

Safety evaluation of peptides and proteins

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For the safety evaluation of a potential therapeutic agent, the toxicologic program is a uniquely designed specific study to predict potential adverse effects in humans. Biotechnology drugs, peptides, and proteins, are fundamentally different from traditional small molecules, and they often cannot substantively be tested in laboratory animals using traditional techniques and packages. For these agents a scientifically based, "case-by-case" approach to the safety evaluation is preferred for meaningful data and prediction of the clinical responses. During the past few years, efforts by world-wide regulatory agencies and pharmaceutical industries have been made to promote international harmonization of regulatory requirements, in seeking the establishment of scientifically based drug testing procedures for the drug development.

In the 1980s, many peptide and protein therapeutic agents such as, colony stimulating factors, interferons, and human growth hormone are produced by the recombinant DNA technology and other biological means. Due to the nature of manufacturing these products, impurities are unfortunately known to present toxic potential. Recently this advanced technology has greatly improved the purity of peptides and proteins, thus has decreased both the immunogenicity and antigenicity problems. These drugs used for the treatment of diseases need to be efficacious and safe.

It is important to recognize that the therapeutic agents are designed to be pharmacological active. These drugs given at high doses may produce undesired side effects resulting from the exaggerated pharmacological activity⁽¹⁾. A full understanding of the mode of pharmacological activity is essential prior to design toxicologic studies. The recent advances in molecular biology helped to understand the basic mechanism of cellular regulatory processes and provided objective basis for the extrapolation of animal data to man.

Objectives of preclinical safety test

The objectives of the preclinical drug safety evaluation are to support the initiation of clinical trials in human and to determine a human risk-benefit ratio which is acceptable for the drug's intended therapeutical use(s): ① to delineate the defined

toxic or adverse effects in animals; ② to evaluate the functional, biochemical, and morphological changes; ③ to support clinical studies with minimal risk; ④ to ensure the safe use in human.

To successfully register a drug, the safety of the product must be demonstrated in both preclinical and clinical studies.

Global requirements for peptide and protein product registration

The regulations regarding the requirements for product registration issued by various agencies are influenced by the legislative background of the countries concerned, which resulted in many differences from many countries. In many instances, health authorities from various countries will differ in their approaches of preclinical drug safety studies. The final decision-making process may not rely solely on the best scientific judgment, but often may involve in political, social, and economic concerns. Also most studies will not be performed without the exact dose, route, and schedule selected for clinical use, or without knowledge of the mode of action, the characteristics of the receptor, and the pharmacokinetic profile of the human patient vs the test species. Thus, preclinical toxicologic data may have to be interpreted with caution. And later, it may turn out that the animal toxicity data generated was not germane to the clinical situation. Guidelines and regulatory requirements need to be changed as our knowledge of science and medicine improves.

Regulatory safety test

The major objective of regulatory agency is to ensure the benefits of the drug outweigh their adverse effects. Regulatory agencies publish drug testing guidelines and/or data requirements for conducting preclinical and clinical studies to get new drug approval. However, these efforts are often duplicated and give rise the unnecessary repetition of studies.

Recently, the International Conference on Harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use is a unique project that brings various regulatory authorities and experts together to seek means to develop scientifically based guidelines to ensure the safety and efficacy of new medicine and to eliminate unnecessary duplicative requirements for registration.

The phases of biological product testing do not differ greatly from the traditional small molecules. In addition, specific properties of these drugs deserve special considerations, such as antibody formation and immunogenicity. The circulating

antibodies are to be detected and analyzed by specific monoclonal antibody which is normally required to monitor the kinetics and biodistribution. The potential immunogenicity following a single and repeated administration can affect the drug's efficacy and safety.

Classification of peptides and proteins for safety test

Three groups of peptides and proteins produced by biotechnological processes can be identified for the requirements of preclinical animal safety studies: ① peptides and proteins are identical to those occurring naturally; normally require limited short term preclinical safety test; ② peptides and proteins are closely related but contained known difference in amino acid sequences; require more extensive preclinical safety test; ③ peptides and proteins are unrelated to that of human peptides and proteins; require preclinical safety testing somewhat similar to that of small chemical molecules and on a "case-by-case" basis.

