ity and pharmacological studies to humans and for determining drug efficacy and safety, as well as for predicting drug interactions when assessing individual variations in pharmacological effects or consideration to patients with hypersensitive reactions. However, while the results of toxicity studies and pharmacological studies in animals with pharmacokinetics similar to those of humans are valuable for extrapolating the results of animal experiments to humans, care is required in interpreting the results obtained in animal species when the metabolites differ significantly from those in humans. Discussion of safety assessment of metabolites is essential, and to this end this article aims to distil the viewpoints on this subject in order to promote understanding of the research undertaken by companies involved in drug development, the drug approval process, and the medical frontline. Such discussion should be useful for drafting of guidelines. However, this article is only a compilation of viewpoints on current issues, and it goes without saying that these should be updated as the science progresses.

The need to study the toxicity and pharmacological effects of major metabolites for safety assessment of metabolites in drug development was outlined in Japan in a notification from the Ministry of Health and Welfare in 1975 (Yakushin No. 526, 1975). However, this notification did not go so far as to indicate the specific studies required for safety assessment, but in general, metabolites have been assessed in single-dose toxicity studies. Since this notification can also be interpreted as requiring the assessment of all metabolites, at the ICH-1 held in 1991 the Japanese authorities suggested that toxicological assessment of metabolites is necessary when the metabolite is present in humans, but not found in animal species used in toxicity studies, when the metabolite is formed in particularly large amounts in humans, and also when the pharmacologic and toxicologic activity of the metabolite is considered significant (Ohno, 1992). However, the amount of metabolites formed that would necessitate safety assessment was not clearly defined. Such assessments in excess of the minimum have been made on a case-by-case basis by the tripartite authorities in the US, EU and Japan. The US Food and Drug Administration (FDA) and Pharmaceutical Research and Manufacturers of America (PhRMA) have since discussed the degree of metabolite formation requiring safety assessment (Baillie *et al.*, 2002, 2003; Hastings *et al.*, 2003). With reference to this background, the toxicity representative of the Pharmaceuticals and Medical Devices Evaluation Center in Japan presented views on the toxicological assessment of unique human metabolites from a personal standpoint (Toxicology Q&A: Question 16; Toxicological evaluation of unique human metabolites, 2003). In addition, a questionnaire survey of the members of the Japan Pharmaceutical Manufacturers Association (JPMA) found that toxicity studies of metabolites are a matter of great concern (Inoue *et al.*, 2005). Further, the FDA released in June 2005 a draft guidance concerning their views on safety testing of metabolites found in clinical studies (FDA CDER, Guidance for Industry, Safety Testing of Drug Metabolites (Draft), 2005).

In the draft guidance, the FDA provided definitions of terms such as unique human metabolites, pharmacologically active metabolites, and major metabolites. They also provided viewpoints on when it is deemed necessary to conduct a safety assessment of metabolites. Circumstances when the safety of metabolites should be considered included when the metabolites are unique to humans; or when they are formed in greater amounts in humans than in animals; or when they exceed 10 percent of (the lower of) the dosage or systemic exposure. The guidance also established the timing and method of performance of such assessments. The four types of metabolite toxicity studies requested by the FDA are primarily: (1) general toxicity studies with direct administration of metabolites (14- ~ 90-day dosing including toxicokinetic (TK) studies, with a modified route of administration); (2) the minimum necessary genotoxicity studies; (3) performance of studies on embryonic-fetal development; and (4) carcinogenicity studies when deemed necessary based on the results of the targeted indication or results from other studies. In addition, the FDA recommends that when metabolite exposure has been inadequate in studies such that safety has not been fully assessed in studies already conducted, a synthesized reference standard should be prepared and the safety of the metabolite assessed using a route of administration whereby adequate exposure can be obtained in an appropriate animal species. The results of these studies should be reported to the FDA prior to the commencement of Phase III clinical studies. The JPMA has submitted public comments on this draft guidance after compiling the views of its members. In particular, the JPMA has pointed to the inadequately clear rationale for the criterion for a major metabolite as being 10 percent of the dose or systemic exposure of the parent. JMPA has also commented on such matters as the cases presented in which the major metabolites in blood and urine differ or in which they do not reflect exposure at

the target site of toxicity, on cases of dosage discrepancies, and also on cases in which manifestations of toxicity are ascribable to the C_{max} or AUC. However, as of January 2007, the FDA draft guidance has not been finalized.

The guidance from the European Medicines Agency (EMEA) on drug interactions (CPMP/EWP/560/95, 1997) recommends that *in vitro* and/or *in vivo* drug interaction studies be conducted for metabolic pathways responsible for 30% or more of total clearance, and that studies may be required even for metabolites with lower levels of exposure that demonstrate toxicity or pharmacological activity.

At present, viewpoints on handling of metabolites vary among reference papers (Baillie *et al.*, 2002, 2003; Hastings *et al.*, 2003; Smith and Obach, 2005, 2006; Davis-Bruno and Atrakchi, 2006; Guengerich, 2006; Prueksaritanont *et al.*, 2006) and guidance documents from different countries concerning the safety assessment of metabolites (CPMP/EWP/560/95, 1997; Iyakushin No. 496, 1998; FDA CDER, Guidance for Industry, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products - General Considerations, 2003, Safety Testing of Drug Metabolites (Draft), 2005, Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling (Draft), 2006). Opinions are divided on what the criteria should be triggering toxicity studies with metabolites. In Japan, as well, the FDA draft guidance has prompted consideration of the standards for safety assessment of major metabolites. Metabolite studies were discussed at Forum 2006 of the 21st Annual Meeting of the Japanese Society for the Study of Xenobiotics held in November 2006 (JSSX Annual Meeting Abstracts, 2006) with emphasis on collaboration between pharmacokinetic and toxicity scientists. The importance of the role played by regulatory sciences in drug development was recognized in such discussions. At the same time in early 2007 the Division of Regulatory Sciences of the Pharmaceutical Society of Japan established a "Drug Evaluation Forum" bringing together industry, government, and university representatives. Academic participation in the discussions allowed pharmaceutical companies and representatives of the Pharmaceuticals and Medical Devices Agency to discuss practical issues while maintaining neutrality and impartiality concerning the process of new drug approval. A significant industry-government-university discussion on the theme of the safety assessment of metabolites took place in January 2007 at the kick-off meeting of this Drug Evaluation Forum. The viewpoints and issues regarding future safety testing of metabolites have now been compiled and are presented in this manuscript. The opinions were gleaned from the discussion at this kick-off meeting concerning the significance and method of metabolite safety assessment with a view to promoting the safety of drugs and identifying their effects in living subjects.

DRUG METABOLISM AND MANIFESTATIONS OF TOXICITY

Drug-metabolizing enzymes and problems associated with metabolites

In addition to exhibiting various effects, drugs administered to living subjects are in many cases excreted after being metabolized in the liver and other organs by enzymes associated with drug metabolism. There are ethnic, individual, gender, and other types of variation in drug-metabolizing enzymes associated with genetic factors, environmental factors and other factors such as clinical condition, concomitant medications, diet, and individual variability.

These considerations reveal major challenges to the safety assessment of metabolites in humans. Moreover, some adverse reactions or adverse effects occurring in humans are associated with the production of "reactive metabolites". However, since reactive intermediates are readily metabolized and may be present only as intermediates in the process of metabolism, it is often difficult to detect reactive metabolites and to supply stable synthesized reference standards. Therefore, in light of the factors exhibiting inter-individual variability referred to above, there are major difficulties with demonstrating the presence of reactive metabolites and with linking them to the mechanisms or manifestations of toxicity. In addition, although the safety of metabolites can in principle be assessed from animal experiments using a parent compound, there are cases in which quantitative or rate-related species differences exists in process of metabolism, or in which unique human metabolites and pharmacologically active metabolites (particularly reactive metabolites) are formed. These cases present significant challenges in assessing the safety of metabolites.

Although the substrate specificity of the CYP1, CYP3, and CYP4 families is relatively well-preserved in both humans and laboratory animals, the CYP2 family exhibits species differences due to its poor substrate correlation (Table 1). In addition, genetic polymorphisms are known to exist among drug-metabolizing enzymes, and effects such as the regulation of enzyme

induction or inhibition by exogenous or endogenous substances have also been identified. Furthermore, it is believed that some of these enzymes are affected by clinical conditions or diseases, concomitant medications, age, smoking, diet, and other circumstances of daily life.

