Regulatory Toxicology and Pharmacology 51 (2008) 237–243

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Safety evaluation to support first-in-man investigations II: Toxicology studies

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ARTICLE INFO

Article history:
Received 25 February 2008

Keywords:
Single dose
Repeat dose
Rodent
Non-rodent
MTD
NOAEL
Genotoxicity

ABSTRACT

Toxicology (single dose, range-finding, repeat dose and genotoxicity) data available in 34 Investigator's Brochures used to support First-In-Man clinical trials over a 10 year period have been evaluated to give an insight into the types of study designs used and how these have changed over the period analysed (1997–2006). Study packages had single dose toxicity studies in the rodent (although there has been a recent trend to reduce the number of these studies), range-finding toxicity studies in the rodent and non-rodent (with only small numbers of the latter used) and key 2–4 week repeat dose toxicity studies in rodent (usually rat) and non-rodent (both dog and monkey). The majority of the latter studies established No Observed Adverse Effect Levels, showed the rodent to be generally less sensitive to target organ toxicity than the non-rodent and showed the liver and then the kidney to be the most common target organs. Genotoxicity assessment included 2 *in vitro* assays (a reverse mutation bacteria and either a chromosome aberration or mouse lymphoma assay) and commonly, an *in vivo* rodent bone marrow micronucleus test. Considerations for general toxicology and genotoxicity study designs are discussed along with the use of appropriate information to help set the clinical starting dose.

1. Introduction

Currently, drug companies tend to perform a fairly standard package of nonclinical studies before commencing First-In-Man (FIM) clinical trial investigations with conventional, chemically synthesised small molecules. Such a package of studies is in agreement with international regulatory guidance as given by the International Conference on Harmonization (ICH, M3). Whether such initial packages will change in the near future is a question for debate considering recent regulatory guidance (CPMP, 2004; CHMP, 2006; CDER, 2006a) suggesting abbreviated nonclinical studies to allow entry into the clinic. Furthermore, ICH, M3 is currently undergoing revision as a result of "the existence of new data and of new approaches, need for a "process allowing for an earlier access to innovative drugs" and "an overall reduction of animals use and suffering" (ICH, M3-R2). Areas relating to the FIM scenario are "the requirement of the toxicity package to support first entry into human", "the need to keep single dose toxicity studies as a fixed requirement prior to first human exposure", "the duration of repeated dose toxicity studies to support the conduct of different phases of clinical trials" and "the timing of completion of the genotoxicity core battery". This manuscript does not propose to enter into such a debate but will examine in some detail the types of studies that have been performed to date by analysing data summarised in 34 Investigator's Brochures (IBs) used to support FIM studies over a 10 year period (1997–2006).

Due to the size of the database examined, the evaluation has been split into 2 publications. This publication examines toxicology (general toxicity and genotoxicity) models performed to support the FIM studies as well as discussing some of the pros and cons of the currently used approaches/study designs. A further publication has examined kinetic (*in vitro*, pharmacokinetic, mass balance and toxicokinetic studies) and safety pharmacology data (Baldrick, 2008).

2. Materials and methods

A total of 34 IBs available to the author were examined for the types of study designs used for toxicology studies. The evaluation was limited to chemically synthesised molecules and excluded biological drugs, vaccines and abbreviated study packages used for some anticancer products. Information on the drug classes examined in given elsewhere (Baldrick, 2008).

From examination of information in the IBs reviewed, study compliance was consistent for pivotal repeat dose toxicology and genotoxicity studies as being performed to Good Laboratory Practice (GLP). Some variation occurred for range-finding studies with a mixture of GLP and non-GLP studies; some studies were performed in GLP laboratories but the study report did not undergo full Quality Assurance review and sign-off.

3. Results

3.1. Single dose toxicity studies

Information on the numbers of single dose (acute) toxicity studies is given in Table 1. A total of 26, 16, 16 and 14 single dose rat

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doi:10.1016/j.yrtph.2008.04.006

Table 1
Single dose and range-finding toxicity studies (34 FIM packages examined)

Study	Comment
Single dose toxicity	
Rat oral	26 studies
Rat intravenous	16 studies
Mouse oral	16 studies
Mouse intravenous	14 studies
Rat range-finding studies	
7-Day repeat dose using 5–6 males + 5–6 females	17 studies
2-Week repeat dose using 3–5 males + 3–5 females	5 studies
Toxicokinetics included	12 studies
Histopathology included	4 studies
Non-rodent range-finding studies	
7 to 14-Day repeat dose using 1–2 males + 1–2 females	6 studies
1–2 Males + 1–2 females ascending dose for up to 4 days	7 studies
1–2 Males + 1–2 females ascending dose (single dose or dosing for up to 6 days) to establish a MTD then new set of 1–2 males + 1–2 females at fixed dose for up to 2 weeks	11 studies
Toxicokinetics included	13 studies
Histopathology included	6 studies

