ISSUES AND PROBLEMS REGARDING THE
PREDICTION OF DELAYED SIDE-EFFECTS
(TOXICITY) OF PHARMACEUTICAL COMPOUNDS

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Before I begin, please allow me to clarify the following: after the abstract was written, the
content of the presentation was substantially changed based on discussion with Dr. T. Yanagita,
Prof. Dr. T. Fujii and other symposiumists. I will present the overall view of “Delayed Toxicity” from the perspective of animal studies. This will be consistent with this symposium and with other subjects to be discussed. A summary of today’s presentation and the abstract will be translated into English as a proceeding, will be available this fall.

1. Is it possible to predict “Delayed Toxicity”?
1) Spectrum of Toxic Effects
The spectrum of toxic effects or side effects in pharmaceuticals, agrochemicals, and chemical substances is generally classified as “local and systemic effects”, “reversible and irreversible effects”, “acute and delayed effects”, “morphologic, functional, and biological effects”, and “allergic and idiosyncratic reactions” (Lu, 1991). The characteristics of the toxic effects are divided into “acute” and “delayed”, depending on the onset of the change.

Cyanide compounds are known to cause acute effects, whereas organic phosphates (insecticides) represent typical chemicals that cause delayed effects (Rosenstock et al., 1991). Organic phosphates also cause delayed neurotoxic effects, and therefore, are used in such research.

2) Safety Pharmacology Data
Before addressing the topic of delayed toxicity, some remarks on the predictability of acute effects are in order. Single-dose animal studies which are conducted to help assess substance safety include acute toxicity studies and safety pharmacology experiments.

The Japanese Pharmaceutical Manufacturer Association (JPMA) conducted a survey of the safety pharmacology experiments and side-effects. Among the pharmaceutical drugs approved by Japanese Ministry of Health and Welfare (JMOHW) between 1987 and 1992, 104 drugs about which literature and other information are available were selected to examine the effects of compounds in safety pharmacology animal studies, and to assess the incidence frequency of clinical side-effects in human (Non-Clinical Safety Evaluation Subcommittee, Japanese Pharmaceutical Manufacturer Association, Database 64, 1994).

Where there is more than 50% “matching” rate between the findings in the safety pharmacology studies in animals and the clinical side-effects in humans, the predictability is considered to be high. For example, vasodilating action in animal experiments is manifested as headache, dull headache, hot flushes, and a feeling of warmth in humans. Hypotensive action in animal experi-
ments is seen as dizziness, light-headedness, malaise, and weakness in humans. Any effects of the central nervous system (CNS) in animals are expected to be seen as anorexia in humans. Acceleration or suppression of gastrointestinal movements in animal studies is expected to be shown as diarrhea, loose stool, and constipation in humans.

Sporadic findings or electroencephalograms (EEG) in animal studies may cause drowsiness in humans. Depression of urine electrolyte excretion in animal studies may cause edema in humans.

In summary, when effects are seen in the CNS, circulatory, or digestive systems in animal studies, side-effects in humans can be predicted relatively easily. However, the CNS findings or observations in animal studies cannot predict what type of side-effects would be found in humans.

According to the survey, the consistency rate was highest in changes in the circulatory system between the animal findings and the side effects in humans. Among them, increase in heart rate in animal studies corresponded most closely with heart pounding and palpitation in humans. By contrast, a decrease in blood pressure in animal studies corresponded poorly and could be manifested as headache/dull headache, dizziness/light-headedness, malaise/weakness, or hot flush/feeling of warmth in humans. Increased blood flow in the common carotid artery and in the femoral artery can be predicted to cause headache/dull headache and hot flushes/feeling of warmth, but direct prediction is difficult.

Even if the phenotypes of side-effects in humans are the same, there are different action mechanisms, clarification of which is desirable. For example, it can be predicted that anorexia would affect the CNS and the motility inhibition of the digestive system and that diarrhea/loose stool would affect the hypermotility of the digestive tract, a bacterioflora disorder, or would depress internal absorption of water. The mechanisms the action that drugs may have is unknown. In order to predict the side-effects of a certain drug, it is considered useful to collect information on side-effects of other drugs with similar chemical structures and pharmacological actions.

3) What is delayed toxicity?

Prof. Dr. T. Fujii introduced earlier in this
symposium. Dr. Yanagita has also explained the difference between side-effects and adverse effects from a clinical perspective. I would like to briefly review their views (Text Table).