Metabolic activation of drugs and manifestations of toxicity

Examples of metabolic reactions and metabolite formations that may lead to manifestations of toxicity are given in Table 2. In metabolic activation, toxicity is manifested as a result of radical formation (active oxygens, active nitrogens), modification of ribonucleic acids (mutagenicity, carcinogenicity), enzyme inhibition and induction, oxidative phosphorylation, electron transport chain inhibition, inhibition of the liberation or uptake of neurotransmitters (binding with transporters etc.), binding with or modification of receptors (receptor function changes), inhibition or acceleration of ion channels, and binding with or modification of proteins, lipids, and other components of living organisms. In addition, the formation of reactive metabolites (pharmacologically active metabolites linked to manifestations of toxicity) is involved in some adverse reactions, presenting a major challenge to the safety assessment of metabolites (Fig. 1). For example, *N*-acetyl-*p*-benzoquinone-imine, a pharmacologically active metabolite of acetaminophen, has been inferred to cause liver damage through allylation of mitochondrial proteins and other effects. Troglitazone, which has a chroman ring, may also cause liver damage through the formation of semi-quinone radical-mediated quinones and quinone methides in the process of oxidation reactions, as well as through the formation of α-ketoisocyanate by an oxidation reaction at the thiazolidinedione ring site (Yamata *et al.*, 2006). In cases in which glutathione conjugates and acyl glucuronide conjugates are detected as metabolites, concerns exist regarding the possibility of reactive metabolite formation, since there have been cases in which manifestations of toxicity such as serious organ damage were triggered by highly reactive metabolites. Many of the drugs for which concerns exist regarding the possibility of relationships between reactive metabolites and liver damage have not been approved in the US, and of the drugs deemed to have reactive intermediates, five have had approval withdrawn or restrictions on use imposed, and another eight have had black box warnings added to their labels (Table 3). However, given the difficulty of assessing the safety of reactive metabolites despite the conduction of toxicity studies using the glutathione conjugate, acyl glucuronide conjugate, or other final metabolites, there is at present no clear basis for ascribing adverse events induced by such drugs to reactive metabolites. Given the difficulty in detecting reactive metabolite-induced adverse events in clinical studies, it is preferable to reduce the human risks associated with the development of compounds by screening for metabolic stability and pharmacologically active metabolites at the exploratory stage of drug development, and minimizing the development of compounds with reactive metabolites that may cause concern.

In a 2006 questionnaire survey sponsored by the Japanese Society for the Study of Xenobiotics, it was revealed that many pharmaceutical companies are making efforts to detect reactive metabolites through screening early to avoid developing compounds that could cause reactions associated with toxic effects of reactive metabolites. For example, companies perform experiments assessing time-dependent enzyme inhibition reactions; covalent binding to tissue, liver microsomal covalent binding, and GSH adduct formation. Such efforts at screening are expected to reduce

Table 1. Classification of Cytochrome P450 Drug-Metabolism Enzymes.

CYP1 Family:	Two subfamilies (CYP1A1, CYP1A2, and CYP1B1 are expressed in humans): AhR-mediated induction of 1A, involvement in metabolic activation of carcinogens by epoxidation or *N*-hydroxylation, and so on
CYP2 Family:	Many subfamilies (10 types of subfamilies are expressed in humans, including CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, CYP2F, and CYP2J): Major enzymes for metabolism of various drugs with different structures as substrates for CYP2C9, 2C19, 2D6
CYP3 Family:	One subfamily; Induced by steroids and macrolide antibiotics; testosterone 6β- hydroxylation is a prototype; CYP3A4, CYP3A5, and CYP3A7 exist in humans; CYP3A is the most abundant species in human liver
CYP4 Family:	Four subfamilies, catalysis of ω- hydroxylation of fatty acids

the risks associated with development of compounds that form reactive metabolites and that may trigger idiosyncratic drug toxicity (IDT, Yamada *et al.*, 2006).

Clinical problems concerning the manifestation of adverse reactions in pharmacotherapy

Concomitant use of drugs is a daily occurrence in medical practice, and the number of prescriptions given to an individual increases with age as well as the incidence of adverse reactions increases with age. Metabolic profiles vary significantly partly as a result of drug-drug interactions, suggesting that metabolites may also be involved in the manifestation of adverse reactions. Accordingly, while the properties of metabolites constitute essential information for the safe use of drugs, assessment of metabolite toxicity in humans should be based on properties such as the amounts of metabolites formed or their activity.

The results of a meta-analysis published in 1998 by JAMA, (Journal of the American Medical Association; Lazarou *et al.*, 1998), found that serious drug-induced adverse reactions occur in approximately 2.2 million patients per year, and that of these patients as many as 100,000 suffer life-threatening adverse reactions. While the extent to which the toxicity of metabolites is involved in drug-induced adverse reactions is unclear, these reactions are recognized as a major clinical problem. Post-marketing "Dear Doctor Letters" written after the time of approval include reported adverse events occurring after the clinical trial stage and those events not previously identified or described as "severe adverse reactions". In the case of quetiapine fumarate, edaravone, pioglitazone HCl, cephocelis sulfate, and troglitazone, new and severe adverse reac-

Table 2. Metabolic reaction or metabolite production leading to toxicity.

N-hydroxylation (hepatic tumor induction by acetylaminofluorene, hepatotoxicity of acetaminophen, methemoglobinemia by aniline, and some others)
Epoxidation (induction of hepatic tumor or hepatotoxicity by aflatoxin, hepatotoxicity by bromobenzene)
Desulfuration (neurotoxicity by organophosphorus insecticides)
Aldehyde or ketone production (toxicity of ethanol, zomepirac, hexon, etc.)
α, β- Unsaturated aldehyde or ketone production (toxicity of acrolein, benzene, 4-hydroxynonenal, etc.)
Quinone or quinoneimine production (toxicity of acetaminophen or diethylstilbesterol)
Sulfoxide production (hepatotoxicity of thioacetamide)
Acylhalide production (toxicity of halothane or chloroform)
Thionoacylhalide production (renal toxicity of hexachlorobutadiene)
Thioketene production (renal toxicity of hexachlorobutadien)
Radical production (hepatotoxicity of carbon tetrachloride or halothane)
Carbonium ion production (carcinogenesis by nitrosamine compounds, induction of hepatic cancer or hepatotoxicity by cycasin)
Nitronium ion production (carcinogenesis by acetylaminofluorene)
Sulfonium ion production (carcinogenesis by 1, 2-dibromoethane)
Metal ion production (renal toxicity of mercury or cisplatin)

tions not noted during the clinical trial stage were subsequently observed in the post-marketing database. In addition, even when the metabolites themselves lack toxicity, interactions in the processes associated with metabolism can sometimes trigger life-threatening drug interactions, e. g., concomitant administration of 5-FU with sorivudine.

To ensure appropriate use of drugs, it is essential to identify fully both factors associated with the drug such as drug receptors, metabolic enzymes, drug transporters, and those that predispose patients such as genetic factors (gene polymorphisms), environmental

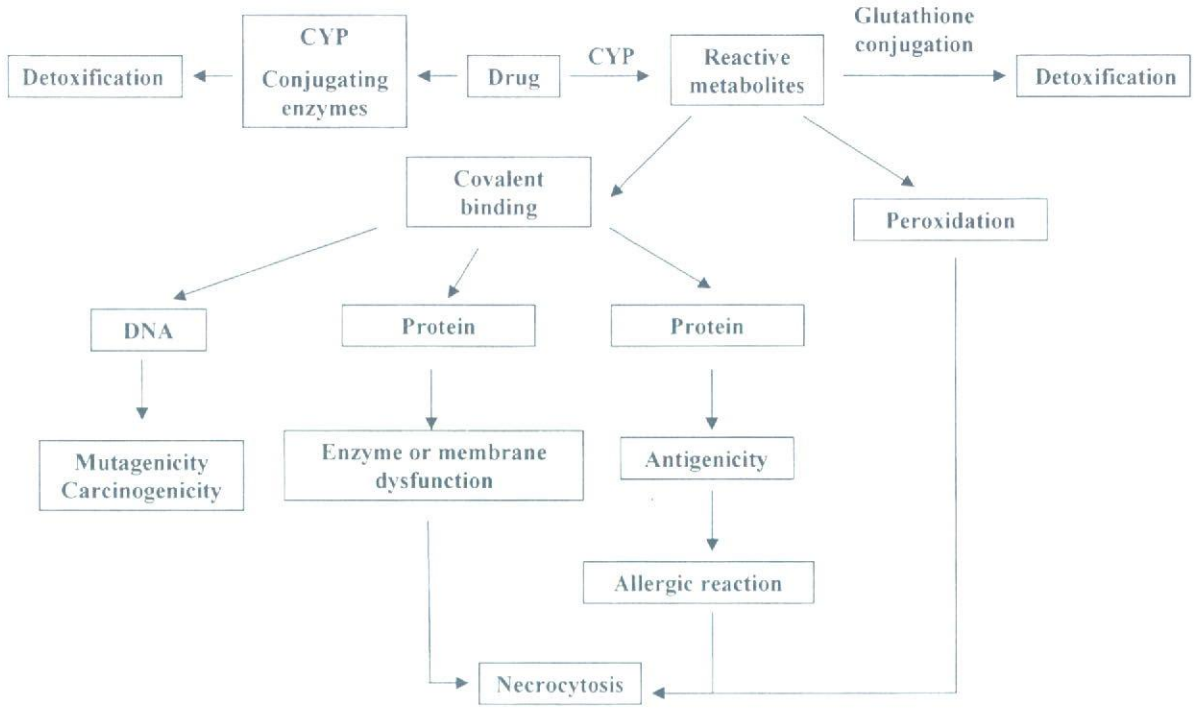

Fig. 1. Metabolic activation and development of toxicity by drugs and chemicals.