Investigative study

The objectives of investigative studies are as follows: ① to provide early toxicological and preclinical screens in discovery phase for preclinical safety studies; ② to assist in the design of preclinical safety studies; ③ to investigate mechanisms of toxicity; such as unexpected pharmacologic effects, expected exaggerated pharmacological effects, and toxicological effects; ④ to investigate reversibility of adverse effects; ⑤ to investigate relevant preclinical issues.

In the past, investigative studies were often initiated to aid in safety evaluation of developing drug candidates at the request of regulatory authority to understand the mechanisms of toxicity. Today, many pharmaceutical companies have an active group involved in investigative studies in animal models not only to address specific safety issues arising from regulatory toxicologic and clinical studies, but also extended its function to interact with preclinical groups at discovery stage in identifying safe drug for development. This approach would reduce the risk of dropouts of potential drug candidates due to toxicity observed during regulatory toxicologic testing and enhance the chances of success. The success of a given toxicologic experiment rests upon the interpretative skills of observers. However, scientific evidence obtained from mechanistic studies would provide better assurance of a safer drug.

Design of preclinical safety test

Because biotechnology is a relative new science, its innovative products are emerging rapidly for the treatment of important diseases. The biotech rules are still to be formulated by various regulatory agencies including FDA. All agencies are now in agreement with developing scientifically justified biotechnology guideline rather than inflexible requirements for preclinical safety test. The design depended upon the intended

clinical indication of the biological product. Animal species, dosage levels, route and duration of tested compound administration are usually based on the pharmacologic activity and the intended dose in human. The lowest dose is usually at a proposed human dose or higher. For the highest dose, a maximum of 100 times the proposed human dose is generally considered more than adequate to demonstrate the safety of the tested compound in both single and repeated dose administration.

Preclinical safety test of recombinant peptides and proteins should be conducted in pharmacologic responsive animal models which will generate useful and meaningful data relevant to safety assessment in humans. It is generally desirable to use the same species of animals as in pharmacologic studies which will allow interpretation of potential adverse effects derived from exaggerated pharmacologic response and systemic toxicity of the tested compounds.

Because of the genotypic and phenotypic resemblance to humans, non-human primates such as rhesus, cyno monkeys, and baboons have often been employed in the crucial efficacy and safety studies for biotechnology products including vaccines and antibody agents, neurotrophins, immunomodulators, hormones, and growth factors^[2].

1 Single dose administration

This study is to elucidate toxicity of the test drug in at least 2 respondent animal species following a single dose treatment.

It was recommended by ICH that requirements for an LD₅₀ determination in rodents or non-rodents can be replaced by well-designed dose-tolerance studies in 2 rodents or one rodent and one non-rodent species.

For the rodent study, it is important to estimate the dose-response relationship, usually 10 ♂ and 10 ♀ are divided into 5 groups of 2 animals per sex per dose group. One group of 2 ♂ and 2 ♀ is served as a vehicle control group. Four groups of 2 ♂ and 2 ♀ will be administered with varied dosage levels of the test compound at reasonable spaced multiples of human dose. The study is designed to determine the maximum tolerated and/or toxic dose, if possible.

Animals should be observed for onset of toxic signs, severity, progression, and reversibility of the clinical signs following dosing. The animals should be observed for at least 14 d. If necessary, clinical pathology determination should be considered and histopathologic examinations should be evaluated on organs and tissues showing macroscopic changes at necropsy.

2 Repeated dose administration

Depended upon the duration of treatment in humans, the length of toxicologic test requirement varies. It is generally agreed as follows:

Duration of human treatment	Duration of toxicity studies
One single dose	14 d
One week	4 wk
One month	3 months
More than one month	6 - 12 months

For those protein drugs where there is evidence for the development of neutralizing antibodies resulted in loss of pharmacologic activities and/or producing undesired antigenicity response, a more restricted case-by-case toxicity studies should be considered.

2.1 Animal species

The repeated toxicity studies are to be conducted in both rodents and non-rodent species which are respondent animal species to the tested drug. A non-human species, eg, rhesus monkey, is preferred as a choice of non-rodent species.

