxicity studies in animals are carried out to identify the intrinsic hazard of a substance to support risk assessment for humans. In order to identify opportunities to minimise animal use in regulatory toxicology studies, a review of current study designs was carried out. Pharmaceutical companies and contract research organisations in the UK shared data and experience of standard toxicology studies (ranging from one to nine months duration) in rodents and non-rodents; and carcinogenicity studies in the rat and mouse. The data show that variation in study designs was primarily due to (i) the number of animals used in the main study groups, (ii) the use of animals in toxicokinetic (TK) satellite groups, and (iii) the use of animals in off-treatment recovery groups. The information has been used to propose a series of experimental designs where small adjustments could reduce animal use in practice, while maintaining the scientific objectives.

1. Introduction

For scientific, ethical and regulatory reasons, general toxicology studies are carried out in animals to help assess the safety and characterise the risks of proposed new substances before they are given to humans. These include short duration toxicity studies, usually up to one month, to support the first clinical trials in humans and longer term studies, typically up to six months, to support phase II and III clinical trials. Longer term toxicity studies, and carcinogenicity studies are not necessary for all products. Information from toxicity studies in animals is used to (i) identify target organ toxicity, (ii) characterise the relationship between exposure to substance and response, (iii) determine whether an observed effect will recover when treatment is withdrawn, and (iv) provide data to allow a risk assessment for man. Additionally, methods for monitoring potential adverse effects in clinical trials may be suggested. Such toxicity studies are also used to establish the safety margins (based on comparison of plasma exposure levels) for human dosing.

Regulatory toxicity studies for pharmaceuticals account for approximately 5% to 7% of the total number of experimental animals used each year in Europe, of which about 95% is rodent use and 5% is non-rodent use (Home Office, 2009; European Commission, 2010). The clinical condition of an animal can indicate that a substance may be causing systemic toxicity and therefore, in toxicity studies it is likely that some of the animals will experience adverse effects. Careful design of toxicology studies to minimise the number of animals used and careful monitoring to minimise harmful effects, whilst achieving the objectives of the study, is a critical part of good scientific practice.

In order to identify opportunities to minimise animal use in regulatory toxicity studies for small molecule pharmaceuticals, a working group was set up by the National Centre for the Reduction, Refinement and Replacement of Animals in Research and the Laboratory Animal Science Association (NC3Rs/LASA) comprising toxicologists that carry out these studies in the UK. Data on study

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Acronyms and definitions

AUC	Area Under the Curve
C_{max}	Maximum plasma concentration of a drug
C_{min}	Minimum plasma concentration of a drug
DBS	Dried Blood Spot
EMA	European Medicines agency
FDA	US Food and Drug Administration
ICH	International Conference on Harmonisation
JMHLW	Japan Ministry of Health, Labour, and Welfare
LASA	Laboratory Animal Science Association
LC–MS	Liquid Chromatography–Mass Spectrometry
MS	Mass Spectrometry
NC3Rs	National Centre for the Replacement, Refinement and Reduction of Animals in Research
TK	Toxicokinetic
T_{max}	Time after administration of a drug when the maximum plasma concentration is reached

design were collected and analysed and this information demonstrated some variation in the number of animals used in general toxicity and carcinogenicity studies. Based on this analysis, recommendations have been made on how small changes to current practice could further reduce the number of animal that are used in regulatory toxicity studies in the future.

2. Factors affecting animal numbers

2.1. Regulatory guidelines

Guidance available from the three major pharmaceutical regulatory authorities (EMA, US FDA and JMHLW), and ICH, were reviewed. Advice on the number of animals to be used for repeat dose toxicity studies is only given by the EMA and JMHLW, although experience with the US FDA suggests their expectations are similar. Guidance on animal numbers also exists in the FDA red book for food materials (FDA, 2007) and OECD guidelines for chemicals. While useful references, these are not directly applicable to the assessment of pharmaceuticals and include different considerations for study design.

For repeat dose toxicity studies, the JMHLW specifies the minimum number of animals that should be used, indicating that for studies in rodents each group should consist of at least 10 animals per sex and for non-rodents each group should consists of at least three animals per sex. The JMHLW guidance also indicates that when interim examinations and recovery investigations are necessary, additional animals should be included (Japan's and ICH Guidelines, 1999).

The EMA guidance (EMA, 2010) does not mention absolute numbers for standard repeat dose toxicity studies and recommendations on animal numbers are given in general terms, providing advice on the factors that should be considered in deciding the size of treatment groups. The guidance indicates that while sufficient animals for meaningful scientific interpretation should be used, ethical and practical considerations are also important. Specific factors that may influence group size are mentioned, such as inclusion of interim examinations and recovery investigations. Guidance on specific group sizes for studies in support of exploratory clinical trials are given in the revision of ICH topic M3 (R2) (ICH, 2009a), suggesting that broad expectations are now the same for all authorities.

The regulatory guidelines are written to enable appropriate judgment to be made about the number of animals required to achieve the scientific objectives of the study. The expectation is that a study will be individually tailored to the substance under development, taking into account factors such as the nature of the substance, the expected effects and their frequency, and whether additional animals may be required to allow specific investigations.

In contrast, the number of animals required for carcinogenicity studies is more explicit. CPMP/SWP/2877/00, Note for Guidance on Carcinogenic Potential, states that, "for each sex there should initially be at least 50 animals per treated group, and one control group of the same number for each sex dosed with the vehicle by the same route" (EMA, 2002). The same guidance also recommends that the starting group size takes into account the strain survival and states: "Approximately 25 animals per sex per group are desired at the scheduled terminal necropsy for histopathological evaluation". Experience shows that discussion with the US FDA is necessary when group sizes reach fewer than 25 animals per sex per group.

2.2. The design of the general toxicology programme

The number of general toxicity studies conducted for a substance is usually determined by the nature of the molecule and the planned clinical development, with toxicity studies performed to support specific clinical milestones. Using knowledge of the pharmacological class of the substance and the proposed clinical plan, both the overall development programme and individual studies should be specifically designed to achieve the stated scientific objectives. Recommended duration of studies to support clinical trials is given in ICH topic M3 (R2). However, there are exceptions to the standard approach which may allow for fewer studies to be carried out, e.g. for antibiotics and for anti-cancer agents nonclinical studies of three months duration may be considered sufficient to support marketing and longer term studies may not be required (ICH, 2009b).

2.3. Strain survival

For carcinogenicity studies, where rodents are dosed over their lifespan, the normal survival of the rodent strain used is an important consideration, as it is essential to ensure that there are adequate numbers of animals at study completion to allow a reasonable evaluation of the end-points. The background survival of a strain can only be determined by reviewing previous data from long term studies in this strain; typically this information is held by laboratories performing a high volume of these studies. The survival of a specific strain of rodent will also vary depending on the housing (single or group housed) and feeding (ad lib or controlled) regimens employed. Where data indicate poor long term survival in a strain (<50% at 2 years) then an alternative strain may be appropriate. This should be considered early in the development programme prior to starting toxicology studies. However if use of an alternative strain is not possible, group size should be increased with the minimum numbers in line with the expected survival (minimum of 20 animals/group) over the period of the study. The impact of survival on group size will be greater as the study duration increases and this is why larger group sizes are employed in carcinogenicity studies.