Table 3. Hypothesis of Hepatotoxicity of reactive metabolites.

Fourteen drugs with reactive metabolites that have warnings regarding hepatotoxicity
Acetaminophen, Carbamazepine, Clozapine, Diclofenac, Disulfiram, Halothane, Leflunomide, Methyldopa, Rifampin, Tacrin, Tamoxifen, Terbinafine, Ticlopidine, Zileuton

Fourteen drugs with reactive metabolites that have warnings regarding hepatotoxicity & have never been approved in USA
Alpidem, Amineptine, Amodiaquine, Cinchophen, Dihydralazine, Dilevaolo, Ebrotidine, Glafenine, Ibufenac, Isoxanine, Niperotidien, Perhexiline, Pirprofen, Tilbroquinol

Five drugs withdrawn or with restrictions on use due to reactive metabolites
Benoxaprofen, Iproniazid, Nefazodone, Tienilic acid, Troglitazone

Eight drugs with black-box warnings due to reactive metabolites
Dacarbazine, Dantrolene, Felbamate, Flutamide, Isoniazid,
Ketoconazole, Tolcapone, Valproic acid

factors, diet, alcohol use, smoking, medical disorders, and concomitant use of medications. The findings of post-marketing observational studies are thus vital for determination of the appropriate use of drugs. In addition, effective and prompt communication of these findings is essential so that the information needed at the medical frontline is supplied to ensure that package inserts, containing basic pharmaceutical data, function as real-time sources of information. To this end, the PMDA framework should be reinforced and detailed post-marketing information gathered for analyses of the pooled data.

NON-CLINICAL SAFETY ASSESSMENT OF METABOLITES

Efforts in Japan

At present, no guidance is available in Japan on the performance of toxicity studies with drug metabolites, so these are handled on a case-by-case basis. The basic viewpoint is that safety verification is required in cases such as the following: (1) those in which metabolites not found in animals occur in humans, (2) those in which the ratios of the metabolites formed in humans and animals differ and metabolites are formed in greater numbers in humans (and where safety cannot be assured on the basis of the metabolite exposure in animal studies), and (3) those in which metabolites considered to have very strong pharmacological or toxicological effects are formed. In cases in which the metabolite of concern is a known substance and its safety profile is clear, or in which the metabolite concerned is deemed to be pharmacologically inactive, or in which the amount formed within the body or exposure to it is believed to be very low, verification of the safety of metabolites may not be required.

The data for new drugs approved in recent years (2000 ~ 2006) were investigated as to the safety assessment of metabolites, study details and rationales, and trends toward assessment of the safety of metabolites. It was found that metabolite safety testing had been conducted on 48 (39%) of the 123 drugs containing new active ingredients (excluding recombinant drugs and biological preparations etc.). Of the 48 products that were investigated for metabolite safety, 44 single-dosing studies (92%) were performed, 7 repeat-dosing studies (15%), and 15 genotoxicity studies (31%) (with some duplication). The most common reason for performing the studies which was given for 44 studies was "It is the major metabolite". Other reasons given were: "It is a unique human metabolite"; "Since virtually no metabolites were formed in rats"; "Since the metabolite was suspected to exhibit genotoxicity"; and "Reason unknown". In the studies investigated, no products were found to have toxicity associated with metabolites that greatly exceeded that of the parent compound. These findings provide further evidence that the number of products for which metabolites exhibit significantly higher toxicity than the parent compound is very small. Nevertheless, these data were obtained for approved drugs, and thus potentially hazardous products suspected of a high degree of metabolite toxicity may have been among the dropouts in the course of drug discovery and development. Future considerations of metabolite toxicity may have been necessary if these dropouts had continued in development.

Safety assessment of metabolites

The process of drug metabolism, excluding prodrugs, generally involves detoxification leading to reduction of pharmacological activity and elimination of the compound. Therefore, cases in which toxicities of a metabolite are completely different from those of the parent compound are believed to be rare. Furthermore, as shown by the examination of new drug approval data referred to above, very few products were noted to have unique human metabolites or to form metabolites that had significantly higher toxicity than the parent compound, thus it is believed that these cases are very rare. However, in some cases in clinical practice there have been concerns regarding manifestations of toxicity ascribable to metabolites, so safety assessment of metabolites found in humans appears to be necessary in these cases. In the FDA draft guidance, the rationale given for the 10-percent criterion in requiring safety assessment of metabolites is based on the amounts of metabolites formed with halothane, felbamate, cyclophosphamide, and acetaminophen; although other examples were provided in which signs and symptoms of toxicity were found with amounts of metabolites less than 10 percent. Accordingly, metabolites found only in humans should basically be subject to safety assessment using some type of method. Furthermore, since in some cases metabolites present in amounts less than 10 percent have been involved in manifestations of toxicity, investigations of the toxicity of these metabolites are called for. In this connection, we have investigated below cases in which toxicity studies of metabolites would be required.

1) Definition and method of assessment of metabolites

Phase I metabolites are formed through reactions

of oxidation, reduction, and hydrolysis, and Phase II metabolites through conjugation reactions. With the exclusion of *N*-acetylization, metabolism involves reactions leading to more polar compounds. Of the conjugate metabolites formed from Phase II reactions, glucuronide conjugates are generally less active than the parent compound. This is thought to be due to the significant molecular modification and increased polarization of aglycones, the decreased affinity of conjugates for receptors and enzymes due to steric hindrance, their reduced membrane permeability (tissue transferability), and the increased ease of their excretion (reduced exposure). It is therefore generally assumed that metabolite toxicity studies using conjugate metabolites are not required. However, cases exist in which there have been concerns over the formation of reactive metabolites in the form of acyl glucuronides or glutathione conjugates, or in which compounds such as irinotecan glucuronide conjugate are deconjugated by enteric bacteria in the intestinal tract after biliary excretion (Takasuna *et al.*, 1996). Thus, adverse reactions need to be fully identified in clinical studies.

Pharmacologically active metabolites are defined as circulating metabolites that have some pharmacological activity. In cases in which metabolites have pharmacological activity similar to or less than the parent compound, it is assumed that safety data have been obtained in the toxicity studies with the parent compound. However, in cases in which the pharmacological activity of the metabolite is greater than that of the parent compound, or in which the metabolite has pharmacological effects different from those of the parent compound, considerations in deciding the necessity for metabolite toxicity studies should include the exposure in humans and any differences between humans and animals in plasma concentration of the pharmacologically active metabolites. In addition, when adverse reactions are noted in clinical practice that cannot be predicted from the parent compound, or when there are strong concerns over the possibility of a relationship between such reactions and a metabolite, the assessment of metabolite toxicity may be needed. Valuable information is sometimes obtained from *in vitro* studies on receptors, enzyme inhibition, or non-clinical *in vivo* metabolite toxicity studies.

Reactive metabolites are intermediate metabolites formed in the process of metabolism that undergo covalent reactions with other physiological substances (proteins etc.). In the safety assessment of reactive metabolites, the investigations undertaken and inferences made from the study results should be based on the test systems in which the reactions occur *in vivo*. It may thus be of little use to determine toxicity through administration to laboratory animals of the final metabolite conjugate instead of the reactive metabolic intermediate. In addition, given the difficulty of chemically synthesizing reactive metabolites formed as metabolic intermediates, *in vitro* toxicity studies incorporating metabolic systems (S9 [9,000 × g supernatant fraction], microsomes, hepatocytes, liver slices) should be considered and studies conducted to appropriately evaluate potential toxicities of the metabolites. However, estimation of the relationships that may exist between clinical adverse events and reactive metabolites is by no means easy; thus, caution should be used when interpreting and extrapolating the *in vitro* findings to clinical findings. Further, consideration of the pathway by which metabolism and elimination occurs is also essential in assessing metabolites. For example, since metabolites excreted in urine are prone to being excreted with polar radicals attached, it is preferable to examine metabolite structure rather than amount of excretion.

Given the differences of opinion found in the public comments studied by the JPMA concerning the 10-percent criterion for safety testing of metabolites described in the FDA draft guidance, and the discrepancy in the value of the metabolite formation ratio established by PhRMA that would require metabolite safety assessment (FDA CDER, Guidance for Industry, Safety Testing of Drug Metabolites (Draft), 2005), it would appear that no clear and agreeable general criterion has been established. Further, studies on humans using radiolabeled forms (RI) are required to determine the metabolite formation, although there are no requirements for these studies in Japan, human RI data have been submitted for many of the products applied for recently. The studies provide important metabolite information used in determining whether or not to conduct animal studies on metabolites or to examine the toxicity of processes of metabolism as they relate to the causes of toxicity obtained from the overall assessment of adverse reactions, signs and symptoms of toxicity, and other manifestations in clinical studies.

