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Special Article

General Guidelines for the Preclinical Toxicology of New Cytotoxic Anticancer Agents in Europe

Joint Steering Committee of the European Organization for Research and Treatment of Cancer (EORTC)* and the Cancer Research Campaign (CRC)†

INTRODUCTION

THE European Organization for Research and Treatment of Cancer (EORTC) and the Cancer Research Campaign (CRC) are two major organizations active in research and development of new anticancer agents in Europe and the United Kingdom, respectively. The two organizations have recognized the importance of coordinating their efforts, and a Joint Steering Committee has been established to coordinate the drug development activity of both. Recently, this Committee signed a collaboration agreement with the US National Cancer Institute (NCI) on anticancer drug development to expand and facilitate this drug development programme. In order to facilitate the mutual participation in drug development projects, the two organizations have adopted very similar preclinical toxicology guidelines for new cytostatic agents.

This paper summarizes relevant background information and describes the philosophy as well as the contents of the guidelines currently in use. It should be noted that more elaborate guidelines and descriptions of the packages are available from the offices of both organizations.

BACKGROUND INFORMATION

No uniform guidelines for the preclinical toxicology of new cytostatic agents entering Phase I clinical evaluation have been published in Europe. In order to enhance clinical evaluation of new drugs in cancer, a disease where new drugs are urgently needed, minimum requirements have been defined which could serve as a basis for phase I studies with new cytostatic agents in cancer research centres throughout Europe.

If clearly defined requirements were available this would enable research organizations to provide for each new drug a data base that would be acceptable to all European countries. It could also help to avoid unnecessary toxicological research, by, for example, pharmaceutical companies, and thereby save time and money in the drug development process.

Thus these toxicology guidelines and requirements should be seen as an attempt to come to generally accepted European standards for anticancer drug development. These should guarantee a high quality of the data generated and thereby maintain safety standards, but at the same time not cause unnecessary delay and increase in cost.

LITERATURE SURVEY

Phase I clinical studies, representing the first administration of a new drug in man, are usually conducted in healthy volunteers. However, for cytostatic agents, Phase I studies are usually performed in cancer patients. This is because, as a result of their mechanism of action, the therapeutic index of anticancer agents is generally low and substantial toxicity is therefore expected as well as the potential for late adverse effects. Starting at what is predicted to be a safe dose, the dose is escalated until the maximum tolerated dose (MTD) is reached for a given schedule of administration. Ideally, the starting dose should be such that the MTD is reached with the minimum number of dose escalations, so as to minimize the number of patients

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required. Thus, a good preclinical toxicology programme should define a safe starting dose in humans. It should also predict which vital organs or functions will be most sensitive to the drug. Several papers have been published on the relationship between preclinical toxicology data obtained in laboratory animals and toxicity observed in phase I clinical trials.

In 1970, Schein *et al.* [1] reported that studies in dogs and monkeys have a high predictive value for those types of toxicity that are usually encountered with cytostatic drugs in man, such as myelosuppression and gastrointestinal intolerance. However, the use of these two animal species in preclinical toxicology has yielded a high percentage of false positive predictions (i.e. toxicity observed in animal studies was not observed in subsequent trials in man). Also, less common types of toxicity in man, such as dermal toxicity and CNS effects, have not been well predicted by studies in these species.

Goldsmith *et al.* [2] compared toxicity data on 30 anticancer drugs from mouse, dog and monkey studies with the clinical observations in man. They concluded that defining the starting dose for Phase I trials as one-third of the toxic dose low (TDL) expressed in mg/m^2 in the most sensitive large animal (either dog or monkey) would have resulted in significant toxicity in the first patient for five of the 30 drugs. It was recommended that quantitative mouse data should be taken into account in determining a safe starting dose for Phase I. In subsequent papers the usefulness of dog and monkey data has been questioned repeatedly and the role of such studies in preclinical toxicology has been changing over the last decade.

Rozencweig *et al.* [3] reviewed 21 chemotherapeutic agents regarding the predictability of toxicological studies in animals for the effects in man. It was concluded that the mouse and the dog are equally relevant with regard to defining a starting dose for Phase I. They suggested that one-tenth of the mouse equivalent LD_{10}, expressed in mg/m^2 (1/10 $MELD_{10}$) could safely be used as the starting dose in Phase I, especially if this dose proved not to be lethal or life-threatening in dogs. The use of mice for the determination of the starting dose for Phase I had earlier been proposed by Guarino *et al.* [4].

In 1980 the Division of Cancer Treatment of the US NCI adopted a new approach for their preclinical toxicology of new anticancer agents. This includes assessment of lethality in mice and toxicity studies at doses up to at least the $MELD_{10}$ in rats and dogs. The results for 16 compounds which had been evaluated in this system were recently reviewed [5]. For six out of seven drugs with completed Phase I evaluation, one-tenth of the $MELD_{10}$ proved to be a safe starting dose in humans. For the seventh drug only mild toxicity was observed at this starting dose. However, some of these seven drugs were entered in Phase I at a lower dose than one-tenth of the $MELD_{10}$, because of toxicity in dogs.

It is clear that the present proposed approach to preclinical toxicology, as described below, will require regular critical evaluation as comparisons between human and animal data become available for new drugs investigated on this basis.

AIMS

The guidelines are intended for the preclinical toxicology of new chemotherapeutic agents for cancer treatment, prior to the commencement of Phase I clinical trials.

