S-8

ICH Alternatives for the Mouse Carcinogenicity Study, with specific comments on Transgenic Animal Models.

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Introduction

The concept of the bioassay for detecting carcinogenic potential was developed at a time when relatively few agents were recognised as being carcinogens. However, during the last 20 years, many investigators have shown that it is possible to provoke a carcinogenic response in rodents by a wide variety of experimental procedures, many of which are considered to have little or no relevance for human risk assessment.

With the initiation of the International Conference on Harmonisation (ICH) in 1991, discussions began on defining the need for carcinogenicity studies, with specific reference to the question of whether the use of rats, but not mice, result in the loss of information on the carcinogenic potential of a compound that would be relevant to human risk assessment.

This article will briefly review the ICH 4 Guidelines relating to carcinogenicity testing, the role currently being undertaken by the International Life Sciences Institute (ILSI) and present an overview of the alternative test systems under investigation.

Review of ICH Guidelines

The International Conference on Harmonisation has been working since 1991 to promote harmonisation of regulatory between the participating regions (Japan, USA and EU).

At the inaugural ICH-1 meeting (Belgium 1991), discussions were held on a number of key issues surrounding carcinogenicity testing such as dose selection, survival problems in rat studies and the need for two species chronic bioassays. It was felt that there was a clear need to define the basis for using two rodent species to detect carcinogenicity.

At the second ICH meeting (ICH-2) held in the USA, the Safety Workshop session entitled "Issues in the assessment of carcinogenic potential of therapeutic agents" discussed the following issues:

1) Guidelines for dose selection
2) Defining conditions which require carcinogenic studies
3) Utility of two rodent species

With reference to the utility of two rodent species, an interim report was presented. This report concluded that "the bioassay, as conducted today, is giving a positive incidence rate of approximately 30 - 50 % for chemicals in the accessible data bases. This contrasts with the 19 pharmaceuticals which have been identified as human carcinogens". Therefore, the findings from non-genotoxic compounds can either be shown to lack relevance for humans because the exposure is excessive compared to human exposure or the response to a carcinogenic challenge is qualitatively different in rodents from that in humans.

At the conclusion of ICH-2, a decision was taken to continue the analysis of available databases and present the information to the Expert Working Group (EWG) with a view to refining the overall protocol for the bioassay.

At the third ICH session (Japan), a full session was devoted to carcinogenicity studies and results

from a survey of 6 pharmaceutical databases were presented (Table 1).

Table 1

ICH-3: Pharmaceutical Databases reviewed

International Agency for Research on Cancer (IARC)	182 compounds
Food and Drug Administration (FDA)	256 compounds
Physicians Desk Reference (PDR)	151 compounds
Japanese Pharmaceutical Manufacturing Association (JPMA)	100 compounds
Committee for Proprietary Medicinal Products (CPMP)	175 compounds
Centre for Medicines Research (CMC)	79 compounds

The data base revealed that the rats were more sensitive than mice and that tumorigenicity only in mice was never the sole reason for regulatory action. Further, findings in rats only were twice as frequent as in mice: all known human carcinogens had been positive in the rat bioassay.

This conclusion resulted in the formulation of a Step 2 document within the ICH process, which was built around a one-species carcinogenicity study conducted in the most appropriate species. Additional studies, including the use of transgenic animals, cell transformation assays and mechanistic studies would support the one-species carcinogenicity study.

Step 3 of the ICH process involved industry review and comments to the ICH EWG, and this process was completed prior to the commencement of ICH-4 held in Belgium in 1997. A Step 4 document was agreed and recommended for adoption by the ICH Steering Committee on 16 July 1997. The document is entitled "Testing for Carcinogenicity of Pharmaceuticals" (ICH document ICB S1B). According to ICH S1B, a "one plus approach" could be preferred to the two year bioassay in rats and mice.

The basic principle is the conduct of one long-term rodent carcinogenicity study plus one additional study that supplements the need for a second long-term carcinogenicity study and provides additional information that is not readily available from the long-term assay. The rationale for selecting the most appropriate species for long-term study should be based on evaluation of specific items, as shown below (Table 2).

Table 2

ICH-4: Choice of species consideration

1) Pharmacology
2) Repeated dose toxicity studies
3) Metabolism
4) Toxicokinetics
5) Route of administration

In the absence of clear evidence favouring one species, it is recommended that the rat be selected.

With regard to the choice of additional *in vivo* tests for carcinogenicity, the guidelines recommend short- or medium-term *in vivo* test systems focusing on models that provide insight into the mechanisms of carcinogenesis such as transgenic rodents (*in vivo*), neonatal rodents (*in vivo*), initiation-promotion models (*in vivo*) and Syrian Hamster Embryonic Cell (SHE) Transformation assay (*in vitro*). It must be noted that a long term carcinogenicity study in a second rodent species is still considered acceptable.