2) Safety assessment in laboratory animals

In cases in which metabolite exposure has already been demonstrated in the toxicity studies with the parent compound, follow-up metabolite toxicity studies are usually considered unnecessary. However, in cases in which plasma metabolite exposure in animals is far less than in humans, the metabolite safety

cannot be assured from the results of studies using the dosages of parent included in the animal studies. Therefore, verification of safety is required when there are concerns over the toxic effects of metabolites. In some cases, metabolite exposure may be able to be increased by changing the dosage of the parent compound administered to animals, the number of doses, the rate of administration, or the route of administration.

Even when the safety of unique human metabolites cannot be verified in animal toxicity studies of the parent compound, the safety will at least be demonstrated in clinical studies for the duration and exposure used in the clinical study in which the metabolites were detected. When this occurs, the safety assessment of unique human metabolites will follow a different strategy from that of the parent compound. The chemical structure of unique human metabolites should be identified and the plasma concentration in humans determined. *In silico* determination of toxicity based on structure-activity correlations may also be useful at this stage. In addition, data on adverse reaction reports from clinical studies is essential, and these may lead to further metabolite safety studies.

While the findings of clinical studies are useful, they are limited to results from non-invasive examinations, whereas in animal studies a final necropsy can be performed, allowing tissue damage to be pathologically identified. Such animal studies are therefore essential, in that data unable to be obtained in humans can be obtained in the animals. When adverse events in humans are noted, it is essential in some cases to attempt to correlate these with the results of non-clinical studies. In addition, in many cases it is useful if scientifically valid metabolite toxicity studies can be conducted. When unique human metabolites exist, it may also be useful in some cases to compare the toxicity of the parent compound to that of the metabolite in single-dose, two-week or other toxicity studies.

3) Issues related to metabolites in clinical practice

Drugs given in low doses clinically are generally assumed to yield low metabolite exposure and correspondingly few concerns regarding manifestations of toxicity. Conversely, drugs given in high doses clinically require attention to the possibility of adverse reactions ascribable to metabolites.

In addition, humans are genetically diverse and exhibit individual variations in levels of expression of metabolic enzymes or in metabolic activity. Furthermore, various types of disorders (hepatic, renal, cardiac etc.) may lead to significant variability in pharmacokinetics from that of normal subjects resulting in variations in the parent compound or metabolite plasma concentrations. In addition, drug interactions may occur resulting in variations in the extent of metabolite exposure depending on other factors such as concomitant medications, diet, or lifestyle-related diseases.

In cases in which the metabolite of concern is formed by metabolic enzymes known to exhibit ethnic variations, differences in exposure to the parent compound as well as metabolites between Japanese and other subjects may exist. In such cases, caution is needed in extrapolating data from clinical studies conducted elsewhere to Japanese subjects.

Timing of non-clinical studies on metabolites

The prompt supply of high-quality drugs to the medical frontline is of crucial importance to patients. Non-clinical studies are of vital importance in ensuring the safe administration of drugs in clinical studies. To this end, the timing of non-clinical studies is essential in the process of drug development. Ideally, during the exploratory stage prior to the first administration to humans (First Human Dose and Phase I clinical studies), screening for metabolic stability and for pharmacologically active metabolites should take place, so that compounds selected will give little cause for concern regarding metabolite toxicity. In addition, *in vitro* metabolic studies on S9, microsomes, hepatocytes, liver slices, and other samples obtained from the liver of humans, mice, rats, dogs, monkeys, and other species will allow metabolic profiles to be identified, metabolic species differences to be investigated, and further metabolite characterization using LC-MS/MS, LC-NMR, and other techniques. Based on these results, the decision on which animal species to use in non-clinical safety studies can be determined by comparing the degree of similarity in human and animal metabolism (metabolite formation, metabolic rate). In addition, to avoid toxicity, it may also be useful to screen for reactive metabolites referred to above and to select compounds with minimal risk of formation of such metabolites.

Human metabolites formed *in vivo* can only be confirmed after administration of the drug to humans. In Phase I clinical studies, human samples (plasma, urine, feces) can be evaluated for the presence of metabolites and the metabolite's potential structure identified. Identifying the major human metabolites and assessing for the presence of any unique human

metabolites is of paramount importance. Once the potential metabolites are identified, then an estimation of their toxicity can be made by comparing the chemical structure of the metabolite with that of the parent compound. When appropriate, estimation of the changes in plasma concentrations of metabolites will also be essential. However, detection of changes in the metabolite concentration over time may require longer-term studies. A fully characterized synthesized reference standard of the identified metabolite will need to be provided, and further identification studies of metabolites separate from the clinical study may also need to be considered.

Studies using RI are useful for investigating the extent of metabolite exposure in humans. Microdosing (MD) studies using very low levels of RI may provide the ratio of the parent compound or metabolite to the total exposure in humans at an early date since the MD studies can often be conducted at an early stage of development. Use of MD studies facilitates the early detection of unique human metabolites or the need for further analyses of the metabolite concentrations, and results from these studies in conjunction with the TK analysis can provide useful information on the assessment of metabolite safety.

In cases in which there are strong concerns over the involvement of metabolites in toxicity in humans, it is necessary to carefully examine the findings available on metabolite safety prior to commencement of large-scale clinical studies (Phase III). The FDA guidance recommends that findings on the safety assessment of metabolites be submitted before commencing Phase III clinical studies, especially when unique human metabolites are found that present toxicological problems. The safety of these unique human metabolites in animals should be verified prior to Phase II clinical studies, and if possible, performance of human RI studies should also be considered early in development.

Prior to filing a new drug application (NDA), the metabolic enzymes and metabolic pathways of major human metabolites of a drug should be determined. The metabolite structure should be identified and the exposure in humans and animals should be determined using blood samples. The pharmacological activity of the metabolite should be assessed as well. Additionally, an understanding of whether or not the metabolites are unique to humans is essential and if so, then additional studies may be warranted.

After launch of a new drug, post-marketing surveillance studies should be carefully performed and are essential to verify drug efficacy and safety in daily medical care as they provide a means to collect information on the appropriate use of drugs unable to be obtained in clinical trials prior to marketing (Table 4). In particular, serious adverse events reported after a new drug launches may include those which non-clinical or clinical study results did not predict. In this connection, for adverse events found in clinical practice, it may be necessary to carefully examine unpublished results and results not available at the time of the application for causal links.

Table 4. Findings of post-marketing surveillance.

Safety and usefulness on the market
Long-term toxicity that affects individuals up to the next generation
Extremely rare, serious, or unpredictable adverse effects
Frequency of adverse effects (time course)
Identification of new types of efficacy
Off-label use
Overdose
Medical economics of drug treatment

Issues concerning methods of metabolite assessment

Studies will sometimes have to be conducted to assess metabolite safety. However, in some cases adequate exposure cannot be obtained even with administration of the metabolite to animals using the route of administration of the parent compound, or only transient exposure can be obtained with intravenous treatment. Exposure to metabolites may require significant modification through such methods as changing the route of administration, the dosage, or the number of administrations, etc. However, notwithstanding the apparent degree of exposure in the systemic circulation, changes in tissue transferability associated with such factors as plasma protein binding, membrane permeability, or affinity for transporters may reduce exposure in the target tissue, resulting in an underestimation of the toxicity. Moreover, the toxicity of reactive metabolites that give cause for concern may not be able to be studied by administering the conjugate metabolite to animals. There are also technical problems, in that chemical synthesis of reactive metabolites and glucuronide conjugates, etc. is often difficult and synthesized reference standards of metabolites are sometimes impossible to prepare. In view of the practical problems such as these, studies of the feasibility of the safety assessment of metabolites are required. In some cases, the profile of toxicity of a metabolite will have to

be determined from numerous factors, including *in silico* predictions from chemical structure databases or *in vitro* studies incorporating human metabolic systems. The safety assessment of metabolites requires consideration of complex factors, and even when safety testing of metabolites is deemed unnecessary, a full explanation of the rationale will need to be provided.