Even in a single dose study, certain anticancer drugs have shown bone marrow or renal toxicities of the delayed type. I will limit my discussion of delayed toxicity to repeat dose studies. Primary toxicity seen in a short-term repeat dose study is not delayed toxicity. The following are signs considered to be delayed toxicity: secondary toxicity that is changed to primary toxicity in a long-term repeat dose study; toxicity that is first seen in a long-term repeat dose study; toxicity that starts to appear toward the end of short- or long-term repeat dose studies; or toxicity that starts in the recovery period. Blood dyscrasias, drug hypersensitivity, and autoimmune disease belong to the category of delayed toxicity. These types of toxicity are irreversible or requires a long time for recovery.

Delayed toxicity is often seen in sensory organs, such as the eyes and ears, and in organs of the nervous system. Drug hypersensitivity caused by damage to the immune system is one form of delayed toxicity (Pohl et al., 1988). In a long-term repeat dose study, hosts would suffer the consequences of sustained cell replication; and homeostasis is disturbed due to hormone or nutritional imbalances or both. This in turn triggers changes in biotransformation and in idiosyncratic drug reactions in immunology and metabolism that cause severe side-effects. The delayed toxicity to be discussed in this panel is limited to those that were not detected as toxic signs in animal studies and that were not found in clinical trials as side-effects, but which were seen as relatively severe side-effects for the first time after the drug was marketed.

Usually, toxic signs are related to drug metabolism, and it is rather difficult to predict the delayed toxicity and to extrapolate the findings in animal studies to humans. This is partially due to species, sex, and age differences. For these reasons, clarification of delayed toxicity requires detailed, thorough, and time consuming research and investigation.

4) Outbreaks of Mass Poisoning

There are several chemical compounds that have cause major problems in Japanese society and adversely affected countless people.

To name a few, “Itai-Itai” (kidney and bone, disease caused by cadmium); “Minamata” disease (CNS toxicity by methylmercury); “SMON” (sub-acute myelo-optico neuropathy by cliquinol). Among them, phocomelia caused by thalidomide and skin disease (general weakness) caused by polychlorinated biphenyl are particularly well-known. Thalidomide and cliquinol are pharmaceutical drugs. In any case, it took a long time to clarify the etiology after the first case had been reported (Lu, 1991). In some instances, the chemical compound causing the side-effects was identified based on an immunological approache, after which animal experiments were conducted to verify the etiological theory.

5) Examples of Delayed Adverse Effects

A) Thalidomide

Malformed babies (phocomelus and limb defects) were born 30 to 40 years ago to pregnant women who took thalidomide, an antipsychotic drugs. The incidence of malformed fetuses caused by this drug is clearly related to species and strain differences. With the most sensitive strains of rats and rabbits used in the studies, there was a more than a 25-fold difference between the dose level and the clinical dose. It has also been shown that particular metabolites have contributed to the malformation (to be discussed later). It is also known that the incidence of malformations in rats increases if thalidomide is dosed at the critical period of organogenesis is much higher than if dosed during the regular organogenesis period (Schardein, 1993).

B) Clioquinol

This drug (Iodochloro hydroxyquin) in gastroenteric forms was used to treat gastrointestinal distress 30 years ago. This caused Subacute Myelo-Optic Neuropathy (SMON) in humans. In dogs and monkeys, only central-distal axonopathy was detected when dosed at the range of the lethal dose for 3 months. No toxicity was observed at all even if animals were administered for 1 year at lower doses (Krinke et al., 1979; Thomas et al., 1984). When cats were used for the experiment at a later date, SMON type findings were observed at relatively low doses. But, as is well known, cats are not used in routine, regulatory toxicity studies.
C) Practolol

Practolol is a hypotensive agent, which has a beta-adrenergic neuron blocking (i.e., beta-blocker) action. Thirty years ago, this drug caused side-effects related to drug hypersensitivities in humans. These included skin rashes (i.e., palmer and/or plantar hyperkeratosis) and ocular changes (i.e., burning eyes, decreased lacrimation) (Pohl et al., 1988; Cocco et al., 1982). These changes had not been observed in rat toxicity studies at all. At a later date, more thorough studies in dogs revealed a reduction of tear flow, lymphocytic infiltration in the lacrimal glands, and a decrease in the amplitudes of the A & B waves in ERG (Tanaka et al., 1983).

