Session 4. Report 1.

TESTING FOR CARCINOGENICITY OF PHARMACEUTICALS :

S1, A ; Conditions which require carcinogenicity studies for pharmaceuticals

S1, B ; Use of two rodent species

S1, C ; Dose selection for carcinogenicity studies of pharmaceuticals

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At the First International Conference on Harmonization in Brussels, November 1991, The Toxicity Testing Program(including short and long-term toxicity testing and carcinogenicity) was discussed concerning with understand the differences between the requirements in the three regions. The need to harmonize procedures for carcinogenicity studies has been a priority, recognizing that a carcinogenicity study is one of the most resource-consuming in terms of animals and time. One of the priorities for the Experts working on this subject was the issue of dose selection for carcinogenicity testing. Traditionally, carcinogenicity studies for chemical agents have relied on maximally tolerated dose as the standard for high dose selection. In the past, the criteria for high dose selection for carcinogenicity studies of human therapeutics have not been uniform among international agencies. In Europe and Japan, dose selection based on toxicity endpoints or attaining high multiples of the maximum recommended human daily dose(greater than 100X on a mg/kg basis)have been

accepted. However, in the US, dose selection based on the maximum tolerated dose traditionally has been the only acceptable endpoint.

In order to achieve international harmonization, the EWG proposed that any one of several approaches may be appropriate and acceptable for dose selection and the published document indicated several ways to provide for a more rational approach to dose selection for carcinogenicity studies for pharmaceuticals. The guideline(S1,C) outlined five generally acceptable criteria for selection of the high dose ; maximum tolerated dose,25 fold AUC ratio(rodent :human), dose-limiting pharmacodynamic effects, saturation of absorption, and maximum feasible dose. The use of other pharmacokinetic- or toxicity-based endpoints in study design is considered based on scientific rationale and individual merits. The availability of multiple acceptable criteria for dose selection provides greater flexibility in optimizing the design of carcinogenicity studies for therapeutic agents. This guideline reached Step 2 status at ICH 2 in Orlando, October 1993, presently this guideline is the final stage of ICH,Step 5, after public consultation and regulatory consensus. For test chemicals of low toxicity,the maximum feasible dose by dietary administration has been considered to be 5% of

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diet. Recent analyses of the types of tumors that are caused in rodents by the chronic administration of various dose levels of chemicals allow a more refined definition of suitable dose ranges that will help improve the relevance of the bioassay for human risk assessment. For most chemicals, orally administered doses need not exceed 1000 mg/kg body weight or the equivalent dietary concentration. This proposed amendment is now being discussed by EWG.

Conditions which require carcinogenicity studies for pharmaceuticals(S1, A) : As the objectives of carcinogenicity studies are to identify a carcinogenic potential in animals and to understand the potential for such risk in humans and carcinogenicity studies are time consuming and resource intensive, they should only be performed when human exposure warrants the need for information from life- time studies in animals. The issues addressed in the guideline include the following : Cause for concern, Duration and exposure, Indication and patient population, Route of exposure, extent of systemic exposure. Consensus was reached under Step 2 by the ICH EWG on this guideline in March 1995.

Carcinogenicity : use of two rodent species : This project differs from most of the other ICH topics, in that it is concerned with making a harmonized change to take into account recent scientific developments and not with harmonizing different requirements between the three ICH regions. The current regulatory requirements for assessing carcinogenic potential of pharmaceuticals in the three regions stipulate chronic studies in two rodent species, usually the mouse and the rat.

Several large data bases of long term rodent carcinogenicity studies conducted over the past 25 years, indicate consistently that about 50% of chemicals tested yield a positive result. Over 100 rodent carcinogens are marketed as human drugs. If these positive results do not reflect a true carcinogenic hazard to humans, this surfeit of positive results presents a dilemma to sponsors, government agencies and the medical profession. The important increases in knowledge of chemical carcinogenesis in recent years has led to the possibility of being able to predict in large measure the outcome of rodent bioassay. For pharmaceuticals, given the availability of exten-

sive information on genetic toxicology and pharmacodynamics (dose-response and specificity) and pharmacokinetics, the predictivity of bioassay should be even better than that for other chemicals. Intense survey and investigation on the data base collected world wide revealed that positive mice studies did not lead to regulatory refusal of any compound, nor were the results mentioned in the labeling, negative mice studies did not carry much weight in assessing the relevance of positive findings, mechanistic data in the rats were prevalent in the risk assessment of these positive rat findings. The guideline reached Step 2 process on May, 1996 after intensive survey and discussion by EWG. The basic scheme comprises one long-term rodent Carcinogenicity study, plus one other study for carcinogenic activity in vivo as followings.

a) Short or medium-term rodent test systems ; models of initiation-promotion in rodents or transgenic rodents, or new -born rodents.

b) A long-term carcinogenicity study in a second rodent species.