FUTURE DEVELOPMENT

In the interest of delivering high-quality drugs to patients as quickly as possible, safety assessment of metabolites was discussed at the kick-off meeting of the Drug Evaluation Forum, with reference to the views of clinicians and other academic representatives, in order to provide useful information for developers and reviewers. In the course of drug development, safety in humans is predicted through means such as metabolite profiling and pharmacological activity screening, clinical pharmacokinetic studies, human RI studies, in addition to the safety studies using metabolite reference standards which are conducted when deemed necessary. Although at present there are no guidelines for metabolite safety testing in Japan, 40 percent of the compounds in licensed products have undergone some form of safety testing of metabolites, and few metabolites unique to humans or with significant toxicity appear to have been found. However, these findings are based only on products approved as new drugs and do not include cases where development of a compound was discontinued. Indeed, it is likely that a large amount of valuable information on metabolites exists in the latter cases. Accordingly, with the cooperation of pharmaceutical companies, it is essential to collect and pool data on the toxicity of metabolites, especially negative data. Establishment of a standard database could be part of the consideration when drafting of guidelines, etc., and preferably a database that will facilitate worthwhile safety assessment. In Japan, as well, pharmaceutical companies would create databases, including negative data, based on consensus regarding their role in contributing to the needs of society through the prompt supply of safe and effective drugs to patients. In addition, on the regulatory sciences front, post-marketing surveillance enables prompt communication of findings to prescribers and patients, and it is also advisable to collect data on metabolites during clinical trials.

It is of vital importance to analyze metabolites using human samples obtained in clinical studies and to determine whether unique human metabolites are major metabolites. However, since these tasks may also constitute a rate-limiting factor in drug development, in some cases drug risk-benefit considerations may require selection of the optimal strategy through use of PMDA consultations or other services. In general, even when unique human metabolites or major metabolites are found in clinical studies, these studies are often single-dose, with smaller dosages than in non-clinical safety studies, or the safety investigations are based on short-term clinical studies with few reported adverse reactions. It therefore cannot always be assumed that the safety of metabolites in humans has been verified. Moreover, after approval, a marketed drug will be used by large numbers of patients and in some cases will be administered long-term. The principal importance of safety studies is identification of the toxicity profile and estimation of safety in humans after administration of high dosages for adequate periods of time. Further, since in the course of drug development the human metabolite profile is often identified around the conclusion of clinical Phase I studies, it is conceivable that development may be slowed when results on metabolites are obtained. On the other hand, MD studies may be helpful in metabolite profiling, and may accelerate assessment of human metabolites. In addition, reactive metabolites, even if a problem in clinical practice, are often unverifiable in ordinary toxicity studies. Provisions should be made for screening systems using human samples that may better evaluate the potential for reactive metabolites and allow a comparison of the findings on reactivity in humans to the findings in animals.

Clinically, a seemingly small finding with use of drugs in humans may have a large impact, so it is essential not to overlook problems that arise at the medical frontline. Future challenges will include how to reduce clinical risks based on considerations of whether clinical problems with less frequency can be detected in the non-clinical studies, or how clinical information should be used to impact basic research. Although compounds supplied to the medical frontline as drugs have been assessed for safety through toxicity studies, post-marketing clinical problems not observed during drug development may occur and may differ considerably from those found during daily usage by large populations. Therefore, feedback to drug researchers of findings from the medical frontline could, for example, be important information useful for improving drug development. Nevertheless, the incidence of actual problems in patients is very low, and these may be problems that cannot be detected by

the non-clinical studies. Accordingly, in the event of adverse effects, it is essential to act as quickly as possible to find ways to understand the mechanism behind the event.

In these terms, the drafting of strict guidelines on the safety assessment of metabolites presents considerable difficulties. It may be preferable to prepare flexible guidelines to speed the development of useful drugs. However, while such guidelines should be scientifically valid, it may be difficult to establish fixed criteria for assessment of the safety of metabolites. The safety assessment of metabolites cannot readily be based on a single theoretical framework, and basically a case-by-case approach is called for. However, some common understanding should be shared. To this end, it is useful to first prepare an outline concept paper. A collective viewpoint is also relevant to any discussion on the need for guidance, etc. A degree of flexibility may also be helpful for drug development and, in turn, society at large. The safety of metabolites should be assessed using scientifically sound methods and take into account signs, symptoms and adverse reactions that may be unpredictable in clinical studies on the parent compound, but may be related to the metabolites. In addition, elimination of scientifically meaningless studies for the safety assessment of metabolites is essential for the prompt supply of high-quality drugs to the medical frontline.

ACKNOWLEDGMENT

Our sincere thanks to those who helped to promote the kick-off meeting of the Drug Evaluation Forum (Masataka Mochizuki, President of Kyoritsu College of Pharmacy; Satoshi Toyoshima, Director of the Center for Product Evaluation, Pharmaceuticals, and the Medical Devices Agency; Kazuhiko Nakashima, Chairperson of the Drug Evaluation Committee, JPMA; Toshihiko Kobayashi, Japan Technical Representative of the Pharmaceutical Research and Manufacturers of America; and Hajime Iwasaki, European Federation of Pharmaceutical Industries and Associations Japan), Taskforce 11 of the JPMA Non-clinical Evaluation Subcommittee of Drug Evaluation Committee, and those concerned at EFPIA-J and PhRMA-J. Our sincere thanks also to Kazuko Soshiki and all members of the Kyoritsu College of Pharmacy, the co-sponsor, for their tremendous efforts in running the Forum. In addition, our sincere thanks to all those who gave pertinent advice and polite comments on numerous occasions in the preparation of this summary.

REFERENCES

Baillie, T.A., Cayen, M.N., Fouda, H., Gerson, R.J., Green, J.D., Grossman, S.J., Klunk, L.J., LeBlanc, B., Perkins, D.G. and Shipley, L.A. (2002): Drug metabolites in safety testing. Toxicol. Appl. Pharmacol., **182**, 188-196.

Baillie, T.A., Cayen, M.N., Fouda, H., Gerson, R.J., Green, J.D., Grossman, S.J., Klunk, L.J., LeBlanc, B., Perkins, D.G. and Shipley, L.A. (2003): Reply. Toxicol. Appl. Pharmacol., **190**, 93-94.

CPMP/EWP/560/95, 17, December (1997): The note of guidance on investigation of drug interactions, Committee for Proprietary Medicinal Products.

Davis-Bruno, K. L. and Atrakchi, A. (2006): A regulatory perspective on issues and approaches in characterizing human metabolites. Chem. Res. Toxicol., **19**, 1561-1563.

FDA CDER Guidance for Industry, Published in Federal Register, March (2003): Guidance for Industry; Bioavailability and Bioequivalence Studies for Orally Administered Drug Products - General Considerations.

FDA CDER Guidance for Industry, Published in Federal Register, June (2005): Guidance for Industry, Safety Testing of Drug Metabolites (Draft).

FDA CDER Guidance for Industry, Published in Federal Register, September (2006): Guidance for Industry; Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling (Draft).

Guengerich, F.P. (2006): Safety Assessment of Stable Drug Metabolites. Chem. Res. Toxicol., **19**, 1559-1560.

Hastings, K.L., El-Hage, J., Jacobs, A., Leighton, J., Morse, D. and Osterberg, R.E. (2003): Letter to the Editor. Toxicol. Appl. Pharmacol., **190**, 91-92.

Humphreys, W.G. and Unger, S.E. (2006): Safety assessment of drug metabolites: Characterization of chemically stable metabolites. Chem. Res. Toxicol., **19**, 1564-1569.

Inoue, T., Harada, Y., Furuta, S., Kawamura, Y., Kurihara, A., Kurokawa, M., Nakazawa, T. and Sagami, F. (2005): The industry's concerns on the inquiries derived from the review of non-clinical studies - Analysis of questionnaire survey data by JPMA, Pharm. Regul. Sci, **36**, 388-397.

Iyakushin no. 496, Jun. 26th, 1998: Guidance for non-clinical Pharmacokinetic studies.

Lazarou, J., Pomeranz, B.H. and Corey, P.N. (1998): Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. JAMA., **15**, 279, 1200-1205.

Ohno, Y. (1992): Toxicity testing: Regulatory Perspectives. Proceedings of the First International Conference on Harmonisation. 1991 Nov.; Brussels, Belgium. Ed D'Arcy PFD and Harron DWG. The Queen's University of Belfast. 186-188.

Prueksaritanont, T., Lin, J.H. and Baillie, T.A. (2006): Complicating factors in safety testing of drug metabolites: Kinetic differences between generated and preformed metabolites. Toxicol Appl Pharmacol., **217**, 143-152.

Smith, D.A. and Obach, R.S. (2005): Seeing through the mist: Abundance versus percentage. Commentary on metabolites in safety testing. Drug Metab Dispos., **33**, 1409-1417.

Smith, D.A. and Obach, R.S. (2006): Metabolites and safety: What are the concerns, and how should we address them? Chem. Res. Toxicol., Dec., **19**, 1570-1579.

Takasuna, K., Hagiwara, T., Hirohashi, M., Kato, M., Nomura, M., Nagai, E., Yokoi, T. and Kamataki, T. (1996): Involvement of β-glucuronidase in intestinal microflora in the intestinal toxicity of the antitumor camptothecin derivative irinotecan hydrochloride (CPT-11) in rats. Cancer Res., **56**, 3752-3757.

The Japanese Society for the Study of Xenobiotics (JSSX, November 29th - December 1st, 2006, Tokyo): 21st Annual Meeting Abstracts 171-177.