2.2 Dose selection

A stepwise dose selection approach is recommended for various duration of studies. For example, a dose range finding study is to evaluate potential toxicity of a test compound in order to select dosage levels for a 28-d safety study. Results obtained from a 28-d study will help to determine dosage levels for longer duration toxicity studies, such as, 3-month and 6-month safety studies. The selection of doses is usually based on: ① the proposed clinical human dose; ② pharmacologically effective dose; ③ pharmacokinetics; ④ results from previous toxicity studies.

At least 3 different dose groups should be employed. The lowest dose is usually a human therapeutic dose or higher which is defined as non-toxic dose level. The highest dose is a toxic dose level usually not to exceed 100 times the proposed human dose. The middle dose is an intermediate level with minimal or no toxicity.

2.3 Dose range finding study

This is to evaluate possible pharmacological and/or toxicological responses associated with repeated administration for a period of 7 - 14 d and to provide scientific basis for the selection of dosage levels for a subsequent 28-d toxicity study.

For the dose range finding study, 4 - 6 groups of 5 - 6 animals per sex per group are usually employed in rodent study and 2 animals per sex per group for non-rodent study. The duration of a dose range study varies from 7 - 14 d. A dose escalation approach is generally recommended for such study to reduce the number of animals employed.

Animals should be observed for pharmacologic and/or toxic signs. Blood samples should be collected periodically for hematology, serum chemistry, and antibodies. It is also important to determine the drug exposure level at various time intervals during the dosing period.

The antibody and/or drug exposure level data may help to interpret adverse effects of the tested compound in animal models.

Necropsy should be performed at the termination. Histologic evaluation should be conducted on major organs and tissues to identify target organ toxicity.

2.4 Subchronic toxicity studies

This is to evaluate possible potential exaggerated pharmacologic effects and/or undesired toxicity. The duration

varies from 28 - 90 repeated administration. These studies are usually designed to define the non-toxic dose, exaggerated pharmacologically effective dose and/or toxic dose. It is important to identify the target organ toxicity and the reversibility of the drug-induced toxicity.

These studies are conducted in responsive animal species. For rodent studies, 4 groups of 15 ♂ and 15 ♀ are dosed for 28 or 90 d. Ten animals per sex per group will be terminated at 28 d following drug administration. In order to investigate the recovery from toxic change the remaining 5 ♂ and 5 ♀ will be allowed for 4 - 6 wk recovery period. For non-rodent study, 5 ♂ and 5 ♀ are dosed for 28 or 90 d. Three ♂ and 3 ♀ will be sacrificed after 28-d dosing and the remaining 2 ♂ and 2 ♀ will be allowed for 4 - 6 wk recovery period.

Three different doses of the tested drug and one vehicle control will be employed. The low dose usually is the proposed human therapeutic dose or higher which should not produce any toxicity. The high dose should produce toxicity and the intermediate dose produce minimal or no undesired effects. The highest dose should not exceed 100 times the proposed human dose which would provide good margin of safety for human use.

Animals should be observed for pharmacologic and toxicologic signs and the onset, severity, progression, and the reversibility of the toxicity. Periodical blood samples should be collected for hematology, serum chemistry, antibody, and drug exposure levels. Urinalysis should be performed for selected animals from each group. Ophthalmologic examination should be conducted in both rodent and non-rodent species prior to and after drug administration. Electric cardiogram should be performed for the non-rodent species at appropriate time intervals. All animals should be necropsied at the termination. Histological evaluation should be performed on organs and tissues of at least the high dose and control groups of rodents and of all groups of non-rodent species.

2.5 Chronic toxicity studies

The chronic studies are to evaluate potential exaggerated pharmacologic effects and/or undesired toxicity in animals following long duration of drug administration. Generally, peptides and proteins are used for over an extended period in patients, long term toxicity studies are required. The duration varies from 6 - 12 months depending on the proposed duration of the treatment in patients. The ICH has agreed that a 6-month, but not 12-month toxicity study in rodents should be performed in support of chronic clinical uses. For the non-rodent toxicity study, there was an agreement in principle that 6-month duration is acceptable. However, under special circumstances that a 12-month study is still required. For protein drugs, the chronic toxicity testing should be scientifically justifiable and on a "case-by-case" basis.