D) Dilevalol

Labetalol, a hypotensive agent with alpha- and beta-adrenergic neuron blocking actions (alpha and beta blockers), has been marketed after confirming its safety in detailed ophthalmic examinations (Poyner et al., 1976). Dilevalol (Baum et al., 1981), a stereo isomer of Labetalol (R.R. isomer), did not show any findings in hepatic functions nor its metabolic enzymes, such as content of cytochrome P-450, arcoxymericine dearchylization activity, methoxymarine demethylation activity, and ploboxymerine depopulation activity (Mitsuzono et al., 1987). But ten years ago, hepatic disorders began to be reported in humans after the product was on the market.

E) Benoxaprofen

Benoxaprofen is a NSAID (Non-Steroid Anti-Inflammatory Drug) of propionic acid, which is a breakthrough drug with dual inhibiting on lipoygenase and cycloxygenase. Benoxaprofen, like other NSAID drugs such as phenylbutazone and piroxicam, has a long elimination half-life from systemic circulation. Animal toxicity studies did not show specific toxicities, and there was no difference in effects among strains of rats (Sano et al., 1981). However, in England, a mortality was reported in clinical use among the elderly patients after the product was marketed. It is speculated that this was attributable to repeated administration of the drug which has a long half-life, and to change in the metabolic patterns of elderly patients.

Renal and gastric disorders in dog studies of NSAIDs have been reported as delayed toxicity, because these changes were observed after more than 6 months of treatment (Contrera, 1991). According to data accumulated by JPMA, it has been shown that these changes could be found in subacute toxicity studies (Igarashi, 1993). It is probable that the dose level was too low in the long-term study mentioned above. Dogs known to be very sensitive to NSAIDs, and they may die during the early phase of a study due to digestive disorders. In Europe and the US, there is a tendency to set the doses rather low, considering the long dosing period. In this instance, the dose levels selected in Japan were high enough to detect toxic signs in the subacute studies. There is a need to design and complete long-term studies with relatively high dose levels, particularly with NSAIDs. These include, but are not limited to, the following: 1) Gradual increase of the dose level over 3 to 4 weeks from the beginning of the study, 2) Dosing of nonfasted animals sufficiently to produce enough excretion of gastric juice, 3) Use of mature animals, i.e. those having reached puberty and with the full ossification of knee joints. With these conditions in subacute studies, it is quite possible with relatively high dose levels to detect renal and digestive tract disorders as the primary toxicity.

F) Bleomycin

Bleomycin, an anti-cancer drug, and BHT (butylate hydroxy-toluene), an antioxidant, are known to cause pulmonary toxicity due to fibrosis (Trall et al., 1974; Witschi, 1990; Adamson, 1984). The etiology of pulmonary disorder by Bleomycin, postulated more than 20 years ago, has been clarified. There have been mechanistic studies conducted to clarify the onset of this disorder in humans and animals (Adamson and Browden, 1974; Ishizuka et al., 1967; Samuels et al., 1976). Pulmonary disorder due to Bleomycin is first triggered by lesions of infiltration in the pulmonary blood vessels. This leads to interstitial edema caused by the increased permeability of the blood vessels due to the changes of endothelial cells and to extravascular filtration of the plasma component.

The side-effects of Bleomycin are related to the levels of tissue concentration, and the anticancer effect also depends on the dose levels. Special care is needed to design studies to control the incidence of pulmonary disorders. It is thought that the toxicokinetic approach in both
plasma (systemic level) and target organs would be very helpful in understanding the toxicity, and to extrapolate the findings to humans. The interstitial pneumonitis caused by Bleomycin has immediate onset with a 5- to 6-week latency period, whereas the pulmonary fibrosis has a delayed onset with a latency period of several months (Luna et al., 1972).

G) Interferon (IFN)

Bone marrow toxicity has been identified in some anti-cancer agents (Ota, 1988). As presented in this symposium, IFN is one such anti-cancer agent. Animal studies of IFN showed few toxicities (Terrell and Green, 1993; Kim et al., 1993; Mannering and Deloria, 1986; Ronneberger and Hilfenhaus, 1983). There are, however, some reports that toxic signs, similar to those in humans, were observed in animals (Terrell and Green, 1993; Frent and Zbinden, 1987; Dowson et al., 1983). Both statements are true, and it is likely that some toxicities would be seen in a prolonged study in monkeys at high dose. The JPMA survey (Non-Clinical Safety Evaluation Subcommittee, Japanese Pharmaceutical Manufacturer Association, Database 64, 1994) indicates fever, weakness, inappetance, vomitus, and diarrhea/soft stool in humans with IFN administration, which suggests similarity to the findings in monkey studies. This agrees with the literature in Europe and the US.