The Pharmaceutical Society of Japan (Jan. 13th, 2007, Tokyo): Kick-Off Meeting of Drug Evaluation Forum - Toward an Innovative Medicine: From Non-clinical to Clinical. Abstracts.

Toxicology Q&A (2003): (Question 16 : Toxicological evaluation of unique human metabolites), Pharm. Regul. Sci, **34**, 794-795.

Yakushin no. 526, March 28th, 1975: Points to Consider for Approval Application Data for New Manufacture or Import Drugs.

Yamada, H., Yamaguchi, J., Iida, I. and Okuyama, S. (2006): Idiosyncratic drug toxicity, Folia Pharmacol. Jpn., **127**, 473-480.

—Reviews—

薬学研究における動物実験代替法研究の重要性とその問題点

大野泰雄

Importance of Research on Alternatives to Animal Experiments in Pharmaceutical Sciences

Yasuo OHNO

National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo, 158-8501 Japan

(Received January 11, 2008)

The Japanese animal protection law was amended in 2005 to include the 3Rs principle in animal experiments. According to this new law, the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labor and Welfare, and the Ministry of Agriculture, Forestry and Fisheries developed announced several guidelines in 2006. These guidelines indicated responsibility of the president of each research institute conducting animal experiments to meet obligating of the animal experiment committee (AEC) and the education to be provided to scientists. About half a year after this notification, I conducted a survey on how these guidelines were put into practice in the pharmaceutical colleges and universities. I received 29 answers from 24 institutes. It seemed that every institute was following, the guidelines, however, there were many institutes where the details were inadequate. For example, questions on the existence of alternative methods and degree of distress and pain were not asked in some questionnaires sent to the AEC. Education on proper conduct of animal experiments (3Rs, methods to evaluate and decrease distress and pain, and methods of euthanasia) was not conducted in many institutes. Further improvement seems necessary.

Key words——animal experiment; alternative; japanese animal protection law; guideline, ethical committee

1. 序言

生物現象を明らかにし，医薬品や化学物質等のヒトへの影響を研究する薬学において，動物実験は基本的研究材料として欠かすことはできない（Table 1）．その結果得られた知見を基に医薬品開発を行う際にも，その有効性と安全性の確認には動物実験に頼らざるを得ないところが多い．また，生命現象を理解することなく，医薬品の適切な使用は行えないが，薬剤師教育の有効な手段としても，動物実験は重要である．

一方，動物実験における動物福祉に関する社会の関心は極めて高く，動物愛護や動物の権利を擁護するため多くの関連団体が設立され，活発な活動が行われている．しかし，Figs. 1-3 に示したように，動物実験への反感の程度はその目的や使用する動物の種類，また，実験の行い方により異なる．適切な説明が行われることにより，必要かつ適切な動物実験に対する同意が増加すると思われる．すなわち，動物実験に係わるすべての研究者及び教育者は，そ

国立医薬品食品衛生研究所（〒158-8501 東京都世田谷区上用賀 1-18-1）
e-mail: ohno@nihs.go.jp

本総説は，日本薬学会第 127 年会シンポジウム S40 で発表したものを中心に記述したものである．

Table 1. Sale of Experimental Animals (JSLAS, 2004)

Species	Conventional	Clean	SPF	Sum
Mouse	232589	1285337	4751033	6268959
Rat	43839	449854	2062193	2555886
Other rodents	48		7468	7516
Guinea-pig	11514	227718	66293	305525
Hamsters	4486	963	32744	38193
Rabbit	23015	69746	29300	122061
Dog	12689	70	0	12759
Cat	260	0	626	886
Monkeys	2248	0	0	2248
Pig	1228	130	0	1358
Goat	0	0	0	0
Sheep	35	0	0	35
Birds	5094	0	12199	17293
Total*	352467	2033893	6961860	9348220

* Include Suncus, ferret, amphibians, and fishs.

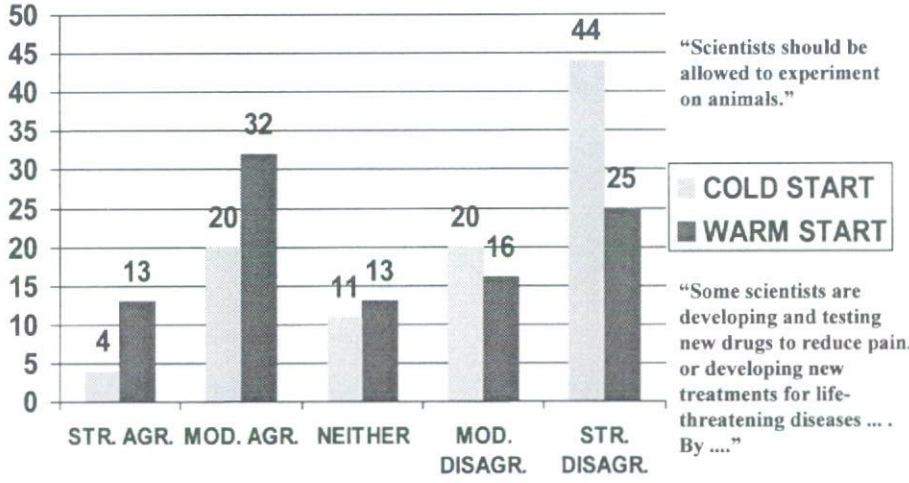

Fig. 1. Agreement on Animal Experiments (Difference Caused by Explanation)

(Aldhous P. *et al.*, New Scientist, 22 May, 1999, pp. 26–31), Light blue column: Explained as "Scientists should be allowed to experiment on animals." Blue column: Explained as "Some scientists are developing and testing new drugs to reduce pain or developing new treatments for life-threatening diseases... . by....", Ordinate: Answer (%), Abscissa: STR. AGR.: strong agreement, MOD. AGR.: moderate agreement, NEITHER: neither, MOD. DISAGR: moderate disagreement, STR. DISAGR.: strong disagreement.

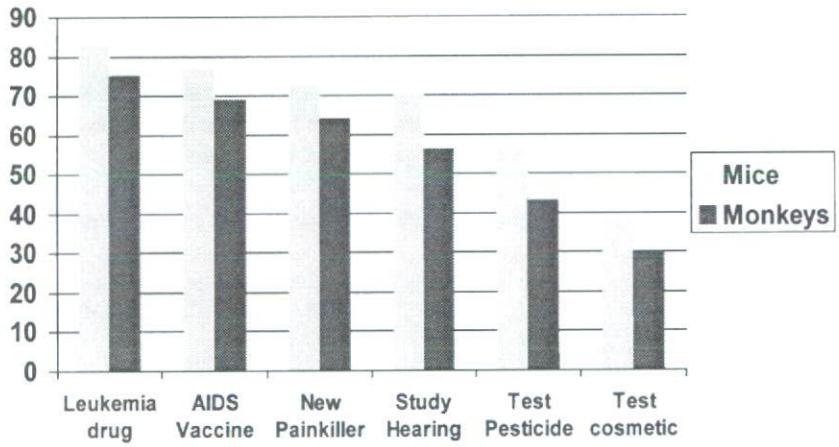

Fig. 2. Response to Animal Experiments (Difference Caused by Animalspecies and Object of Research)

(Aldhous P. *et al.*, New Scientist, 22 May, 1999, pp. 26–31), Yellow column: mouse, Blue column: monkey, Ordinate: Rate of agreement (%), Abscissa: Object of research.

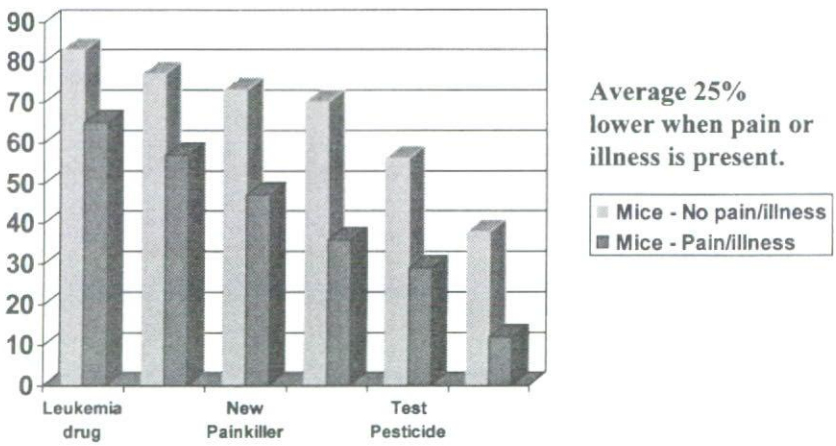

Fig. 3. Response to animal experiments (Difference by pain and illness)

(Aldhous P. *et al.*, New Scientist, 22 May, 1999, pp. 26–31), Yellow green column: Research without pain and illness, Red column: Research with pain and illness, Ordinate: Rate of agreement (%), Abscissa: Object of research.