The basic design is similar to that of subchronic studies with the exception of an increase of number of animals and frequency of laboratory tests. For rodent study, generally 20 ♂ and 20 ♀

per dosage group are used, 3 different dosage levels are employed with one vehicle control group. Fifteen ♂ and 15 ♀ will be terminated at the end. For recovery study, 5 ♂ and 5 ♀ per dosage group are assigned as recovery groups 4–6 wk following the end of dosing period. For a 12-month study, depending on the nature of test drug, an additional 5–10 animals per sex per dose group is added for a 6-month interim sacrifice which is usually incorporated in the study design. For non-rodent study, 7 ♂ and 7 ♀ per dose group are employed in a 6-month study, 4 ♂ and 4 ♀ are sacrificed at the end of 6-month study. Three ♂ and 3 ♀ will be allowed to recovery for a period of 4–6 wk. In a 12-month study, 3 ♂ and 3 ♀ per dose group is added for a 6-month interim sacrifice.

Three different doses of the test compound and one vehicle group will be employed. The lowest dose usually is the proposed human dose or higher and should not produce any undesired adverse effects. The highest dose should produce toxicity and the intermediate dose should produce minimal or not toxicity. The highest dose should not exceed 100 times the proposed human dose which would provide good safety margin for human use.

Animals should be observed for toxic signs and the onset, severity, progression, and the reversibility of toxicity. Periodical blood samples should be collected at appropriate time intervals for hematology, serum chemistry, antibody, and drug exposure levels. Urinalysis should be performed for selected animals from each group. Ophthalmologic examination should be conducted at appropriate intervals in both rodent and non-rodent species. Electric cardiogram should be performed in non-rodent species at designated time intervals. All animals should be necropsied at the terminations. Histological evaluation should be done on organs and tissues at least in the control and high dose groups for rodents and in all groups for non-rodent species.

2.6 Carcinogenicity study

For a drug that is used for over 6 months carcinogenicity studies are generally required. The rat and mouse species are generally used in carcinogenicity studies for small chemical molecules. Since rodents are not respondent animal species for some peptides and proteins, such as interferon and stem cell stimulating factor, carcinogenicity testing can not be meaningfully conducted in these species. Therefore, rodent carcinogenicity studies are not required. There are proteins such as tissue growth factors which might promote tumor growth, short term *in vitro* studies might be warranted.

3 Reproductive studies

To determine the potential adverse effects on mammalian reproduction, the study design should include the exposure of mature adults and stages of development from the conception to sexual maturity. The observation should cover one complete life circle as defined by the ICH and endorsed by the FDA, European Community and Japanese Ministry of Health and Welfare⁽³⁾.

The members of the Steering Committee have recommended it including details of experimental procedures for adoption by the regulatory agencies in European Communities, Japan, and USA⁽⁴⁾. These guidelines provide a basis from which an investigator can devise a strategy for testing according to available knowledge and the state of art.

The endorsement of the ICH guideline allows standardization of study protocols and deletion of unnecessary procedures. The ICH guideline also allows flexibility in developing the scientifically justifiable testing strategy. The overall aim of ICH described reproductive toxicity studies is to identify any adverse effects on mammalian reproduction with subsequent comparison of these effects with all pre-existing pharmacokinetics, pharmacologic and toxicologic data⁽⁵⁾.

If there is evidence for adverse effects on spermatogenesis the effects on male fertility test should be performed. If women of child-bearing age are to be included in the clinical trials then the embryotoxicity/fetal development studies should be investigated.

The ICH guideline for reproductive toxicity testing covers 6 stages of the reproductive cycle as described below:

① ICH stage A — Premating to conception This stage evaluates the adult male and female reproductive functions, development and maturation of gametes, mating behavior, and fertilization

② ICH stage B — Conception to implantation This stage evaluates the adult female reproductive functions, preimplantation development, and implantation.

③ ICH stage C — Implantation to closure of the hard palate This stage evaluates the adult female reproductive functions, embryonic development, and major organ formation.