New- born rodent(mouse) model is briefly introduced in this proceedings, because other speakers introduce other models.

Extensive studies had been done by Prof,K-,Fujii and colleagues(1970-1991) using ICR and CD1 mice as the newborn mouse tumorigenesis assay(NMTA). Prof. Fujii pointed out that the MNTA had several advantages. (I) A small amount of a chemical used for animal test was effective in inducing tumors in the MNTA system, when the maximum tolerated dose was administered properly at newborn age within 24 h of birth. (ii) The technique of s.c. administration was a simple method, and the animal can be handled easily,(iii) Pharmacokinetics and metabolism of a chemical in mice at newborn age and those of adult age vary from chemical to chemical ; it is generally accepted that metabolic activity of a chemical at newborn age was in the developing stage, and delay of excretion of a chemical in metabolic pathway results in prolongation of the existence of a chemical in the animal body. Such prolongation of chemical metabolism would have a greater chance of changing normal cells into mutant cells. (iv) 1 year was sufficient for developing tumors. (v) Newborn mice were easy to manage and handle, and the space and

Table 1. Protocol for New Born Mouse Tumorigenesis Assay by Prof. K. Fujii.

Strain : ICR (ICR/Ha, ICR/Jcl), CDF1 (BALB/c×DBA/2)F1
Time of administration : Within 24 hrs after birth (a few exceptions)
Route of Administration : s. c. in the nape of the new born animals
Dosages : MTD or equivalent dose to the highest dose of long-term carcinogenesis study.
Observation Period : One year.
Pathology : Complete necropsy of all animals (Moribund, dead, and alive), histomorphological evaluation of all organs and tissues.
Statistical analysis : 2×2 for χ^2 . Positive results ; determined by $P < 0.01$ or $P < 0.05$

Table 2. 45 Chemicals Tested with NMTA by Prof. Fujii.

2 Aromatic Hydrocarbon, 5 Aromatic amine and related compounds, 3 Azo dyes and their related compounds, 10 Nitrosoamine, nitrosourea and their related compounds, 3 Steroids, 4 Tryptophan metabolites and their related compounds, 4 Naturally occurring compounds, 4 Pyrolysates and 10 Miscellaneous compounds

Table 3. Sensitivity, specificity, predictive value, prevalence, false positive and false negative rates of the NMTA and those of the adult rat (ARCT) or the adult mouse carcinogenesis test (AMCT).

	NMTA	NMTA	ARCT
	AMCT	ARCT	AMCT
Sensitivity	84.6*(22/26)	92.0 (23/25)	95.8 (23/24)
Specificity	87.5 (7/ 8)	90.0 (9/10)	75.0 (6/ 8)
Predictive value	95.6 (22/23)	95.8 (23/24)	92.0 (23/25)
Prevalence	76.5 (26/34)	71.4 (23/25)	75.0 (24/32)
False positive rate	12.5 (1/ 8)	10.0 (1/10)	25.0 (2/ 8)
False negative rate	15.4 (4/26)	8.0 (2/25)	4.2 (1/24)

Asteriks indicates percentage and numbers in parentheses indicates the number of chemicals compared (cited from K. Fujii 1991)

cost required to keep them was less than that required to keep a rat.

Several disadvantages pointed out were (i) The route of administration to newborn mouse was limited to s.c. or i.p., and the oral route may not be used. It had been generally documented in the guidelines for carcinogen bioassay that the route of administration of chemical to rodent should be the same as possible route of human environmental hazards, (ii) Metabolism of a chemical in newborn age was different from that in adult age. (iii) Target tissues or organs of mice in the carcinogenesis test were not

extrapolative for those in humans. (iv) It was difficult to check the exact time of delivery of pregnant mice. His conclusion was that compared with the results of the NMTA and those of the adult mouse or rat carcinogenesis test, it was reasonable to conclude that the NMTA was one of the most reliable methods for detecting carcinogenic chemicals, when a small amount of the chemical was used for rodent carcinogenesis test, and the most advisable method of treatment for the NMTA was 4 weekly s.c., injection of a chemical at the maximum tolerated dose starting at newborn and suckling age, and observation for

1 year was sufficient to detect a carcinogenic chemical.

NCTR(National Center for Toxicology Research),FDA,USA is now conducting validation studies using B6C3F1 mouse.

The present data indicates however, that those animals are sensitive only for unequivocally genotoxic compounds, a category in the pharmaceuticals for which carcinogenicity studies usually not required. Further investigation with non-genotoxic pharmaceuticals would be necessary in near future.

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