The aims of the studies contained in this package, based on the use of mice and rats, are:

1. to provide a safe starting dose for clinical studies in humans;
2. to define the most likely targets of toxicity;
3. to determine the extent to which cumulative toxicity occurs with repeated administration;
4. to check the reversibility of toxicity.

If possible, the studies will be extended with a limited preclinical pharmacokinetic study, the results of which can be used to facilitate dose escalation during subsequent Phase I studies [6, 7]. If the new drug is an analogue of an existing clinically used agent, additional studies on the specific toxicities associated with that drug class may be needed. Additional studies may also be required in cases where serious toxicity is found for a novel structure.

GOOD LABORATORY PRACTICE

All tests and procedures should be carried out in conformity with the FDA Regulation for Good Laboratory Practice [8]. The final report will be subject to inspection by the local Quality Assurance Unit. Further guidelines to improve the quality of the studies are given by the 'OECD Guideline for Testing of Chemicals' and the 'Guidelines for Acute Toxicity Testing' issued by the European Communities [9, 10].

PROTOCOL DESIGN

Dosing solutions

Preclinical toxicology is done with the clinical formulation of the drug. If a special solvent is required, appropriate checks on the possible toxicity of the solvent are made. Samples of stock solutions and dosing solutions (or suspensions) should be stored under appropriate conditions for subsequent analyses of drug content. The sponsor will be responsible for the organization of these analyses.

Animals

The studies will be done with male animals, unless the intended use of the drug is endocrine-related and for use specifically in female cancers or there is evidence for differences in sensitivity to toxic effects of the drug between males and females. In that case the most sensitive sex will be employed. Female animals should be nulliparous and non-pregnant. Animals are transferred to the testing laboratory 7–14 days before the start of each part of the study. After the acclimatization period the animals are checked for ill health. All animals are housed under the standard conditions of the testing laboratory and have free access to a standard lab diet and tap water.

Single dose intraperitoneal study in mice

Groups of 10 mice will receive a single intraperitoneal injection of the drug at five appropriately spaced dose levels to determine the LD_{10} and LD_{50}. A preliminary dose-finding study with small numbers of animals would normally be undertaken.

Animals will be observed daily for a period of 28 days after dosing and are weighed weekly. Mortality will be recorded and the time of death noted as precisely as possible. A *post mortem* examination is done on all animals dying intercurrently and those sacrificed at the end of the study. Samples of selected organs will be preserved for possible future histological examination.

Single dose intravenous study in mice

This study will be performed as described above for the intraperitoneal route.

Single dose oral study in mice

If the oral route is proposed for clinical application a single dose oral study should be done, as described above for the intraperitoneal route. Special attention will be given to possible toxic effects on the gastro-intestinal mucosa.

Single dose study in mice to assess toxicity by haematology, histopathology and bone marrow cytology

The aim of this study is to assess the toxicity of the drug by haematology, histopathology and bone marrow cytology after a single dose. The dose level should be selected such that distinct toxicity is elicited without causing extensive mortality, i.e. close to the LD_{10}. This study is conducted as a separate study. The route of administration will normally be that most relevant to the clinical study.

Groups of animals will be sacrificed at regular intervals during a 28-day period after dosing. Samples will be taken for the assessment of haematological parameters (haematocrit; haemoglobin; erythrocyte, leucocyte and platelet counts; additional parameters only if indicated), for histopathology (brain, gastrointestinal tract, heart, kidneys, liver, lung, spleen, testes/ovary; additional organs if indicated) and for bone marrow cytology from these animals and from animals found dead during the study. A control group, receiving the drug vehicle only, will be included in this study.

Multiple dose intraperitoneal study in mice

The aim of this study is to assess the toxicity of the drug by haematology, histopathology and bone marrow cytology during repeated dosing and a subsequent 28-day recovery period. The dose level should ideally be selected such that distinct toxicity is elicited without causing extensive mortality. Therefore, a preliminary dose-finding study is done for this purpose. In the main study, the animals are injected with the drug (or vehicle for the control group) daily for 5 days during 4 consecutive weeks. Alternative dose schedules may be employed in this study, however, depending on the drug profile.

Groups of animals are sacrificed at regular intervals during the dosing period and the 28-day follow-up period. Samples are taken for haematology, histopathology and bone marrow cytology, as described for the single dose study.

Toxicity check in rats and Phase I starting dose

The starting dose for the first clinical trials is taken to one-tenth of the LD_{10} in mice (1/10 $MELD_{10}$, mg/m^2), provided that this dose is totally non-toxic in rats. This is demonstrated for the single and multiple dose protocol, as employed in the mouse studies, with haematology, histopathology and bone marrow cytology assessments of possible drug effects. If 1/10 $MELD_{10}$ is toxic in rats, this part of the study should be repeated at a lower dose to establish a non-toxic dose level in rats.

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ABBREVIATIONS/GLOSSARY OF TERMS

EORTC	European Organization for Research and Treatment of Cancer
CRC	Cancer Research Campaign (U.K.)
NCI	National Cancer Institute (U.S.A.)
MTD	maximum tolerated dose
TDL	toxic dose low, i.e. the dose which produces minimal toxic effects
LD_{10}	dose which is lethal to 10% of the animals
$MELD_{10}$	mouse equivalent LD_{10} (comparing doses on a mg/m^2 basis)
LD_{50}	dose which is lethal to 50% of the animals

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