④ ICH stage D — Closure of the hard palate to the end of pregnancy This stage evaluates the adult ♀ reproductive functions, fetal development and growth, and organ development and growth.

⑤ ICH stage E — Birth to weaning This stage evaluates the adult female reproductive functions, neonate adaptation to extrauterine life, and preweaning development and growth.

⑥ ICH stage F — Weaning to sexual maturity This stage evaluates the postweaning development and growth, adaptation to independent life, and attainment of full sexual function. This stage is generally treated only when the intended use of the pharmaceutical product is in children.

3.1 Study design

The ICH guideline is to perform a study for effects on fertility and early and later embryonic development and fetal and pup development. ICH guideline also allows flexibility for Segment I study by reducing the duration of premating treatment period in male animals from 60 d to 28 d, provided that the results of a 28-d toxicology study in the same species do not show adverse effects on male reproductive organs.

Adequate number of animals should be employed to allow meaningful interpretation of the data. Usually 3 dosage levels

are used. The lowest dose should not produce any adverse effect in parent animals, fetuses or offspring. The highest dose should produce some toxicity and the intermediate dose should be the geometric mean of the highest and lowest dose.

It is generally desirable to use species of animals as in pharmacological and toxicological studies. In doing so, it will allow interpretation of potential adverse effects derived from exaggerated pharmacological responses and systematic toxicity of the test drug. For peptides and proteins the choosing of animal species is very important. In many instances, reproductive studies were performed in non-relevant animal species, the results obtained from these studies are difficult to interpret. It is not unusual that reproductive studies might be carried out in a single species, eg, in non-human primate, for certain protein. It is quite obvious that there is of little or no value by employing non-responder animal species in reproductive studies.

3.1.1 Fertility and early embryonic development to implantation

This is to identify the adverse effects resulting from the treatment from before mating, through mating, and implantation. Reproduction performance is evaluated for male and female reproductive functions, development and maturation of gametes, mating behavior, fertilization, preimplantation development and implantation.

This study is normally conducted in the rat. Depending on available pharmacological and toxicological data, ♂ are treated for 28–60 days to cover the period of spermatogenesis. Mature female are treated for 14 d to cover 3 estrous cycles. Males and ♀ then are mated and treatment continuing during the mating period. Treatment of ♀ continues throughout the period of pregnancy. This will permit evaluation of functional effects on the fertility of male and female animals.

3.1.2 Prenatal and postnatal development, including maternal function

This is to detect adverse effects on the pregnant/lactating female and on the development of the concepts and the offspring following exposure of the female from implantation through weaning. Since manifestations of effect induced during this period may be delayed, observations should be continued through maturity.

The study is usually performed in one animal species, preferably in the rat. Females are treated for a period from implantation to the end of lactation. They are allowed to deliver and rear their offspring to weaning, observations should be made on the duration of pregnancy and parturition. At the terminal necropsy, examinations should be made on the implantation and abnormalities. New borns should be evaluated with pre- and post-weaning and growth, sensory functions and reflexes, behavior, maturity, and fertility.

3.1.3 Effects on embryo-fetal development

This study is to detect adverse effects on the pregnant female and development of the embryo and fetus consequent to

exposure of the female from implantation to closure of the hard palate.

This study is usually conducted in 2 species; one rodent, preferably rats; one non-rodent, preferably rabbits. The treatment period is from implantation to the closure of the hard palate. Female should be sacrificed and examined about one day prior to parturition for corpora lutea, numbers of live and dead, embryonic development and growth, major organ formation, fetal viability and abnormalities.

3.2 Reproductive studies in non-human primate

Reproductive studies are rarely been carried out in non-human primate species. However, non-human primate has been employed for special drug of protein nature, eg, stem cell factor, which demonstrates its pharmacological activity only in monkeys. Because of limited supply and high cost, they must be utilized conservatively. In general, small number of animal per dose level is used for reproductive studies.

In fertility study, menstrual cycles and hormone levels (estrogen, progesterone, luteinizing hormone, and follicle stimulating hormone) are monitored for 90 d for ♀. In ♂ the spermatogenesis and testosterone levels are evaluated during a minimum 60 d of drug treatment period. In teratology study, pregnant ♀ are treated during the period of organogenesis (gestation days 20–50). A cesarean section is performed on day 100 gestation for assessment of fetal abnormalities. In prenatal and postnatal study, pregnant ♀ are administered the test drug from gestation days 90–150 of the late gestational effects for evaluation of any abnormal neonatal neurological and/or behavioral changes.