In general pharmacology studies, the rectal temperature in rabbits and the clinical observations, rectal temperature, and EEG in monkeys were mentioned, but there were no similar findings in humans. This suggests that the dose level was probably not high enough. Recently, the CNS toxicity of IFN in humans has been investigated (Merimsky and Chaitchik, 1992; Meyers, S. et al., 1991; Meyers, O. et al., 1991; Rohatiner et al., 1983). Even though there are some subtypes of IFN (i.e., alpha, beta, gamma), the toxicity or the onset of side-effects in humans and animals is considered “similar”. Because the CNS toxicity of not only IFN but also cytokines including interleukins (Ronneberger and Hilfenhaus, 1983; Bocci, 1988; Tocco-Bradley et al., 1986) has been of concern, it is essential to carefully consider the clinical doses, the patients to be treated, and the dosing duration. This, in turn, suggests that depending on clinical usage, there may be a chance of a side-effects even if the drug is a biological compound of human origin.

It is highly possible to detect toxicity in animal studies, if the drug affects the CNS directly and specifically. But, if the drug affects neuropeptides or changes the blood flow volume, the toxicity might be apparent only in humans whose cerebrum is much more developed than in animals. In such cases, it is not easy to predict the possible side-effects in humans from animal experiments.

H) Estrogen

There are about 20 compounds that are known to cause lesions in both humans and animals (Scales et al., 1992). In pharmaceutical compounds, only synthesized female hormones and similar compounds are reported to cause lesions (Baggs et al., 1991; Marselos and Tomas, 1992; Montandon and Williams, 1994). Certain compounds may have an increased incidence of lesions in either sex of animals, or in rats or mice, or both, but whenever the benefit of dosing to patients obviously exceeds the risk, they will be permitted to be used as drugs. In so doing, the drug must be non-genotoxic and its incidence rate, severity, magnitude, dose response, onset time, and onset mechanism of side-effects must be carefully reviewed. There are 50 to 60 pharmaceuticals that have been used clinically in spite of false positive findings (Griffith, 1988; Physicians’ Desk Reference (49th ed.), 1995).

I) Etombolut

Sight disorder/paropsia and renal disorder have been reported for etombolut, an antituberculosis drug (Russo and Chaglasian, 1994; Harcombe et al., 1991). Isoniazid is also known to cause paropsia.

J) Coralgil and lipodosis

Known examples of drugs that exhibit drug-induced lipodosis include: the coronary vasodilator. Coralgil; the antimalarial drugs, chloroquine and quinacrine; the anorectics, chlorphen- termine and fenfluramine; the antidepressants, imipramine and imipramine; the psychopharmaceuticals, chlorpromazine and thioridazine; the cholesterol synthesis inhibitors, azacholesterol, triparanol, and booxide; and the antiarrhythmic, amiodarone and perhexiline (Reasor, 1989; Hayashi et al., 1985).

However, some of these drugs have also
been observed to exhibit delayed toxicity. For example, in the case of coralgic, phospholipidosis of the liver in animal studies expresses itself as cirrhosis in humans (Watanabe and Tashiro, 1974). Adverse effects of perhexiline (Hayashi et al., 1981) have included neural symptoms, diarrhea, respiratory disorders, and so forth which are observed in animal toxicity studies, but since these symptoms are gradually reduced when administration of the drug is discontinued, the tendency is to assess them as not constituting serious toxicity. In clinical cases, however, problems encountered have included prolonged duration of recovery following discontinuation of drug administration, as well as accumulation of the drug in organs. Serious lesions have also been observed in humans.

As mentioned previously, immune toxicities are also detected as delayed toxicity. Immunity is a protective mechanism of the body against extrinsic/xenobiotic materials, including drugs (Pohl et al., 1988). It is well-known that T-cells of lymph system are involved with immunologic responses. Abnormal acceleration or suppression of immunity can translate into toxicological problems. Immunological controls are closely related to homeostasis, hormones, and the neuro-transmitting system. Simple investigations on T-cells will not be sufficient to fully understand the immune mechanisms. The incidence of immune toxicity has wide species difference, which makes the extrapolation to humans very difficult. Allergic reactions to drugs include not only skin reactions, but also interstitial nephritis in the kidneys (Type IV) and acute nephritis (Type I). Considerable progress has been made recently in researching of immune toxicity, and a part of the testing method has been implemented in the guidelines (U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 1993). Many drugs are expected to be studied in this field of research by many scientists.