Occasionally values presented do not add up to 34 studies as parameter is not stated in the IB.

oral, rat intravenous, mouse oral and mouse intravenous studies, respectively, were seen among the 34 packages of data reviewed. For non-rodents, 2 packages contained a single dose oral dog toxicity study. The high dose level for single dose toxicity studies was derived as a limit dose (2000 mg/kg), by notable clinical signs or deaths (as found in preliminary investigations with small numbers of animals) or by the limit of practicable dosing formulation. Standard assessment comprised observation for deaths, clinical signs, bodyweights and necropsy examination; in addition, 2 rat oral studies measured toxicokinetics, 2 measured clinical pathology and one included control animals. Results showed that an oral limit dose of 2000 mg/kg was achieved on 17 occasions in rodents; the limit dose by the intravenous route was usually limited by mortality.

3.2. Range-finding toxicity studies

As can be seen from Table 1, range-finding work to support the pivotal GLP study in the rat usually took the form of a 7-day investigation using groups of 5 males + 5 females. Occasional modifications to this design included a slightly higher or lower number of animals/group or a 2-week duration of treatment. Standard parameters measured were clinical signs, bodyweight, food consumption, clinical pathology (haematology and clinical chemistry), organ weights and macroscopic examination. Toxicokinetic assessment was sometimes included and (rarely) histopathology.

A wide range of study designs were utilised for range-finding work to support the pivotal GLP study in the non-rodent (Table 1). However, in all cases only small numbers of dogs or monkeys were used. The most common design involved giving ascending doses to groups of one or 2 animals of both sexes (either as a single dose or over periods ranging from 3 to 6 days) to establish a Maximum Tolerated Dose—MTD (ie a level at which higher dosing would not be tolerated based on e.g., clinical signs or bodyweight loss); on some occasions (but not always) a washout period of up to a few days was allowed between ascending dose levels. Once established, a new set of one or 2 animals of both sexes were then dosed at this MTD for periods ranging from 5 to 14 days. A modification on this design was to include an additional set of animals given vehicle during the ascending dose period and then using them for the MTD phase. Other designs included an ascending dose phase without the MTD phase or giving animals repeated doses over 7 or 14 days only. As in the rat, standard parameters measured were clinical signs, bodyweight, food consumption, clinical pathology (haematology and clinical chemistry), organ weights and macroscopic examination. Toxicokinetic assessment was sometimes included and (rarely) histopathology; ECG evaluation was not usually performed due to the low number of animals involved.

3.3. Repeat dose toxicity studies

Information on the study designs used in pivotal GLP repeat dose toxicity studies is given in Table 2. For rodents, the majority

Table 2
Repeat dose toxicity studies (34 FIM packages examined)

Parameter	Comment
Species	
Rat (CD)	24 studies
Rat (Wistar)	5 studies
Mouse (CD-1)	2 studies
Dog (Beagle)	16 studies
Monkey (Cynomolgus)	16 studies
Monkey (Marmoset)	2 studies
Study duration	
4 weeks	29 rat studies + 30 non-rodent studies
2 weeks	5 rat studies + 4 non-rodent studies
Recovery (non-dose) period	9 studies (usually 4 weeks, rarely 2 weeks)
Dose route	
Oral	29 studies (gavage in rats and gavage/capsules in dogs)
Intravenous	3 studies
Subcutaneous	2 study
Number of animals	
Rat: 10 males + 10 females	27 studies (15 males + 15 females used in 2 studies and 12 males + 12 females used in one study)
Mouse: 12 males + 12 females	2 studies
Non-rodent: 3 males + 3 females	28 studies (4 males + 4 females used in 2 studies)
Number of recovery animals	5 males + 5 females for rats and 2 males + 2 females for non-rodents for the control and high dose level groups only
Number of dose groups	
One control + 3 drug-treated	29 studies
One control + 4 drug treated	5 studies (one or both species)
Age at start of treatment	
Rat	5–9 weeks (generally approximately 6 weeks)
Dog	4–10 months (generally approximately 6 months)
Monkey	1.25–5 years
Dose volume	
Rat	Generally 10 mL/kg (one study used 4 mL/kg twice daily)
Dog	5 or 10 mL/kg (4 studies used capsules)
Monkey	4–10 mL/kg
Vehicle	
Carboxymethylcellulose (0.5–1%)	10 studies
Methylcellulose (0.5–1%)	7 studies
Hydroxypropyl-beta-cyclodextrin (10–15%)	3 studies
Corn oil	2 studies
Parenteral	5% dextrose, 5% glucose, isotonic acetate buffer, phosphate buffer, saline for injection
Others (used in one study)	PEG 400, methylcellulose (0.55) + Tween 80 (1%), water, Tween 80 (0.55) + methylcellulosedextrose (0.5%)

Occasionally values presented do not add up to 34 studies as parameter is not stated in the IB.