4 Toxicokinetics

The objective of toxicokinetics is to determine the exposure levels, duration of exposure to a test drug in different animal species at different dosage levels following a single and/or repeated administration.

In toxicologic studies, toxicokinetics usually is an integrated component which would provide useful data to assess exposure at steady state and to demonstrate that a dose-related exposure has been achieved during the study. Toxicokinetic data may assist an assessment of systematic exposure to the administered drug and/or its metabolites. It not only provides information regarding the toxic effects and differences between animal species and human but also serves as basis for dose selection for repeated dosing. Toxicokinetic data derived from these studies would contribute to the design of long term toxicology studies and the interpretation of the results of the toxicology studies and its extrapolation from animal to man.

The ICH has recommended ICH Harmonized Tripartite Guideline on the Note For guideline on Toxicokinetics to be used for drug testing; the Assessment of Systemic Exposure in Toxicity studies published in 1997 Oct 27.

The toxicokinetic studies should be performed in at least 2 responder species following single and repeated administration of

the test drug. It is preferable that one rodent and one non-rodent species (usually monkeys) are employed for biotechnology products. These studies can be conducted with radiolabelled compound to determine absorption, distribution, metabolism (if possible), and elimination.

Toxicokinetic studies may be performed concomitantly with toxicity studies in separate satellite groups or in a totally independent kinetic study. For peptides or proteins the non-human primate is usually recommended to be used for such studies. In a review of the selection of animal models for drug kinetics and metabolism studies, Caldwell found that there are few biochemical differences between the rhesus monkey and humans than other species. Caldwell concluded that the monkey was the best animal model for predicting kinetics and metabolism in human^[7].

If the test drug is to be administered other than iv route, a cross-over design with single and repeated administration should be conducted by 2 different routes to determine the bioavailability, to compare the maximum blood concentration, area under the curve, volume distribution, clearance, half life, and tissue distribution, if possible. A minimum of 2 doses, low and high doses which are employed in toxicity study should be used. A brief cross-over study design for monkey species is presented in the following:

Group	Monkeys	Dose	Dose 1	Dose 2
1	3	low	IV	SQ
2	3	low	SQ	IV
3	3	high	IV	SQ
4	3	high	SQ	IV

Dose 1 is a single dose administration. Dose 2 is to be administered for 7 consecutive days 7-14 d after Dose 1.

5 General pharmacology studies

These studies are to investigate potential undesired side effects and to provide information for determining counter measures against adverse effects. If similar side effects are encountered in clinical trials, then the mechanisms of the side effects should be investigated. In general, the following studies are recommended to be performed prior to conducting clinical trials.

① Effects on cardiovascular and respiratory system

This study is to investigate the action of the test drug on blood pressure, heart rate, electric cardiogram, cardioutput and respiratory effects in the rat and non-rodent (monkey species is usually preferred).

② Effects on central nervous system and behavior

This study is to investigate the action of the test drug on spontaneous motor activity, motor incoordination, spasm, sedation, spinal reflexes, convulsions, pain, body temperature and etc. The rat and mouse species are usually used if they are respondent species to the tested drug.

③ Effects on digestive system This study is to investigate the effect of the test drug on the transport capacity in the gastrointestinal tract of the mouse or rat.

6 Drug interaction studies

The drug-drug interaction studies are to investigate the possible potential adverse effects following concomitant medications. Although there is no drug interaction guidelines at the present time, such studies should be conducted routinely when the therapeutic index or ratio (toxic dose to therapeutic dose ratio) of drugs is narrow. There is a rapid growing list of drugs that can interact or interfere with other drugs' pharmacological activities, pharmacokinetics and metabolism. There is no standard protocol for such interaction studies. The study design is usually based on the pharmacological and toxicological profiles of the drugs intended for concomitant medications. It is important to initiate *in vitro* studies during the early phase of drug development to investigate potential routes of metabolism which may provide useful information prior to conduct *in vivo* studies. A pharmacokinetic screen is also very useful in determining altered serum concentrations of one drug which is under the influence by another drug when they are administered concomitantly.