There are some findings once considered to constitute delayed toxicity. Drug-induced chronic nephropathy is caused by analgesic antipyreptic, which has been confirmed in animal studies. Drug-induced acute nephropathy caused by antibacterial drugs is found at the renal tubules. This can be readily detected in animal studies, and can occur in humans as well.

Drugs confirmed to cause delayed toxicities in animals and humans have been announced publicly. But it is also true that many toxic signs in animal studies can still be neither explained nor related to the onset of side-effects in humans.

6) Bioactivation of Toxicants

When there are differences in production of metabolites between animals and humans, and when toxicity is not found in animals, it is difficult to detect the delayed side-effects in humans. Some of the examples of drugs that are very toxic in human metabolites are hepatic necrosis by acetaminophen, cancer and hepatic necrosis by cycasin, hepatic and renal necrosis by furosemide, hepatic necrosis by isoniazid, cancers by sofrole (Lu, 1991), intramyelina edema by 5-fluorouracil (Zhang et al., 1993) and many more. It is known that, as metabolites, phthaIyl-1-glutamic acid in thalidomide, and 5-fluorouridine and 2-fluoro-beta-alanine in 5-fluorouracil are toxic in humans.

7) Correlation between Animal Toxicity and Side Effects in Humans with New Drugs (JPMA survey)

The Non-Clinical Safety Evaluation Subcommittee of JPMA conducted a survey of 139 pharmaceutical drugs with new effective ingredients which had been approved by JMOHW in the 5-year period from 1987 to 1991. A total of 468 repeat toxicity studies in animals and the incidence rate of side-effects in humans were examined (Non-Clinical Safety Evaluation Subcommittee, Japanese Pharmaceutical Manufacturer Association, Database 65, 1994). There was not much correlation between the toxicities in animals and the side effects in humans (Table 1). Some of the findings in the animal studies that were reported to correspond to a higher than 5% incidence rate of side-effects in humans are abnormalities in the CNS, digestion, red blood cells, white blood cells, reticulo-endothelial, platelet-blood coagulation, and hepatic functions. The incidence of findings that are three times less than that of animal toxicity (i.e., relatively narrow margin) were abnormalities in digestion, white blood cells, reticulo-endothelia, platelet-blood coagulation, and hepatic function. This means that these parameters in animal studies are considered to be useful in predicting side-effects.
Table 1. The incidence of animal toxicity and side-effects in humans for new drugs (JPMA Survey).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Toxicity</th>
<th>Side-Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat</td>
<td>Dog</td>
</tr>
<tr>
<td>General activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nervous system</td>
<td>40.2</td>
<td>52.9</td>
</tr>
<tr>
<td>Cardiovascular system</td>
<td>1.3</td>
<td>3.0</td>
</tr>
<tr>
<td>Digestive system</td>
<td>13.4</td>
<td>27.0</td>
</tr>
<tr>
<td>Skin/Fur</td>
<td>20.1</td>
<td>18.4</td>
</tr>
<tr>
<td>Urinary organ system</td>
<td>2.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Death</td>
<td>25.5</td>
<td>21.8</td>
</tr>
<tr>
<td>Abnormal body weight</td>
<td>64.4</td>
<td>41.4</td>
</tr>
<tr>
<td>Abnormal food and water intake</td>
<td>56.9</td>
<td>35.6</td>
</tr>
<tr>
<td>Abnormal eyes</td>
<td>2.1</td>
<td>6.3</td>
</tr>
<tr>
<td>Abnormal blood pressure</td>
<td>1.3</td>
<td>3.4</td>
</tr>
<tr>
<td>Abnormal ECG</td>
<td>0.4</td>
<td>12.1</td>
</tr>
<tr>
<td>Abnormal urinary function</td>
<td>41.8</td>
<td>28.2</td>
</tr>
<tr>
<td>Abnormal erythrocyte mass</td>
<td>38.9</td>
<td>29.3</td>
</tr>
<tr>
<td>Abnormal leucocyte/reticuloendothelial system</td>
<td>23.4</td>
<td>14.9</td>
</tr>
<tr>
<td>Abnormal platelet/coagulation system</td>
<td>16.7</td>
<td>16.1</td>
</tr>
<tr>
<td>Abnormal GOT, GPT, GGTP, bilirubin</td>
<td>24.3</td>
<td>21.3</td>
</tr>
<tr>
<td>Abnormal ALP, LDH, lipids, protein</td>
<td>48.5</td>
<td>36.8</td>
</tr>
<tr>
<td>Abnormal BUN, creatinine, electrolytes</td>
<td>30.5</td>
<td>21.3</td>
</tr>
<tr>
<td>Other abnormal clinical pathology parameters</td>
<td>17.6</td>
<td>8.6</td>
</tr>
<tr>
<td>Total studies</td>
<td>246</td>
<td>179</td>
</tr>
</tbody>
</table>