の活動が社会の同意により許容され，支持されることにより，初めて可能となっていることを認識し，このような社会の動静に敏感に対応し，科学的・法的・倫理的に適切な研究を行わなくてはならない．

2. 動物実験の実施に関する指針

わが国においても科学研究においても動物福祉の尊重の必要性が認識され，平成17年6月の「動物の愛護及び管理に関する法律」（動愛法）の改定[1]で動物実験代替法に関する3R（Replacement，Reduction，Refinement）の原則が法に組み込まれた．また，平成18年6月までに，法令の目的を達成するため，環境省（環境省告示　第88号「実験動物の飼育及び保管並びに苦痛の軽減に関する基準」），[2] 文部科学省（文部科学省告示　第71号「研究機関等における動物実験等の実施に関する基本指針」），[3] 厚生労働省（厚生労働省通知　科発0601002号「厚生労働省の所管する動物実験等の実施に関する基本指針」）[4] 及び農林水産省（農林水産省通知「農林水産省の所管する研究機関における動物実験等の実施に関する基本指針」）[5] から実験動物の飼育・管理や動物実験についての指針が示された．日本学術会議からも動物実験に関する詳細指針「動物実験の適正な実施に向けたガイドライン」[6] が示された．

3Rの達成は一研究者の努力だけでは不可能であり，研究機関全体としての対応が不可欠である．そこで，上記指針には実施機関（研究機関）の長の責任が明確に示された（文部科学省告示　第71号「研究機関等における動物実験等の実施に関する基本指針」）．すなわち，実施機関の長は当該機関における動物実験等の実施に関する最終的な責任を有し，本指針に定める措置その他動物実験等の適正な実施のために必要な措置を講じること，と定められている．必要な措置とは，1）動物の愛護及び管理に関する法律（昭和48年法律第105号．以下「動物愛護管理法」という）や実験動物の飼養及び保管並びに苦痛の軽減に関する基準（平成18年環境省告示第88号以下「飼養保管基準」という．）並びに各省庁からの指針その他の動物実験等に関する法令等の規定を踏まえ，動物実験等の施設等の整備及び管理の方法並びに動物実験等の具体的な実施方法等を定めた規程を策定すること，2）動物実験委員会の設置，3）動物実験計画の承認，4）動物実験計画の実施結果の把握，5）教育訓練等の実施，6）自己点検及び評価，並びに7）適切な方法で動物実験等に関する情報公開を行うこと，と定められている．動物実験委員会は実施機関の長の諮問を受け，動物実験計画が本指針及び機関内規程等に適合しているか否かの審査を行い，その結果を実施機関の長に報告するものであり，また，動物実験計画の実施結果について，必要に応じ助言を行うものとされている．この委員会は1）動物実験等に関して優れた識見を有する者，2）実験動物に関して優れた識見を有する者，3）その他学識経験を有する者で構成される．また，動物実験実施に際しては，科学的合理性を確保し，目的を達成するに必要な適正な動物実験等の方法の選択や代替法の選択，苦痛の軽減について考慮するとともに，適切に維持管理された施設及び設備において動物実験等を実施するよう定められている．

3. 薬学会及び薬学系研究機関における動物実験への対応

日本薬理学会では以前より動物実験を巡る社会状況に対応するために動物実験指針の改訂作業を行い，法や通知の改訂の結果も取り入れ，これに反する研究結果を学会誌から排除することが明示された（大野泰雄 2007）．[7] しかしながら，薬学会では薬学雑誌の投稿規定に「ヒトならびに動物実験に関する倫理基準として，――，動物実験に関する報告も所属機関の定める動物実験ガイドラインに基づいて行われるのみならず，文部省（現文部省科学省）の策定したガイドライン，No. 141，1987年：“大学などにおける動物実験”に従って下さい」と古い指針を引用している．J. Health Sci. の投稿規定では「2」動物を対象とした論文は，所属機関の定める動物実験ガイドラインのみならず文部科学省など公的機関の策定したガイドラインに従って実施されたものに限り投稿を受け付けます．なお，当該論文はこれらのことを本文中に明記してください」とされているのみである．

一方，平成17年の日本薬理学会で動物実験結果をポスター発表した研究者にインタビューした結果では，医学部関係者の多くが，動物実験についての教育や動物実験委員会での審議について，明確に回答したのに対し，薬学系大学からの発表者の中には曖昧な回答者が多くいた．中には動物実験委員会が

Table 2. Suvey on the Existence of Committee for Animal Experiment in 2004

Institution		Exist	Not exist	%
Medical school	*	9	0	100
Pharmaceutical school	*	4	2	67
Agricultural school	*	1	0	100
CRO	*	1	0	100
Research institute	*	1	0	100
Private company	**	59	10	86

* Result of question to young scientists presenting posters at the annual meeting of The Japanese Pharmacological Society in 2004. ** Mutai *et al* (2004) Iyakuhin Kenkyu 35, 196–201.

存在しないとの回答もあった (Table 2：大野泰雄 2005)．[8] 平成 6 年に改訂された文部科学省の通知「大学等における動物実験について（文部省国際学術局長通知）」[9] では動物実験委員会の設置が明確に求められていたことから，動物実験委員会が存在しないとは考えられない．実際は存在しているが，適切に機能していないか，あるいは動物実験についての教育が若手研究者に十分に行われていないと推定された．今回，新たな動物愛護と管理に関する法令が改正され，省レベルの動物実験指針が示されたことから，薬学部における動物実験がどのように変わ

Table 3. Results of Survey on Animal Experiments in Pharmaceutical Schools

Number of answers 29
Number of Institutes 24

1) Type of Institutes

	Yes	Number of answers	%
State university	9	24	37.50
with Medical School	9	24	100.00
with veterinary School	3	24	33.33
Private University			
with Medical School	3	24	20.00
with veterinary School	0	24	0.00
Pharmacutical College	7	24	29.17

2) Position in the Pharmaceutical School

	Yes	Number of answers	%
students	0	29	0.00
Research assistant	2	29	6.90
Associate professor	7	29	24.14
Porfessor	20	29	68.97

3) Dose your school conduct animal experiments?

Yes	Number of answers	%
28	28	100.0

4) Approximate number of animals used in a year

	Sum	Number of answers	Mean
Mouse	119832	17	7049
Rat	39610	18	2201
Hamster	72	12	6.0
Rabbit	424	14	30.3
Dog	20	13	1.5
Moneky	0	13	0.0
Others	810	10	81.0

5) Do you conduct animal experiment?

Did you conduct animal experiment recently?

Yes	Number of answers	%
27	29	93.1

6) Do you know guideline notified by the Ministry?

Yes	Number of answers	%
27	29	93.1

If you knows,	Yes	Number of answers	%
7) Do you have the guideline?	23	28	82.1
8) Can you acces the guideline?	24	28	85.7

9) Do you know the guideline issued by Japanese Science Council?

Yes	Number of answers	%
27	29	93.1

If you knows,	Yes	Number of answers	%
10) Do you have the guideline?	21	27	77.8
11) Can you acces the guideline?	23	27	85.2

12) Does your institute have institutional guideline on animal experiments?

Yes	Number of answers	%
28	29	96.6

If there is,	Yes	Number of answers	%
13) Do you have the guideline	24	27	88.9
14) Can you acces the guideline?	24	26	92.3

15) Is there Animal Experiment Committee in you institute?

Yes	Number of answers	%
29	29	100.0

If there is,	Sum	Number of answers	Numbers/Committee
16) number of committee members	259	27	9.59
members from own institute	216	26	8.31

17) Members?

	Sum	Number of answers	施設当たりの人数
General public	20	16	1.25
Veterinarian	5	12	0.42
Expert of animal care*	10	14	0.71
Expert of animal experiment*	117	21	5.57
Expert of alternatives	3	10	0.30
Others	71	18	3.94

*: Other than veterinarian

18) How often do the committee meeting held?

	Sum	Number of answers	平均	
Number of times in a year	60.5	23	2.63	回

19) How often do you submit application of animal experiment to the committee?

	Yes	Number of answers	%
At each purchase	3	23	13.0
At each planning	19	23	82.6
Once in a year	13	23	56.5
twice in a year	1	23	4.35

20) Descriptions of application form

	Yes	Number of answers	%
Purpose of experiments	27	28	96.4
Study director	28	28	100.0
Study practitioner	28	28	100.0
Animal species	28	28	100.0
Animal number	26	28	92.9
Alternatives	20	28	71.4
Pain	21	28	75.0
Method to decrease pain	25	28	89.3
Reason not to decrease pain	21	28	75.0

21) Does your institute educate ethics on animal experiment?

Yes	Number of answers	%*1
26	29	92.86

If education is conducted,

22) To whom and how many hours do you educate?