7 Mutagenicity test

This is to evaluate the genotoxicity potential. There is no definitive guideline from regulatory agencies as to the scope and timing of conducting genotoxicity studies for peptide and protein therapeutics. The FDA does not require genotoxicity studies prior to Phase I clinical trials whereas minimal genotoxicity studies are required by both the European Community and Japan. For global product license application (PLA), the following genotoxicity studies are generally conducted:

① *In vitro* bacterial mutation assay employing salmonella typhimurium/histidine reversion^[8] with or without the complementary *Escherichia coli*/tryptophen reversion test^[9].

② *In vitro* mammalian cell gene mutation assay (MCGM) employing the mouse lymphoma cell^[10].

③ *In vivo* micronuclei test in mouse bone marrow^[11].

No single genotoxicity test is capable of detecting all genotoxins. A core battery test which offer different genetic targets should effectively identify a compound for its genotoxic potential.

8 Immunogenicity testing

This is to identify the immune responses that would produce adverse effects and/or invalidate conclusions that might be reached in toxicological studies. Peptide and proteins tend to be immunogenic in non-homologues species, thus immunogenicity testing data generated from laboratory animals has limited usable information because they are foreign to these animal species.

Antibody responses are frequently seen in laboratory animal models after 2-4 wk of treatment. However, the antibody development in these test animal models might not be necessarily neutralizing the pharmacological activities of the drug. The

immunogenicity is not necessary the primary concern in clinical use of protein drugs, such as interferons (Hobson WC, Fuller GB. Species selection for safety evaluation of biotechnology products. Proceedings of Satellite Symposium to the IVth International Congress of Toxicology. 1986, Tokyo, p 55 - 71) and human growth hormone^[12].

Interferon induces antibody formation in a significant proportion of patients depending on the dose and route of administration. This might be the dose-limiting factor for interferons in clinical uses. Generally the intravenous route showed to be less immunogenic than other routes. The presence of circulating antibodies might influence the pharmacokinetics and pharmacodynamics of the protein, ie, a speedy elimination of the protein or neutralization of its pharmacological activities. A careful analysis of drug exposure levels and antibody levels is very important to the interpretation of toxicity data.

Depending on the nature of immunogenicity injury, study on immunogenicity of peptides or proteins needs much more complicated experimental design. Some proteins showed a form of immunogenicity injury was due to the active mediator release, for example, histamine from mast cells by stem cell stimulating factor. Whereas other proteins might induce antigen-antibody complexes which could lead to destructive organ and tissue damages.

9 Monoclonal antibodies

Monoclonal antibody (Mab) technology has been developed rapidly during last 10 years. The usage of monoclonal antibodies has been expanding tremendously as diagnostic and therapeutic agents in clinics and hospitals. Monoclonal antibody such as OKT3 murine monoclonal is used to reverse acute rejection in kidney transplantation. In some other areas, the Mab may be conjugated to a toxin or therapeutic drug which will produce desired therapeutic effect. Depending on the nature of the usage and the frequency of administration the toxicological testing requirement varies greatly by the regulatory agencies.

It is important to recognize that, in theory, the Mab has a specific antigenic site for the diagnostic and therapeutic target. However, other cells and/or tissues might also react to the circulating Mab, thus produce undesirable toxicity due to cross reactivity. To initiate clinical trials, FDA requires *in vitro* human tissue-binding site studies (Points to Consider, in the Manufacture and Testing of Monoclonal Antibody Products for Human Use).

10 Local irritation study

This is to evaluate the local response of tissues at the site of injection following single or repeated drug administration. Most drugs derived from peptides and proteins are administered by parental routes. The local tolerance and irritation studies should be conducted prior to clinical studies.

Usually one species should be used and the rabbit is of choice. The actual concentration of final formulation should be tested. Observation of the injection sites should be performed

during 24 - 96 h after administration. If irritation and/or lesions occurred, histopathologic evaluation should be carried out and a special reversibility study should be performed.

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175-181
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