in humans.

JPMA also examined the correlation in the abnormal clinical pathology values between animal studies and side-effects in humans. There were findings of both false positive and false negative findings (Table 2). However, parameters such as white blood cell count, GOT, GPT, ALT, LDH, UN, creatinine, and urine protein are considered to be useful in checking the correlation of findings in the animal studies with the side-effects in humans.

In summary, it is difficult to predict the delayed side-effects in humans from animal experiments.

2. Things to be considered in conducting animal toxicity studies in order to improve the predictability of the delayed side-effects in humans

1) The ultimate goal and value of animal toxicology is to be able to predict the possible side-effects in humans, and to help to make effective approaches toward characterizing them. Toxicological characterization must be carried out with careful and strict evaluation of pharmacological effects of drugs. This pharmacological profiling must include effects on not only the systems which the treatment is intended to affect, but also on other systems as well. In other words, it is important to clarify the onset location and the pathological specificity.

2) Characterization of adverse pharmacological and deteriorating effects might affect the importance of certain toxicological aspects. It is desirable to determine the dose-effect/response, the effective duration, any residual effects and, if possible, the onset mechanism of toxicity.
Table 2. Correlation of clinical pathology parameters affected in animal testing and side-effects in humans.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Animal species</th>
<th>Comments (P&lt;0.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematology</td>
<td>Rat</td>
<td>False negative</td>
</tr>
<tr>
<td>Leucocytes counts</td>
<td>Dog</td>
<td>False positive</td>
</tr>
<tr>
<td>Blood chemistry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GOT/AST</td>
<td>Dog</td>
<td>False negative</td>
</tr>
<tr>
<td>GPT/ALT</td>
<td>Dog</td>
<td>False negative</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Rat</td>
<td>False negative</td>
</tr>
<tr>
<td>LDH</td>
<td>Rat</td>
<td>False negative</td>
</tr>
<tr>
<td>Urea N</td>
<td>Dog</td>
<td>False negative</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Rat</td>
<td>False negative</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>Dog</td>
<td>False positive</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
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</table>


3) To characterize the drug, it is essential to run proper studies for absorption, excretion, and metabolism and to investigate if there are any species differences in drug metabolism. It is equally important to know the drug metabolism at the site of onset.

4) The type of experimental mammals to be used in toxicity studies are rodents, rabbits, dogs, and primates. Closeness to human phytogenesis or embryology does not guarantee analogous findings in toxicology or metabolism. There are also differences in sensitivity to toxicity between juveniles and aged animals. Prior to the human clinical trials in infants, the safety must be fully confirmed in adults.

One should recognize that it is not appropriate to rush to do studies in juvenile animals without first studying the species differences and before the clinical trials in adults are well-evaluated.

5) It is also important recognize that the toxicity of new compounds does not always follow the chemical structure and does not follow the toxicity of other pharmacologically similar drugs. Each drug must be studied and investigated as a novel and separate compound.

6) In extrapolation to humans, one should remember the quantitative and qualitative differences in metabolism among species, the species differences in pharmacological sensitivity, and the difficulties of conducting toxicity studies in animal species that can prove the treatment effects.

7) To distinguish the false positives and false negatives from the true findings, it is imperative to establish a background database with positive and negative controls. It is a prerequisite to select parameters that have the reproducibility and precision of the measured values. It is essential to study and clarify the factors preventing us from predicting and extrapolating the side-effects and to eliminate those factors, one by one. It is also important to accumulate and analyze the results of many experiments and the literature.