The Notes at the end of the guidelines stress the importance of evaluating the new methods in terms of relevance to humans and applications to risk assessment. These models might be promising tools for carcinogenicity testing and assessment in future, but must be evaluated on their value and relevance in risk assessment.

Position of ILSI

An international collaborative effort was initiated in 1996 to evaluate the new models suggested in the ICH Safety topic S1B. This programme is being co-ordinated by the International Life Sciences Institute (ILSI) and involves more than 60 laboratories. The collaborative programme will evaluate 21 compounds, which are either carcinogens (human or rodent) or non-carcinogens utilising a number of alternative test systems (Table 3).

Table 3

ILSI: Compounds for the Collaborative Study

Genotoxic human carcinogens	Cyclophosphamide Melphalan Phenacetin
Immunosuppressant human carcinogens	Cyclosporin A
Hormones	Diethylstilbestrol Estradiol
Rodent carcinogen/putative human non-carcinogens (epidemiology)	Phenobarbital Clofibrate Reserpine Dieldrin Methapyrilene
Rodent carcinogens/putative human non-carcinogens (mechanism)	Haloperidol Chlorpromazine Chloroform Metaproterenol WY-14643 DHEP Sulfamethoxazole
Non-carcinogens	Ampicillin D-mannitol Sulfisoxazole

The main aim of the programme is to determine whether data from these alternative assays can improve the process of human risk assessment. The studies are being conducted currently and detailed evaluation is not expected until late 2000. Huntingdon Life Sciences is taking part in the ILSI collaborative programme funding studies to evaluate metaproterenol in the p53 +/- transgenic mouse, neonatal mouse and SHE assay.

The Alternative Test Systems

Transgenic Animals

The strains being developed have the advantage that the numbers of animals used and the time required for the bioassay (6 months daily dosing) are reduced. These strains have either the addition or deletion of genes thought to be central to the development of neoplasia in humans:

Oncogenes: Transgenic models utilising oncogenes are the TG-AC (able to detect both genotoxic and non-genotoxic human carcinogens) and Tg-rasH2 (able to detect known human genotoxic carcinogens).

Tumour suppressor genes: Transgenic animals utilising tumour suppressor genes is the p53 test system (able to detect known human genotoxic carcinogens).

DNA repair gene activity: This is represented by the XPA -/- test system, expressing homozygous absence of the DNA repair genes.

Neonatal mice

This system has been in use for more than 30 years and revolve around the basic concept that developing tissues are more susceptible to the carcinogenic effects of chemicals. The test has been demonstrated to be highly sensitive to genotoxic carcinogens. This is explained by the fact that the higher rates of cell replication during rapid growth of the neonate will amplify the biological effect of DNA modification.

The ILSI protocol involves oral dosing of the test material to CD-1 mice on Days 8 and 15 of age. The duration of the ILSI study is one year following weaning of offspring at 28 days of age.

Initiation-promotion models

The basic premise of this model is the determination whether a substance acts as an initiator or promotor.

Initiator: The test substance is administered in a single dose or over a period of several days or weeks. After several weeks of wash-out, a promotor (eg phenobarbital) is administered and some months later, the number of preneoplastic or neoplastic changes are examined.

Promotor: The procedure is reversed to investigate whether a substance is a promotor. Following administration of a known initiator (eg diethylnitrosamine), the test substance is administered intermittently by the oral route at different dosages over a period of several months.

Syrian Hamster Embryo (SHE) cell transformation assay

This assay was discussed at the 1995 ICH meeting as a potential "alternative" test system. However, following subsequent discussions, the Step 4 document issued in 1997 did not include this system. It has been included in the ILSI programme.

Since the early 1960's the SHE assay has been used successfully for predicting rodent carcinogenicity with more than 500 chemicals from a wide variety of chemical classes having been tested. The concordance between the standard SHE assay and the rodent bioassay is approximately 80%. By modifying the standard SHE assay protocol (pH 6.7), the predictive ability has improved to 85% concordance.

In the SHE assay, cells are cultures with test material for up to one week and the colony is then

examined for clonal transformation.

Conclusion

The ICH process has provided an opportunity to look at alternative test systems in risk assessment and evaluate the contribution they may be able to offer in a "weight of evidence" approach. This approach offers a degree flexibility which is a positive improvement to the traditional two species bioassays, for as stated in the ICH-4 tripartite guideline "given the complexity of the process of carcinogenesis, no single experimental approach can be expected to predict the carcinogenic potential of all pharmaceuticals for humans".

References

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