	Total hours	Number of answers	average (h)	target of education (%)*2
Students	60	24	2.50	92.3
Post graduate students	41.5	22	1.89	84.6
Research stuffs	27.5	17	1.62	65.4
Others	2.5	2	1.25	7.7

23) Caliculum?

	Yes	Number of answers	%*3
Animal protection law	21	24	80.8
Physiology of experimental animals	17	24	65.4
Methods of animal care	22	24	84.6
3Rs principles	18	23	69.2
Evaluation of pain	18	23	69.2
Methods to decrease pain	19	23	73.1
Method of euthanasia	21	23	80.8
Alternative methods	15	23	57.7
Others	11	17	42.3

24) Have your institute received inspection on animal experiments by third party.

Yes	Number of answers	%
3	28	0.11

If you have,

25) By whom the inspection was conducted?

	Yes	Number of answers	%*4
Stuff	2	2	0.67
Outsiders	2	2	0.67

*1: calcuated on the number of institutes that conducted animal experiments.
*2: calculated on the total number of answers (26).
*3: caluculated on the number of instititues that conducted education on animal experiments.
*4: calculated on the number of inspected institutes.

ったかを知るため，平成19年の2–3月にかけてe-mailによるアンケート調査を行った．その結果は平成19年の薬学会年会でのシンポジウムで発表した．以下はその結果を示したものである．

アンケート調査の内容はTable 3に回答とともに示した．アンケートは筆者の友人である薬学部職員に直接e-mailで行い，29人（重複があったため24施設）から回答を得た．その結果をTable 3に示す．

動物実験委員会の設置状況を示したものであるが，今回はすべての動物実験実施施設に設置されていた．委員の数は6–10人が最も多かったが，16–20人というのも2施設あった．なお，委員の構成は動物実験の専門家が中心で一般人とその他が加わっているところが多く，獣医師や動物飼育及び動物実験代替法の専門家を委員としているところは少なかった．また，動物実験届けが年に1回のみの施設が56.5%を占めており，包括的な内容での審議が行われているものと推定される．一方，動物実験届けへ

Table 4. Classification of Pain and Distress Caused by Biomedical Experiments

Category A: Experiments involving either no living materials or use of plants, bacteria, protozoa, or invertebrate animal species.	Biochemical, botanical, bacteriological, microbiological, or invertebrate animal studies, tissue cultures, studies on tissues obtained from autopsy or from slaughterhouse, studies on embryonated eggs. Invertebrate animals have nervous systems and respond to noxious stimuli, and therefore must also be treated humanely.
Category B: Experiments on vertebrate animal species that are expected to produce little or no discomfort.	Mere holding of animals captive for experimental purposes; simple procedures such as injections of relatively harmless substances and blood sampling; physical examinations; experiments on completely anesthetized animals which do not regain consciousness; food/water deprivation for short periods (a few hours); standard methods of euthanasia that induce rapid unconsciousness, such as anesthetic overdose or decapitation preceded by sedation or light anesthesia.
Category C: Experiments that involve some minor stress or pain (short-duration pain) to vertebrate animal species.	Exposure of blood vessels or implantation of chronic catheters with anesthesia; behavioral experiments on awake animals that involve short-term stressful restraint; immunization employing Freund's adjuvant; noxious stimuli from which escape is possible; surgical procedures under anesthesia that may result in some minor post-surgical discomfort. Category C procedures incur additional concern in proportion to the degree and duration of unavoidable stress or discomfort.
Category D: Experiments that involve significant but unavoidable stress or pain to vertebrate animal species.	Deliberate induction of behavioral stress in order to test its effect; major surgical procedures under anesthesia that result in significant post-operative discomfort; induction of an anatomical or physiological deficit that will result in pain or distress; application of noxious stimuli from which escape is impossible; prolonged periods (up to several hours or more) of physical restraint; maternal deprivation with substitution of punitive surrogates; induction of aggressive behavior leading to self-mutilation or intra-species aggression; procedures that produce pain in which anesthetics are not used, such as toxicity testing with death as an end point; production of radiation sickness, certain injections, and stress and shock research that would result in pain approaching the pain tolerance threshold, *i.e.* the point at which intense emotional reactions occur. Category D experiments present an explicit responsibility on the investigator to explore alternative designs to ensure that animal distress is minimized or eliminated.
Category E: Procedures that involve inflicting severe pain near, at, or above the pain tolerance threshold of unanesthetized, conscious animals.	Use of muscle relaxants or paralytic drugs such as succinyl choline or other curariform drugs used alone for surgical restraint without the use of anesthetics; severe burn or trauma infliction on unanesthetized animals; attempts to induce psychotic-like behavior; killing by use of microwave ovens designed for domestic kitchens or by strychnine; inescapably severe stress or terminal stress. Category E experiments are considered highly questionable or unacceptable irrespective of the significance of anticipated results. Many of these procedures are specifically prohibited in national policies and therefore may result in withdrawal of federal funds and/or institutional USDA registration.

From guidance on the classification of pain and distress (2004) by The Japanese Association of Laboratory Animal Facilities of National University Cooperations (http://www.med.akita-u.ac.jp/~doubutu/kokudou/rinri/pain.pdf)

Table 5. Methods of Euthanasia (US Assoc. Veterinarians)

Species	Acceptable	Conditional acceptable
Cat	Barbiturates, inhalant anesthetics, CO_2, CO, potassium chloride in conjunction with general anesthesia.	N_2, Ar.
Dog	Same as above.	N_2, Ar, penetrating captive bolt, electrocution.
Non-human primates	Barbiturates.	Inhalant anesthetics, CO_2, CO, N_2, Ar.
Rabbit	Barbiturates, inhalant anesthetics, CO_2, CO, potassium chloride in conjunction with general anesthesia.	N_2, Ar, cervical dislocation (<1 kg), decapitation, penetrating captive bolt.
Rodents and other small manmals	Barbiturates, inhalant anesthetics, CO_2, CO, potassium chloride in conjunction with general anesthesia, microwave irradiation.	Methoxyflurane, ether, N_2, Ar, cervical dislocation (rats <200 g), decapitation.
Ruminants	Barbiturates, inhalant anesthetics, potassium chloride in conjunction with general anesthesia, penetrating captive bolt.	Chloral hydrate (IV, after sedation), gunshot, electrocution.
Swine	Barbiturates, CO_2, potassium chloride in conjunction with general anesthesia, penetrating captive bolt.	Inhalant anesthetics, CO, chloral hydrate (IV, after sedation), gunshot, electrocution, blow tothe head (<3 weeks of age).

AVMA Guidelines on Euthanasia (2007).

の記載内容については，代替法の有無，動物に与える苦痛の程度，及び苦痛軽減措置を取らない理由について記載しないところが25％以上あった．また，実験の目的そのものを記載しないとする回答が1件あった．動物実験倫理に関する教育については，92％の施設で行っており，行っていないところは29件の回答の内2件であった．教育内容については，3Rの原則や動物の苦痛の評価，苦痛の軽減方法，安楽死の方法，また，動物実験代替法について教えていないところが多くあった．なお，第3者による査察・調査が行われている施設が3施設あり，その内2施設では外部の者に依頼していた．

4. 結論

薬学部における動物実験実施状況と教育に関する今回のアンケート調査結果はすべての薬学部を網羅しているものではないが，大まかな傾向はつかめたものと思われる．動愛法の改定により3Rの原則が盛り込まれ，それを実行あるものとするため，指針が文部科学省をはじめとする行政機関から通知され，多くの施設で指針に基づき適正に行われていた．しかし，指針に示された動物実験委員会での適正な審議や研究者教育の内容に不十分なところもあると考えられた．それらの施設では今後の改善が望まれる．なお，倫理的な動物実験を行うための参考として，国立大学法人動物実験施設協議会が作成した苦痛の分類と米国獣医師会編が作成した安楽死の方法について，それぞれTable 4とTable 5に示した．

REFERENCES

1) Animal protection law, revised in June 2005
2) Ministry of Environment: Criteria on the housing, care, and reduction of pain of experimental animals. Notification No. 88. April, 2006.
3) Ministry of Education, Culture, Sports, Science and Technology, Basic principles of animal experiments in research institutes. Notification No 71, 1 June 2006.
4) Ministry of Health, Labor, and Welfare, Basic principles of animal experiments in research institutes that are administered by MHLW. Notification Kahatu 0601002, 1 June 2006.
5) Ministry of Agriculture, Forestry, and Fisheries, Basic principles of animal experiments in research institutes that are administered by MAFF. 1 June 2006.
6) Science Council of Japan, Guidelines for Proper Conduct of Animal Experiments. 1 June, 2006.
7) Ohno Y., *Folia Pharmacol. Jpn.*, **129**, 5–9 (2007).
8) Ohno Y., *Folia Pharmacol. Jpn.*, **125**, 325–329 (2005).
9) Ministry of Education, Culture, Sports, Science and Technology, Animal Experiments in Universities. Revised in 1994.