3. Practical Approaches to Increase Predictability

1) Unlike 10 to 15 years ago, animal toxicity studies and human clinical trials must follow GLP and GCP, respectively. These regulations must be followed, and the studies should be conducted to allow reconstruction of the study findings. Along these lines, improvement in quality is expected for study directors and study monitors. This will make the test results understandable by a third party, and reconstruction of the study becomes possible.

In primary pharmacology, the test material should be shown to have a dose response, and high affinity and specificity at the action site. The pharmacokinetics also needs to be examined, and its reproducibility confirmed. The precise design for a toxicity study can be made with clear pharmacological and metabolic profile data of the compound. Every time tragedies are caused by a drug, guidelines for toxicity studies have been implemented and updated by the regulatory authority; these guidelines have been effective to a certain degree. With improvements in scientific technology, the requirements discussed at the ICH will be kept to a minimum, and the data and resources should be used most effectively to clarify the onset mechanisms of toxicity. From this perspective, some comments are listed here.

2) At the ICH, it is said that there was no difference in the incidence of toxic signs, regardless of the dosing durations: whether 6 months or
12 months. This means that the longer administration period does not necessarily increase the predictability or the detection rate of these signs (D'Arcy and Harron, 1991; Igarashi, 1993). In carcinogenicity studies, the goal is to detect the carcinogenic potential (i.e., the delayed toxicity), it is, needless to say, important that more than 18 months of dosing be required to see if potential lesions occur.

3) When the drug is dosed to humans for the first time, evaluation of safety pharmacology and acute toxicity from the point of “mechanistic toxicity”, and selection of tests and observation parameters that have a higher potential for extrapolation and predictability are necessary.

In evaluating the repeat dose toxicity studies and the side-effects humans, efforts must be made to coordinate and to examine the false positive and the false negative from the aspect of both toxicity studies and medical technology. In the toxicity studies, emphasis is placed on findings of histopathological examinations, which is, of course, not feasible in humans.

There is a need of establishing clinical pathology tests and functional examinations that can detect findings specific to the animals used in the study.

4) It is desirable to have immunotoxicity testing as a part of the repeat dose toxicity study battery in place of antigenicity studies, which are required only in Japan.

In immunotoxicology, attention must be paid to the behavior of neuro peptides and hormones besides T-cells (Kawamura, 1994; Nagura and Ohtani, 1994).

5) The Expert Working Group at the ICH is discussing elimination of the mouse from the rodent species to be used in carcinogenicity studies, and use of the rat only. Only limited information is obtained from the mouse in carcinogenicity studies, and there are more false positive findings that make extrapolation to humans more difficult. Use of the mouse in carcinogenicity studies should be discontinued, and the same resources should be used for other studies. One of example is the use of transgenic animals designed for that purpose.

6) In toxicity studies of biotech compounds (bio pharmaceuticals), selection of animal species that respond to the treatment is necessary. Also, immune chemistry and immuno histochemistry techniques should be added in the study evaluation.

Use of the same dosing regimen as in clinical use and conduct of a bridging study should be required. This would help clarify the differences in the findings from “routine” toxicology studies.

7) In organically synthesized drugs (chemical pharmaceuticals), use rat and dog or monkey in the efficacy and pharmacology studies with the same administration route and regimen as the toxicity studies, keeping the same species. It is desirable to confirm the level of reactions among the species used.

8) It may also be necessary to assume actual clinical use and to investigate the drug interaction with metabolizing enzymes. Then, extrapolation of the results to humans would aid in the prediction of delayed toxicity.

Recently, progress has been made to create a program using Quantitative Structure-Activity Relationship (QSAR) in predicting toxicity (Moriguchi and Hirono, 1993; Tanii, 1994; Schultz et al., 1994; Debnath et al., 1993). When the quantitation of congeneric QSAR is established, progress is expected in mechanistic studies of toxicity in drugs. The predictability of side-effects in human is expected to improve when the program is finished by initiating input of in-vitro toxicity data and completing the input of in-vivo toxicity data.

4. Discussion

Q: What is your thought on species difference?
A: In aminoglucomide antibiotics, auditory disorders are well-known, and the incidence has been reported in guinea pigs. It was not reported in rats, so I conducted an experiment in rats. There was a difference in sensitivities among F344, SD and Wistar rats in that order (Matsuzawa and Suzuki, 1985). As you know, there have been differences in the incidence rate of lesions and the types of cancers in different rats and mouse strains. It is extremely difficult to select the proper strains for carcinogenicity studies of drugs and to learn how to extrapolate the results to humans.
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